THEME SECTION

Evolution and ecology of marine biodiversity: mechanisms and dynamics

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Contribution to the Theme Section 'Evolution and ecology of marine biodiversity'

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Introduction

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ABSTRACT: The Theme Section on marine ecology and evolution resulted from a special symposium honouring Roger N. Hughes for his highly significant contributions as a scientist and teacher in the field of marine research. Contributions to this collection focus on evolution, animal behaviour, and population and community ecology, i.e. those areas to which Roger Hughes has contributed most notably.

KEY WORDS: Evolution · Ecology · Biodiversity

In the current scientific world obsessed with metrics such as citation indices, it might be more accurate to use an index of the 'reach' of a person's influence on the wider scientific community. This Theme Section was generated in tribute to the contribution and influence of Roger Hughes in the field of marine ecology. Roger has always described himself as a naturalist. As a small boy in Lancashire, he wandered the neighbouring fields, streams and ponds, observing wildlife. In his professional life, he has remained an observer of whole animals, with feeding behaviour a special interest. A wide range of taxa have been brought under the spotlight, including molluscs, crabs, bryozoans, fishes, and onychophorans. Evolutionary principles have inspired the major thrust of his work encompassing life-history and behavioural ecology, molecular biogeography, and phylogeny.

The papers collected in this Theme Section are diverse and reflect the extent of Roger Hughes' interests and the manner in which he has inspired former students and colleagues to pursue different avenues of specialisation. The papers within this Theme Section can be defined around the following key areas: evolutionary ecology, animal behaviour population, and community ecology, ranging from theoretical studies to the application of ecology for the management of natural resources.

Evolutionary studies

Roger Hughes' research has focussed on evolutionary processes, primarily using marine organisms as model subjects (and particularly those that are clonal). Shell shape has important implications for survival in relation to predation and physical processes such as wave action. Here, Walker & Grahame (2011) studied the relationship between shell shape and fitness in relation to brood size and found that subtle variation in shell shape is a potential predictor of fitness. Using another model organism, cyclostome bryozoans, Pemberton et al. (2011) examined the phenomenon of polyembryony (the splitting of a single sexually produced embryo into many clonal copies). They investigated the possibility that sperm limitation reduced female reproductive success at low population density using local colony density as a proxy for sperm supply. However, for colonies with broods, a score of colony weight and density suggested that sperm supply does not influence female reproductive success. Cannicci et al. (2011) extend the focus on evolutionary processes with a meta-analysis that investigates the current hypothesis that terrestrialisation of crabs from a water to a more land-based existence is reflected in the evolutionary tendency towards larger egg size and small brood size. They found no consistent evidence to sup-

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port this theory and instead hypothesise that major evolutionary steps at each ontogenetic stage are necessary to enable such a transition. Okamura et al. (2011) studied the variation in bryozoan zooid size in relation to fluctuations in temperature. The advantage of using modular organisms is that the response to temperature can be considered to be more consistent among replicates that are cloned and therefore genetically identical. Okamura et al. (2011) show how use of an index that relates zooid size to temperature can usefully inform palaeoecological studies that aim to gain insights into past temperature fluctuation.

Animal behaviour

A key area of Roger Hughes' interest was the study of how animal behaviour influences community structure. This is picked up in a number of contributions. Davenport et al. (2011a) studied the diet selection of sessile intertidal anemones that are ubiquitous on many rocky intertidal shores. Often cited as sessile predators or consumers of dissolved organic material, Davenport et al. (2011a) demonstrate that anemones consume a considerable amount of carrion that is dislodged or advected into the intertidal zone through wave action. Murray et al. (2011) investigated the factors that determine foraging decisions in the top predator in the system (humans). They studied the behaviour of a fishing fleet that targeted static benthic prey (scallops) and show how the fleet rapidly depleted the scallop population at the beginning of the fishing season and then adjusted its fishing behaviour to maintain catch rates. Davenport et al. (2011b) used the physics of fluid mechanics and direct observation to propose a physical mechanism that explains how emperor penguins exit seawater with sufficiently high velocity to propel them onto land. They propose that the air release from beneath the penguin's plumage reduces drag and hence increases velocity at the critical stage of ascent. Understanding animal movement and behaviour is particularly important in the context of fisheries management. Dando (2011) reports on a tagging study of flounder, a species that is assuming much greater commercial importance in inshore UK fisheries. The study demonstrated a surprisingly high level of site fidelity. Tagged fish made migrations up to 35 km west of their home estuary and then returned post-spawning. Gibson et al. (2011) examined depth fidelity in juvenile plaice *Pleuronectes platessa* and found a strong relationship between size and depth, such that smaller plaice occupy the shallowest depths. The study raises questions regarding the mechanism by which the fish are able to discriminate habitat characteristics at such a high resolution. Manríquez & Castilla (2011) studied the behaviour of competent larvae of the gastropod *Concholepas concholepas* and provide important insights into their larvae transport. The larvae display diurnal behavioural patterns and use byssus threads to attach themselves to buoyant particles in the water column or to air bubbles, or could utilise surface tension to maintain their position. Larvae become highly aggregated within surface foam slicks that form at fronts — areas of enhanced food supply.

Population and community ecology

Empirical studies of the importance of keystone species and the loss of biodiversity are important for improving our understanding of the role of species in ecosystem processes. Effects of biodiversity loss have been shown in a number of empirical studies (e.g. Emmerson et al. 2001) to be idiosyncratic. Crowe et al. (2011) demonstrated how the effect of removal of grazing and ecosystem engineering species is highly context dependent, which confirms the emerging view that environmental context is a key factor in the interpretation of such studies. Species invasions are another important process by which ecosystems can be modified. McGaw et al. (2011) report on the population demographics of green crabs — which have invaded many parts of the world through larvae transported in ship ballast water or via transfers of species used in aquaculture — in British Columbia, an area which they have recently invaded. The body size attained by the individuals was much larger than for populations elsewhere. This is likely explained by competitive release in the new environment. In their study of the impact of fishing on seabed habitats and associated biota off the coast of South Africa, Atkinson et al. (2011) found that fishing disturbance was linked to changes in community structure. Species that characterised the more heavily fished areas tended to be more mobile and smaller in body size than species from less intensively fished areas. Fishing disturbance has very negative influences on biogenic reefs, but our understanding of the ecological importance and temporal variability of such features is poor. In this theme, Hughes et al. (2011) have studied the taphonomy of serpulid worm tube reefs, which are a habitat subject to protection under European Union conservation legislation. The study revealed that the biogenic remains of the worm reef complex provide an important substratum for associated fauna. This material persists for periods in excess of 5 years. Hughes et al. (2011) hypothesise that these worm reefs can be ephemeral on a decadal timescale. Finally, Hart & Pearson (2011) focus on one of the most sensitive habitats in the world oceans, namely seamounts. They studied speciation on seamounts

across the globe. Poor sampling made it impossible to test the relationship between endemism and seamount age, although they did find that species number had a domed functional response to seamount age. They hypothesise that island biogeography provides a robust framework for constructing future sampling regimes for seamounts.

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Contribution to the Theme Section 'Evolution and ecology of marine biodiversity'

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Shell shape variation and fitness variables in the gastropod *Littorina saxatilis*

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ABSTRACT: On shores on the east coast of Yorkshire, England, the gastropod *Littorina saxatilis* shows consistency in shell shape variation in samples taken from the upper portion of its intertidal range. Using analysis of shell dimensions by standard multivariate techniques and extended eigenshape analysis, this variation can be dissected into different aspects, and related to the 'Raup growth parameters'. Some of the shell variation is shown to be related to the relative proportions of body and brood mass, and to an estimate of reproductive effort. This implies an association (perhaps indirect) between subtle variations in shell shape and fitness variables.

KEY WORDS: Shell shape · Fitness · *Littorina* · Trade-offs

INTRODUCTION

The intertidal environment is one which demands of the organisms that live in it the successful resistance of considerable stress. Primarily marine organisms are exposed to desiccation, extremes of temperature, wetting by rain and wave action to a greater degree than are marine inhabitants of the sublittoral or the pelagic. They are favourite subjects for the study of adaptation in terms of morphology, physiology, life history and phenotypic plasticity. Among intertidal animals, shelled gastropods are very attractive precisely because they have a shell which is so vital in the life of the animal, while presenting a set of features convenient for study. Among shelled gastropods, *Littorina saxatilis* (Olivi) has attracted a great deal of attention. Shell shape is very variable in this species; it is considered the most variable in the genus *Littorina* (Reid 1996). There is evidence that at least some of this variation may be adaptive in resisting wave action (Grahame & Mill 1986) or desiccation (Grahame et al. 1990). Atkinson & Newbury (1984) related such variation to life history strategy, as did Hart & Begon (1982). Hughes (1995) took an experimental approach, showing that when *L. saxatilis* was starved, growth was reduced; this was more severe in reproductive snails, indicating relative protection of allocation to reproduction.

Such experiments lead to considerations of how much a currently observed state or feature may be due to phenotypic plasticity. Studies using *Littorina* species have played their part here too. Current understanding is that what are called 'genetic determination' and 'plastic response' are both involved, with the former being at least as important and usually most important in determining shell shape. For example, a recent estimate is that 72 to 99% of adaptive shape variation in *L. saxatilis* on Galician shores was due to ecotype irrespective of growth environment (Conde-Padín et al. 2009).

For many of these studies, measurement of shape is crucial. Frequently, workers have used points on the shell between which linear dimensions are measured and analysed using conventional multivariate analytic methods (e.g. Caley et al. 1995, Grahame et al. 2006). An alternative approach, but starting perhaps with the same points, is to use a geometric morphometric method such as thin-plate spline analysis (Conde-Padin et al. 2007). A potential difficulty with such approaches is the placing of the points: an outline of a gastropod shell may not offer many truly homologous points which can be unambiguously determined across

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a series of shells. It is appealing in this circumstance to use an outline-based method, perhaps Fourier coefficients, applied to *Littorina* spp. by Dytham et al. (1992), or the more recently developed eigenshape and extended eigenshape methods (MacLeod 1999, Krieger et al. 2007).

In *Littorina* (as in the great majority of molluscs), the realised shape of the shell is determined by incremental growth at the margin of the mantle, morphologically the snail's anterior. Raup (1966) developed a model for shell growth that is able to describe the form of molluscs using 4 parameters: *W*, *T*, *S* and *D*. Imagining the aperture of a gastropod to be where the shell is formed by incremental growth, the shape of the aperture is measured by *S*, the 'generating curve'. This generating curve rotates round a coiling axis, and if it moves along this axis, a shell with a spire is formed (e.g. the turbinate form of *Littorina*); the rate of movement along the axis is the translation rate, *T*. The area enclosed by the generating curve gets larger according to the expansion rate, *W*. Finally, the generating curve may move outward away from the coiling axis at a rate *D*. This model is admittedly simple and general (Raup 1966), yet it adequately describes much of the realised shape of molluscs, leaving aside questions such as sculpturing and shell thickness. For real shells to work, changes in 1 parameter may have to be accommodated by changes in others (e.g. Clarke et al. 1999). The Raup growth model provides a framework for considering mollusc shell form which is less abstract than the statistics of multivariate analysis. The 2 can be synthesised, at least to a degree, by using data sets where at least some of the linear measures can be used to estimate Raup growth parameters.

This paper explores the variation in shells of *Littorina saxatilis* collected from a very restricted part of its range, viz. the high intertidal of 3 shores on the east Yorkshire (UK) coast. We use 2 of the shape-analytic approaches referred to above, namely principal component analyses (PCA) of linear dimensions of the shells, and the extended eigenshape method of MacLeod (1999). We consider the composite variables generated by PCA in relation to Raup parameters, and also in relation to the results of the eigenshape analysis. To seek biological significance, we relate the variation of the shell to estimates of allocation to body and to brood size, thus getting an approximate estimate of reproductive effort.

MATERIALS AND METHODS

Littorina saxatilis were collected from 4 sample sites on 3 shores on the east coast of Yorkshire in December and January; the species breeds all year at these locations (Hull et al. 1999). The sites were Selwicks Bay (British National Grid [BNG] TA254707) and Thornwick Bay (BNG TA233724), ~2.5 km apart on Flamborough Head; and Old Peak (BNG NZ984021), ca. 41 km northwest along the coast from Flamborough Head. At this site, we used 2 sample locations (A and B) about 250 m apart. Collections were from pits and crevices in either bedrock cliff (Flamborough) or high-shore large boulders (Old Peak), taking snails from within about 1 to 2 m^2 of rock. Thus in this work we refer only to variation within the H morph referred to by Hull et al. (1999).

In the laboratory, snails were killed by brief immersion in boiling water and either processed immediately or frozen (–25°C) and worked with subsequently. For each snail, the body was removed from the shell, ensuring that the entire body was removed. For gravid female *Littorina saxatilis*, shell, body (less operculum) and brood pouch were dried at 60°C for 24 h and then weighed to 0.01 mg. Brood pouches were used if they appeared to be full of embryos. Those few which had a small number (~10 or fewer) of embryos were discarded. No other control over brood pouch size was attempted. Great care was taken to exclude males and any females of *L. arcana* Hannaford Ellis.

For morphometrics, shells were carefully oriented in the way standard in our laboratory. Essentially, this method requires that the columella axis is horizontal, and at 90° to this, the axis across the shell is made horizontal at its widest extent. This results in the aperture facing upwards with its plane at some degrees off the horizontal. In this orientation, shells were imaged digitally. We used the software TpsUtil and TpsDig (Rohlf 1996) to place points on the shell images, again using the pattern which is standard in our laboratory. From the coordinates of these points, we took linear dimensions as shown in Fig. 1. Estimates for the Raup parameters *W*, *T* and *S* can be derived from these dimensions: *W* is ww1/ww2, *T* is cl/ww1, and *S* is al/aw, where ww1 and ww2 are whorl widths 1 and 2, cl is columella length, al is aperture length, and aw is aperture width (Fig. 1; see also Clarke et al. 1999). We cannot adequately estimate *D* from our data.

Analysis of the linear dimension data was carried out using PCA in either SAS (SAS Institute 1990) or R (R Development Core Team 2010), transforming the raw measures to logarithms of the ratio of any given dimension divided by the geometric mean of all linear dimensions. This procedure has been adopted to minimise the effect of size as such on the analysis, following Grahame & Mill (1989). It was further explored by Darroch & Mosimann (1985) and by Jungers et al. (1995), who referred to it as the '*DM_LOG*' approach. Here, we denote transformed variables with a subscripted t. We calculated an index of aperture size, taking the area of

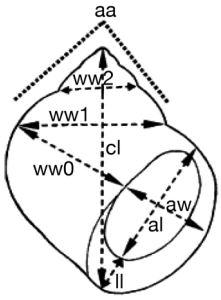

Fig. 1. *Littorina saxatilis.* Shell dimension variables used for the principal component analysis approach to shape measurement, and from which estimates of the Raup growth parameters may be calculated (see 'Materials and methods'). The dimensions taken are columella length (cl), lip length (ll), aperture length and width (al, aw), whorl widths 0, 1 and 2 (www0, ww1, ww2), and the apical angle (aa). Modified after Grahame et al. (2006)

the aperture estimated from its length and width and dividing this by the square of the geometric mean size. This in effect expresses aperture area in the same framework as the linear dimensions standardised by geometric mean size; we call this variable the aperture index. Thus we used 2 variables expressing properties of the aperture: its circularity or shape (which relates to *S*) and aperture index (which relates to its size as a linearised estimate).

PCA yields a set of eigenvalues, i.e. numbers which express the variance in the data in a diminishing series, and each of which has an associated eigenvector consisting of coefficients relating the standardised variables to the components. We will thus refer here to coefficients of the eigenvectors. For each shell in the analysis, a score may be calculated which relates it to one or another principal component (PC); thus, we will refer to PC scores.

In a further analysis of outline shape, we made black and white versions of the shell images and outlined these in TpsDig (Rohlf 1996) using 450 coordinate points and defining 1 apical landmark point. This landmark served to provide a common starting point for an extended eigenshape analysis of the outlines (MacLeod 1999, Krieger et al. 2007). If such a landmark is not used, much of the variation detected in the analysis concerns rotation of the outlines. Eigenshape analysis generates vectors of numbers referred to as eigenscores, values which relate the shells to the axes defined in the analysis. In this respect, they are analogous to PC scores, and must be distinguished from the eigenvalues and the eigenvectors of a conventional PCA.

With estimates of shape, we could place each shell on an axis—either a PC axis, or an axis of eigenscores—reflecting its shape relative to other members of the set. For each shell, we also had estimates of the supposedly biologically important variables of body and brood masses, as surrogates for somatic and reproductive function. We could now seek relationships between shape of the shell and body or brood mass. A difficult problem here is that all the variables are capable of change: there is no invariant standard to which to relate, e.g. body mass. We addressed this by standardising body and brood masses by shell mass to obtain ratios that we refer to as body index and brood index, respectively, and then standardising brood mass by body mass, obtaining another ratio referred to as reproductive effort (RE). This is necessarily a proxy measure of RE, which should be determined as the fraction of assimilated energy directed towards reproduction (e.g. see Hughes & Roberts 1980).

Concerning multiple testing, which is an issue when, for example, we are seeking correlations between the tissue indices and shape, we are mindful of the considerations of Rosenthal (1978) and Moran (2003) in paying attention to the pattern of observation of 'significant' correlations. We have also included Bonferroni corrections.

RESULTS

Sample sizes and basic statistics are shown in Table 1. The snails were all of very similar mean size; as expected, the rather small changes in mean columella length (cl) are reflected in somewhat larger variations in the mass measures. PCA using shell dimensions (excluding shell mass) showed considerable consistency between the samples. For each sample except that from Selwicks, there were 3 components with eigenvalues >1. At Selwicks, PC3 had an eigenvalue of only 0.86.

Within any sample, the components ought to be uncorrelated, and this was found to be the case. However, if there is consistency in the variation between the samples, there ought to be similarities between components in the different analyses. Spearman rank

Table 1. *Littorina saxatilis.* Statistics for size and mass of snails. Values are means ± SE

Site	n	Columella length (mm)	Body mass (g)	Brood mass (g)	Shell mass (g)
Selwicks	38	8.08 ± 0.27	0.011 ± 0.00071	0.0022 ± 0.00020	0.059 ± 0.0053
Thornwick	56	8.36 ± 0.17	0.014 ± 0.00087	0.0031 ± 0.00027	0.088 ± 0.0062
Old Peak A	40	7.53 ± 0.12	0.011 ± 0.00051	0.0033 ± 0.00026	0.054 ± 0.0036
Old Peak B	18	9.62 ± 0.26	0.016 ± 0.0011	0.0059 ± 0.00095	0.15 ± 0.016

correlation coefficients calculated using the coefficients for the variables on the components showed that there were indeed sometimes high correlations between those for the different samples. We carried out a multidimensional scaling analysis of the coefficients for the variables on the PCs, using 'cmdscale' in R. This gave an ordination shown in Fig. 2; this is a representation of the similarities between the PCs, which fall into 3 clear groups. Statistical data relating to Fig. 2 are shown in Table S1 in the supplement at www.int-res.com/articles/suppl/m430p103_supp.pdf.

The strongest relationships were for the body index and PCs for the second group of vectors in Table S1, i.e. Selwicks PC1, Old Peak B PC3, Old Peak A PC2 and Thornwick PC2. These are characterised by having highest positive values for $ww0_t$, and highest negative values for either lip length (ll_t; 3 cases) or $ww2_t$ followed by ll_t (Old Peak B PC3). RE values related convincingly at 2 locations to PCs in this same group and for 1 location to a component in the first group (that for Old Peak A PC1).

In view of the fact that there is a high degree of consistency between the PCs and the relationships of standardised tissue variables to them, we next carried out a PCA on the shell dimension data pooled across samples, and calculated Spearman rank correlations between the standardised tissue variables and the shell scores on these 'global' components (Table 2).

Table 2 shows that there are 5 (of a total possible 12) relationships with PC1 that are significant at $p \leq 0.05$, and all are negative, reflecting a decrease in the standardised tissue variable with shape along this component. PC2 shows only 1 such relationship, for body index at Selwicks ($p < 0.001$). The remaining relationship shown is for brood index at Old Peak B, which is not significant ($p = 0.070$). PC3, which accounts for relatively little of the total variation (18%) shows 6 relationships with standardised tissue variables significant at $p \leq 0.05$. Where the correlation is with body index, it is negative; where it is with RE, it is positive, and there are no relationships with brood index. A Bonferroni correction for multiple testing on the rows in Table 2 reduces the number of significant correlations; a table-wide correction would eliminate significance.

Table 3 shows the coefficients for the variables making up the eigenvectors. Here we will refer to the transformed variables (see 'Materials and methods'), denoted with a subscripted t. PC1 is mainly related to changes in $ww2_t$ and cl_t contrasted with the apical angle (aa). Of the Raup parameters *T*, *W* and *S*, only *W* correlates with PC1 ($\rho = 0.696$, $p < 0.001$). However, the derived variable aperture index (see 'Materials and methods') does correlate with this PC ($\rho = 0.472$, $p < 0.001$). Fig. 3 shows example shells from the negative and positive ends of PC1, together with a plot of *W* and aperture index on the PC.

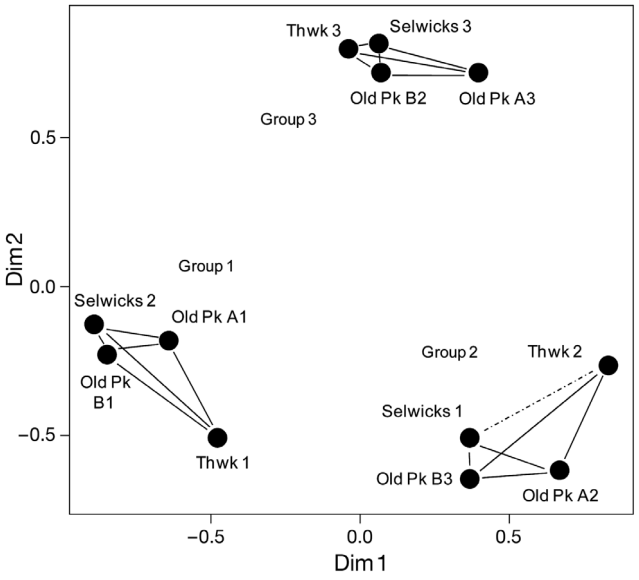

Fig. 2. *Littorina saxatilis.* First 3 principal components calculated from each of the 4 samples plotted on the first 2 dimensions of a multidimensional scaling analysis of the component vectors. Solid lines connect the symbols for the vectors between which the Spearman rank correlation is significant at $p \leq 0.05$; the broken line shows the 1 instance where the correlation is weaker than this ($p = 0.083$). Numerical data are given in Table S1 in the supplement

Table 2. *Littorina saxatilis.* Spearman rank correlations (ρ) between tissue indices and shell principal component (PC) scores for the components calculated across all samples. Correlations with $p \leq 0.003$ are shown in **bold**. This represents adjusting α to 0.003. Correlations with $p > 0.05$ are not shown

	Brood index		Body index		Reproductive effort (RE)	
	ρ	p	ρ	p	ρ	p
PC1 (2.98, 37%)						
Thornwick	**−0.426**	**0.001**	−0.383	0.004	−0.278	0.038
Old Peak A	−0.380	0.016	–	–	−0.363	0.021
PC2 (2.27, 28%)						
Selwicks	–	–	**0.612**	**<0.001**	–	–
Pooled	0.188	0.023	**0.388**	**<0.001**	–	–
PC3 (1.47, 18%)						
Selwicks	–	–	−0.371	0.022	0.323	0.048
Thornwick	–	–	−0.275	0.040	–	–
Old Peak A	–	–	**−0.537**	**<0.001**	**0.474**	**0.002**
Old Peak B	–	–	**−0.699**	**0.001**	–	–
Pooled	–	–	**−0.508**	**<0.001**	0.180	0.027

Table 3. *Littorina saxatilis.* Variable coefficients in the eigenvectors (see 'Materials and methods') of the principal components (PCs) calculated across all samples. ww0, ww1, ww2: whorl widths 0, 1 and 2; cl: columella length; ll: lip length; al: aperture length; aw: aperture width; aa: apical angle. Subscript t indicates transformed variables (see 'Materials and methods')

Variable	PC1	Variable	PC2	Variable	PC3
$ww2_t$	−0.524	ll_t	−0.572	al_t	−0.506
cl_t	−0.443	$ww2_t$	−0.015	cl_t	−0.331
$ww1_t$	−0.233	cl_t	0.067	aw_t	−0.222
$ww0_t$	−0.028	aa	0.110	$ww2_t$	−0.146
ll_t	0.223	al_t	0.330	aa	0.074
aw_t	0.249	ww1t	0.417	ll_t	0.252
al_t	0.274	$ww0_t$	0.428	$ww1_t$	0.471
aa	0.536	aw_t	0.437	$ww0_t$	0.522

As PC2 shows relatively little relationship with tissue variables, we neglect it here. PC3 contrasts al_t (and to a lesser extent cl_t and aw_t) with $ww0_t$ and $ww1_t$. Fig. 4 shows that along PC3, *W* increases, *T* decreases, and both aperture index and *S* change—apertures become smaller, and the estimate of *S* declines towards 1, thus apertures become more circular. All of these relationships are significant, as ρ values have $p < 0.001$ in these instances.

The results of an extended eigenshape analysis on the same shells were compared with the PCA, and similar features emerged in terms of relationships of shell to standardised tissue variables, both on a location-by-location and a global basis. We will not deal with the first, but will discuss the global analysis. First of all, we found that using the eigenscores (EScs) of the shell outlines and correlating these with the shell scores on the PCs, there were many correlations which were very small and a few which were comparatively large. The strongest by far was that between ESc1 and PC1 (Table 4). After this, there is a comparatively weak correlation between ESc3 and PC2, and rather stronger ones between ESc3 and ESc4 and PC3. For the remaining EScs up to ESc10, there were 4 more correlations with PCs which were significant at $p \leq 0.05$; 2 of these involved PC3, with ESc8 ($\rho = 0.173$, $p = 0.033$) and ESc9 ($\rho = 0.358$, $p < 0.001$). Thus in a total of 30 correlations between PCs and EScs, 4 involved PC3, and 4 were with the other 2 PCs. As with Table 2, Bonferroni correction based on the columns in Table 4 leaves significant correlations, while an analysis-wide correction would eliminate any significance.

Considering likely relationships between body, brood and shell sizes, PC1 showed evidence of such a relationship, with the standardised tissue variables decreasing along the PC (Table 2). However, the correlations are only significant at Thornwick and Old Peak A (and here for brood index and RE only). For these instances, this means that the tall shells exemplified by negative scores on this PC had relatively larger tissue masses than the squatter ones with positive scores (Fig. 3). This relationship is not evident in the pooled data correlations (Table 2).

ESc1 was strongly identified with PC1, and correlated with brood index ($\rho = 0.418$, $p = 0.007$) and RE ($\rho = 0.422$, $p = 0.007$) at Old Peak A. Since the correlation between ESc1 and PC1 is itself negative, positive correlations between ESc1 and standardised tissue variables are consistent with negative ones between those variables and PC1. At Thornwick, the correlations with ESc1 were comparatively very weak at $p \sim 0.06$. As with PC1, the relationships are not evident in the pooled data correlations.

Finally, we found that ESc4 and PC3 were evidently strongly related (Table 4). At Old Peak A and B there was evidence of the expected correlations with standardised tissue variables, although it was weak for body index at Old Peak B ($\rho = 0.281$, $p = 0.080$). For the

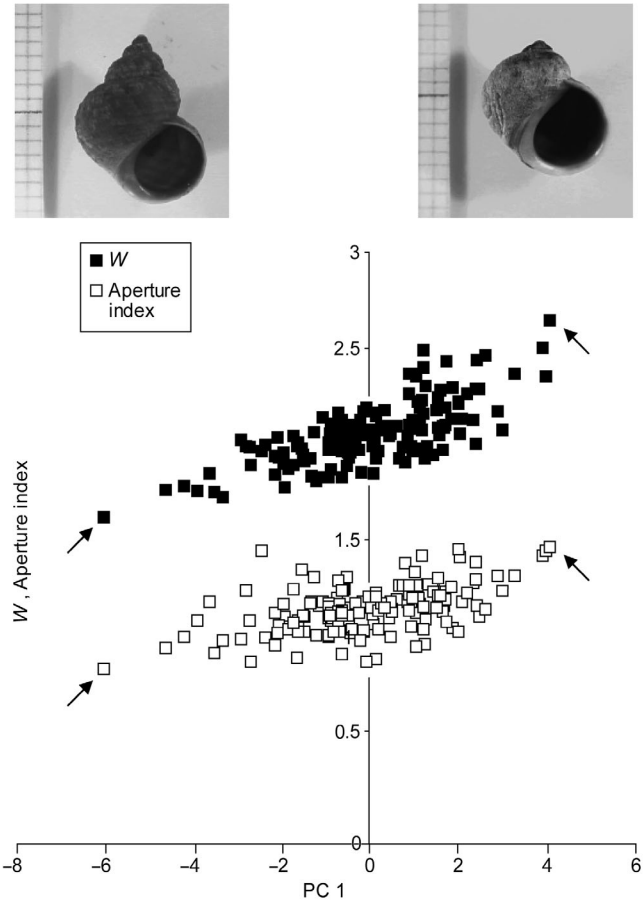

Fig. 3. *Littorina saxatilis.* Estimates of the Raup parameter *W* and aperture index, plotted on scores for principal component (PC) 1. Example shells from the extremes of the component are shown; their positions on the plot are indicated by arrows

pooled data, the correlation between ESc4 and body index was 0.268 (p = 0.001), and the correlation with RE was −0.262 (p = 0.001). Again, because the correlation between ESc4 and PC1 was negative, the signs of these correlations are consistent with those in Table 2.

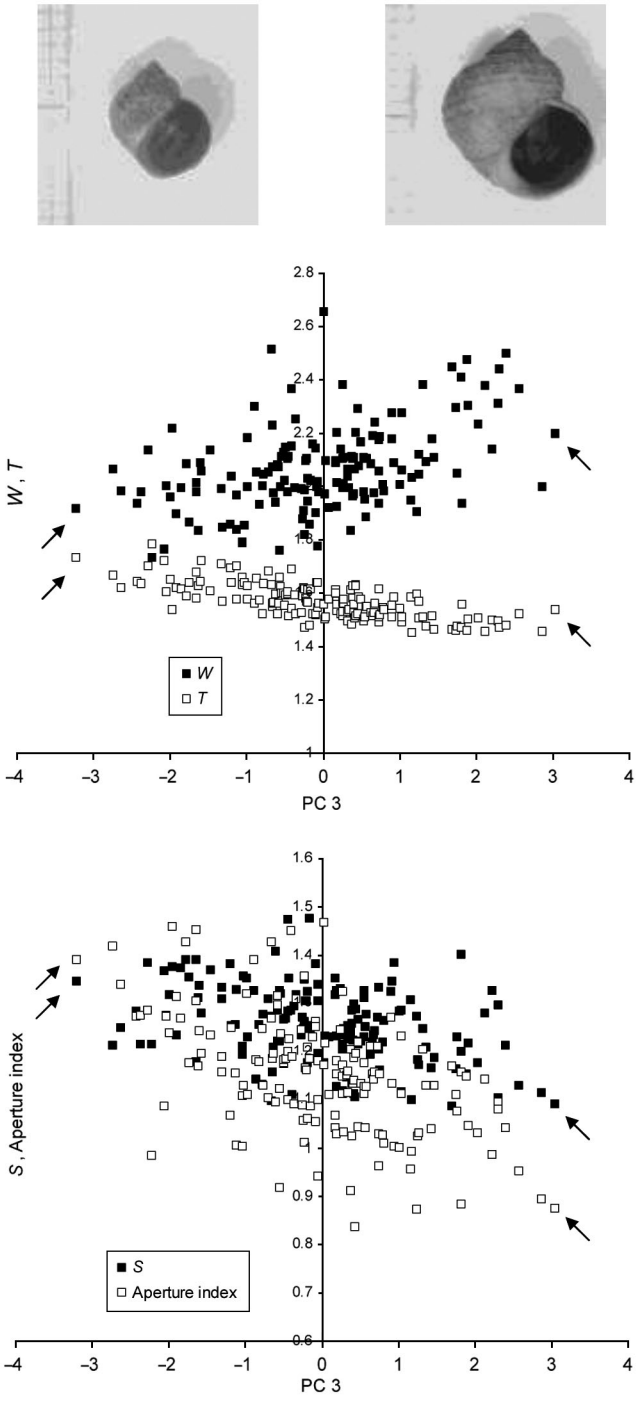

Fig. 4. *Littorina saxatilis.* Estimates of the Raup parameters *W*, *T* and *S* (see 'Materials and methods'), and also the metric of aperture index, plotted on scores for principal component (PC) 3. Example shells from the extremes of the component are shown; their positions on the plots are indicated by arrows

We illustrate the evident relationship between body index, RE and shape in Fig. 5, for the pooled data plotted on PC3. Brood index itself does not correlate with this PC, so that while the body index declines (ρ = −0.508, p < 0.001, Table 2), RE estimated as the ratio of brood mass to body mass increases (ρ = 0.180, p = 0.027, Table 2).

Fig. 6 shows shell models calculated from the extended eigenshape analysis for EScs1, 3 and 4, revealing different aspects of the shell variation. PC1 is associated with variation in *T*, and ESc1 shows fluctuations in shell outline which appear symmetrical. EScs3 and 4 are correlated with PC3; while the PCA points towards changes in *W*, *T* and *S*, as well as aperture index, the eigenshape analysis of shell outlines appears to localise the variation asymmetrically on the lower whorls of the shell.

DISCUSSION

In 4 samples taken from 3 locations on the east Yorkshire coast, the shells of the high-shore form of *Littorina saxatilis* show consistent patterns of variation. Using shell dimensions which allow estimates of variables reflecting the parameters Raup (1966) used to describe molluscan shell growth, and comparing these with the results of PCA, we found that PC1 (the major aspect of variation in shell shape) is entirely related to changes in the whorl expansion rate, *W*. As the shells become relatively taller, with a lower *W*, the amount of tissue enclosed by the shell increases. We base this on finding an increase in the mass of tissue (either body or brood) as a ratio of the shell mass.

PC1 is evidently related to the axis of variation represented by ESc1 in an extended eigenshape analysis, as the correlation between the ordering of the shells on PC1 with that on ESc1 is very high (Table 4). As in the case of PC1, where everything that was happening appeared to be related to 1 Raup variable (*W*), the variation on ESc1 is comparatively simple: there is an apparently symmetrical alteration in outline of the

Table 4. Spearman rank correlations between eigenscores (ESc) 1, 3 and 4 (see 'Materials and methods') and principal component (PC) scores, with both sets of scores calculated across all samples. Correlations with p ≤ 0.013 are shown in **bold**. This represents adjusting α to 0.013. Correlations with p > 0.05 are not shown; therefore ESc2 has been omitted from the table

	PC1	PC2	PC3
ESc1	**–0.925, p < 0.001**		
ESc3		0.193, p = 0.017	**0.248, p = 0.002**
ESc4			**–0.297, p < 0.001**

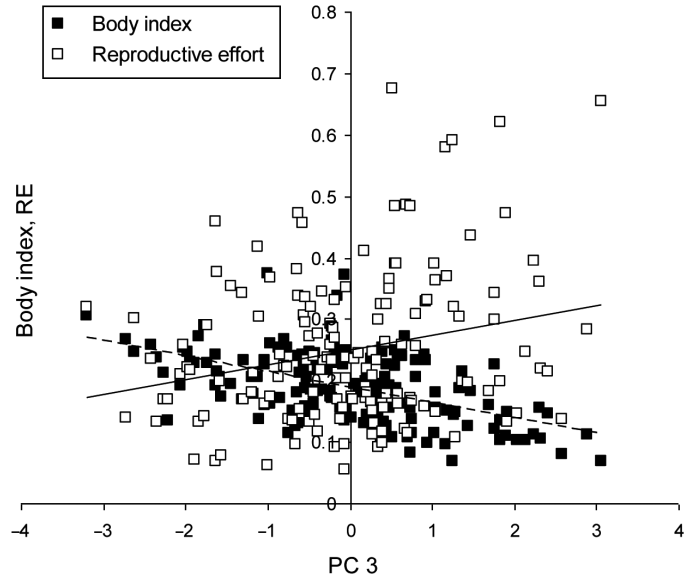

Fig. 5. *Littorina saxatilis.* Estimates of body index and reproductive effort (RE) plotted on principal component (PC) 3. Solid line: least squares slope for RE; dashed line: least squares slope for body index

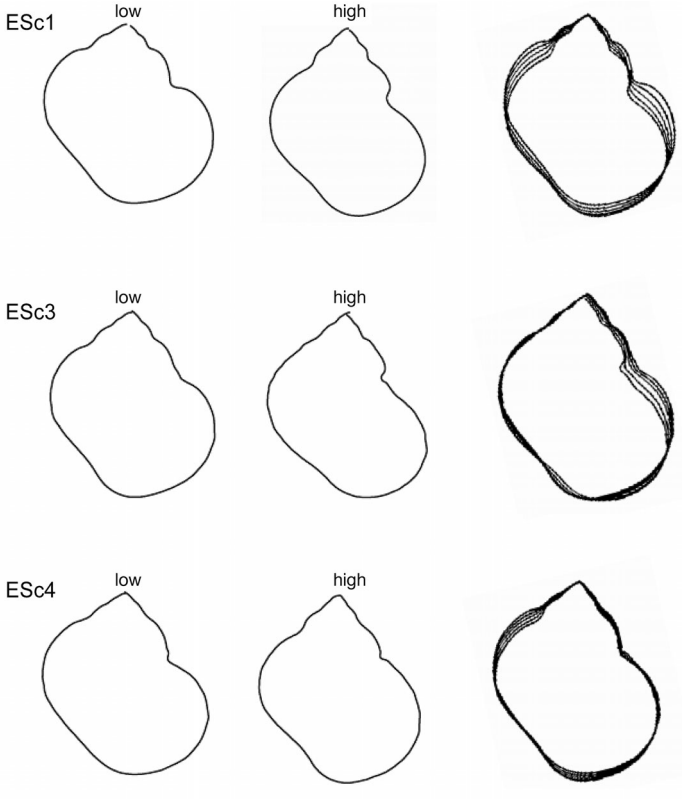

Fig. 6. *Littorina saxatilis.* Models of shell shape calculated from eigenscores (ESc) 1, 3 and 4 in an extended eigenshape analysis. The 2 extreme models are shown together with a superimposition of these with 3 intermediates

shells (Fig. 6), again associated with changes in relative tissue mass estimates.

We did not explore PC2, as there seemed to be comparatively little biological interest there. PC3, however, is interesting, with changes in *T*, *W*, *S* and aperture index. Paradoxically perhaps, with changes in both *T* and *S* the variation in shell form along PC3 appears less extreme than it does along PC1 (cf. Figs. 4 & 3). This apparent paradox could be resolved if greater variability in the Raup parameters in some way buffered the overall shape changes. A point of difference between the 2 PCs is that in the case of PC1, small *W* is associated with a small aperture index (Fig. 3), while for PC3 the reverse is true (Fig. 4). On PC3, larger *W* is associated with smaller and rounder apertures (*S* nearer 1), whereas there is no consistent change in *S* on PC1.

A common property of these 2 PCs is that on both, smaller *W* is associated with a relatively larger body (judged by standardising body mass by dividing it by shell mass). However, and this may be a point of great biological interest, on PC3 there is only the slightest hint of a corresponding change in standardised brood mass; the correlation is –0.130 ($p = 0.109$). This underlies the change in our estimate of RE, which increases along PC3 (Fig. 5) as *W* increases, and *T*, *S* and aperture index decrease (Fig. 4). There is a great deal of scatter in the points for RE (Fig. 5); nevertheless, we used the equation of the line of best fit for RE shown in the figure to obtain conservative estimates of a plausible change in RE. These are conservative because we used PC3 values of –2.5 and 2.5, well within the range of the data, and because there are many points above the line at around PC3 score of 2.5. The estimates are of RE values varying between 0.19 and 0.31, representing an increase of about 63% of the smaller value. This implies a major shift in allocation to brood mass along the axis of variation represented by PC3. This is evidently a trade-off between the alternative functions of body and brood: presumably the reduction in body mass could be less severe if brood mass were similarly allowed to reduce along this axis of variation. It is worth emphasising that this is occurring on a very small spatial and indeed ecological scale: these data represent just 1 morph of *Littorina saxatilis* collected over a very small spatial scale, though replicated at 4 sample sites.

At least some of what we have found may be due to the individual experiences of the snails as they grew and matured; thus, there may be a phenotypic component involved. We cannot test this using our data, but it would seem likely on the basis of what is understood that there must be some genotypic component involved as well. A further caveat is that the dynamics of turnover of eggs and juveniles in the brood pouch of

Littorina saxatilis are poorly understood, with embryos of early or late developmental stages present in widely varying proportions in different females (e.g. see Hull et al. 1999). In this circumstance, it is likely that a substantial amount of variation may be introduced into our estimate of reproductive effort from this source. This may explain at least some of the scatter evident in Fig. 5.

We suggest above that PC3 represents more complex interactions of the Raup parameters. Something similar seems to be the case with ESc4, which is correlated with it, and also with ESc3, which is also correlated with PC3 (although these 2 ESc axes are themselves uncorrelated; $\rho = 0.022$, $p = 0.790$). Whereas the shell models for ESc1 show what appears to be symmetric variation in outline between the left and right sides of the shell, that is not the case for ESc3 and ESc4 where the outline varies more on the right and the left side, respectively (Fig. 6). This is counterintuitive from a consideration of the Raup growth model, which suggests that the 2 'sides' of the shell should mirror one another. We cannot resolve this here, beyond making 2 suggestions. The first is that what appears in the shell models for ESc3 and ESc4 is in some way an artefact introduced by lack of control at the imaging stage, although if this were the case it would seem to be remarkable that there should then be such a strong signal in terms of the enclosed body and brood mass quantities. It would also seem to be the case that if positional error were involved, the shells on the 2 EScs should be correlated in order, but they are not.

The second suggestion is that there may be ontogenetic changes in the way the shell grows, perhaps to do with the need to accommodate a brood, so that there are biologically important (and variable) changes in allometric relations during growth. In this connection, it is worth noting that PC1 shows no correlation with the geometric mean size of the shells along it, while PC3 does ($\rho = 0.248$, $p = 0.002$), as does ESc3 ($\rho = 0.169$, $p = 0.037$), although the correlation of size with ESc4 is not significant ($\rho = -0.143$, $p = 0.078$). The possibility that the changes suggested by ESc3 and ESc4 may be a real phenomenon can be investigated by taking a wider size range of shells than was used here, which should reveal ontogenetic changes, and also perhaps by investigating males, which do not brood, or the high shore *Littorina arcana*, which also does not brood.

This work is an exploration of shell shape in a very small subset of the possible gastropod shapes, a subset restricted to 1 species living in a particular habitat. Our findings lead us to 2 different sorts of conclusion: firstly, we have demonstrated small and subtle changes in shape and its variation which yet seem to be associated with features of importance in the life of the animals. It would be fruitful to explore whether this is direct or through some intermediate variable(s). Secondly, in terms of methodology, the 3 ways of working with shell shape which we have employed—using PCA on linear dimensions between points on the shells, relating these to Raup growth parameters, and using extended eigenshape analysis—are complementary approaches which promise further enhancement of our understanding of the significance of shell shapes.

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Does sperm supply limit the number of broods produced by a polyembryonous bryozoan?

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ABSTRACT: Polyembryony, the splitting of a single sexually produced embryo into many clonal copies, seems to involve a disadvantageous combination of sexual and asexual reproduction, but persists in a diverse range of organisms. It has been suggested that embryonic cloning in cyclostome bryozoans (colonial, sessile marine invertebrates that mate by the release, dispersal and uptake of water-borne sperm) may be a response to sperm limitation. The cyclostome *Crisia denticulata* inhabits its subtidal rock overhangs. Cloned larvae are produced by a colony in a series of independent brood chambers (gonozooids). Offspring from different brood chambers are genetically distinct and are, thus, the outcome of separate fertilisations. We investigated the possibility that sperm limitation reduced female reproductive success at low population density, by assessing the relationship between local colony density, as a proxy for sperm supply, and the number of brood chambers possessed by colonies, as a proxy for fertilisation success. In the patchily distributed population of *C. denticulata* we studied, the number of brood chambers varied enormously between colonies of the same size, and large colonies entirely lacking brood chambers were frequent, suggesting the occurrence of low fertilisation success within many colonies. However, in colonies with broods, only 17% of the variation in the number of broods per colony could be explained jointly by colony weight and local population density score, with population density being a non-significant predictor in the model. This suggests that sperm supply, as such, does not strongly influence female reproductive success and may, therefore, not be important for the maintenance of polyembryony, at least in the studied population. The wide variation in allocation to female function still requires explanation.

KEY WORDS: Sex allocation · Polyembryony · Sperm limitation · Population density · Bryozoa · *Crisia*

INTRODUCTION

Polyembryony, the splitting of a single sexually produced embryo into clonal copies, appears to represent a mixture of sexual and asexual reproduction that largely forsakes the respective benefits of the 2 modes. Producing polyembryonous offspring forgoes the genetic diversity inherent within a sexual brood, but nevertheless breaks up the successful parental genotypes and 'bets' instead on a single, unproven genotype (Craig et al. 1997). Despite such an apparent handicap, routine polyembryony appears to have evolved numerous times, being reported in some rust fungi, algae, plants and animals (metazoan phyla include cnidarians, platyhelminths, arthropods, bryozoans, echinoderms and chordates; see Craig et al. 1997, Hughes et al. 2005).

The present work focuses on a bryozoan of the order Cyclostomata, a group in which all representatives, with the possible exception of the Cinctiporidae (Boardman et al. 1992), are thought to be polyembryonous (Ryland 1970). Bryozoans are colonial and hermaphroditic, built from replicated zooids budded from a metamorphosed, sexually produced larva. Zooids filter feed with a ciliated, tentacular lophophore. In bryozoans, sperm are released into the water, but eggs are retained, and fertilisation is internal. In the class Gymnolaemata, sperm are known to be brought into contact

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with acting female zooids through the feeding current (Temkin 1994), but sperm collection in the Cyclostomata has not yet been described. Oogenesis in cyclostome bryozoans has been studied most fully within the genus *Crisia*, in which branching erect colonies consist of rigid internodes of several zooids in a biserial arrangement, joined by flexible, non-calcified joints (nodes). Many oogonia initially form in the developing internode, but most degrade, their host zooids differentiating into autozooids, which produce sperm; on some internodes a developing zooid (rarely >1) differentiates into a gonozooid, in which the lophophore is transitory (Borg 1926, Ryland 2000). Within the expanded brooding space of the cyclostome gonozooid, polyembryony occurs when a primary embryo repeatedly buds off clumps of cells that, with slight variation between taxa, develop into independent larvae (Harmer 1893, Robertson 1903, Borg 1926). A single gonozooid of *C. denticulata* can release 10s of larvae upon dissection, and earlier developmental stages are generally also present, indicating ongoing production of young (authors' unpubl. obs.); Borg (1926) notes the common production of >100 embryos and larvae from the primary embryo of *Crisiella producta*. Molecular techniques have confirmed that embryos within individual gonozooids of *Crisia denticulata* are genetically identical, but different gonozooids on the same colony house genetically distinct embryos, resulting from separate fertilisation events (Hughes et al. 2005).

Several circumstances have been suggested that should favour the evolution and maintenance of polyembryony in animals, but most of these do not seem applicable to algae, plants, or colonial animals, such as cyclostome bryozoans, that mate at a distance by the remote transfer of male gametes (see Pemberton et al. 2007). More applicable explanations envisage polyembryony as making 'the best of a bad job' in the face of constraints on mating opportunities (Craig et al. 1997). Data from a study of *Crisia denticulata* failed to support a suggestion by Ryland (1996) that polyembryony might be a response to limited gene flow in sessile species in which 'potential mates will differ little, and sexual reproduction may produce larvae with genotypes no less fit within the immediate vicinity than their parent'. A large proportion of the total genetic variability encompassed by 2 widely separated populations of *C. denticulata* was present within patches of colonies in small-scale rock overhangs (Pemberton et al. 2007); thus, potential mates would not be genetically similar.

Ryland (1996) also predicted (echoing an earlier suggestion made for red algae with aflagellate gametes by Searles 1980) that low sperm output from typically small cyclostome colonies, when combined with a sparse adult distribution, gave a low probability that a colony could capture enough sperm to fertilise all the eggs it could potentially produce. This appeared to tie in well with reports in the contemporary literature of sperm-limited reproductive success in many free-spawning taxa because of rapid sperm dilution (e.g. Levitan & Petersen 1995). Here, we address the suggestion by Ryland (1996) that polyembryony is advantageous because it enables cyclostomes to replicate the zygotes resulting from the capture of relatively few sperm. In circumstances where sperm were limiting, the level of brooding would reflect sperm supply. We test the prediction that the number of brood chambers (gonozooids) decreases as sperm become limiting at low population density in *Crisia denticulata*. If so, this would support Ryland's (1996) premise that sperm limitation is important for the maintenance of polyembryony.

MATERIALS AND METHODS

Data collection. Colonies of *Crisia denticulata* were collected by wading and snorkelling at Wembury, near Plymouth, Devon, England (UK national grid reference SX518482). *C. denticulata* were found on the underside of rock overhangs in the shallow subtidal. Individual overhangs were ca. 1 to 6 m in length and ca. 0.5 to 2 m from top to bottom. Each overhang, which might contain one to several thousand colonies, was separated horizontally from adjacent overhangs by ca. 2 to 30 m.

Colonies collected during 2001 (August to October) were those also studied by Pemberton et al. (2007) and were spatially mapped, allowing estimation of local colony density. The location of collected colonies within individual overhangs was established from the *x*, *y* position of 5 × 5 cm divisions on strung quadrats held against the rock wall. The density of *Crisia denticulata* across all sampled overhangs was estimated at the level of the 5 × 5 cm quadrat divisions (score 0 = no colonies present; 1 = <50% cover of *C. denticulata*, generally 1 or 2 colonies; 2 = >50% cover, generally 3 or more colonies). Focal colonies for further analysis were collected, transported individually back to the laboratory and preserved in ethanol as described by Hughes et al. (2005). Further details of the collection sites, including maps, are provided by Pemberton et al. (2007).

Colonies from 11 overhangs representing a range of local densities were haphazardly selected from the ethanol-preserved 2001 collections. Only specimens with well-defined holdfasts were included to ensure the complete colony was measured. Following the removal of epifauna and any other adherent material, colonies were dried at room temperature and weighed

on an analytical balance. Autozooids made up the vast majority of the weight of a colony, with a much smaller proportion being composed of rhizoids (a further zooidal polymorph with a purely structural function). Gonozooids contributed an insignificant proportion to the total weight, so they were not removed before weighing, nor were final weights adjusted. Regression analysis of a small preliminary data set showed autozooid number to be highly correlated with colony weight ($r^2 = 0.986$). There were approximately 120 autozooids mg^{-1} dry weight. Numbers of gonozooids were recorded. An index of local population density was estimated by adding the 0, 1, or 2 density scores of the relevant 5 × 5 cm quadrat divisions surrounding each focal specimen. (The score of the 5 × 5 cm square in which the focal colony itself fell was not adjusted, i.e. the focal specimen was included in this calculation.) This potentially allowed estimates of local population density at various spatial scales to be made. However, independent investigation of density dependence across a range of within-overhang spatial scales was not possible, as density measures were highly positively correlated (e.g. 0.25 × 0.25 and 1 × 1 m sided squares, r_S = 0.918, p < 0.0001). Therefore, we consider here only the summed scores within a 1 × 1 m square surrounding the focal individual (total area = 1 m^2).

A supplementary collection was made from March to June 2002, but without mapping of colonies to allow estimation of local population density. The weight and number of brood chambers of colonies was determined as for the 2001 samples.

Statistical analysis. Following data collection it became clear that the majority of colonies lacked gonozooids (see 'Results'). Such a dominance of zero values deviated from normality assumptions of parametric statistics and presented an unsuitably large number of ties for rank-based non-parametric tests. Statistical analysis, therefore, progressed in 2 stages. The initial analysis investigated differences in weight and (for 2001) local density between colonies with and without gonozooids. The second part to the analysis focused solely on colonies from the 2001 collection that possessed gonozooids, to explore any possible 3-way relationship between local density, colony weight and gonozooid number.

RESULTS

Data from collections in 2001

Local density varied considerably, from isolated individuals to dense stands of *Crisia denticulata*; the summed density scores for a 1 m square around a focal colony ranged from 1 to 108. Colony dry weight varied by >2 orders of magnitude (0.0022 to 0.4581 g); 88 of the 134 colonies scored (i.e. 66%) possessed no gonozooids, and these non-brooding colonies occurred across almost the entire density range; the maximum number of gonozooids on a single colony was 84 (Fig. 1A).

Colonies that lacked gonozooids weighed less (Mann-Whitney, $W = 4230.5$, p < 0.0001), i.e. had fewer autozooids, and were found at lower local density (Mann-Whitney, $W = 4777.5$, p = 0.0112) than those colonies possessing 1 or more gonozooids. However, in the 43 colonies that did possess gonozooids and for which a density score was available, no relationship existed between weight and local density (r = 0.080, p = 0.611). For these colonies, multiple regression revealed a statistically significant overall relationship between log gonozooid number (as the dependent variable), local density and weight (ANOVA, $F = 5.33$, p = 0.009, regression equation: log gonozooids = 0.504 + 0.00343[density] + 1.79[weight]) (Fig. 2). However, the trend was extremely weak, with both independent variables together accounting for only 16.9% (= R^2_{adj}) of the variation in log gonozooid number. Local density was a non-significant predictor in the multiple regression ($t = 1.69$, p = 0.098), while the effect of colony weight was significant ($t = 2.63$, p = 0.012).

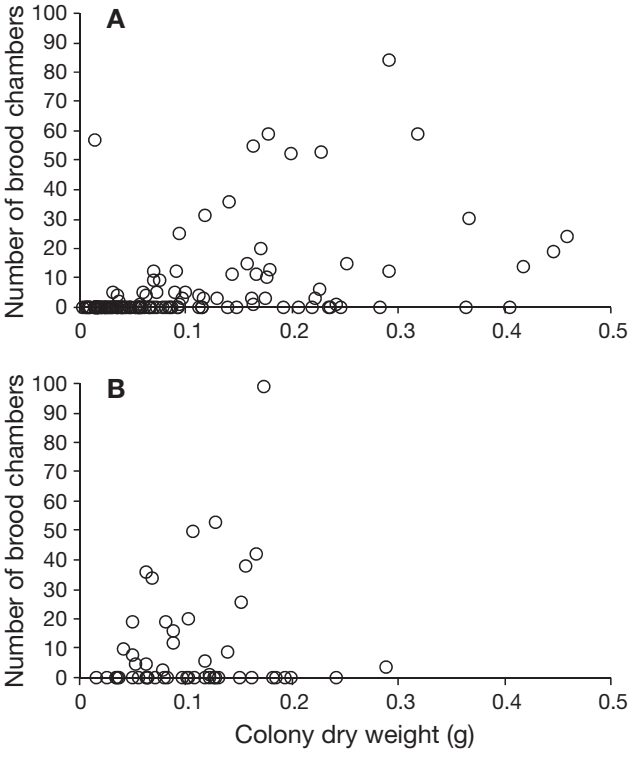

Fig. 1. *Crisia denticulata.* Relationship between colony dry weight and the number of brood chambers per colony: (A) data from 2001 and (B) data from 2002

Data from collections in 2002

Fifty-one colonies were scored. Colony weight varied 19-fold (0.0149 to 0.2873 g); 29 of the 51 colonies scored (i.e. 57%) possessed no gonozooids; the maximum number of gonozooids on a single colony was 99 (Fig. 1B). Colonies that lacked gonozooids were not different in weight from those possessing 1 or more gonozooids (Mann-Whitney, $W = 580.0$, $p = 0.8866$).

A ranking of the combined 2001 and 2002 colonies by weight was divided into 9 groups with increasing weight, each of 20 or 21 colonies. With increasing weight a clear increase in the proportion of colonies with brood chambers was evident (binary logistic regression, the probability that slope = 0 was <0.001) (Fig. 3). Nevertheless, 35 to 60% of the colonies above approximately 50 mg (ca. 6000 autozooids) lacked brood chambers.

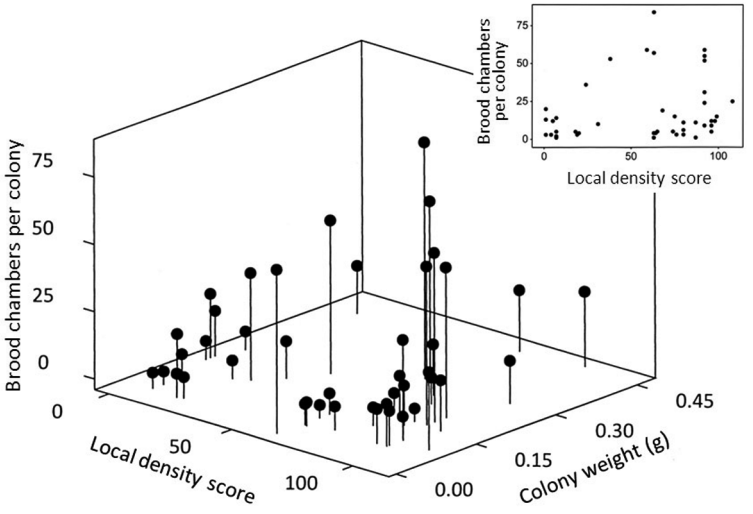

Fig. 2. *Crisia denticulata*. Relationship of number of brood chambers per colony, colony dry weight and local population density score (1 m^2 scale), 2001 data; inset, bivariate scatter plot of brood chambers per colony versus local density score, from the same data set. Only colonies with at least 1 brood chamber were plotted

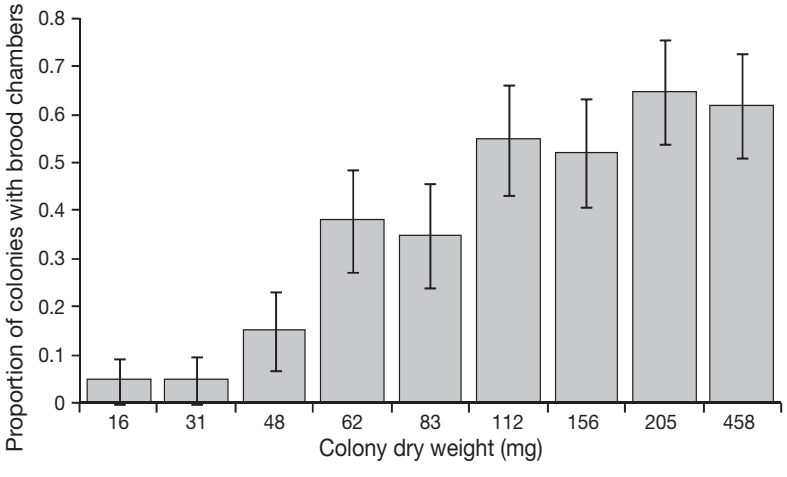

Fig. 3. *Crisia denticulata*. Proportion (±SD) of colonies with brood chambers in 9 weight categories (n = 20 or 21) formed by ranking the colonies of the combined 2001 and 2002 data. Values on the *x*-axis represent weight (mg) of the largest colony in each category

DISCUSSION

Although in some species the zooids are single-sex, bryozoan colonies as a whole are hermaphroditic (Ryland & Bishop 1993). Gonochorism (i.e. single-sex colonies) has been reported in a very few bryozoan species (e.g. Robertson 1903), but in these cases the alternative of sequential hermaphroditism requires careful assessment (Borg 1926). A striking feature of the data presented here for *Crisia denticulata* is the large proportion of colonies that lack brood chambers altogether, even in the largest size categories. A similar observation (framed as the finding of equal numbers of similar-sized colonies with and without ovicells [i.e. gonozooids]) was presented as evidence of gonochorism in *Crisia franciscana* in the published abstract by Beauchamp (1984). Three possible patterns of gender allocation with colony growth are presented in Fig. 4. The data for *C. denticulata* resemble Pattern A, in which female investment ranges from zero to high levels across the range of post-maturation colony sizes. Thus, *C. denticulata* colonies with no, one, or only a few brood chambers are present at relatively large colony size (Fig. 1). Although colonies of *C. denticulata* without brood chambers could logically be referred to as male (presuming they do produce sperm), they are probably better regarded as the extreme of a continuum of female investment within a hermaphroditic population. It is not known whether the observations underlying Beauchamp's brief report on *C. franciscana* related to a situation like Pattern A in Fig. 4, or to Pattern C, in which a clear separation between fully male and fully female sexual investment exists. However, our own data gave very little support for the existence of truly separate sexes in *C. denticulata*.

The extreme variability in levels of female investment in *Crisia denticulata* requires explanation. The probability that a colony has any brood chambers at all is clearly influenced by colony size (Fig. 3), and, with a single exception, colonies with a dry weight <25 mg (n = 39) did not possess brood cham-

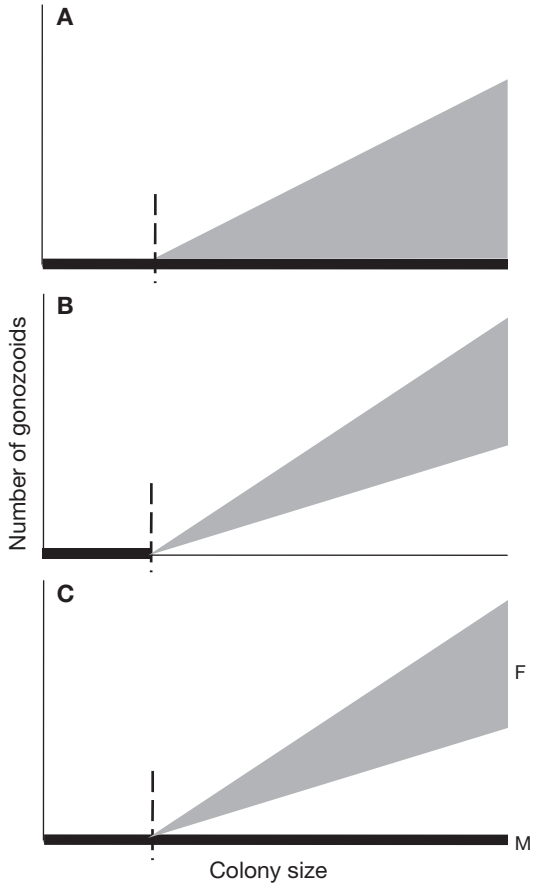

Fig. 4. Some possible patterns of gender allocation in an organism such as *Crisia denticulata*. Thick line along the *x*-axis represents colonies lacking gonozooids. Dashed vertical line represents onset of sexual maturity, here presumed to occur at a set colony size. (A) Simultaneous hermaphroditism accompanied by extreme variability in female investment, resulting in a proportion of colonies lacking gonozooids at all sizes. (B) Simultaneous hermaphroditism accompanied by only moderate variability in female investment, so that all post-maturity colonies have at least some gonozooids. (C) Gonochorism (with moderate variability in female investment, F); post-maturity colonies without gonozooids are males (M)

bers, suggesting that the smallest colonies analysed were predominantly immature as females. Colony weight was a significant predictor of the number of brood chambers per colony in the 2001 data set (with the statistical analysis constrained to those colonies with 1 or more chambers). Nevertheless, the wide variation in the number of brood chambers per colony, even when comparing colonies of similar size, is notable in both the 2001 and 2002 data (Fig. 1). The main possibility we wished to investigate here was that the brooding activity of colonies was influenced by the sperm supply experienced by each, under the hypothesis that the most isolated colonies are undergoing sperm limitation, restricting the production of broods. Local population density surrounding each focal specimen varied considerably from the absence of neighbours to dense stands of *C. denticulata*. This could have partially explained the variation in brooding observed and would relate to the maintenance of polyembryony within the cyclostomes. In fact, there was very little indication of an effect of estimated colony density on the number of brood chambers in colonies possessing any chambers at all, although colonies without brood chambers occurred at a lower median density than those with chambers. It seems that, at most, local population density, as estimated here, had only a minor effect on female reproductive investment. Thus, our study of the effect of local density in this population of *C. denticulata* presents little direct evidence that sperm limitation is a likely explanation for polyembryony.

Since Ryland (1996) speculated on the possible role of polyembryony in counteracting sperm limitation, evidence has been accumulating that the dynamics of fertilisation in species that, like *Crisia denticulata*, retain their eggs and release sperm into the surrounding water—so-called spermcast mating species (Pemberton et al. 2003, Bishop & Pemberton 2006) or egg-brooding free-spawners (Johnson & Yund 2004)—may differ greatly from the external fertilisation models based largely on broadcast-spawning echinoderms (McCartney 1997, Bishop 1998, Yund 2000, Pemberton et al. 2003, Johnson & Yund 2004, Bishop & Pemberton 2006). The essence of Ryland's (1996) argument that sperm production in cyclostome bryozoans will be low compared to the better studied large-bodied external fertilisers is almost certainly correct. It is much less certain, given their ability to gather sperm from very low concentrations (e.g. Pemberton et al. 2003, Phillippi et al. 2004, Yund et al. 2007; for colonial ascidians and cheilostome bryozoans), that cross fertilisation is unreliable in suspension-feeding spermcasters. Accordingly, sperm limitation may not have been experienced within the range of local population densities investigated in the present study. However, caution may be required in extrapolating fertilisation data from other taxa, as direct tests have not been performed on any cyclostome species; cyclostomes have a relatively simple form of filter feeding (Nielsen & Riisgård 1998), which may have significance for efficiency of sperm capture and avoidance of sperm ingestion (J. S. Ryland pers. comm.). Also, some species of cyclostome are found at much lower population densities than *C. denticulata* (Hayward & Ryland 1985, J. S. Ryland pers. com.). Future work should address different spatial scales of potential density dependence, both in natural populations and artificial experiments, to test experimentally at what level of sperm supply female reproductive output becomes compromised.

In searching for an explanation for the prevalence of polyembryony within the group, we cannot completely discount the possibility that this reproductive mode was an adaptation in the common ancestor of cyclostome bryozoans and that its persistence is attributable to phylogenetic constraint (Hughes et al. 2005). However, the trade-off between the number of gonozooids per colony and the number of cloned larvae produced per gonozooid would appear highly susceptible to adjustment over evolutionary time. If polyembryony were an evolutionary relic, presently disadvantageous because of the lack of genetic diversity amongst progeny, the routine production of multiple gonozooids each producing only a few larvae (or ultimately 1) would be predicted, given sufficient sperm supply. In fact, despite the absence of a clear effect of local density, the overall pattern of occurrence of gonozooids within the study population of *C. denticulata* indicates that fertilisation success within a colony is often low, suggesting that the 'amplification' of relatively rare fertilisations remains a viable explanation for the current maintenance of polyembryony in cyclostomes. A parallel argument is provided by Ryland (2000), who reported considerable variation in the position of gonozooids along fertile internodes of various *Crisia* species, including *C. denticulata*, in contrast to the statement by Borg (1926). On this basis, Ryland suggested that the development of gonozooids was governed by rare fertilisation events.

The pattern of female investment noted here may not be restricted to *Crisia denticulata*; Harmer (1896, p. 72) remarked that, in most cyclostomes, 'ovicells (gonozooids) are not present in a very large proportion of the colonies which may be examined'. This situation is not new. Studying fossilised encrusting cyclostomes from the Mesozoic era, McKinney & Taylor (1997, p. 552) noted: 'Most colonies that reached the size at which reproduction could occur within their species failed to produce brood chambers.' In 15 Mesozoic species, the percentage of colonies possessing brood chambers ranged from 2.5 to 88% (McKinney & Taylor 1997); the percentage for *C. denticulata* in the present study (37% over both data sets) falls near the centre of the distribution of values for the fossils. In contrast, Harmer (1896) reported that all colonies of the present-day species *Lichenopora verrucaria* possessed a brood chamber, the development of which was initiated very early in colony growth, at the 3-zooid stage. Clearly, the determinants of the very wide range of female investment in one species documented here and the exact nature and mechanism of gender allocation in crisiids and other cyclostomes require further study. Such work would benefit from a parallel investigation of sperm production by autozooids, which would involve techniques additional to the simple external observations reported here.

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Role of the embryo in crab terrestrialisation: an ontogenetic approach

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ABSTRACT: Strategies permitting amphibious brachyurans to successfully occupy land environments have long been studied, with a focus on both the adult-terrestrial and larval-water-dependent stages. However, the ontogenetic approach to terrestrial adaptations in crabs has not considered the strategies of embryos, even though natural selection should act on all stages of development. We review the state of the field of reproductive adaptations of terrestrial crabs through both an extensive meta-analysis, aiming at testing the current hypotheses suggesting evolutionary trends towards an increase in egg size and decrease in total egg clutch during the conquest of land, and the presentation of novel data on bimodal respiration of crab embryos. Published studies on the morphological characteristics of eggs and on the reproductive traits of 121 marine, freshwater and terrestrial species of brachyurans could not confirm the currently hypothesized trends. Our meta-analysis confirms that the conquest of land by brachyurans implies strong selective pressures at all developmental levels, leading towards fundamental evolutionary steps, such as the air respiration of embryos. Our novel data on aquatic and aerial respiration in the embryos of an intertidal and a shallow subtidal species confirm recent data showing that early life stages of crabs are able to breathe in air, although they are apparently water dependent. An ontogenetic approach is needed to formulate new hypotheses regarding trends in terrestrial adaptations at all brachyuran life stages and to highlight other embryonic processes related to terrestrialisation, such as excretion, which is of the utmost importance in terrestrial adult crabs.

KEY WORDS: Terrestrial adaptations · Crab embryo · Respiration · Reproductive strategy · Maternal care · Developmental pathways · Marine–terrestrial ecotone · Bimodal respiration

INTRODUCTION

Strategies permitting brachyuran crabs to successfully occupy a variety of terrestrial and semi-terrestrial environments have long been studied (for reviews see Bliss 1968, Burggren & McMahon 1988, Greenaway 1999). Previous research has demonstrated how the adult stages of terrestrial and semi-terrestrial crabs evolved integrated strategies, encompassing morphological (Hartnoll 1988, Vannini et al. 1997, Fratini et al. 2005), respiratory (Innes & Taylor 1986, Farrelly & Greenaway 1993, 1994, Morris 2002), excretory (Morris 2001, Weihrauch et al. 2004), ecological (Wolcott 1988) and behavioural adaptations (Warner 1967, Cannicci et al. 1996, Vannini et al. 1997), to adjust to the relatively new environment. On the other hand, larval stages of the great majority of terrestrial brachyuran are still strongly dependent on either marine environments or freshwater habitats, often presenting no adaptations to cope with truly terrestrial conditions (Anger 1995). This generalised amphibious life-cycle strongly affects the life-history patterns of terrestrial and semi-terrestrial species, leading to critical consequences for their reproductive ecology (Wolcott 1988) and for the parental behaviour of reproducing females (Diesel 1992, Diesel & Horst 1995). In contrast to the vast amount of data collected about the adaptive strategies used by the adult and larval stages of

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amphibious brachyurans to cope with the different environmental challenges, an ontogenetic approach has never been applied to eggs and embryos.

Charmantier & Wolcott (2001) called for an ontogenetic approach to adaptive strategies in ecological and physiological studies, citing Bartholomew (1987) and Burggren (1992). These authors followed the simple rationale that natural selection acts on all stages of development and that ontogenetic strategies, defined as the adaptations of the different stages of development of diverse taxa, should be studied at all stages of development, including the egg and embryo. Research into the terrestrial adaptations of the eggs and embryos of amphibious crabs is both regrettably absent and, in our opinion, of extreme importance in understanding the evolutionary pathways leading to the conquest of land, for at least 2 reasons. First, there is a strong link, in crabs, between the mother and the embryos, and, secondly, embryos represent the link between the terrestrial adult stage and the water-dependent larval stage of most species (Anger 1995). Female crabs do, in fact, take care of their embryos until larval hatching, undertaking energy-costly parental care (see Fernandez & Brante 2003 for a review). Carrying mothers and embryos form an ecological unit whose biology is shaped and often limited by the ecological requirements of the embryos. As a consequence, an ontogenetic approach, aimed at understanding the eco-physiological traits of embryos during their developmental stages, is needed to highlight the adaptive strategies of amphibious carrying mothers and the overall ecological needs of the species themselves.

Unfortunately, very little is known about the ontogenetic adaptations of embryos of terrestrial and semi-terrestrial crabs, and the few hypotheses that have been formulated about embryonic adaptations actually only deal with the optimisation of egg size and egg clutch volume to cope with a low oxygen tension. The rationale behind these hypotheses is that, although it is difficult to ascertain if the interstices of the egg masses of terrestrial and intertidal crabs are occupied by water or air, problems of anoxia should arise in any case. Thus, a morphological adaptation to prevent low oxygen tension of the interstitial medium should be an increase of average egg size, since this trait could become adaptive, resulting in the enlargement of the interstices within the mass (Strathmann & Chaffee 1984, Strathmann & Hess 1999). Enlarged interstices, in fact, will both favour the fluxes of the respiratory medium and prevent their water-filling by capillarity, a critical issue for air-breathing embryos. This trend in egg size increase from marine to terrestrial crabs has been confirmed by Anger (1995), although the core of his review involved larval adaptations to retention strategies and not embryos. Another biological adaptation to anoxia within egg mass interstices was suggested by Hartnoll et al. (2007), who pointed out that terrestrial crabs may reduce the production of eggs at each spawning event, thus reducing clutch volume and, consequently, problems due to hypoxia at its centre. Both the above hypotheses tacitly imply that the embryonic phase of terrestrial and semi-terrestrial crabs remains strongly water dependent, with the need for a constant supply of water for metabolic processes such as respiration and excretion. The main aim of the present article is provide an overall review about the state of the field of reproductive adaptation of terrestrial crabs, by means of: (1) testing the suggested hypotheses on the characteristics of egg and egg clutch of terrestrial crabs, through a meta-analysis on a large dataset of egg characteristics of marine, freshwater and terrestrial crabs, and (2) suggesting a new interpretation of the existing information on the basis of novel data on the capability of crab embryos to breathe air.

MATERIALS AND METHODS

Meta-analyses on egg characteristics. We searched for research and review papers dealing with the reproductive output of crab species colonising any kind of environment. Papers presenting any sort of data related to the dimension and number of eggs, even if inferred from the total egg mass, have been included in our dataset. The response variables directly collected or inferred from the original papers were: (1) the average diameter (in µm) of eggs produced, (2) their average number in a single clutch and (3) the total volume (in mm^3) of the average egg mass volume. We found 38 papers on the egg production of crabs, which provided data on 121 species, since some papers were comparative studies on >1 species. To analyse the influence of adult habitat and larval development on the response variables, we divided the 121 selected species into distinct classes. Regarding adult habitat, we differentiated between marine species (M), intertidal species (IT), terrestrial species from marine or intertidal ancestors (T(M)), freshwater species (F) and terrestrial species from freshwater ancestors (T(F)). From this particular analysis we excluded the Chinese mitten crab *Eriocheir sinensis*, since the adults of this species are truly freshwater dwellers, but the ovigerous females reach the sea for spawning and can be considered marine in this part of their life-cycles. *E. sinensis* was, however, included in the rest of the analyses, since it was possible to clearly define its larval development and its taxonomical status. For larval development we considered 3 categories:

(1) species having larvae with full development in water environments (export strategy, E), (2) species that retain larvae with a shortened development cycle in particular habitats (retention strategy, R) and (3) species with direct development of larvae in the egg (direct development, D). To test for possible effects of phylogenetic relatedness on the response variables, the species were also grouped at the family level, in a third analysis. If the source papers did not mention biological (such as average carapace width; CW), ecological, or taxonomic characteristics, we searched for other source papers to complete the dataset.

Given the well-known relationship between female dimensions and egg production in crabs, to standardise data for the statistical analyses, we used the ratio between egg size and number, respectively, and the average CW of the species. For total egg clutch volume we used the ratio between the square root of the egg mass volume and the CW for each species, due to the allometric relationships between these 2 characteristics (Hines 1982). By means of 3 distinct PERMANOVA (permutational analysis of variance) designs (Anderson 2001), we tested the null hypotheses of: (1) no influence of adult habitat, (2) no influence of larval development characteristics and (3) no differences among different families and, within them, among different adult habitats. In the first 2 cases, two 1-way designs were applied, while, in the third case, a 2-way design, with 'family' as orthogonal and fixed and 'adult habitat' as fixed and nested in family, was applied only to a sub-set of species, belonging to the families most represented in our dataset. Post hoc pair-wise tests were also applied when possible.

Water and air breathing of the embryos of 2 East African crabs. Oxygen uptake rates of embryos of 2 crab species in water and air were measured in the KMFRI Laboratory of Gazi (Kenya). The developmental stage of the embryos was assessed by optic microscope. Ovigerous females carrying eggs at different developmental stages were collected in the field. The truly intertidal, air-breathing mangrove sesarmid *Perisesarma samawati* was collected from the muddy substratum of the eulitoral *Rizophora mucronata* belt of the forests where it is active during low tides (Gillikin & Schubart 2004). The shallow subtidal swimming crab *Thalamita crenata* was captured in the tidal creeks bordering the mangroves during its maximum peak of activity, nocturnal flood tides (Cannicci et al. 2000). Specimens were brought to the laboratory and maintained in plastic tanks until embryo development was completed. The tanks for both species contained a 5 cm layer of moist mud with seawater for *P. samawati* and continuously aerated seawater for *T. crenata*. A daily turnover of fresh mud and seawater were supplied. *P. samawati* fed on mud, while *T. crenata* was provided with small sesarmid crabs, as prey, every other day. No mortality was recorded during the experiment.

Embryo respiration in water and air was measured on known numbers of eggs carefully separated from the pleopods of ovigerous females and placed in a closed respirometry system. Hamilton microlitre precision syringes (volume: 250 µl; Hamilton Bonaduz AG), filled with seawater or ambient air, were used as chambers to measure the aquatic and aerial respiration, respectively.

Oxygen saturation was recorded with an oxygen micro-optode sensor (needle-type, fibre-optic microsensor, flat broken tip, diameter: 140 µm) introduced through the top of a Hamilton syringe by a hole in the low-bleed high-temperature septa and connected to a Microx TX2 (PreSens GmbH). Prior to insertion, optodes were calibrated with air-equilibrated seawater (100%) and with oxygen-free water (0%) obtained with a solution of 1% of sodium sulfite (Na_2SO_3) in seawater. Oxygen consumption was determined by measuring the decline in oxygen saturation in the known volume of water and/or the air surrounding the eggs in the chamber over a known period of time.

Between 100 and 150 eggs were used for each water respiration test. The eggs were immersed in seawater of between 100 and 200 µl in volume for a fixed time of between 20 and 40 min, depending on the different rates of respiration during embryo development. During each measure the syringe was constantly and gently inverted, keeping the eggs continuously moving to avoid clumping. In tests for respiration assays in air, due to the higher concentration of oxygen than in water, we used masses of 200 to 250 eggs, a volume of 60 µl and a longer measurement time to obtain a significant decline in oxygen saturation. Recordings were initiated after the berried female had been exposed to ambient air for 60 min. This approach standardised the degree of dryness of the egg. During air respiration tests no attempt was made to stir the syringe, which was immersed in a cylinder filled with water, to avoid accidental ingression of ambient air. Repeated measurements were performed without eggs both in air and water to evaluate possible external factors affecting oxygen consumption. Finally, to obtain the net oxygen uptake of the embryos, values of water oxygen consumption (13.7 ± 5.7% of the experimental consumption rate) were also subtracted. All respiration tests were performed at an average temperature of 30.5 ± 2°C.

The embryos involved in the respiration measurements were observed by optic microscope after each test, to assign them to 1 of the 5 developmental stages described in Simoni et al. (unpubl. data) and then stored in 7% formaldehyde by volume. Images of the embryos stored in Kenya were scanned at a resolution of 3600 dpi and analysed with the software Image J at the Department of Evolutionary Biology in Florence

(Italy) to count the experimental eggs and measure their diameter. Changes in the volume of eggs during embryo development in both experimental conditions were indirectly measured by the formula $4/3(\pi r^2 R)$, where r (minor radius) = SA (short axis)/2 and R (major radius) = LA (long axis)/2.

A full factorial 3-way PERMANOVA was used to test for differences in oxygen uptake, with the factors 'embryo stage', 'respiratory medium' and 'species' all fixed and orthogonal. Post hoc tests were performed when appropriate. All analyses were performed using PRIMER V6.1 (Clarke & Gorley 2006) and the PERMANOVA+ for PRIMER routines (Anderson et al. 2008).

RESULTS

Meta-analyses on egg characteristics

Our in-depth literature review allowed us to confirm that no relationship is present between egg size and species size, with large species frequently opting for

Table 1. PERMANOVA, examining differences in egg size (egg diameter in µm), egg number and volume of egg clutch (in mm^3) among crabs with different adult habitats (1-way), with different larval export/retention strategies (1-way) and belonging to different families and with different adult habitats (2-way). M: marine; F: freshwater; IT: intertidal; T(M): terrestrial from direct marine ancestors; T(F): terrestrial from direct freshwater ancestors. E: export of larvae in the sea; R: retention of larvae with abbreviated development in specific environments; D: no larval phases, direct hatch of a small crab. For the first 2 PERMANOVAs, post hoc *t*-tests are also shown for significant interactions among factors, while for the 3rd one, significant interactions were too numerous to show; they are discussed in the text

	Egg size		Egg number		Clutch volume	
Adult habitat						
Source	df	Pseudo-*F*	df	Pseudo-*F*	df	Pseudo-*F*
Adult habitat	4	21.93**	4	29.82**	4	2.1822
Residual	97		88		73	
Total	101		92		77	
Post hoc *t*-test						
Groups	*t*	p	*t*	p		
F, T(F)	0.61	0.5543	1.58	0.1335		
IT, F	9.78	0.0001	12.35	0.0001		
IT, T(F)	10.97	0.0001	14.82	0.0001		
M, F	5.59	0.0001	5.00	0.0002		
M, IT	0.55	0.5871	0.80	0.4277		
M, T(F)	4.88	0.0001	5.09	0.0001		
M, T(M)	0.58	0.5640	0.68	0.4969		
T(M), F	2.19	0.0489	6.94	0.0002		
T(M), IT	1.47	0.1491	0.75	0.4579		
T(M), T(F)	2.32	0.0405	9.90	0.0001		
Larval export/retention						
Source	df	Pseudo-*F*	df	Pseudo-*F*	df	Pseudo-*F*
Larval export/retention	2	65.19**	2	140.2**	2	1.66
Residual	100		91		75	
Total	102		93		77	
Post hoc *t*-test						
Groups	*t*	p	*t*	p		
E, R	8.99	0.0001	11.58	0.0001		
E, D	8.66	0.0001	13.42	0.0001		
R, D	1.31	0.2144	1.707	0.1103		
Family						
Source	df	Pseudo-*F*	df	Pseudo-*F*	df	Pseudo-*F*
Family	4	15.11**	6	30.72**	5	1.62
Adult habitat(Family)	6	16.79**	6	18.27**	5	1.31
Residual	55		62		45	
Total	65		74		55	

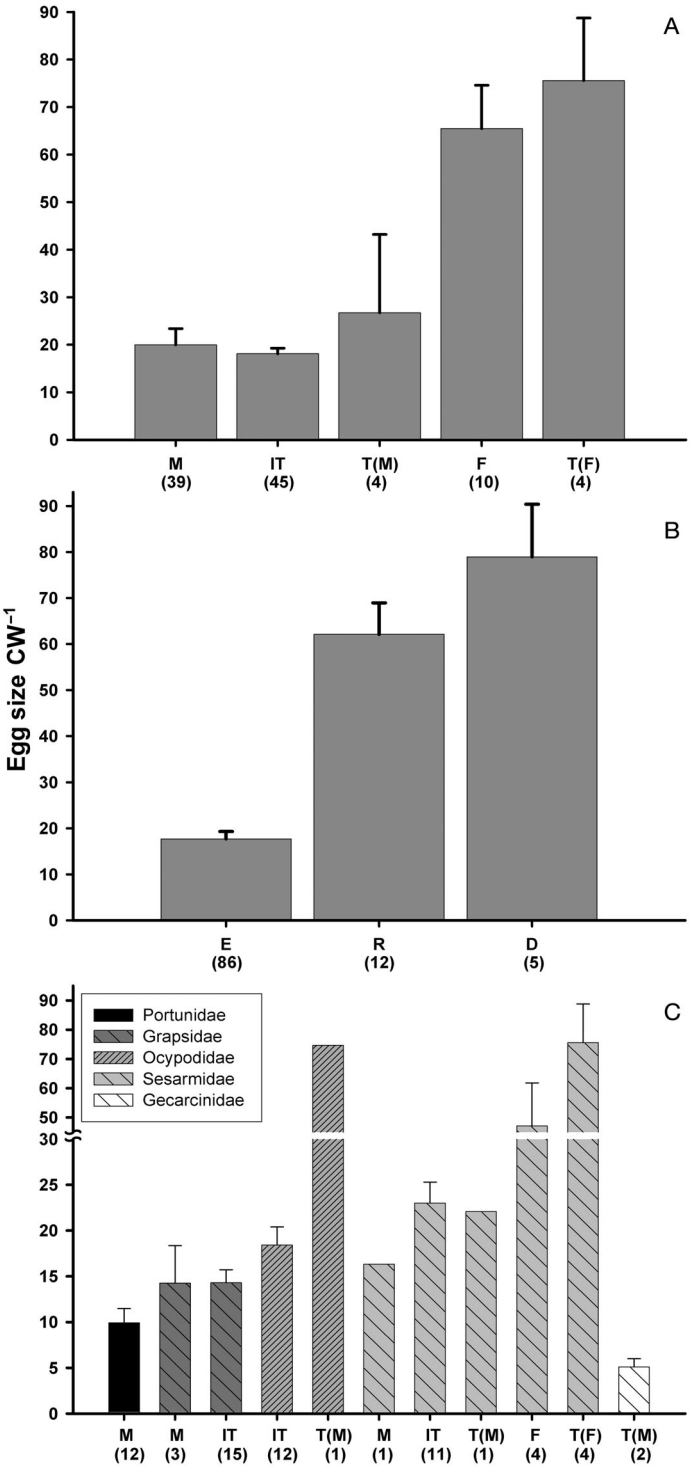

Fig. 1. Trends in egg size of bachyuran crabs based on published data. Ratio between the average egg size (diameter; in µm) and the average carapace width (CW; in mm) of crabs grouped on the basis of (A) the life-style of the adults, (B) their larval retention strategy and (C) within their families and the life-style of the adults. M: marine; F: freshwater; IT: intertidal; T(M): terrestrial from direct marine ancestors; T(F): terrestrial from direct freshwater ancestors; E: export of larvae in the sea; R: retention of larvae with abbreviated development in specific environments; D: no larval phases, direct hatch of a small crab

small eggs (Table S1 in the supplement at www.int-res.com/articles/suppl/m430p121_supp.pdf).

The habitat of adults influenced egg size (Table 1, Fig. 1A), with freshwater crabs and terrestrial species from freshwater ancestors bearing eggs not dissimilar to each other, but significantly larger than marine, intertidal and terrestrial species from direct marine ancestors (Table 1, Fig. 1A). On the other hand, no significant differences in egg size emerged among these latter categories. Also the type of larval development appears to be significantly related to egg size (Table 1, Fig. 1B). Species that evolved an export strategy of larvae produce smaller eggs than both those with larval retention and those with direct development, which display eggs of similar size. Significant differences in egg size were found among the 7 most well-represented families, but also among species with different ecological characteristics within the families (Table 1, Fig. 1C), confirming that the habitat of adults strongly influences egg size, across families. The post hoc pair-wise tests showed significant differences between the families characterised by freshwater-related species and all others. Potamonidae had significantly larger eggs than all other species, while no differences were found between families that only included marine species, such as the Portunidae and Majidae, and those including mainly intertidal, such as the Grapsidae, and truly terrestrial species from marine ancestors, such as the Gecarcinidae.

The results of the analyses on egg numbers produced per single clutch were perfectly comparable to the above-mentioned results on egg size. Freshwater and freshwater-related species showed a significantly lower number of eggs per clutch with respect to the species whose adults colonise marine and intertidal habitats, as well as to the terrestrial species with marine origins (Table 1, Fig. 2A). The large-egg species that evolved larval retention and direct development strategies produced less eggs than the crabs characterised by an export strategy of larvae with full development (Table 1, Fig. 2B), confirming an evolutionary trade-off between egg number and size. As for the egg size, we found differences in egg number produced per clutch both at the family level and within the various families, when species colonising different habitats were present (Table 1, Fig. 2C), confirming the influence of this factor on the response variable.

The volume of the egg mass produced by the various species analysed, on the other hand, showed no relationship with the habitat chosen by the adults, with the strategy of retention/dispersion of larvae, or with the phylogenetic relationships among species (Table 1, Fig. 3). The volume of the clutch was only related with the average CW of the species.

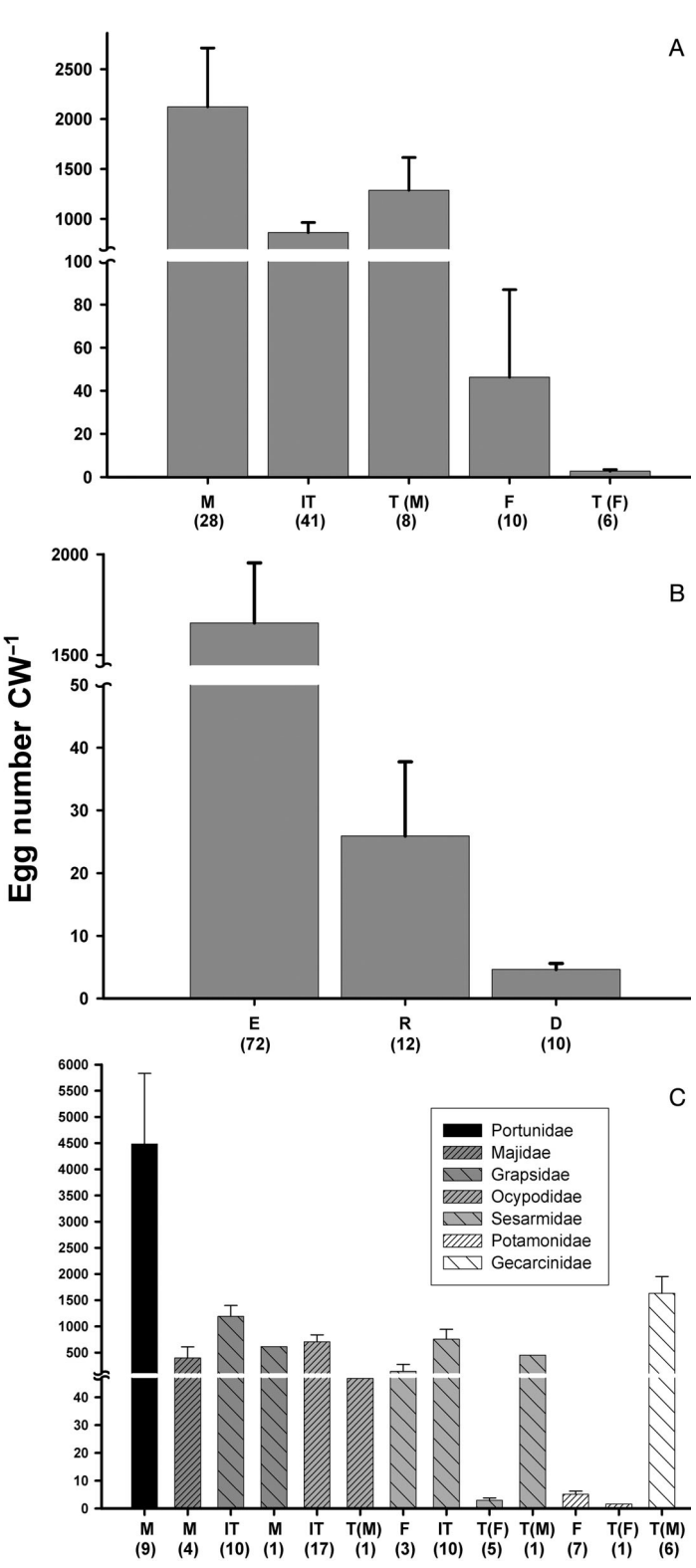

Fig. 2. Trends in egg number produced by bachyuran crabs based on published data. Ratio between the average egg number and the average carapace width (CW; in mm) of crabs grouped on the basis of (A) the life-style of the adults, (B) their larval retention strategy and (C) within their families and the life-style of the adults. For abbreviations see Fig. 1

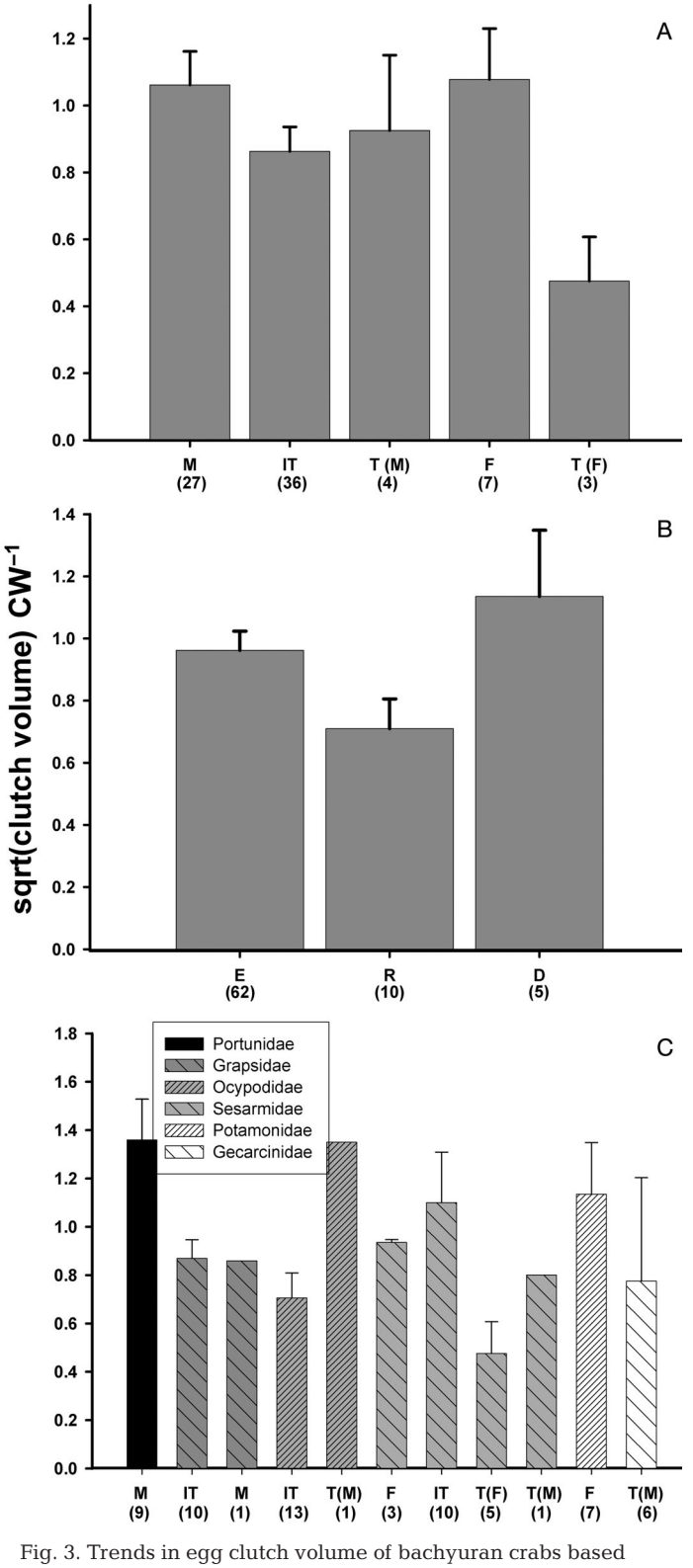

Fig. 3. Trends in egg clutch volume of bachyuran crabs based on published data. Ratio between the square root of the average volume of the egg clutch (mm^3) and the average carapace width (CW; in mm) of crabs grouped on the basis of (A) the life-style of the adults, (B) their larval retention strategy and (C) within their families and the life-style of the adults. For abbreviations see Fig. 1

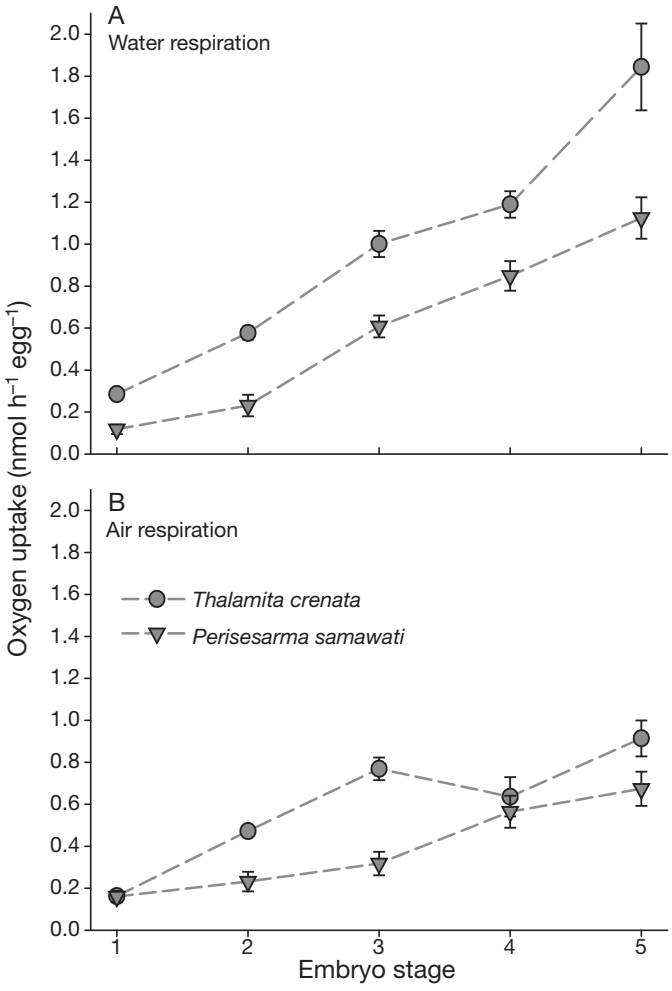

Fig. 4. *Thalamita crenata*, *Perisesarma samawati*. Oxygen uptake of embryos. (A) Water and (B) air respiration rates by embryo stage (n = 6 to 10, ±SE; when the error bar is not visible, error is within the symbol size)

Table 2. *Thalamita crenata*, *Perisesarma samawati*. PERMANOVA examining differences in respiration rate of embryos at different stages and in different respiratory media. Post hoc *t*-tests are also shown for significant interaction among factors

Source	df	MS	Pseudo-*F*	p
Medium, Me	1	3771500	67.42	0.001
Species, Sp	1	3973300	71.03	0.001
Stage, St	4	5038400	90.06	0.001
Me × Sp	1	425090	7.60	0.006
Me × St	4	695930	12.44	0.001
Sp × St	4	217730	3.89	0.005
Me × Sp × St	4	96833	1.73	0.145
Residual	163	55942		
Total	182			
Post hoc *t*-test				
Sp × St:	*P. samawati*		*T. crenata*	
	1 = 2 ≠ 3 ≠ 4 ≠ 5		1 ≠ 2 ≠ 3 = 4 ≠ 5	
Me × St:	1	2	3	4	5
	air=water	air=water	air≠water	air≠water	air≠water

Water and air breathing of the embryos of 2 East African crabs

The embryos of the intertidal Sesarmidae *Perisesarma samawati* and of the swimming crab *Thalamita crenata* were shown to be able to take up oxygen from both air and water (Fig. 4). Aquatic and aerial respirations show a typical exponential increase during ontogenesis in both species. PERMANOVA confirmed that the progressive development of embryonic complexity was paralleled by both higher oxygen consumption and higher metabolic rate, except in the first 2 stages of *P. samawati*, which showed similar respiration rates, and in the third and fourth embryonic stages of *T. crenata*, mostly due to a drop in oxygen uptake in air by the developing embryos (Table 2, Fig. 4). Oxygen uptake in air, for almost all embryonic stages, resulted in a 2-fold lower rate with respect to water, while *T. crenata* embryos showed a higher oxygen uptake than those of *P. samawati*, regardless of the medium (Table 2).

DISCUSSION

Which external forces can select for the morphology of eggs and reproductive output in terrestrial brachyurans? As for any other aquatic organism, the major physical challenges, as discussed by Martin & Strathmann (1999), are desiccation, UV radiation, large temperature variability and reduction of mechanical support. In brachyurans, these challenges are mainly endured by berried females, who give mechanical support to and control the micro-environment of embryos until larval hatching. Crabs, in fact, adopt highly effective parental care, and the evolution of egg-carrying forms established a strong link between mother and offspring. This egg-carrying phase has significant consequences for the life style and activity regime of the berried intertidal and terrestrial females. Most brooding females of the terrestrial and semi-terrestrial Ocypodidae of the genus *Uca* and *Ocypode*, for instance, are known to rarely leave their burrows, where they can control and limit embryo desiccation and avoid exposure to both ultraviolet radiation and high temperatures (reviews in Crane 1975, Wolcott 1988). On the other extreme, the well known migrations performed by *Gecarcoidea natalis* of Christmas Island serve a 2-fold purpose, since copulation occurs only at the end of the seaward journey (Adamczewska & Morris 2001). Migration is thus undertaken both to brood the egg masses in burrows on the coast, i.e. in a more marine and less stressful habitat, and to hatch the larvae in the sea.

Another selective force for reproductive traits is surely oxygen availability for embryos, both in marine and terrestrial environments. Few studies have addressed the quantification of the oxygen uptake and metabolism of developing embryos in marine crustaceans. These studies have established the central role of oxygen in shaping various life-history traits, such as egg and clutch size, development duration, hatching success and larval quality. Embryo development is accomplished through an exponential increase in oxygen consumption, which occurs to maintain an efficient metabolism for the increasing complexity of the organism during ontogenesis (Taylor & Leelapiyanart 2001). Small differences in oxygen uptake are present during the first stages of development, whereby single or a few undifferentiated cells gain oxygen by simple diffusion through the chorion. In contrast, major differences in oxygen uptake rates between developmental stages have been observed at higher levels of organization, due to the development of a complete circulatory system and the differentiation of organs and tissues (Taylor & Leelapiyanart 2001). At later stages, simple diffusion appears insufficient to provide the required amount of oxygen, and an active water intake occurs in combination with the development of efficient osmoregulatory processes and the onset of heart beating (Spicer & Morritt 1996, Reiber & Harper 2001, Taylor & Seneviratna 2005, Seneviratna & Taylor 2006).

Fluctuations of oxygen availability are also dependant on the size of the embryo mass and the relative position of the eggs. The oxygen level is rapidly depleted in the centre of the clutch, with respect to the external layers, although a series of parental care actions are provided to maintain elevated water refurbishment and circulation. Adjustments in embryonic metabolic rates also occur independently of the surrounding oxygen availability and appear to be stage dependant, with a higher plasticity of oxygen consumption evident with progression in development (Naylor et al. 2001, Baeza & Fernández 2002).

Given the strong importance of oxygen availability, the traits of eggs and egg masses of terrestrial and semi-terrestrial species were supposed to be shaped by anoxia, due to the hypothesised, although never tested, low oxygen tension within the egg masses exposed to air. However, our analyses proved that both evolutionary trends hypothesised for egg size and egg mass dimension by Strathmann & Hess (1999) and Hartnoll et al. (2007), respectively, are not supported by an extensive dataset on crab reproductive features. In fact, no dimensional trend related to eggs along the terrestrialisation evolutionary pathways could be gleaned from published data, since the eggs of terrestrial species are not larger than those of their marine relatives. The eggs of freshwater-related species indeed proved to be larger than those of the marine-related and terrestrial ones, but this could be explained by evolution towards a shortened larval phase, ranging from a reduced number of larval stages to the direct hatching of young crabs (Bliss 1968, Anger 1995). Although differences in egg size could be detected among families, these could be ascribed to the homogeneity of habitat preferences among species belonging to the same family, a common feature in crabs. The Portunidae and Majidae species we could analyse, for instance, were all marine, while Gecarcinidae and Potamonidae were all truly terrestrial and freshwater related, respectively. More interesting findings were achieved in families, such as the Grapsidae, Ocypodidae and Sesarmidae, in which group species belonging to different habitats. In these cases, we found intra-family differences in egg size, which support the strong link between egg size, freshwater environment and the larval retention strategy. The data available from literature, in summary, strongly suggest that egg size is mainly related to the characteristics and fate of larvae and not to the level of terrestrialisation of the adult.

Accordingly, the number of eggs produced per clutch is strongly related to larval dispersion/retention strategies, showing that crabs have to face the evolutionary trade-off existing between the dimension of single eggs and the number of eggs produced per reproductive event, as highlighted by Hines (1982). Thus, the dependences of egg number from adult habitat and developmental strategy mirrored the ones discussed for egg size, with freshwater-related and direct-development strategy species producing significantly less eggs than all other categories. As a result, the overall energy allocated to a single spawning event, expressed as total clutch volume, was not influenced by any ecological or evolutionary factor, but by allometric constraints alone (Hines 1982). Thus, published data do not support the hypothesis that terrestrial crabs may reduce their reproductive effort per single event (Hartnoll et al. 2007), showing that average clutch volume is similar among marine, freshwater and terrestrial species.

We suggest that the above-mentioned hypotheses fail to explain the actual trends found in nature for 2 reasons. The first is that the authors underestimated the capability of air breathing of crab embryos, thus basing their theories on the assumption that the egg masses should occupy water-based micro-environments and, consequently, overestimating the levels of anoxia within the interstices. The second reason is the influence of the terrestrial environment on maternal care.

The leading benefit for evolving a terrestrial development is the 30-fold abundance and 10 000 times higher diffusiveness of oxygen in air than in water.

Thus, the opportunity to adopt aerial respiration could indeed reduce the problems described for marine species due to the limiting oxygen demand within masses of developing embryos (Strathmann & Hess 1999). Our novel results on embryonic respiration in the semi-terrestrial *Perisesarma samawati* and in the shallow subtidal *Thalamita crenata* support the hypothesis of true bimodal respiration in the early life stages of brachyurans, even in species still strongly related to the sea. The embryos of the 2 East African crabs proved, in fact, to uptake oxygen from both media, with *T. crenata* showing an overall higher oxygen demand than *P. samawati*. Since the eggs of the 2 species are similar in dimension, this difference can only be ascribed to differences in the overall metabolic rates of the 2 species, an issue which should be addressed in further studies. Our data also highlighted an important evolutionary trend towards air-breathing embryos, since the comparison of intertidal and shallow subtidal species revealed the failure of fully efficient oxygen uptake in air for the latter. Embryos of *T. crenata*, in fact, develop completely in water, with a few sporadic emersions during low tides. While their oxygen uptake in water increases through all developmental stages, they cannot maintain successful respiration in the air, showing the water dependence of the ontogenetic stages.

These novel data confirmed the results of a recent study on an amphibious Jamaican sesarmid, *Armases miersii*, by Simoni et al. (unpubl. data) and show, for all 3 species, that oxygen uptake in air, for almost all embryonic stages, results in a 2-fold lower rate with respect to water. Simoni et al. (unpubl. data) interpreted this interesting result in 2 contrasting ways. The first hypothesis is that the embryos are not completely able to extract enough oxygen from the air to complete full development out of the water. Consequently, they would respond to the lack of oxygen diffusion at a tissue level with a correspondent decrease in metabolic rate, by means, for instance, of bradycardia. On the other hand, the low oxygen uptake in air could be the direct consequence of the lower metabolic cost of extracting oxygen from air than from water. Thus, water respiration implies an active and expensive process, in terms of energy, to maintain normoxia within the eggs (Simoni et al. unpubl. data).

Regarding the influence of the terrestrial environment on maternal behaviours, it seems that, compared to water, the higher availability of oxygen in the air reduces the energetic costs of maternal care to maintain a normoxic condition within the egg mass. A crucial energetic cost of parental care in marine crabs is oxygen provision to the clutch through active ventilation or water circulation (Fernández et al. 2000). Early studies addressed the importance of oxygen availability to embryos and the consequent behaviour of ovigerous females (Wheatly 1981, Naylor et al. 1997, 2001). These works, which principally focus on the requirements of the embryo, describe the maternal behaviour of *Carcinus maenas* and *Cancer pagurus* and reveal an active control of the egg mass milieu independent of environmental conditions. An increased oxygen demand, due to temperature variation or local hypoxia, is rapidly supplied through the intensification of abdominal flapping and providing efficient ventilation. On the other hand, a series of studies by Miriam Fernández and colleagues have investigated parental care in terms of oxygen provision to the egg mass, focusing on the role of the mother (Fernández et al. 2000, 2002, 2003, 2006, Baeza & Fernández 2002, Ruiz-Tagle et al. 2002, Brante et al. 2003, Fernandez & Brante 2003). These studies have noted the high metabolic expenditure associated with active and continuous parental care. Brooding females perform a series of behaviours such as abdomen flapping and pleopod beating that frequently intensify with embryo development. These behaviours have important consequences for the adult, in terms of both egg losses and oxygen consumption, used as proxies for mechanical and energetic costs, respectively. At the interspecific level, the energetic investment of berried females appears to be size dependent. While larger species undergo higher metabolic expenditure, smaller species do not show increased metabolic demand, even if brooding behaviours are present. However, the direct benefits of parental care appear evident when the oxygen/water exchange is evaluated inside the egg mass. These studies demonstrated a clear difference in oxygen concentrations at the periphery of the mass with respect to the centre. On-line and continuous measurements of oxygen availability have shown the effectiveness of water exchange by abdominal flapping. This behavioural strategy increases hatching success through 2 mechanisms: the ability to lay larger egg masses than those laid by non-ventilating mothers and the synchronization of development time between the embryos at the centre of the mass and those laid at the periphery.

Although bimodal respiration of crab embryos seems a common feature, including in shallow-water species, it does not imply a real independence from water of the egg clutches. In the only available study on the water dependence of embryos of the amphibious crab *Armases miersii* the authors found that, even if embryos could breathe in air throughout their development, the eggs of water-limited females could not produce healthy larvae (Simoni et al. unpubl. data). These results led the authors to suggest that other important physiological pathways could be limited if water cannot be provided to the embryos, such as, for instance, excretion and osmoregulation processes. Unfortunately,

nothing is known about the excretion of crab embryos, although they are thought to rely on ammonia just like both the larvae and adults (Greenaway 1999, Weihrauch et al. 2004). In particular, no data are yet available on the ontogeny of osmoregulation or on excretory patterns in terrestrial crabs, although Charmantier & Charmantier-Daures (2001) pointed out that 'a wider knowledge of osmoprotection and osmoregulation of embryos may also lead to renewed insight on adaptation to terrestrial conditions, particularly in […] decapods'. Due to this huge gap in our knowledge, the effects of these physiological traits on the water dependence of embryos are, at present, difficult to ascertain. Simoni et al. (unpubl. data) found that air-breathing embryos of *A. miersii* experimentally deprived of water could not develop normally, resulting in an elevated number of misbehaving, dead and morphologically deformed larvae at hatch. The authors suggested that abnormal embryo development could be caused by their nitrogen excretion mechanisms, supposedly still strongly water dependent, although they cautioned that further studies must be carried out to corroborate this supposition.

In conclusion, amphibious and shallow-water crabs have bimodal embryos capable of oxygen uptake from both water and air, as demonstrated in recently published studies and by the novel data presented here. This evolutionary step towards land completely changes our understanding of the reproductive biology of land crabs and, in particular, the brooding strategies and parental care of their females. Indeed, existing hypotheses regarding the evolutionary trends in egg morphology, reproductive output and egg mass characteristics of terrestrial crabs should be rethought in light of the ability of their embryos to breathe in air throughout their development. However, amphibious crab embryos did not prove to be totally independent of water; they are still vulnerable to the stressing factors peculiar to emergent habitats. Thus, the behaviour and parental care performed by brooding mothers remain extremely important in providing a vital microhabitat in which embryo development can occur.

The current state of the field, outlined here, is a fascinating starting point for future ontogenetic approaches to the study of terrestrial adaptations in crabs. Since the scant available data come from subtidal and intertidal species, an increase in our knowledge of the respiratory traits of embryos in truly terrestrial crabs is probably the first step to take in order to ascertain whether the trend toward full air breathing in embryos is consistent. A further step would be to extend the ontogenetic approach to other physiological processes taking place during embryo development. Greater understanding of processes such as osmoregulation and nitrogen excretion is, in our opinion, needed to assess the degree of water dependence of embryos and eggs. In parallel with the physiological analyses of embryos, new hypotheses on the evolutionary trends shaping parenting behaviour and the characteristics of maternal care in land environments should be tested to develop a more complete evolutionary scenario of the ongoing terrestrial invasion of crabs.

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Bryozoan growth and environmental reconstruction by zooid size variation

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ABSTRACT: The modular growth of cheilostome bryozoans combined with temperature-induced variation in module (zooid) size has enabled the development of a unique proxy for deducing seasonal temperature regimes. The approach is based on measures of intracolonial variation in zooid size that can be used to infer the mean annual range of temperature (MART) experienced by a bryozoan colony as predicted by a model of this relationship that was developed primarily to infer palaeoseasonal regimes. Using the model predictions effectively requires a highly strategic approach to characterise the relative amount of within-colony zooid size variation (by adopting random or very systematic measurements of zooids that meet a stringent set of criteria) to gain insights on temperature variation. The method provides an indication of absolute temperature range but not the actual temperatures experienced. Here we review the development of, support for and applications of the zooid size MART approach. In particular, we consider the general issue of why body size may vary with temperature, studies that validate the zooid size–temperature relationship and insights that have been gained by application of the zooid size MART approach. We emphasise the potential limitations of the approach, including the influence of confounding factors, and highlight its advantages relative to other proxies for palaeotemperature inferences. Of prime importance is that it is relatively inexpensive and quick and allows a direct estimate of temperature variation experienced by an individual colony. Our review demonstrates a strong and growing body of evidence that the application of the zooid size MART approach enables robust interpretations for palaeoclimates and merits broad recognition by environmental and evolutionary biologists and climate modellers.

KEY WORDS: Cheilostomes · Mean annual range of temperature · MART · Body size–temperature relationship · Palaeoclimates

INTRODUCTION

Patterns of growth in plants and animals have long been used to gain insights into past environments. Variable accretion of structural material in trees, fish otoliths and the shells of bivalve or gastropod molluscs, for example, can be used to retrospectively extract ambient environmental conditions such as rainfall, temperature or food availability (e.g. Falcon-Lang 2005, Zazzo et al. 2006, Hallmann et al. 2009). Organismal attributes that favour such analyses include a continuous record of growth and the sequential development of discrete and measurable features that vary consistently with respect to a single environmental variable and remain fixed, thereby permitting the retrieval of environmental conditions relevant to particular time periods. Benthic colonial invertebrates can provide an especially appropriate system for such retrieval, since, with some exceptions (e.g. sponges), they comprise distinct, individual modules (zooids) that are produced iteratively throughout the lifetime of the colony. The sclerochronological analysis of modular

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growth can provide inferences for both intra- and interannual environmental variation, information that is not readily available from analyses of short-lived, unitary organisms that are commonly used as proxies, such as foraminifers or ostracodes. Furthermore, coloniality is often associated with polymorphism, with modules specialised for different functions within a colony. These attributes, viz. modular iteration, polymorphism and individual colony longevities ranging from months to many years, may enable joint insights into environmental conditions and associated life history variation (O'Dea & Okamura 2000a, O'Dea & Jackson 2002). Such insights are generally difficult to achieve through investigations of longer-lived unitary organisms, such as bivalves or brachiopods, since the morphologies of these organisms do not readily provide a record of functional allocation during their lifetime. Surprisingly, however, the unique contribution of colonial invertebrates for retrospectively deducing environmental and life history variation has not been widely recognised.

Bryozoans are colonial, suspension-feeding invertebrates that are common members of benthic assemblages (McKinney & Jackson 1989). There are some 6000 described extant species of bryozoans (Gordon et al. 2009), most of which belong to the order Cheilostomata. Colonies of cheilostomes comprise asexually budded zooids that are reinforced by skeletal walls composed of calcitic and/or aragonitic carbonate (Rucker & Carver 1969, Smith et al. 2004). Typically, cheilostomes display zooid polymorphism (McKinney & Jackson 1989). The majority of zooids are specialised for feeding (autozooids), whilst a smaller proportion function in reproduction (ovicells) and defense (avicularia). The carbonate skeleton ('zooecium') confers preservation of colony features, including zooid polymorphism, and bryozoans are well represented in the fossil record (McKinney & Jackson 1989). Once the skeletal walls of a new zooid are secreted, there is no further expansion of zooid surface area (O'Dea & Okamura 2000b). This gives the zooid a determinate size that has been shown to be controlled to a significant extent by the ambient water temperature at the time the zooid was produced. Bryozoan colonies therefore record the range of temperatures experienced during their lifetime as intracolonial variation in zooid size (Fig. 1). Such temperature-induced variation in size is also observed in unitary animals and is generally known as the 'temperature–size rule' (Atkinson 1994).

The above-mentioned features make cheilostome bryozoans unique amongst colonial taxa in offering opportunities for inferring environmental conditions and biotic responses in the present day as well as over geological time. Other colonial taxa such as corals, hydroids, ascidians and other non-cheilostome bryozoans either do not produce carbonate skeletons, show indeterminate growth of their polyps or zooids, or exhibit little to no polymorphism and therefore preclude gaining additional insights on how life histories may respond to environmental conditions.

Recognition of the unique opportunities afforded by cheilostome bryozoans for the retrieval of environmental information led to the development of a method that allows the estimation of the mean annual range of temperature (MART) based on variation in zooid size within cheilostome colonies. The method is based on using model predictions for how zooid size varies with MART and thus requires that zooids meet a stringent set of criteria, in keeping with assumptions of the model, and that a strategic sampling protocol is adopted to target appropriate zooids randomly or very systematically. The method informs on absolute seasonal variation in temperature but does not indicate the actual temperatures. Thus polar and tropical bryozoans will converge on similar low MART values.

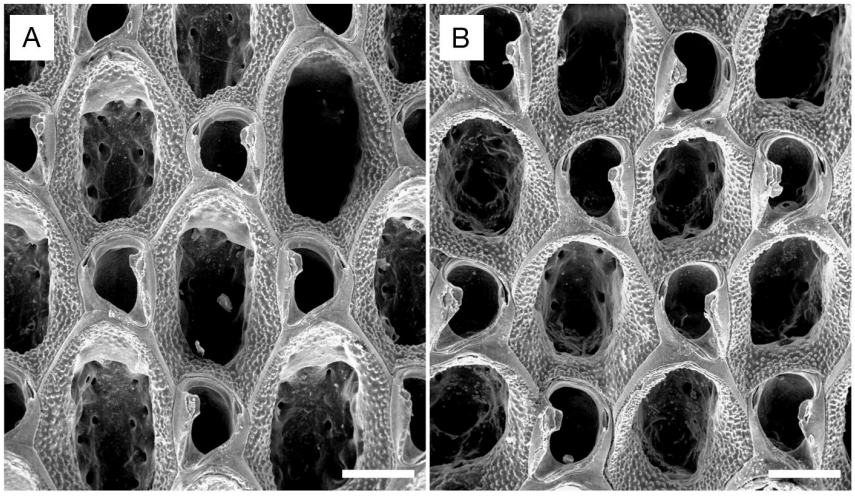

Fig. 1. *Cupuladria exfragminis.* Seasonal variation in zooid size. Scanning electron micrographs of a recent colony from the Gulf of Panama. Size difference between zooids that developed during (A) upwelling (cold) and (B) non-upwelling (warm) conditions. Same magnification for the purpose of comparison. The skeletal walls of both autozooids (large orifices) and avicularia (small orifices) are evident. Scale bar = 150 µm. Photos by R. Dewel

In this paper we describe this zooid size MART approach, review studies that validate the zooid size–temperature relationship on which the technique depends and summarise research that has made use of the approach in order to demonstrate the insights that can be gained by its adoption. We also emphasise the potential limitations of the zooid size MART approach, describe confounding factors that must be borne in mind and suggest directions for future studies. However, because the approach is based on temperature-induced variation in zooid size, we first describe the temperature–size rule and address the mechanism(s) that may underlie the temperature–size relationship. Examination of these issues leads us to conclude that the zooid size MART approach provides a unique and independent proxy that will enable more robust interpretations when incorporated as part of the toolkit used for environmental and evolutionary studies.

VARIATION IN BODY SIZE WITH TEMPERATURE

The inverse relationship between zooid size and temperature conforms to a general pattern observed in ectotherms known as the 'temperature–size rule' (Atkinson 1994). This pattern is expressed as phenotypic plasticity in response to temperature variation demonstrated by negative thermal reaction norms. As with any 'rule', there are instructive exceptions, but the overall weight of evidence for the temperature–size rule (Atkinson 1994, Angilletta et al. 2004, Kingsolver & Huey 2008) lends additional and important support for temperature-driven variation in zooid sizes. What has been unclear, however, is what mechanisms may underlie this nearly universal relationship, whether there exists an adaptive basis for thermal sensitivity in body size, and whether the mechanisms and/or adaptive explanations are inclusive across taxa. Central to this issue is the association of the temperature–size rule with a life-history puzzle (Berrigan & Charnov 1994): that good conditions result in faster growth to a larger size but that temperature has contradictory effects on growth and size, with higher temperatures driving faster growth to a smaller size.

Hypotheses proposed for the temperature–size rule include both adaptive and non-adaptive scenarios (Atkinson 1994, Angilletta et al. 2004). However, demonstrations that the shapes of thermal reaction norms can readily evolve in response to selection (see Kingsolver & Huey 2008 for review) suggest that selection maintains the temperature–size rule. A problem common to many of the hypotheses proposed to explain the temperature–size rule is that they are too restrictive to apply to the diversity of taxa that follow the rule (see Angilletta et al. 2004, Kingsolver & Huey 2008 for recent review). Mechanistic explanations have included: (1) the production of smaller adult stages because developmental rate is more strongly influenced by increasing temperature than growth rate (van der Have & de Jong 1996); (2) the related hypotheses that cell (van Voorhies 1996, Woods 1999) or body (Chapelle & Peck 1999) size is limited by oxygen diffusion.

Atkinson et al. (2006) recently addressed the explanation that the temperature–size relationship may relate to oxygen concentrations by examining the thermal responses of the bryozoan *Celleporella hyalina* to 2 temperatures (10 and 18°C) and 2 oxygen concentrations (21 and 10%, representing normoxia and hypoxia, respectively). They found that smaller zooids were produced under hypoxia regardless of temperature (although size was also influenced directly by temperature), providing evidence for the expected adjustment of size in response to oxygen requirements. This adjustment is anticipated because increasing temperatures increase metabolic rates and thus oxygen demands, but these metabolic oxygen demands increase faster with temperature than diffusion in the organism's oxygen uptake and transport system. Smaller size will therefore decrease diffusion distances and increase the relative surface to volume ratios for oxygen uptake. In addition, size variation may regulate respiratory activity, with a decrease in size reducing activity at higher temperatures via reductions in mitochondrial volume density and in cristae density (see review by Atkinson et al. 2006).

Atkinson et al. (2006) also obtained evidence that the temperature–size rule is not a fundamental response of cells. Larval parenchyma cells were larger at the lower temperature, but temperature had no effect on the size of epithelial cells of the tentacle. Similar results have been obtained in other empirical studies that have shown that larger body sizes at lower temperatures can be caused by an increase in cell size in some systems (e.g. in nematodes or some *Drosophila* populations) but not in others (including other *Drosophila* populations; see Angilletta et al. 2004 for review). As mentioned earlier, Atkinson et al. (2006) also found that an inverse temperature–size relationship characterised zooids, but this was not the case for tentacle length. Thus, the temperature–size rule did not apply universally at the cell or organ (e.g. tentacle) level.

Their results led Atkinson et al. (2006) to propose that temperature-induced size changes at different levels of organisation are part of a range of acclimation mechanisms, including variation in body size, that will optimise functional capacity (e.g. of mitochondria and tissues) to maintain scope for aerobic activity. They suggest that these acclimatory processes occur within a temperature range whose limits are determined by when oxygen partial pressures of body fluids fall. This

occurs at the so-called 'pejus' limits, when the capacity of oxygen supply mechanisms is unable to support oxygen demand (Pörtner 2002). Thus, at high temperatures, excessive oxygen demand results in insufficient oxygen levels in the body fluids, while at low temperatures, the aerobic capacity of mitochondria may become limiting. Beyond these limits aerobic scope disappears, and the adoption of anaerobic metabolism will support time-limited survival. If these acclimatory responses to maintain scope for growth underlie the temperature–size rule, they could at least in part account for the puzzle of the inverse temperature–size relationship despite increased growth rate with warmer temperatures.

As discussed below and exemplified by the study of Atkinson et al. (2006), cheilostome bryozoans demonstrate intraspecific thermal sensitivity that reflects the temperature–size rule. They also demonstrate variation in zooid size within (see 'Evidence for relationship between temperature and zooid size') and among closely related species (e.g. species of *Haplopoma*; Ryland 1963) living in geographic regions that are characterised by different temperature regimes. The extent to which the latter reflects phenotypic plasticity versus selection requires investigations of thermal reaction norms. It should be noted that the zooid size approach to MART is not complicated by these issues since it is based on intracolonial zooid size variation. However, the occurrence of the temperature–size rule at the modular level amongst colonial organisms raises the question of the adaptive significance of body size when environments change over the lifetime of colonies. Because zooids remain fixed in size, any adaptive basis for size at the time of budding will be ephemeral in seasonal environments. For bryozoans, an adaptive basis for phenotypic plasticity in zooid size may nevertheless apply if at least some regions of a colony are in optimal condition as a result of zooid-size matching to the prevailing thermal regime. Alternatively, smaller zooids may simply result if the developmental rate is more strongly influenced by increasing temperature than growth rate (van der Have & de Jong 1996).

ZOOID SIZE MART APPROACH

The zooid size MART approach is based on a predictive model that allows seasonal variation in temperature regimes to be estimated from the empirically derived relationship between intracolonial zooid size variation and the MART (O'Dea & Okamura 2000b). The model was developed by undertaking morphometric analyses of 157 colonies of 29 cheilostome species ranging from tropical to polar regions and is based on a regression of the mean coefficient of variation (CV) of zooid size and the MART with 95% confidence limits within ±1°C across the entire temperature range (see Fig. 2c in O'Dea & Okamura 2000b). Zooid size was estimated by measuring the maximum distance between the proximal and distal skeletal walls, to estimate zooid length, and the maximum distance between the lateral skeletal walls, to estimate zooid width (Fig. 2). These maximum distances infer growth along a straight line trajectory aligned perpendicular to zooid margins that are farthest apart (see Fig. 2). Zooid frontal area is then calculated as the product of zooid length and width. The mean maximal difference between summer and winter temperatures over a number of years for the depths at which the bryozoans were collected (see O'Dea & Okamura 2000b for further discussion) was used to estimate MART. Algebraic rearrangement of the regression provides a means of predicting MART as: MART = –3 + 0.745b, where b is the mean intracolonial CV of zooid size.

The dependability of the zooid size MART approach is a function of several factors, including the data that were originally used to develop the model, variation in response to MART amongst taxa, other agents of zooid size variation and rigour in applying the approach. In developing the model, data on zooid sizes were required to meet a stringent set of criteria. Zooid size values were based on 20 randomly selected autozooids per colony. However, to minimise variation due to factors known to influence zooid size, the autozooids were required to show uninhibited growth (i.e. were not perceptibly deformed), to be part of the basal series of zooids (i.e. not frontally budded) and to be located outside of the zone of astogenetic change (Fig. 3). This zone is created during the early growth of colonies, when zooid generations show pronounced incremental increases in size. Beyond this zone zooid sizes are relatively stable (Boardman & Cheetham 1969). There were also criteria for choosing colonies and species for measurement. For colonies, these included offering at least 30 ontogenetically complete autozooids and avoiding measuring colonies whose shapes were compromised by e.g. irregularities in the substratum or observable competition from other organisms. For species these included: offering clearly delimited zooid margins for measurement; no evidence of distortion in dried material; avoidance of 'spot' colonies that undergo determinate growth to a very small size (Winston & Håkansson 1986, Bishop 1989); the availability of at least 5 replicate colonies per species. The CVs were averaged for each species at each locality. Since the model uses estimates of zooid size to infer seasonal temperature regimes, it is important that, as far as possible, these estimates are based on applying a similar set of criteria to those that were used to develop the model.

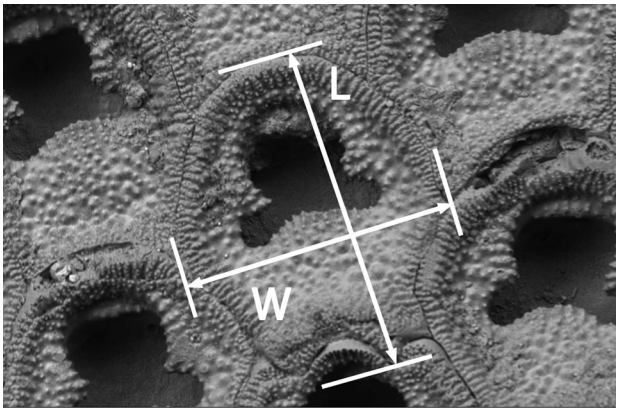

Fig. 2. *Floridina regularis*. Scanning electron micrograph of a colony from the Yorktown Formation (Chuckatuck, Virginia, USA) showing length (L) and width (W, 150 µm) of a zooid. Measurements are based on maximum dimensions of zooids that meet the criteria for zooid size mean annual range of temperature (MART) analysis, see Table 1

O'Dea & Jackson (2002) later developed an alternative method for zooid size MART analysis. Instead of conducting random sampling of 20 zooids per colony, they undertook highly systematic sampling of zooids that met the above criteria. This entailed measuring sequential generations of disto-laterally budded zooids in cupuladriid bryozoans (Fig. 4). They characterised 4 such zooid profiles per colony and found that the mean maximum and minimum values correlated well with the MART experienced by the colonies living in contrasting seasonal regimes. This zooid profiling approach requires particular care to avoid measuring the somewhat smaller zooids that occur at bifurcations or just distal to bifurcations in the normal budding series. Such zooids are encountered more frequently near the centre of the colony, as row bifurcations allow zooids to populate space as the colony extends radially. In such cases, the path of the profile was altered (Fig. 4). This zooid profiling technique has the advantage over randomly sampling zooids for MART estimation in that recording continuous changes in zooid sizes allows the demonstration of annual growth increments and insights on growth rates and colony longevity (e.g. Fig. 5 of O'Dea & Jackson 2002). Table 1 summarises the set of rules that must be met when conducting either random or systematic measurements of zooids for zooid size MART analysis. These rules reflect the various criteria mentioned above.

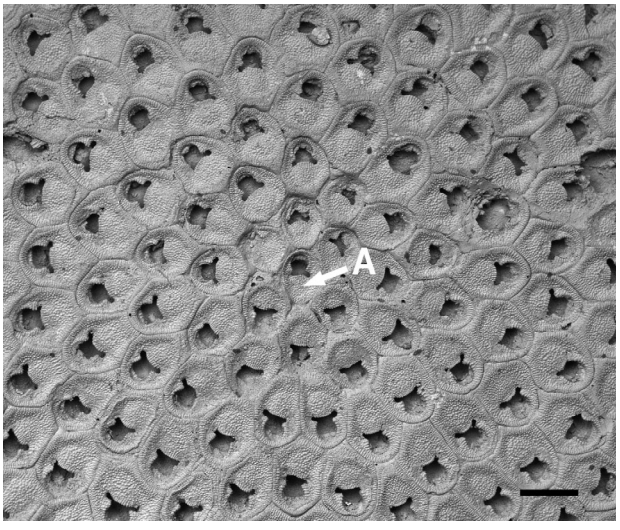

Fig. 3. *Floridina regularis* (as in Fig. 2). Zone of astogenetic change. Scanning electron micrograph of ancestrula (A) and region of early colony growth showing a gradual increase in zooid size throughout the first few generations. Scale bar = 250 µm

EVIDENCE FOR THE RELATIONSHIP BETWEEN TEMPERATURE AND ZOOID SIZE

The zooid size MART approach is founded upon the negative relationship between zooid size and temperature that has been demonstrated in both controlled laboratory and field situations and over spatial and temporal scales. In the following 4 subsections we review and evaluate the growing body of evidence that supports this relationship.

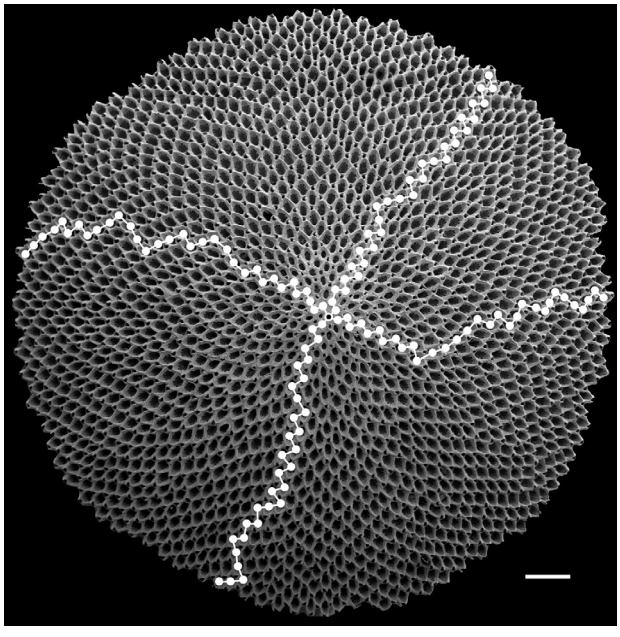

Fig. 4. *Cupuladria* aff. *biporosa*. Zooid size profiling. Scanning electron micrograph. White lines = paths of 4 profiles from the central ancestrula to the colony margin. Dots = zooids chosen for measurement according to strict rules as outlined in text. Scale bar = 1 mm. Reprinted from O'Dea & Jackson (2002) with permission from Elsevier

Laboratory studies

Controlled laboratory studies have consistently demonstrated that zooids increase in size at lower temperatures. Menon (1972) showed decreases in both the lengths and widths of zooids of *Electra pilosa* and *Conopeum reticulum* as laboratory temperatures increased (temperature regimes: 6, 12, 18 and 22°C for *E. pilosa*; 12, 18 and 22°C for *C. reticulum*). Hunter & Hughes (1994) found that *Celleporella hyalina* produced significantly smaller zooids at 18°C than at 8°C and that this response occurred irrespective of food supply (10 vs 100 cells ml^{-1} of *Rhodomonas baltica*). A later study provided further and independent evidence for an inverse relationship between temperature and zooid size in *C. hyalina*, with autozooids being smaller when colonies were reared at 10°C than at 18°C, even when the partial pressure of oxygen was altered from normal to hypoxic conditions (Atkinson et al. 2006). This study also demonstrated that, like length and width, autozooid volume varies inversely with temperature. Amui-Vedel et al. (2007) found that *Cryptosula pallasiana* produced longer and wider zooids at 14°C than at 18°C under equal food concentrations (100 cells ml^{-1} of *R. baltica*). The aforementioned studies were all conducted on temperate encrusting species that were growing on glass or plexiglass slides whose flat surfaces will minimise variation in zooid dimensions arising from topographic complexity.

O'Dea et al. (2007) took advantage of the naturally high rates of colony cloning in the free-living, tropical species *Cupuladria exfragminis* to observe the effects of temperature upon zooid size amongst genetically identical clones. Colonies from the Gulf of Panama were halved and the resulting clonal replicates exposed in culture to either 29°C, the normal temperature for the Gulf of Panama, or 24°C, a temperature commonly observed during episodes of upwelling. The 5° reduction in temperature resulted in a 25% increase in zooid surface area. When normal, warmer temperatures were restored, new zooids reverted to the smaller size associated with 29°C.

Growth in the field

Temperature-induced variation in zooid size has been directly investigated in the field in the encrusting species *Conopeum seurati.* This was achieved by measuring the maximum lengths and widths of focal colonies that colonised glass slides on 19 occasions over 15 mo in the Severn Estuary and conducting simultaneous measurements of temperature, salinity and food availability, as estimated from chlorophyll *a* (chl *a*) concentration (O'Dea & Okamura 1999). Sampling intervals were approximately biweekly during periods of rapid growth but less frequent in winter when growth slowed considerably. General linear model analysis revealed that temperature consistently accounted for most of the variation in zooid size (40.5%) with larger sizes occurring at lower temperatures (e.g. Fig. 1). Salinity, colony identity (genotype) and an interaction between temperature and salinity also influenced zooid size (by 21.1, 3.2 and 22.0%, respectively). Factors that had no significant effect on zooid size were food availability (chl *a* concentration), colony growth rate and the reproductive status of colonies based upon the presence or absence of embryos, eggs or oocytes (O'Dea & Okamura 1999).

Other studies that have measured zooid size variation in the field were conducted on colonies established on natural substrata. Lombardi et al. (2006) demonstrated that the upright bifoliate species *Pentapora fascialis* produces larger zooids during the colder winter than during the warmer summer near Plymouth (UK) and Tino Island in the Mediterranean. In contrast,

Table 1. Rules for choosing zooids, colonies and species appropriate for zooid size mean annual range of temperature (MART) analysis on cheilostome bryozoans

Zooids	Colonies	Species
1. Must be autozooids (not kenozooids, vibracula, avicularia, etc.)	1. With >30 ontogenetically complete autozooids, preferably many more	1. Should possess clear autozooid margins
2. From outside zone of astogenetic change	2. Not growing on highly irregular surfaces	2. Autozooid margins are not obscured by expansion of polymorphs (e.g. avicularia) or frontal budding
3. Not abnormal in size/shape (due to e.g. physical damage, biotic interaction, position at or just distal to bifurcation in budding series or local regeneration following colony fragmentation)	3. Growth not impeded by competition with other sessile organisms or epibionts	3. Not distorted by e.g. drying
4. From basal series (not frontally budded)	4. Replication of colonies within species (≥5)	4. Colonies can achieve large size (not 'spot' colonies with determinate growth to small size)
5. EITHER: Randomly choose 20 autozooids conforming to zooid rules 1–3		
6. OR: Measure successive generations of autozooids conforming to zooid rules 1–3 (zooid profiling)		

the average zooid lengths of *Cryptosula pallasiana* from south Wales were significantly longer in July than in January, although there was no significant difference in zooid width (Amui-Vedel et al. 2007). These field results counter the zooid size–temperature relationship that was observed for *C. pallasiana* in controlled laboratory studies (see 'Laboratory studies'), implying that factors other than temperature may have influenced zooid dimensions. O'Dea & Jackson (2002) analysed the growth of free-living cupuladriid bryozoans from tropical American regions that experienced strong seasonal upwelling with closely related species from non-upwelling environments. They adopted the zooid size profiling approach to characterise the sizes of zooids in successive generations in a colony (Fig. 4). Strong cyclical patterns of increasing zooid sizes were characteristic of colonies that experienced the seasonal flux of cold water associated with upwelling, when temperatures can drop by >10°C within 1 wk (D'Croz & O'Dea 2007; see also Fig. 1). In contrast, colonies from non-upwelling environments revealed no significant variation in zooid size throughout their zooid size profiles.

In addition to those studies that compared zooid size directly with temperature, there are several that have done so indirectly, including an early observation that led to the development of the zooid size MART approach (Okamura 1987). Autozooids of *Electra pilosa* were observed to vary in size over the year in the Menai Straits, North Wales. The evidence was derived by dividing the total number of zooids per colony by colony size, allowing an estimate of mean zooid size per colony at different times of the year (Okamura 1987). Zooid size appeared to be inversely related to temperature, with smaller zooids produced during the warmer summer months. There was no apparent link between zooid size and productivity (e.g. spring and autumn phytoplankton blooms in the Menai Straits; Jones & Spencer 1970, Al-Hasan et al. 1975).

Other studies have taken a related approach using zooid densities as a proxy for size. O'Dea & Okamura (2000a) exploited the production of annual growth lines in the upright bifoliate species *Flustra foliacea* to temporally constrain measures of zooid densities in colonies from Wales, Denmark and Nova Scotia. They found that zooid densities varied cyclically throughout the year in apparent synchrony with seasonal variation in temperature, such that the lowest densities and hence the largest zooids occurred during the cooler spring and autumn periods. Records of planktonic primary productivity, in particular the bimodal spring and autumn phytoplankton blooms that characterise the Menai Straits and the Skagerrak (Pettersson 1991, Blight et al. 1995), indicated that food availability could not have significantly affected zooid size. O'Dea (2005) investigated how zooid densities in a colony of the upright bifoliate species *Pentapora foliacea* related to stable oxygen isotope data gathered during a previous investigation by Pätzold et al. (1987). The study demonstrated covariation between zooid density and contemporaneous $\delta^{18}O$ values derived from analyses of zooid skeletal walls, such that lower densities, and hence larger zooid sizes, were associated with higher $\delta^{18}O$ values indicative of cooler waters (O'Dea 2005).

Geographical variation in the present day

Geographical variation in zooid sizes has been noted for the encrusting, cave-dwelling species *Haplopoma sciaphilum*, which produces large zooids in Sweden where it is colder, smaller zooids in the North Adriatic where it is warmer, and yet smaller zooids in France where it is warmest (Silén & Harmelin 1976). Similarly, mean zooid lengths of *Pentapora fascialis* showed a significant and negative correlation with mean annual temperature for colonies collected from 9 sites in the Mediterranean and the UK (Lombardi et al. 2006). Conversely, Novosel et al. (2004) found no systematic differences in zooid lengths between 2 sites in the North and South Adriatic that could be explained by temperature. Their study was highly inclusive, analysing 14 cheilostome species. However, the sites were each characterised by varying and complex environmental regimes, including fluctuating salinities at 1 site due to large freshwater inputs via the Zrmanja River and an underground system of karst canals acting as temporary freshwater springs. Substantial fluctuations in salinity are likely to have confounded the zooid size response to temperature (see 'Confounding factors' below). Furthermore, the wholly inclusive random selection of zooids for measurement, without due care, may have resulted in measures of zooids with aberrant growth or those from the zone of astogenetic change. This may explain why the authors were required to adopt a non-parametric approach to analyses since occasional large variances in zooid length would be expected if early stage or distorted zooids were inadvertently measured.

Deep-time variation

Environments vary not only over space but also over time. The first study to explicitly use zooid size in bryozoans to estimate relative changes in seawater temperatures over geological time scales focused on 8 species common to both the Pliocene Coralline Crag Formation in the eastern UK and in the present-day waters around the UK (Okamura & Bishop 1988). Specimens

in the collections of the Natural History Museum, London, were identified that provided at least 5 autozooids for measurement that were unobstructed in growth and outside the zone of astogenetic change. Mean zooid sizes were significantly smaller in 5 of the 8 species in the Coralline Crag than the present day, corroborating previous estimates (see Okamura & Bishop 1988 for references) that the Coralline Crag was deposited in a sub-tropical sea considerably warmer than today (see also Williams et al. 2009 for further evaluation of the Coralline Crag environment). Replicating the same methods and many shared species, A. O'Dea (unpublished data), found that zooid sizes in the younger Pleistocene Red Crag formation were midway between those of the Coralline Crag and modern day, corroborating the inferred slightly warmer conditions of the Red Crag relative to the present day and cooler conditions relative to the Coralline Crag (Head 1998). In contrast, Berning et al. (2005) found that zooid areas were notably smaller in the Late Miocene cheilostomes from the putatively cooler Guadalquivir Basin in the eastern Atlantic than they were in the same species in 2 warmer and nearly coeval Mediterranean assemblages, suggesting that factors other than temperature may have influenced size (e.g. freshwater input from the Guadalquivir River).

Confounding factors

When applying the zooid size MART technique, it should always be borne in mind that water temperature can be influenced by factors other than seasonal variation, such as upwelling or depth. In these cases, while the bryozoan responses should represent a true indication of temperatures experienced, the minimum and maximum zooid sizes may reflect temperature variation as a result of ocean currents. Systematic sampling of species that produce annual growth lines may provide a means of recognising multiple drivers of zooid size. For instance, this may be the case if successive zooid generations demonstrate >1 peak in size over the course of a single year.

Intracolonial zooid size can also be influenced by other environmental factors besides temperature, e.g. by distortion arising from microenvironmental variation such as irregularities of the substratum over which colonies are growing (Boardman et al. 1969) or following injury or abrasion. More pervasive environmental influences on zooid size include variation in food availability, salinity or flow regimes.

There are conflicting results regarding the influence of food on zooid dimensions. For instance, food availability was not associated with variation in zooid size in *Electra pilosa* and *Conopeum seurati* in the field (Okamura 1987, O'Dea & Okamura 1999) nor with variation in zooid density in *E. pilosa*, *C. reticulum* or *Celleporella hyalina* in laboratory experiments (Menon 1972, Hunter & Hughes 1994) or in *Flustra foliacea* in the field (O'Dea & Okamura 2000a). However, analysis of morphological responses of *E. pilosa* in controlled laboratory studies demonstrated that food concentration produced predictable yet non-linear effects on zooid size (Hageman et al. 2009). At very low food levels, stunted colonies with small zooids developed. At low to intermediate food concentrations, zooid sizes increased with food levels up to a threshold food concentration (~7.51 µg l^{-1} chl *a*) where maximum zooid sizes were produced. At food concentrations above this threshold, zooid sizes diminished and then stabilised at a size smaller than the maximum size observed at the threshold. The magnitude of food-induced variation in zooid size was associated with these zooid size trends. Thus, maximum variance was coincident with the maximum size, and minimum variance was observed for zooids of submaximum size above the threshold food concentration. Intermediate levels of variation were associated with zooids experiencing low to threshold food concentrations.

If the responses of *Electra pilosa* apply broadly, they imply that food-induced variation in zooid size will be minimal above some threshold food concentration while greater variation in zooid size in response to food levels can be expected at the threshold food concentration and at levels below. These latter concentrations are estimated to be within the range of concentrations reported for the natural environment and typical of waters from which the *E. pilosa* material was originally collected (0.256 to 16.0 µg l^{-1} chl *a*; Hageman et al. 2009). This suggests that variation in food levels may at times confound temperature effects by enhancing or reducing seasonal temperature effects. Previous studies that have reported no effect of food either used food concentrations that were higher than the threshold concentration for *E. pilosa*, or they did not report food levels. Hageman et al. (2009) therefore speculated that studies revealing no food effect may have been conducted well above the critical value to invoke variation in zooid size.

The study by Hageman et al. (2009) has implications for inferences of temperature regimes made by the zooid size MART approach, with variation in food levels potentially confounding inferred regimes. However, there are several caveats. (1) It is not clear how food-induced changes in zooid size in *Electra pilosa* when fed an algal monoculture might equate to food-induced changes in zooid size when a diversity of different food types is available in natural environments. (2) Before the experiment is repeated on other species, it is impossible to discount the possibility that the trends

observed for *E. pilosa* may not be universal. (3) Changes in food concentrations from 10^3 to 10^4 cells ml^{-1} (estimated as equivalent to 1.251 to 12.51 µg l^{-1} chl *a*) and from 10^4 to 10^5 cells ml^{-1} (12.51 to 125.1 µg l^{-1} chl *a*) were associated with +14.8% and −2.7% changes in zooid area, respectively. These effects are much smaller than the effect of temperature on *Conopeum seurati*, which explained 40.51% of the variation in zooid size when accounting for variation in food levels ranging from near 0 to ~45 µg l^{-1} chl *a* (O'Dea & Okamura 1999).

As discussed earlier, oxygen may also influence zooid size and indeed may provide a mechanistic explanation for the temperature–size rule. The majority of shallow water and shelf environments that support bryozoan growth can generally be expected to be near oxygen saturation at any given temperature due to atmospheric mixing and photosynthesis. This indicates that the systematic variation between seawater temperature and oxygen content should not be confounded by e.g. oxygen depletion as a result of biological processes that might indirectly influence zooid size. However, the effects of salinity are potentially more complicated due to the inverse relationship between oxygen concentration and salinity levels. This relationship prompted O'Dea & Okamura (1999) to conclude that salinity, probably as a result of its effect on oxygen solubility, significantly influenced zooid size in *Conopeum seurati*, accounting for 21.13% of the variation in zooid size. However, Spicer & Gaston (1999) highlighted that oxygen partial pressure drives the diffusion gradient in organisms (along with respiratory pigments). At higher salinities, oxygen solubility will be reduced, but the oxygen partial pressure should not be affected. Thus, the changes in zooid size in *C. seurati* (21.13%) may be caused by the direct effect of salinity rather than the covariance of salinity and oxygen solubility (or concentration). In any case, the fact that O'Dea & Okamura (1999) retrieved temperature as the most significant factor in driving zooid size even under temperate estuarine conditions suggests that salinity effects may rarely swamp temperature effects if temperature and salinity generally covary.

Flow regime can also influence zooid size. A field transplant study in the relatively simple hydrodynamic regime of the Rapids at Lough Hyne, Ireland, demonstrated that zooids of *Membranipora membranacea* decrease in size with increasing flow (Okamura & Partridge 1999). The equivalent growth rates of colonies regardless of flow provided evidence that flow-induced miniaturisation may enable effective suspension feeding by creating favourable flow microenvironments. This may be achieved by size alterations that place lophophores into slower flow regimes of the boundary layer, thereby avoiding excessive flows that are detrimental to feeding (Okamura 1985, Eckman & Okamura 1998).

Finally, a number of studies have demonstrated that zooid size variation is influenced by genotype. This has been shown in laboratory studies of *Celleporella hyalina* and *Electra pilosa* (Hunter & Hughes 1994, Atkinson et al. 2006, Hageman et al. 2009). Genotype has also been inferred to influence zooid size amongst sexually produced colonies that developed under field conditions, accounting for 3.16% of the variation in zooid size of *Conopeum seurati* (O'Dea & Okamura 1999). Such variation demonstrates the variation in thermal reaction norms amongst individuals referred to earlier, and which, for instance, would allow selection and conformation to Bergmann's Rule.

ZOOID SIZE MART APPROACH: APPLICATIONS AND INSIGHTS

The zooid size MART approach has been applied to several situations to interpret palaeoenvironmental conditions. Estimates of MART from encrusting cheilostomes from the Pliocene Coralline Crag and the Miocene 'Faluns' (O'Dea & Okamura 2000b) suggest that northwest European seas were considerably more equable (less seasonal) than they are today, a conclusion upheld by open ocean palaeoenvironmental proxies and oceanic modelling from these times (e.g. Cronin & Dowsett 1996, Lécuyer et al. 1996, Knowles et al. 2009 and references therein). More recently, the zooid size MART approach has been applied for the first time across broad spatial scales focusing on the Pliocene North Atlantic, especially on the time in and around the mid-Pliocene warm period (Knowles et al. 2009). Cheilostome assemblages from the Coralline Crag Formation (UK), the Yorktown Formation (Virginia, USA), the Duplin Formation (South Carolina, USA), the Lower Tamiami Formation (Florida, USA) and the Cayo Agua Formation (Panama) were used to reconstruct MART estimates and to investigate patterns of heat transferral from equatorial to mid-latitude regions. Knowles et al. (2009) corroborated previous evidence for upwelling in the southern Caribbean and in Florida. Their study also indicated that the warm current flowing northeast from the Caribbean resulted in reduced seasonality along the eastern seaboard of the USA, and that it was deflected across the Atlantic further north from the Cape Hatteras area of North Carolina where it is deflected today. The study also showed that seasonal variation in the southern North Sea was greater than that experienced today.

Free-living cupuladriid bryozoans have a rich fossil record in the Caribbean extending back to the time

before the Isthmus of Panama closed and have been used as key proxies for inferring environmental change during the closure of the Isthmus via the zooid size MART approach. Today, seasonal upwelling brings cool, nutrient-rich waters along the Pacific coast for 3 mo each year (D'Croz & O'Dea 2007). In contrast, no upwelling occurs along the Caribbean coast (D'Croz & Robertson 1997). The zooid size MART approach was initially applied to cupuladriids from the present-day by profiling the sizes of zooids from the centre of colonies to their margins (Fig. 4; O'Dea & Jackson 2002). Strongly fluctuating patterns in zooid size were documented in colonies from the upwelling Gulf of Panama but not in geminate species from the non-upwelling Caribbean coast, and resulting estimates of MART obtained were within an accuracy of ±1°C of actual MARTs as derived from multiple oceanographic data (see O'Dea & Jackson 2002 for references). Subsequent estimates of MART from nearly 150 fossil cupuladriid colonies suggest that strong seasonal upwelling was a permanent feature of the Caribbean when the inter-oceanic Central American Seaway connected the Pacific and Caribbean (O'Dea et al. 2007). The end of upwelling in the Caribbean occurred rapidly during the final stages of isthmus closure, even though the formation of the isthmus was a slow geological process (Coates & Obando 1996). When the Caribbean became isolated from the Tropical Eastern Pacific, there was a synchronous shift from heterotrophic-dominated to auto- and mixotrophic-dominated benthic communities, consistent with a rapid decline in planktonic productivity and nutrient levels. The high-resolution estimates of upwelling derived from the zooid size MART approach have revealed that 'nutriphilic' coral, bryozoan and molluscan taxa went extinct some 1 to 2 Ma after the end of upwelling, thus challenging the conventional wisdom that cause and effect coincide in deep-time.

Polar material has been the focus of recent zooid size MART analysis. Clark et al. (2010) undertook a pilot study to examine cheilostomes from the Early Pliocene Weddell Sea, Antarctica. Although relatively few colonies were found to meet criteria for analysis, the material provided MART estimates (range = 4.8 to 10.3°C) that were consistently greater than the seasonal variation in temperature in the present day in the Weddell Sea (2°C; Whitehouse et al. 1996). Although zooid size MART estimation does not provide an indication of absolute temperatures, the results from this investigation suggest that summer maximum temperatures may have approached or exceeded 8°C in the Early Pliocene (given a minimum of −1.8°C when seawater freezes), similar to the present-day seasonal temperature variation in coastal surface waters of southern Patagonia.

Another study used a modified version of the zooid size MART approach to reconstruct MART from fossil cheilostome assemblages of 2 consecutive units of the lower Setana Formation (1.2 to 1.0 Ma, Kuromatsunai, Hokkaido, Japan; Dick et al. 2007). A 'specimen-limited MART' (SL-MART) approach was developed that simulates additional MART estimates from available data to enable use of small datasets when few specimens are available for study. The results suggested more pronounced seasonality in the lower than in the upper Setana Formation, but it was concluded that greater sample sizes were advisable for reconstruction of palaeoseasonality. The SL-MART technique is therefore perhaps best used as a means of gaining preliminary insights into palaeoenvironmental conditions.

In a very recent study, Knowles et al. (2010) undertook the first investigation to examine how results from the zooid size MART approach and stable isotope analyses relate to the actual measured ranges of temperature experienced by cheilostome bryozoans as recorded by a datalogger. They found that the MART implied by zooid size variability in *Pentapora foliacea* (overall mean of 6.8 and 6.9°C at 2 sites in Wales) gave a good approximation to the recorded range in temperature (overall mean range = 7.8°C, based on a mean minimum recorded temperature of 8.2°C and a mean maximum recorded temperature of 16.0°C), while the temperature ranges reconstructed by oxygen stable isotopes were narrower. The latter result appeared to reflect secondary skeletal thickening that homogenised the temperature signal by time-averaging. However, the good approximation to maximum recorded temperatures by oxygen stable isotopes (range = 14.8 to 16.9°C) demonstrated that zooid size variation and stable isotope analyses can provide independent and valuable proxies for inferring temperature regimes, with data from stable oxygen isotopes relating MART values derived from zooid size variation to absolute temperatures.

ADVANTAGES AND LIMITATIONS OF THE ZOOID SIZE MART APPROACH

The zooid size MART approach offers several advantages. (1) It represents a relatively inexpensive and quick method to infer palaeoclimate regimes. (2) MART values can be used directly as estimates of temperature variation in environments from which the individual colonies were collected. In contrast, estimates of mean zooid size provide no environmental information on their own but require comparisons with mean zooid sizes of colonies from different environments (e.g. as in Okamura & Bishop 1988, Berning et al. 2005, Lombardi et al. 2006). (3) The approach can be used to charac-

terise the ecology of present-day environments that are difficult to access or for which relevant temperature data are unavailable. (4) The zooid size MART approach provides information on the environment experienced by an individual colony that can be related to the life history of that individual colony (e.g. allocation to reproduction, defense and growth), thereby enabling direct correlation of life history responses with environmental regimes. We reiterate that bryozoans are the only colonial invertebrates that enable such insights as a result of their unique combination of traits: intracolonial polymorphism, the production of carbonate skeletons and determinate zooid sizes.

Like any technique, the zooid size MART approach has limitations that are important to appreciate. (1) It requires appropriate material for analysis — a necessity inherent to all methods of assessing temperature regimes. (2) Adherence to the set of rules outlined in Table 1 is critical, since violating assumptions of the approach is likely to give misleading results. For instance, ignoring the strict criteria for measurement, such as measuring distorted zooids or zooids within the zone of astogenetic change, will compromise inferences. We have extensively explored how factors other than temperature may influence zooid size, concluding that these are both less pervasive and exert weaker effects than temperature. (3) It assumes that colonies grow throughout the year (or that growth spans the period of maximum and minimum temperatures). Lack of growth during, for instance, periods of low food availability (e.g. in winter) may result in an inferred MART that is lower than the actual temperature.

There are various means of recognising when inferences for MART may be compromised, including aberrant estimates obtained from a small number of colonies. For instance, a species that consistently estimates a smaller MART relative to other species may be ceasing growth during winter periods. This could be indicated by the presence of annual growth check lines (Fig. 5), and such material could then be avoided. Also, zooid size changes resulting from variation in salinity could be misinterpreted to represent temperature effects on size. In environments where temperature and salinity covary, such as in temperate estuarine conditions, temperature appears to exert an overwhelming effect on zooid size variation (O'Dea & Okamura 1999). However, salinity could be confounding in environments where temperatures remain relatively constant. For instance, coastal salinities may vary due to seasonal rainfall in the tropics or ice melting in polar regions. In such cases, assemblage information may help to inform on the type of environment in which bryozoans lived, with MART values unexpected for such environments suggestive of possible salinity effects.

RECOMMENDATIONS FOR FUTURE STUDIES

The foregoing discussion has reviewed the development and application of the zooid size MART approach and the negative relationship between zooid size and temperature that provides the foundation for the approach. Here we suggest future areas for research to address outstanding questions and to identify extensions and further developments of the zooid size MART approach.

Further exploration of the mechanisms that underlie the zooid size–temperature relationship and its generality is merited. Atkinson et al. (2006) have clearly demonstrated that zooid sizes in *Celleporella hyalina* can be influenced by both temperature and oxygen levels. Are such responses general amongst cheilostome bryozoans? Is there an adaptive basis for the size change, and how might understanding this help us to interpret size variation over time and space? Their finding that colony volumes do not conform to the temperature–size rule is intriguing, as it suggests that zooids, but not colonies, are the units that show equivalent responses to those displayed by solitary organisms. Does this vary amongst species according to their degree of colony integration or mode of life? Do zooids in poorly integrated colonies (e.g. uniserial runners that lack zooid polymorphism) show stronger temperature-induced variation in zooid size than zooids in highly integrated colonies (multiserial forms with a high degree of zooid polymorphism)?

The body of evidence to date suggests that cheilostome zooids generally respond to temperature. Nevertheless, phylogeny can influence many organismal responses (Harvey & Pagel 1991), and temperature-induced zooid size variation has been demonstrated for relatively few cheilostome species.

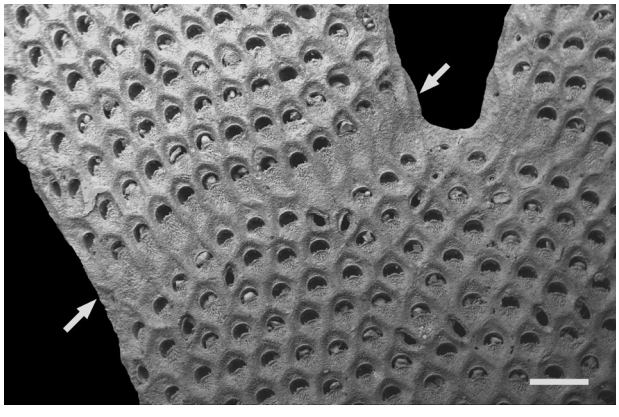

Fig. 5. *Melicerita* sp. Growth cessation during the winter. Scanning electron micrograph of a fragment from the Coralline Crag Formation (Suffolk, UK) showing skeletal thickening typical of an annual growth check line (white arrows). Scale bar = 250 µm

Although the apparent ubiquity of the temperature–size rule suggests that phylogeny will play a minor role, further investigation is warrented. If phylogenetic history proved important in determining how zooids respond to temperature, then more strategic sampling and analyses would be advisable. For instance, analyses could be adopted to deal with correlated evolution as a result of common phylogenies (Harvey & Pagel 1991), and taxa could be targeted or avoided to minimise phylogenetic constraints (Gould & Lewontin 1979).

Exploring whether an approach based on temperature-induced variation in module size can be extended to other groups could prove to be highly productive and might provide key insights on very ancient environments. Cyclostome bryozoans are the most obvious candidate for such exploration, although their mode of growth would require focusing on different character(s) for measurement (e.g. the aperture). If cyclostomes proved applicable, the use of the zooid size MART approach to reconstruct ancient environments might be extended from the Upper Jurassic (155 Ma; when cheilostomes originated) to the Lower Ordovician (480 Ma; when cyclostomes originated).

Finally, refinement of the zooid size MART approach might enable more accurate and powerful estimates of temperature regimes (Dick et al. 2007). This could certainly be achieved by increasing the number of data points in the model to incorporate more species and to extend geographic cover. It could be argued that more accurate estimates might be gained by more precise measurements of zooid size. For instance, a pilot study confirmed that greater precision can be gained through length and width measurements obtained from scanning electron microscopy (SEM) since SEM images can reduce error in size estimation relative to measurements made using a stereomicroscope (Knowles 2009). However, a requirement to use SEM images for measurement is not warranted for 3 reasons. (1) It would then be advisable to develop a new model using SEM-based measurements. (2) A highly attractive aspect of the zooid size MART approach is that it is easy, inexpensive and requires only standard laboratory equipment. (3) The reliability (e.g. regression model with 95% confidence limits within ±1°C) and accuracy (e.g. concordance with known temperature regimes or other proxies) of the zooid size MART approach appears to us very reasonable in view of background noise inherent to both new data and the model. We note, however, that SEM images can be very helpful to delineate zooid boundaries, particularly in fossil specimens (e.g. Knowles et al. 2009), and for this reason, making measurements on SEM images can be highly justified as a means of improving data collection.

CONCLUSION

Our review demonstrates a strong and growing body of evidence that the zooid size MART approach is a unique and independent proxy for environmental reconstruction. The above-described investigations of bryozoan growth in laboratory and field conditions are nearly unanimous in confirming the inverse relationship between zooid size and temperature. Furthermore, the apparent ubiquity of the temperature–size rule lends additional support. Temperature consistently provides, either directly or indirectly, a pervading and dominant influence on zooid size. The potential for other environmental factors to influence size should nevertheless always be borne in mind when using the zooid size MART approach. However, incorporating other proxies may help to deal with such confounding factors, and palaeoenvironmental reconstruction is considerably strengthened when multiple independent proxies are used. Cheilostomes may provide further potential in this respect, since studies indicate the feasibility of combining the zooid size MART approach with stable isotope analyses of the same specimens (O'Dea 2005, Knowles et al. 2010), provided the mineralogy of the specimens has been well constrained (Smith et al. 2004) and diagenesis is absent (T. Knowles et al. unpubl. data).

We conclude that the zooid size MART approach represents a robust proxy for environmental reconstruction that warrants equal consideration for use as that given to traditional proxies such as stable isotope analyses or alkenones. In addition, because the zooid size MART approach entails sampling over discrete periods of time, insights into the actual annual temperature range are possible. In this respect, the approach offers equal or greater precision than analyses of traditional proxies based on combined samples deriving from a number of years (e.g. geochemical signals from foraminifera) and which may be further compromised by bioturbation. Furthermore, because it is a relatively inexpensive and quick method, the zooid size MART approach can provide a means of gauging environmental variation and of identifying where or when more expensive proxies might be adopted. It therefore merits broad recognition by environmental and evolutionary biologists and climate modellers. Finally, its incorporation in multiproxy toolkits used in palaeoenvironmental research will enable more robust interpretations. Ultimately, such multiproxy-based research with strategic focus on palaeoclimate change will enable better understanding and prediction of how the earth system has responded in the past and how it may therefore respond to future climate change.

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Common sea anemones *Actinia equina* are predominantly sessile intertidal scavengers

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ABSTRACT: Coelenteron contents of anemones *Actinia equina* collected from 2 shore heights (upper and lower) on 3 shores (exposed, semi-exposed and sheltered) in south-west Ireland were investigated in summer 2009. Diets of exposed and semi-exposed shore anemones were dominated by small mussels *Mytilus edulis*, mostly (>90%) with broken/cracked shells. The diet of anemones on the sheltered shore was dominated by insects, particularly on the upper shore. On all shores, larger food items (isopods, mussels, insects) were broken or fragmented; only small gammarid amphipods, crab megalopae, midges and mosquitos were found whole. Laboratory behavioural experiments showed that *A. equina* were unselective at small prey size, but were limited by their own size, which restricted the maximum size of prey that could be ingested. Whole mussels were ingested, but egested alive after mean elapsed times of 0.75 to 1.95 h (depending upon anemone size). In contrast, mussels with cut adductor muscles, and incapable of shell valve closure, were fully digested, empty shells not being egested until 8.70 to 8.95 h after ingestion. Whole, live mealworms *Tenebrio molitor* were eaten by anemones, but were egested after a mean 1.30 h with no signs of digestion or fragmentation. Mealworms with mechanically perforated exoskeletons were held in the coelenterons of *A. equina* for a mean of 5.90 h and all soft tissues were digested. It was concluded that *A. equina* predominantly scavenges on macrofaunal carrion, as well as preying upon smaller food items.

KEY WORDS: Sea anemones · *Actinia equina* · Scavengers · Carrion

INTRODUCTION

The beadlet sea anemone *Actinia equina* (L.) is a common intertidal actinian anthozoan on Atlantic rocky shores of all exposure types in Western Europe; a conspecific (*A. mandelae* Wirz & Delius) also occurs on the west African coast and has been studied extensively (e.g. Kruger & Griffiths 1997, 1998). *A. equina* is most abundant on the lower shore, particularly on exposed coasts, but is also found on the middle and upper shore, especially in rock pools, as well as in damp crevices. Upper and lower distributional limits of mean low water springs (MLWS) and mean high water neaps (MHWN) were given by Quicke et al. (1985), indicating that the species is an intertidal specialist (though it is subtidal in the microtidal Mediterranean; Chintiroglou & Koukouras 1992). Much of its biology was reviewed by Shick (1991).

The feeding ecology of *Actinia equina* is generally assumed to be similar to that of many other sea anemones that are characterized as opportunistic omnivores (Van-Praët 1985, Shick 1991) and generalists (Kruger & Griffiths, 1998). Technically, beadlet anemones are sedentary, since they are capable of very slow movement, but in terms of feeding they are regarded as sessile sit-and-wait predators that appear to feed on whatever falls onto the tentacles and oral disc (Shick 1991). While some anemones have long tentacles and actively catch benthic prey, *A. equina* has short tentacles and appears to be a suspension feeder (Chintiroglou & Koukouras 1992). Adhesive spirocysts (as well as nematocysts) on the tentacles are involved in food capture, while killing of prey apparently takes place in the coelenteron, where nematocysts are the only cnidae present on the mesenteries (Van-Praët 1985). There is a considerable literature

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devoted to digestion and absorption of food (see Van-Praët 1985 for review).

The most quantitative account of the diet of *Actinia equina* in Europe has been provided by Chintiroglou & Koukouras (1992), but their information was derived from subtidal Mediterranean animals, most of which (70%) had empty coelenterons. They also implied that beadlet anemones exhibited food preferences, although earlier studies had indicated that *A. equina* simply ate items that fell onto the tentacles and oral disc (e.g.Van-Praët 1985). The study reported here was primarily designed to investigate the diet of intertidal *A. equina* living at different heights on shores of various wave exposure levels. It was hypothesised that diets would vary amongst shores and shore heights because of stochastic processes determining the availability of food items. As a result of the findings, subsequent laboratory feeding experiments were conducted to determine (1) whether anemones could select food items of different sizes, (2) whether they were predators or scavengers, and (3) whether they could distinguish between inorganic and organic material. Additional data concerning anemone appetite were also collected for comparison with the quantities of material found in the coelenterons of anemones taken from the field.

MATERIALS AND METHODS

Collection and sampling. Specimens of *Actinia equina* were collected from 3 shores in south-west Ireland in summer 2009 (Fig. 1) on 3 different days. Specimens for coelenteron contents analysis were collected from the high water neap (HWN) level and low water leap (LWN) level at Garrettstown (51° 38′ 61″ N, 8° 35′ 1″ W), Bullens Bay (51° 38′ 48″ N, 8° 32′ 52″ W) and Tralispean (51° 29′ 51″ N, 9° 13′ 45″ W). Between 19 and 26 anemones were collected from each site/shore height combination. Garrettstown is an exposed, mussel-dominated (cf. Lewis 1964) rocky shore (Ballantine Scale 3; Ballantine 1961). Bullens Bay is a moderately exposed shore (Ballantine Scale 4) situated in a sheltered inlet east of the Old Head of Kinsale, which shields the shore from prevailing south-west winds. Its lower shore exhibits mixed barnacle/mussel domination. Tralispean is completely sheltered from prevailing winds (Ballantine Scale 7) and the lower shore has a mixed fucoid and barnacle cover, with low densities of larger mussels. In all cases, anemones were collected on falling tides within a few minutes of the tide leaving them, to minimise time available for digestion of coelenteron contents. On the days of collection, Garrettstown and Bullens Bay both experienced moderate wave action with swell and surge; at Tralispean waves were absent.

Coelenteron contents analysis. All specimens (n = 123) were preserved in 70% ethanol immediately and stored individually in 5 × 5 cm ziplock bags until taken to the laboratory. Anemones were dissected and examined under a stereoscopic dissecting microscope (Nikon). Dissection involved cutting the specimens in half from the oral disc to the basal disc in Petri dishes with ~2 mm depth of 70% ethanol. Any other items in the ziplock bag were also moved to the Petri dish in case the specimen had egested prey after exposure to alcohol (Sebens 1981). First the alcohol was searched for possible food items; next the tentacles of the specimen were inspected and finally the coelenteron contents were examined for food items by scraping. This was achieved by removing all gut contents including

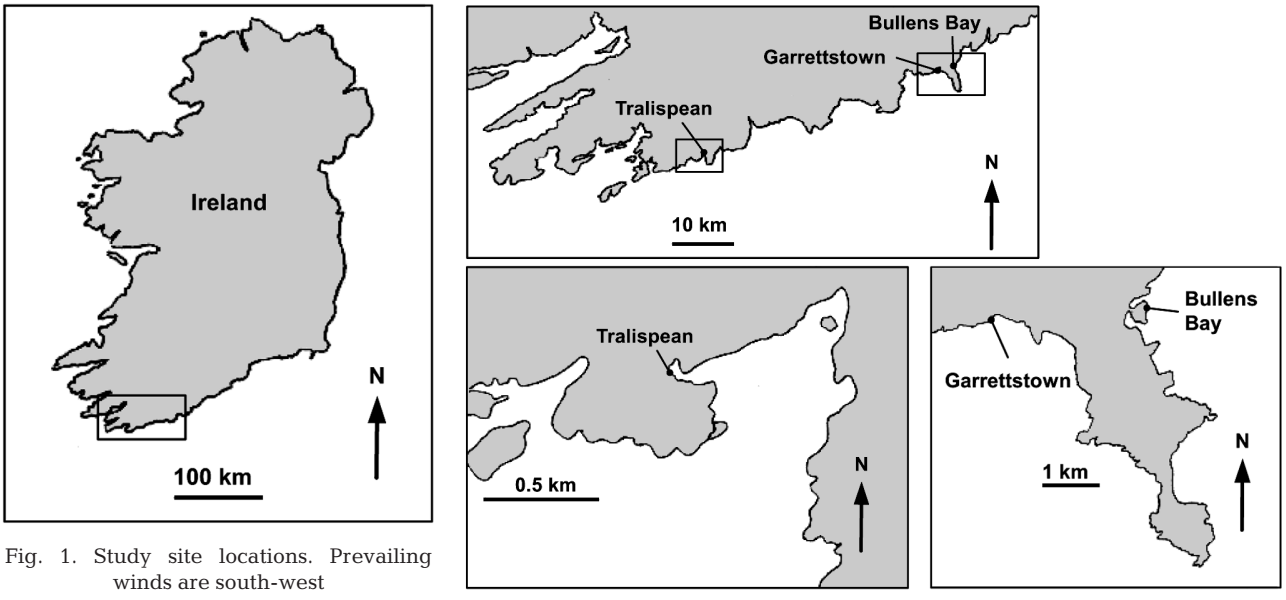

Fig. 1. Study site locations. Prevailing winds are south-west

food bolus, mesenterial filaments, mesenteries, gonad and any juvenile anemones present. Food items were identified to the lowest possible taxonomic level and counted. Counting of fragmented animal material was carried out carefully to determine the minimum number of animals that a set of fragments corresponded to. It was impractical to estimate ingested biomass as much material was substantially digested.

Behavioural experiments. Anemones used in behavioural experiments were gently removed from the substratum at either Garrettstown or Bullens Bay (depending upon weather), taking care to ensure that the pedal disc was not damaged. They were held in aerated seawater (salinity 34 psu) in individual polystyrene containers that each contained a pebble to allow the anemone to attach itself. The containers were held in large glass aquaria and the seawater was changed weekly. They were maintained on a diet of crushed small mussels *Mytilus edulis*.

Prey size selection: For feeding experiments, the specimens of *Actinia equina* were divided into 3 size classes; small (<15 mm basal disc diameter), medium (15 to 30 mm basal disc diameter) and large (>30 mm basal disc diameter). Before feeding experiments, specimens were not fed for 2 d to standardize appetite. Selection of small prey of different sizes was investigated by feeding brine shrimp *Artemia* of different sizes as proxies for zooplankton to 5 anemones of each size class kept individually in plastic beakers (800 ml) filled with aerated seawater (35 psu) at 19.7 to 20.8°C. Each anemone size (5 replicates per size class) was given a known number of *Artemia* of various sizes (newly hatched, mean length 0.625 mm; larger nauplii, mean length 1.28 mm; subadult, mean length 2.76 mm; adult, mean length 3.1 mm). Each anemone was offered either 50 newly hatched, 40 nauplii, 30 subadult or 15 adult *Artemia* by 1 ml pipette. After 2 h the seawater was removed from the beaker and any remaining brine shrimps were counted to allow calculation of the number eaten.

Selection of prey of various larger sizes was investigated using whole, live mussels *Mytilus edulis* of a range of known shell lengths. Individual anemones (5 each of size categories small, medium, large) were each held in a beaker and offered a large mussel by dropping it onto the oral disc. If not ingested within 30 min, the mussel was removed and a slightly smaller size mussel offered. This process was repeated until the anemone ingested the offered mussel within 30 min, at which point it was assumed that the upper prey size threshold had been reached.

Appetite: Ten *Actinia equina* of wet body masses from 1 to 5.4 g were used to evaluate appetite (in terms of satiation ration). The anemones were not fed for 1 wk and their wet masses (when out of water and contracted) established. Each was then offered pieces of mussel flesh (each ~0.15 g) by placing the pieces on the anemone's tentacles using forceps. If the anemone extended its tentacles after ingestion (defined as the moment at which the ingested flesh was no longer visible in the mouth), another piece of mussel flesh was offered. This process was continued until the anemone showed no signs (e.g. transfer of flesh to the mouth) of ingesting a piece of flesh 15 min after it was placed on the tentacles. The individual satiation ration was calculated from the summed ingested flesh mass.

Ingestion and egestion of mussels: *Mytilus edulis* were also offered to *Actinia equina* to determine how long it would take them to process (ingest, digest, egest) mussels. Single whole, live mussels (close to, but below the upper prey size threshold already determined) were fed to 5 anemones in each of the 3 size classes. Mussels were placed on the oral disc of the anemones and were completely ingested within 30 min. The anemones were periodically observed until they egested the mussel shells. To further elucidate processing of mussels, the experiment was repeated using mussels in which the adductor muscles had been cut, so that the shell valves remained open and the flesh exposed to the contents and structures of the coelenteron.

Survival of ingestion: Single intact mussels were ingested by each of 10 large anemones that had been starved for 48 h. After 2 h they were inspected and all had egested mussels in the interim. The mussels were placed in a glass dish, covered with seawater (salinity 34) and inspected under a binocular microscope for signs of life (protrusion of foot, movements of shell valves).

Experiments with insect material: Larvae (mealworms) of the darkling beetle *Tenebrio molitor* were used as proxies for insect material ingested by *Actinia equina*. Ten large anemones, not fed for 48 h, ingested whole, live mealworms of 12 to 15 mm length. They were observed until the mealworms were ingested. The experiment was repeated with a further 10 anemones, but in this case the mealworms were killed and holes made with a sharp scalpel in each of the body segments before they were ingested by the anemones. In both experiments, the mealworm material was inspected subsequent to egestion under a binocular microscope to determine the state of digestion.

Experiments with plastic pellets: Fasted anemone of each size class were separately fed each of 3 types of negatively buoyant 'food grade' alkathene pellets (length 2.18 mm): (1) clean, (2) pellets coated with a biofilm (pellets held in sunlit unfiltered seawater for 7 d), and (3) pellets coated with mussel extract (extract coat left to air dry for 24 h before experiments). Each anemone was offered one pellet of each type by dropping it onto the oral disc and observed for 30 min to determine whether the pellets were ingested.

Table 1. Vacuity coefficient and mean number of food items in non-empty coelenterons of *Actinia equina* sampled in SW Ireland in June 2009

Site (shore height)	Vacuity coefficient (%)	Mean no. animals per coelenteron
Garrettstown (exposed)		
High shore	8.6	2.86
Low shore	15.7	2.29
Bullens Bay (semi-exposed)		
High shore	9.0	2.40
Low shore	4.0	5.87
Tralispean (sheltered)		
High shore	14.8	1.67
Low shore	11.5	2.17

RESULTS

Coelenteron contents analysis

Vacuity coefficients and numbers of animal food items. Empty coelenterons were found in anemones sampled from all shores and shore heights (Table 1), though there was no obvious pattern in incidence. The overall vacuity coefficient across all 3 shores and 2 shore heights was 9.2%. Although over 90% of coelenterons contained food items, the numbers of individual items found per coelenteron was low (Table 1), mean values ranging from 1.67 (high shore, Tralispean) to 5.87 (low shore, Bullens Bay).

Taxonomic composition of contents. All identifiable taxa found in the coelenterons are listed in Table 2. With the exception of allochthonous terrestrial plant and insect material, almost all other items were of marine benthic or benthonic origin; planktonic or nektonic items were generally not found. The barnacle cyprids and crab megalopae observed are settlement stages; they could either have been caught when in the plankton, or when moving over rock surfaces. Most of the marine material was probably of autochthonous rocky intertidal origin. However, some were definitely allochthonous; the tubiculous polychaete *Sabellaria alveolata* occurs in reefs of sandy tubes several hundreds of metres from the Garrettstown collection site. No material of anthropogenic origin was found. Although small pieces of rock were often present, they were almost always partially covered by coralline algal layers.

Quantities of food items. Table 3 summarizes the relative occurrence of the major groups of organisms found within the coelenterons. Molluscs (particularly *Mytilus edulis*) dominated the diet at the exposed shore and the lower part of the semi-exposed shore. They made up a higher proportion of the diet on the lower shore than the upper shore. In contrast, at the sheltered site (Tralispean), molluscs were unimportant and coelenteron contents were dominated by crustaceans (predominantly crab megalopae) and insects (especially on the upper shore, where 41.5% of coelenteron contents were made up of winged insects).

Table 2. *Actinia equina.* Taxonomic composition of items found in coelenterons on 3 shores in SW Ireland in June 2009

	Category (identified whole or from fragments)	Lowest identifiable taxa		
Marine plants	Coralline algae	*Lithothamnium* sp.	*Corallina officinalis*	
	Fucoid algae	*Fucus* sp.		
	Chlorophytes	*Enteromorpha* sp.		
Terrestrial plants	Seeds, leaf fragments			
Marine animals				
Crustaceans	Isopods	*Idotea balthica*	*Jaera marina*	*Campecopea hirsuta*
		Idotea granulosa	*Janira* sp.	
	Amphipods	*Gammarus* sp.		
	Cirripedes	*Semibalanus balanoides* (cyprids)	*Elminius modestus* (adults)	
	Decapod crabs	Portunid megalopae	*Carcinus maenas*	
Molluscs	Gastropods	*Littorina saxatilis*	*Littorina littorea*	
		Littorina obtusata	*Melaraphe neritoides*	*Patella vulgata*
		Skeneopsis planorbis	*Gibbula umbilicalis*	*Patella ulyssiponensis*
Bivalves		*Mytilus edulis*	*Anomia ephippium*	*Lasaea rubra*
Annelids	Polychaetes	*Sabellaria alveolata*		
Echinoderms	Asteroids	*Asterias rubens*		
Bryozoans	Colony fragments			
Cnidarians	Hydrozoan fragments			
Terrestrial animals				
Insects	Dipterans	Midges, mosquitos, hoverflies	*Musca domestica*	
	Hymenopterans	Vespidae (wasps)		
	Coleopterans	Hydrophilidae (Dung beetles)	*Cercyon* sp.	

Table 3. Relative frequency (%) of major items found in coelenterons of *Actinia equina* on 3 shores in SW Ireland in June 2009. Note that 'macroalgae' includes coralline algae as well as other taxa, and that 'molluscs' includes *Mytilus edulis* as well as other bivalves and gastropods

Taxa	Garrettstown (exposed)		Bullens Bay (semi-exposed)		Tralispean (sheltered)	
	High shore	Low shore	High shore	Low shore	High shore	Low shore
Macroalgae	16.3	12.5	7.5	5.0	–	20.9
Coralline algae	11.6	5.5	–	–	–	3.0
Molluscs	37.3	75.5	11.3	62.3	7.2	6.0
Mytilus edulis	29.0	72.2	5.7	44.6	4.8	4.5
Crustaceans	20.9	4.1	54.6	14.1	36.6	38.7
Insects	–	–	7.5	7.8	41.5	17.9

Condition of contents. In considering the nature of the contents, it has to be recognized that they are not comparable with stomach contents of predators such as fish, prawns or squid that have plug flow guts, because the coelenteron contains fully digested material as well as newly ingested food items. Turnover time of different food items is known to differ (Kruger & Griffiths 1998), which will also influence coelenteron content. Algae were invariably present as fragments or, in the case of coralline algae, as crusts on rock fragments. Molluscan material varied in condition. Gastropod shells were usually whole (though the contents were digested), but the majority of bivalves (>90%) had cracked or broken shell valves and in some cases soft tissue was still present. The exoskeletons of some crustaceans, particularly gammarid amphipods and crab megalopa, were largely intact, but many isopods of the genus *Idotea* were present as pieces. Many insects (except midges and mosquitos) were present as pieces (e.g. head, thorax or abdomen), rather than as whole animals. Overall, the biomass of ingested and digested material present within the coelenterons was very low by comparison with the quantities of material that the anemones are capable of ingesting (see section below).

Behavioural experiments

Prey size selection. All sizes of *Artemia* were ingested (Table 4). Anemones captured and ingested material <1 mm size, but the proportion of newly hatched *Artemia* ingested was lower than that of larger brine shrimps. There was an upper limit to the size of food item ingested as anemones could not ingest mussels with shell lengths greater than about 60% of pedal disc diameter (Fig. 2).

Appetite. All 10 anemones ingested mussel flesh, none rejected it and the wet satiation ration ranged from 34.7% of wet body mass in a 1 g anemone to 13.1% in a 5 g anemone (Fig. 3). Visually, these quantities of food greatly exceeded the amounts of material found during inspection of the coelenterons of wild-caught anemones.

Ingestion and egestion of mussels. Intact mussels (capable of shell valve closure) and mussels with cut adductor muscles (incapable of closure) were processed in markedly different ways (Table 5). Intact mussels were egested by anemones a median of 0.93 h after ingestion, apparently undigested. Mussels with cut adductors were egested much later (overall median 7.73 h) and their flesh had been digested, so that only the shells remained. Median elapsed times between ingestion and egestion were different to an highly significant extent (Kruskal-Wallis test: $p = 0.004$).

Survival of ingestion by mussels. Although egested intact mussels sometimes had a coating of mucous material, they resumed normal activity (foot and shell valve movement; reactions of the mantle edge to touch)

Table 4. Ingestion of *Artemia* by *Actinia equina*

Anemone size	Pedal disc mean diameter (mm)	SD	% ingested
Newly hatched *Artemia* (mean length 0.62 mm), 50 offered to each anemone			
Large	33.0	2.7	56.0
Medium	18.8	2.4	50.8
Small	12.0	2.9	66.4
Artemia nauplii (mean length 1.28 mm), 40 offered to each anemone			
Large	32.1	5.5	80.0
Medium	23.5	2.7	84.5
Small	8.7	4.5	64.0
Artemia subadults (mean length 2.76 mm), 30 offered to each anemone			
Large	32.3	2.9	90.7
Medium	21.9	1.9	92.0
Small	11.1	4.4	79.3
Artemia adults, (mean length 3.10 mm), 15 offered to each anemone			
Large	32.1	10.1	84.0
Medium	26.2	4.4	92.0
Small	13.0	4.4	77.3

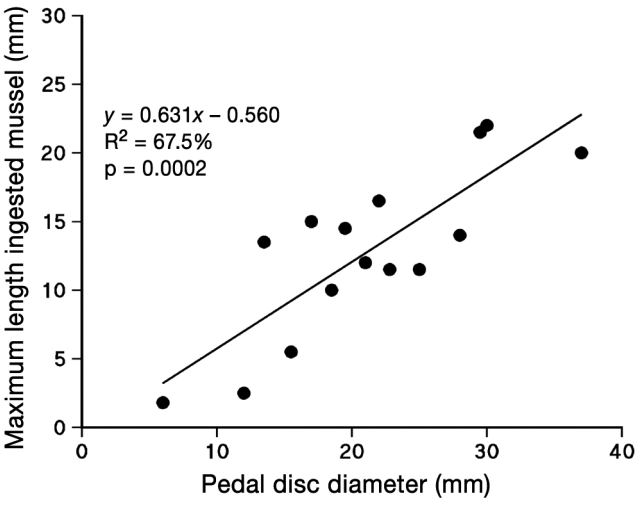

Fig. 2. *Actinia equina* and *Mytilus edulis*. Relationship between anemone size (pedal disc diameter) and maximum length of ingested mussels

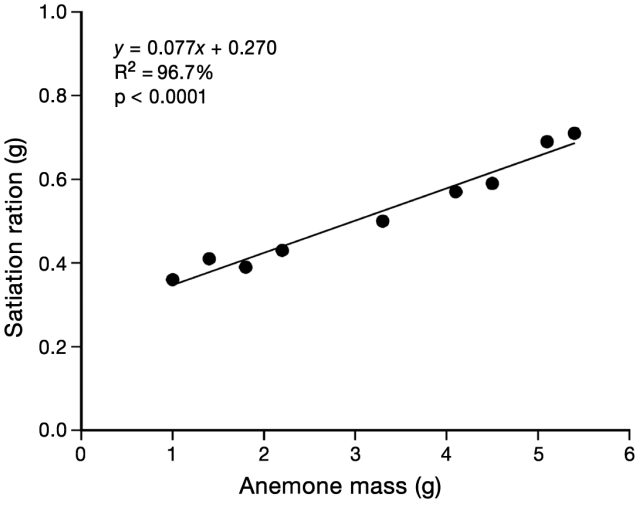

Fig. 3. *Actinia equina* and *Mytilus edulis*. Relationship between anemone wet mass and satiation ration (flesh wet mass) measured under laboratory conditions

Table 5. *Actinia equina* and *Mytilus edulis*. Time interval between ingestion and egestion of mussels by anemones: comparison of processing of intact mussels and mussels with cut adductor muscles

Anemone size	Mean anemone pedal disc diameter (mm)	SD	Mean length ingested mussel (mm)	SD	Median interval between ingestion and ejection (h)	SD
Intact mussels						
Large	32.8	5.51	17.6	1.56	2.12	1.17
Medium	26.7	2.50	13.9	0.48	1.58	2.10
Small	10.6	2.06	5.7	1.44	0.86	0.35
Mussels with cut adductors						
Large	32.0	3.26	17.60	0.89	7.73	2.21
Medium	23.7	4.16	12.83	0.29	6.93	2.18
Small	12.7	3.23	7.00	3.37	3.73	1.60

Table 6. *Actinia equina*. Ingestion of alkathene pellets (length 2.18 mm)

Anemone size	No. of pellets ingested (out of 5) Clean pellets	Pellets with biofilms	Pellets coated with mussel extract
Small	2	1	2
Medium	1	1	4
Large	0	0	4
Total (out of 15)	3	2	10

on return to seawater. Mussels with cut adductors were egested much later as shells; their flesh had been digested.

Experiments with insect material. Of the 10 anemones fed live intact mealworms, 5 did not ingest them, but released them from the tentacles (dead as a result of drowning) within 15 to 30 min. All 5 anemones that ingested live intact mealworms egested them after a median 1.3 h. The egested mealworms were dead, but intact, with no signs of digestion or fragmentation. Some were coated with mucous material.

Of the 10 anemones fed dead, perforated mealworms, 4 discarded the mealworms, which were not ingested. The remaining 6 ingested their perforated mealworms and held them in the coelenterons for a median 5.9 h before egestion. The elapsed times between ingestion and egestion differed between the 2 groups to a highly significant extent (Kruskal-Wallis test: $p = 0.006$). The egested mealworms each exhibited a whole, perforated exoskeleton (i.e. not fragmented), but a completely watery, liquid interior, indicating full digestion of the internal organs.

Experiments with plastic pellets. Anemones ingested few clean pellets or pellets only coated in a biofilm (20 and 13%, respectively); the presence of a biofilm did not make the pellets more attractive (Table 6). However, 67% of the pellets coated with mussel extract were ingested, indicating that such extract enhanced

attractiveness of the plastic material. All ingested pellets were egested in less than 1 h.

DISCUSSION

From the results presented it is evident that the coelenteron contents of *Actinia equina* are strongly dependent on shore exposure and shore height. This would be expected from an omnivorous sessile suspension feeder that simply ingests available material delivered stochastically to it by tides, currents and wave action. Chintiroglou & Koukouras (1992) divided the diet of Mediterranean subtidal sea anemones into categories of 'preferential' (making up >50% of total food items), 'secondary' (10 to 50%) and 'accidental' (<10%). These terms originate in fish diet composition studies (e.g. Deniel, 1975), but we believe they are misleading in the context of a species that cannot actively seek out preferred prey. Chintiroglou & Koukouras (1992) found 70% of subtidal *A. equina* in Greek waters to have no material in their coelenterons (in summer). The diet of the remaining 30% was dominated (45%) by 'organic detritus', with insects, crustaceans and molluscs being the most important identifiable items. Clearly the intertidal *A. equina* reported on here received a more plentiful summer diet, as the mean vacuity coefficient was only 9.2%. Low-shore coelenteron contents were dominated by mussels (72.2 and 44.6% at exposed and semi-exposed sites, respectively; Table 3), while high-shore anemones contained proportionately fewer mussels (29.0 and 5.0% at exposed and semi-exposed sites, respectively). Marine mussels (Mytilidae) on rocky shores have long been known to compete for space and food and to exhibit self-thinning (e.g. Hughes & Griffiths 1988, Guiñez 2005), a process by which mussels are ejected from 2- or 3-dimensional beds. It is also known that storms and heavy wave action make ejection of mussels much more likely (e.g. Harger & Ladenberger 1971). In consequence, on high-energy shores with extensive low-shore mussel beds, small mussels are continually being ejected and presumably battered against rocks by wave action. Rocky shore mussels are also extensively preyed upon by shore crabs *Carcinus maenas*, and mussels damaged by crabs are likely to add to the numbers available to anemones. Since mussels have a high body density, because of their calcareous shell valves, it is likely that fewer will be thrown up by wave action to high-shore *A. equina*, hence the difference in importance to anemone diet. The data for the sheltered shore at Tralispean reinforce this hypothesis: with negligible wave action and no low-shore mussel mats, mussel occurrence was low and similar on low and high shores (4.5 and 4.8%, respectively).

The importance of mussels *Mytilus edulis* to the diet of *Actinia equina* on Irish coasts mirrors the data presented by Kruger & Griffiths (1998) for *A. mandelae* at the south-western Cape, South Africa. They found an overwhelming dominance of bivalve molluscs (of several species, including 2 mussel species), but also reported amphipods, isopods and insects.

The incidence of insects in the diet of *Actinia equina* in south-west Ireland is also explicable in terms of exposure. Garretstown, the most exposed site studied, is subject to prevailing onshore south-west winds, presumably carrying few insects; hence, no insects were recorded from coelenterons either on the high shore or low shore at this site. The Bullens Bay semi-exposed collection site was about 200 to 250 m away from arable farmland and the prevailing south-west winds are offshore. Insect incidence in coelenterons was 7.5% on the high shore, 7.8% on the low shore. The highly sheltered shore at Tralispean was adjacent to pasture land, hedges, shrubs and trees, all rich in insect life. Prevailing winds were offshore. At Tralispean, insect incidence in coelenterons was 41.5% on the high shore nearest to the terrestrial habitat and lower on the low shore (17.9%). Insects that are carried onto seawater can rarely escape because of surface tension and soon drown. Presumably tidal rise and fall will carry them within reach of anemones' tentacles.

It has generally been accepted that *Actinia equina* preys upon other animals; Shick (1991) emphasised that anemones with short, thick tentacles prey upon macrofauna. Our field data, and laboratory experiments with *Artemia* as prey items, indicate that *A. equina* is certainly capable of catching, killing and digesting small planktonic and benthonic crustaceans such as gammarid amphipods, barnacle cyprids and crab megalopa. Whether it is a predator of larger animals (e.g. large isopods of the genus *Idotea*, which occurred in pieces within the coelenteron) is less clear, and it we consider it unlikely that *A. equina* is a predator of mussels and insects. Mussels of all sizes almost always had cracked or broken shells and there seems no obvious means by which *A. equina* could inflict such damage; shell valve breakage before ingestion seems far more likely. Although small insects were often found whole within the coelenteron, large insects were not. In any case, it seems most likely that insects were drowned and were carrion before ingestion. Insects are fragile and even gentle wave action is likely to break them up in the intertidal habitat.

The coelenteron of anemones is compromised in function because it also has a role in respiration, with seawater flowing to and fro between the coelenteron and the exterior (though not during digestion) (Shick 1991). In consequence, the available evidence indicates that digestion is extremely local to the surfaces of

the mesenteries, where enzymes are secreted and macromolecules absorbed (Van-Praët 1985). The bulk fluid of the coelenteron does not feature the low pH and high enzyme concentrations of a typical carnivore such as a fish (Kapoor et al. 1975). From the field evidence presented here, it seemed likely that much of the anemones' diet in south-west Ireland consisted of dead, dying or damaged animals in which the anemones' mesenteries had direct access to soft tissues. This indicated that *Actinia equima* is predominantly a scavenger on macrofauna, an unusual attribute in a sessile animal.

The finding that *Actinia equina* ingested intact whole mussels, but subsequently egested them alive, them is novel and unexpected, principally because it contradicts a single study conducted more than 230 yr ago (Dicquemare 1773), but referred to in relatively recent reviews (e.g. Shick 1991). In Dicquemare's study, live mussels of about 13.5 mm ('6 lines') shell length with closed shells were fed to *A. equina* of unknown size and '40, 50 and 60 hours after, the shells were thrown up at the mouth empty and perfectly cleared'. No more detail was given and it is not clear from the text (either in English translation or the original French) whether the anemones tested were in the field (in which case it is not obvious how the observer could be sure that the egested shells corresponded to the ingested mussels) or, if in the laboratory, whether or not aquarium experiments were conducted with freshly caught *A. equina*, or anemones that had been starved for long periods (up to 12 mo in some of Dicquemare's experiments). The implication from Dicquemare's study (identified by Shick 1991) was that anemones held mussels within the coelenteron until the mussels were forced (by anoxia?) to open their shell valves and were subsequently digested. Clearly this did not happen in our study.

Because mussels with cut adductor muscles were fully digested (as was mussel flesh offered in the appetite trials), the experimental data reported here indicate that *Actinia equina* does not prey upon intact mussels, but scavenges on dead or damaged animals. A scavenging role was confirmed by the experiments with beetle larvae (mealworms). Again, the anemones could ingest, but not digest, intact *Tenebrio molitor*, yet could digest mealworm soft tissues completely when these were made available by perforation of the integument.

Since *Actinia equina* did not digest intact mussels or insects, why did they ingest them in the first place? It has been well known since the pioneering work of Pantin & Pantin (1943) that sea anemones' feeding responses are triggered by chemotactile stimuli (see van Praët 1985, and Shick 1991 for extensive reviews), and the experiments reported here demonstrated that plastic pellets coated with mussel extract were ingested (though soon egested again). It is probable that *A. equina* ingests all material that provides a positive combined tactile and chemical signal. This will include pieces of macroalgae, fragments of rock with coralline algal films, whole animals and fragmented animals. Presumably all will be subject to digestive processes amongst the mesenteries, but material that does not provide feedback in terms of assimilated molecules will soon be egested.

The beadlet anemone is an intertidal specialist that reaches its highest densities on wave-cut platforms on the lower portion of Irish shores. The intertidal habit is subject to waves and surge, the water being laden with sand, stones and debris, as well as containing planktonic, nektonic and benthonic animals that can be dashed against the rocks. Offshore winds bring insects, seeds and terrestrial plant debris. Benthic animals and plants are dislodged, abraded and triturated, creating a dilute 'soup' that contains food resources ranging from macromolecules to whole animals (live and damaged). *Actinia* sp. were known to take up dissolved organic material (e.g. Pütter 1911, Chia 1972), to assimilate energy from bacteria and microalgae (van Praët 1985), and to predate on a range of organisms (e.g. Chintiroglou & Koukouras 1992, Kruger & Griffiths 1998). Our study demonstrates that its diet is heavily influenced by shore height and wave exposure, but also that *A. equina* scavenges (rather than preys) on the larger food items that it ingests.

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Functional response of fishers in the Isle of Man scallop fishery

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ABSTRACT: To implement effective fisheries management, it is important to understand the variables influencing the distribution and intensity of fishing effort. The functional response of consumers to the availability of prey determines their impact on prey populations. The relationship between predator and prey observed in nature also applies to fishers and the populations they target. The present study focuses on the behaviour of a scallop dredging fleet fishing for *Pecten maximus* around the Isle of Man during a single fishing season. The functional response was investigated by examining the relationships between catches and fishing effort, scallop abundance and other variables. Scallop abundance was depleted rapidly during the first month of fishing. The increased patchiness of scallops towards the end of the season probably reduced their catchability, but fishers were able to maintain catch rates at intermediate abundance levels. The functional response did not conform to a particular type, but there was latent fishing capacity in the fishing fleet even at the highest levels of abundance. Therefore, reducing the number of vessels would not necessarily reduce fishing mortality unless combined with a reduction in the fishing power of individual vessels.

KEY WORDS: Functional response · *Pecten maximus* · Fisher behaviour · Optimal foraging · Isle of Man · Scallops

INTRODUCTION

Understanding the variables that influence the foraging decisions of fishers is a prerequisite to implementing effective management strategies that achieve sustainable use of marine resources. The functional response describes the same relationship in ecology as that between prey abundance and catch rates in fisheries science (Johnson & Carpenter 1994, Eggleston et al. 2008). Relationships between predator and prey populations have been widely described in terms of numerical and functional responses (e.g. Holling 1961, Hassell et al. 1976, Jeschke et al. 2002, Griffen 2009) over both the short term (Caldow & Furness 2001, Wong & Barbeau 2005) and long term (Jaksic et al. 1992, Höner et al. 2002). While the numerical response (Solomon 1949) describes the increase in predator numbers with increasing prey densities, the functional response (Solomon 1949) describes the consumption of prey by predators at different prey densities and often takes one of 3 main forms (Holling 1961).

In a type I functional response, the prey consumption rate (C = number of prey items per total time) increases linearly with prey availability (N) up to a critical level. Thus, the type I functional response model, where a = encounter rate, is simply:

$$C = aN \quad (1)$$

This model applies up to a critical value of N above which there is a plateau in C (see Gascoigne & Lipcius 2004, Jeschke et al. 2004). Holling's type II functional response model, the disc equation (Holling 1959), is based on the assumption that handling time per prey item is constant and that the total feeding time is the sum of time spent searching for prey and handling prey. The type II functional response, where t = handling time per prey item and a = encounter rate, is described by:

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$$C = \frac{aN}{1 + atN} \quad (2)$$

Therefore, the type II response is hyperbolic in nature. However, prey capture may be more efficient at higher prey densities. Thus the encounter rate may be expressed as aN^2, giving a sigmoidal, type III response (Real 1977):

$$C = \frac{aN^2}{1 + atN^2} \quad (3)$$

If attack rate is expressed as aN^m, then type I, II and III functional responses can be described by the equation:

$$C = \frac{aN^m}{1 + atN^m} \quad (4)$$

where m is a coefficient allowing for variation in encounter rate with prey density. If $m = 1$ and $t = 0$, the response is type I; if $m = 1$ and $t > 0$, the response is type II; and if $m > 1$, the response is type III. If $m > 1$, the encounter rate varies with N, and therefore a becomes the attack rate coefficient (Smout et al. 2010). t and a are assumed to be constant in the basic functional response equations, which in many cases will not be true (Hassell et al. 1976, Caldow & Furness 2001). The functional response may deviate from the 3 distinct types due to the cost of foraging (Abrams 1982), or other factors, but in any case it is a major determinant of the population dynamics of predator and prey populations (e.g. fishers and targeted species).

The nature of the functional and numerical response of fishers to the target species will determine the potential impact that fishing activity has on the exploited populations. Fisheries management generally involves the restriction of fishing effort spatially, temporally or through technical measures (e.g. mesh size constraints), or limiting landings (e.g. total allowable catches). These restrictions necessarily change fishing patterns, but their effect depends on the other variables influencing the distribution and magnitude of fishing effort, such as the locations of target stocks relative to fishing ports, weather, vessel size, fishers' knowledge, and prior fishing patterns. Therefore, understanding the key drivers of a particular functional or numerical response to prey availability will assist management decisions that aim to achieve conservation of pressure stocks.

A number of studies have examined fisheries in an optimal foraging context. These include studies of both artisanal (Beckerman 1983, Begossi 1992, Béné & Tewfik 2001) and mechanised (Gillis et al. 1993, Rijnsdorp et al. 2000a,b, Gillis 2003) fisheries. Johnson & Carpenter (1994) examined fish and angler interactions within a framework of numerical and functional responses. Furthermore, Eggleston et al. (2008) identified the aggregate functional response of fishers in a Caribbean spiny lobster *Panulirus argus* fishery and the implications for management. Such an approach would be valuable in identifying appropriate management strategies in other fisheries. The lack of spatio-temporal information of sufficient resolution could prevent such an approach being applied, and a number of the difficulties encountered, such as seasonality, are highlighted by Johnson & Carpenter (1994). However, with the advent of satellite monitoring of individual vessels, examining the functional response of fishers will become much more widely applicable in the future, and several studies have examined fishing effort using satellite monitoring data (Mills et al. 2007, Lee et al. 2010, Gerritsen & Lordan 2011). Dynamic-state variable models have been used in fisheries science to examine high-grading (Gillis et al. 1995), targeting decisions (Babcock & Pikitch 2000) and effort allocation (Poos et al. 2010), but to parameterise such models, it is first necessary to understand which variables influence fisher behaviour.

The present study focused on a scallop dredging fleet that fishes in the waters around the Isle of Man, in the Irish Sea (Fig. 1). Fleet dynamics are relatively simple in that they target only 1 species within the open fishing season, and all vessels within the fishery are fitted with satellite vessel monitoring systems (VMS). Furthermore, the fishery does not usually interact with other species of commercial value and there is no incentive for high-grading (a process whereby a legal catch is discarded in the expectation of catching larger individuals that are more valuable). The method of fishing is also relatively simple. The principal modifications to the dredging technique occur by adjusting tension on sprung tooth bars or by altering vessel speed. Thus, we examined the functional response of fishers in response to changing scallop abundance during a single fishing season and sought to identify the type and primary drivers of the functional response to prey availability.

MATERIALS AND METHODS

Study area and fishery. The Isle of Man has a territorial sea, extending 12 nautical miles (n miles, 22.2 km) from the baseline, with an area of 3965 km^2. The great scallop *Pecten maximus* is targeted around the Isle of Man between 1 November and 31 May. Fishers targeting *P. maximus* did not target other species during the study period. *P. maximus* is generally sedentary but moves to evade predators (Thomas & Gruffydd 1971). Despite *P. maximus* being fished for several decades (Mason 1957), the species has increased in abundance during recent years (Beukers-Stewart et al. 2005), which is possibly related to warming sea temperature (Shephard et al. 2009).

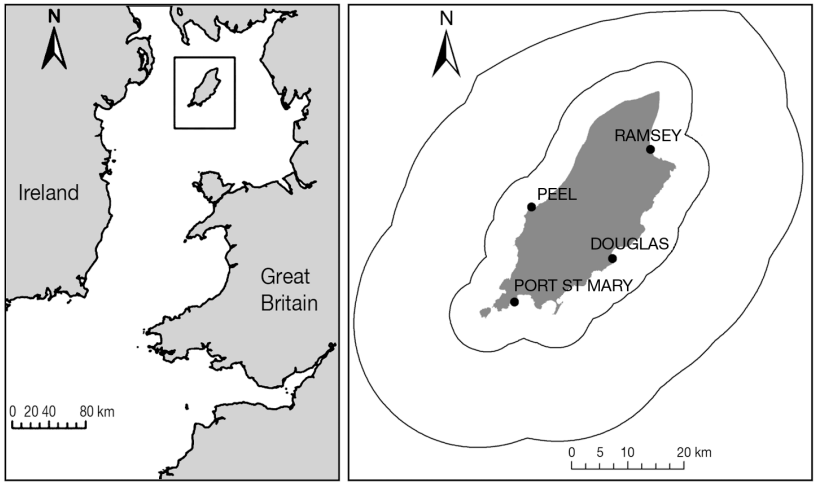

Fig. 1. Isle of Man. Fishing ports and boundaries of the 3 and 12 nautical mile zones are shown

The Isle of Man has exclusive fisheries control out to 3 n miles from its coastline; responsibility for fisheries management is then shared with the UK from 3 out to 12 n miles. *Pecten maximus* is caught using toothed Newhaven dredges, each of which is 0.76 m wide. Local fishing regulations during 2007/2008 dictated that a total of 10 dredges per vessel could be used in the inner, 0 to 3 n mile zone, while 16 dredges per vessel could be used in the outer, 3 to 12 n mile zone. During the study period, fishing time was restricted by a curfew to 12 h d^{-1} within the inner zone and 16 h d^{-1} in the outer zone. Fishing for *P. maximus* is prohibited from the beginning of June until the end of October.

The fishery is prosecuted by vessels originating from the Isle of Man as well as by vessels from the UK. The Isle of Man's fishing vessels are based in 4 ports: Douglas, Peel, Port St. Mary and Ramsey (Fig. 1). We focused on fishing activity by the Isle of Man fleet that occurred within the 12 n mile territorial sea, as less comprehensive data are available for other vessels. The great scallop fleet consists of around 25 vessels, although this number varies between years. In this study, data from all 24 Manx vessels known to be fishing during the study period were used. Vessels ranged from 9.23 to 18.4 m in registered length, with maximum continuous engine power (MCEP) ranging from 60 to 372 kW. Vessels towed between 4 and 8 dredges on each side of the vessel. Toothed bars with 8 teeth of ~110 mm in length are mounted on a pivot linked to springs. The tension on these springs is usually reduced on cobbly or rocky fishing ground so that less force is required to rotate the tooth bars backwards, reducing the quantity of stones picked up. Once dredges have been hauled, they are emptied onto deck manually by inverting 1 dredge at a time. Once the dredges have been redeployed, or the vessel is steaming, catches can then be sorted. The minimum landing size for *Pecten maximus* is 110 mm at the widest point; thus, smaller scallops are returned to the seabed.

VMS and logbooks. Data were obtained from VMS, which is fitted to all fishing vessels >15 m (overall length) in the European Union. However, all dredging vessels operating within 3 n miles of the Isle of Man during the study period were required to have operational VMS transceivers, meaning all Manx vessels were fitted with VMS. UK vessels fishing exclusively between 3 and 12 n miles from the Isle of Man were required to have operational VMS transceivers if they were >15 m in length. Consequently, the level of fishing activity by UK vessels ≤15 m in length and not fishing within 3 n miles of the Isle of Man is unknown. Records were received at ~2 h intervals from all vessels in the study fleet and included latitude and longitude, vessel course and speed recorded using differential global positioning system receivers.

Fisheries logbooks are returned to the Isle of Man Government by all Manx fishers landing to the Isle of Man or UK. UK vessels fishing within the Isle of Man's Territorial Sea and landing catches to the UK submit logbook returns to the UK authorities only, and these were not available for use in this study. Random checks are conducted by fisheries officers to ensure that logbooks have been completed correctly. Logbooks detail gear type and dimensions (i.e. number of dredges used), fishing time and catches for each fishing trip, but do not provide data on catches per tow. Catches are reported in terms of the number of bags of scallops landed. When full, these bags are estimated by fishers and processors to weigh 40 kg. Therefore, the number of bags landed was used in the present study as the unit of catch size. To check that logbooks were representative of actual landings, the reported landings were checked against a sub-sample of landings as recorded by a scallop processor who independently records the number of bags of scallops bought, from whom and on what date, the price paid per kg, and the wet meat weight per bag. Data on landings from 416 fishing trips by 8 vessels landing to 1 processing factory were obtained. Prices were given as £ kg^{-1} of meat (adductor muscle and gonad) landed. The prices paid to these vessels were assumed to reflect the prices paid to the entire fleet at any given time. The relationship between the number of bags and the meat weight landed was also examined. Logbook data were linked to VMS data using a unique vessel and date identifier in order to spatially reference reported catches.

Estimating fishing time. Fishing time vessel^{-1} d^{-1}, f_v, was estimated by plotting the speed frequency distributions of all VMS data from Manx vessels. The speed values falling between the lowest frequency classes on either side of the central mode were examined to identify fishing activity. Thus, the range of fishing speeds was identified as 1.2 to 3.4 knots (kn; 2.2 to 6.3 km h^{-1}). A fishing zone was demarcated around all VMS points indicating a speed of 1.2 to 3.4 kn using a 1 km buffer; any data points indicating a vessel speed of <1.2 kn falling within this zone were also considered to indicate fishing activity, as vessels may stop to empty dredges or perform maintenance tasks. Given the 2 h polling interval, excluding these points would result in underestimates of fishing time. Vessels may at times fish at >3.4 kn; however, it is not possible to distinguish fishing and non-fishing activity at these speeds. f_v was calculated by subtracting the time of the earliest fishing activity (f_{min}) from the time of the latest fishing activity (f_{max}) for each vessel on each day. Total active time, T_A, from first to last VMS record, was calculated by subtracting the minimum time, $T_{A,min}$, from the maximum time, $T_{A,max}$, as indicated by the first and last VMS records from a fishing trip on any one day. All VMS records were included except those indicating a speed of 0 while in port, as on many occasions VMS transceivers continued transmitting records while vessels were inactive in port.

Fishing times reported in fishing logbooks (f_l) were compared to f_v for each fishing trip. An average of f_v and f_l was used as the estimate of fishing time in hours, f:

$$f = \frac{f_l + f_v}{2} \quad (5)$$

If f_l was not reported or f_v could not be calculated as there was only a single VMS record, then the alternative measure was used. When f_l was not reported and there was a single VMS record only, this was assumed to represent 2 h of fishing. Where fishing continued outside the territorial sea, f may have exceeded 16 h, although this was rare. Values of f >16 h occurred in only 39 fishing trips, and for 21 of these $f \leq 18$ h.

The area dredged per fishing trip was defined as:

$$A = uwf \quad (6)$$

where w = width of dredges deployed (km) and u = mean vessel fishing speed (km h^{-1}). Therefore, catch per unit effort (CPUE) = S/A, where S = number of bags (B) of scallops.

Other variables. The departure and return ports of each vessel fishing trip were determined using a combination of VMS data and fisheries logbooks. A square of 2 × 2 km was drawn around each port. When the first or last VMS record fell within any of these boxes, they were deemed to indicate the departure or return ports, respectively. Where no VMS points fell within the port areas, due to a VMS transceiver fault for instance, the departure and return ports recorded in logbooks were used. Cost–distance rasters (1 × 1 km cell size) were generated over the range of fishing activity with each port included as a point source. Thus, in each cell over the range of fishing activity, the distance of that cell from each port was stored. The mean distance of all VMS points defined as fishing activity was then calculated from the appropriate ports using the cost–distance raster files.

UK wave model data (Met Office 2009) was obtained from Met Office hindcast archives and is based on a 12 km grid at 3 h intervals. The wave model is forced by wind fields derived from the Met Office numerical weather prediction model and includes swell wave, wind wave, and total wave height, direction and period. The wave data point closest to each VMS record in space and time was associated with that record. Seawater temperature data were derived from mean monthly temperature at Port Erin, Isle of Man (Isle of Man Government Laboratory 2007, 2008). Vessel capacity units (VCUs) were used as a proxy accounting for the differences between vessels, where VCU = vessel length × breadth + (0.45 × engine power) (Pascoe et al. 2003). VCUs were then used to predict the width of fishing gear deployed by vessels from the UK, since no data were available on the actual width of fishing gear deployed by these vessels. Fishing time and the area dredged were calculated as for Manx vessels.

Functional response. Three possible sets of definitions of the time components of the functional response in relation to scallop fishing activity were identified. Firstly, handling time in scallop dredging operations could be considered as the time spent processing each catch unit on deck, t; searching time is then the time spent dredging. Total available time, T_T, would then be the 16 h permitted by the Isle of Man's curfew outside the 3 n mile zone. Transit time, J, could be added as an additional parameter or not be considered as part of the functional response. However, no data are available on the time spent handling the catch on deck. A second approach then is to consider handling time per catch unit, t, to be equivalent to the time spent dredging and handling the catch on deck (i.e. fishing time per catch unit; thus, $f = St$) and the remaining time ($R = T - f$) is then the sum of time spent preparing to go to sea, transit, landing the catch and non-fishing activity, where T_T = 24 h. Finally, T may be defined as the total active time as derived from VMS records, T_A, and will vary between vessels and days with transit time, J, equivalent to searching time. Therefore, the functional response was examined with parameters defined according to the second and third scenarios.

C is therefore the catch rate per vessel ($C = S/T$), and the unit of C is B V^{-1} h^{-1}, where V = vessel, and is distinct from CPUE for which the unit is B km^{-2}. Since f also includes handling time, the area dredged will be over-estimated slightly, although the time spent deploying, hauling and emptying dredges is relatively small compared to the time spent dredging.

Statistical models. Generalised additive models (GAMs) were used to examine the relationship between CPUE and several predictor variables in order to generate a standardised abundance index. A similar approach was used to examine the relationship between C and a range of predictor variables. GAMs were fitted using the 'mgcv' package (see Wood & Augustin 2002, Wood 2008) in R. Detailed descriptions of the use of the 'mgcv' package and associated methods are given by Wood (2006) and Crawley (2007). GAMs were assessed using the deviance explained and the Akaike information criterion (AIC) and generalised cross validation (GCV) scores.

RESULTS

Overview of the study fleet

Total landings of scallops by the study fleet were 25 673 bags of great scallops, amounting to ~1027 t. The Manx fleet swept over 530 km^2 of seabed during the fishing season, 13% of the total area of the territorial sea, although many areas of seabed will have been swept more than once. Over 50 km^2 of seabed were swept by the Manx fleet during the first 10 d of fishing. The general area over which fishing occurred covered 48% of the territorial sea. Fishing effort was particularly high on the west coast and off the northeast coast, and was higher in inshore areas. Vessels from Port St. Mary, Peel, Ramsey and Douglas fished mean distances of 22.7, 21.3, 20.1 and 17.6 km from their home ports, respectively.

Fishing time

The modal fishing speed was 2.4 kn (Fig. 2). Frequency minima below and above the central mode were at 0.2 and 4.8 kn; these are outside the normal range of dredging speeds. The effect of setting different upper fishing speeds on estimated fishing time was examined.

When 3.6 kn was used as the maximum vessel speed that was classified as fishing activity, fishing time was over-estimated relative to f_l for both mean (Fig. 3a) and total (Fig. 3b) times. Therefore, 3.4 kn was selected as the threshold value. True fishing time is likely to be underestimated when derived from VMS data, as any fishing activity occurring <2 h before the first VMS record or <2 h after the last VMS record will be unrecorded. Increasing the value of f_v for each fishing trip by 0.75 h resulted in a strong correlation with f_l for mean (Fig. 3c) and total fleet (Fig. 3d) fishing time, which suggests that f provides a suitable relative measure of fishing time; however, since both f_l and f_v may differ from true fishing time, f cannot be considered an absolute measure.

The width of dredges deployed by Manx vessels did not differ significantly between vessels fishing within the 3 n mile zone and those fishing outside of this area, although 5 of the vessels that fished did not fish within the 3 n mile zone. The model including an interaction term (VCU × zone) was fitted first. Neither the interaction term nor the zone term were significant ($p \geq 0.726$), while both the intercept and VCU term were highly significant ($p < 0.001$). Parameters were removed from the model until the lowest AIC was obtained. The final model was the simple linear relationship between VCU and width of dredges, w, (intercept = 3.705, slope = 0.029). Hence, the linear relationship between VCU and w was used to estimate the width of dredges deployed by UK vessels.

The difference between C calculated using T_T and T_A was examined using linear regression. There was a strong linear relationship between C_{TT} and C_{TA} (Fig. 4). Therefore, the proportion of the total time used seemed to have little impact on C. Whether T is fixed or included as a variable in the functional response model, the impact will be predominantly on the absolute values of C, not the relative values. Hence, C was calculated with T = 24 h.

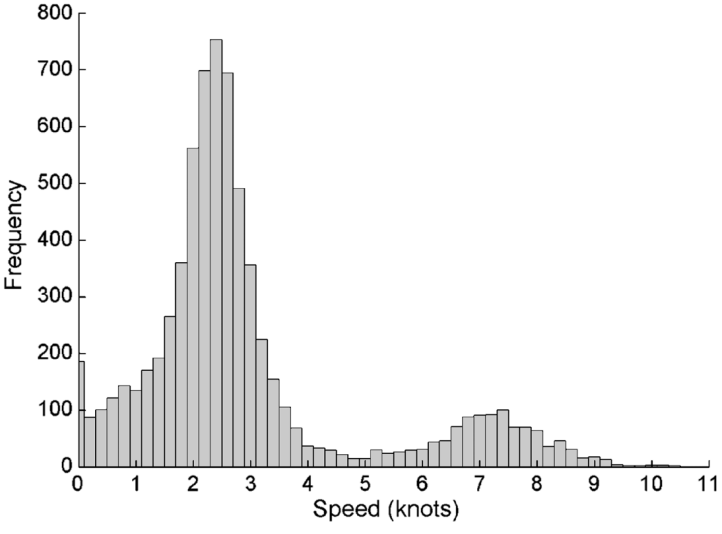

Fig. 2. Vessel speed frequency distribution derived from vessel monitoring system records from the Manx scallop dredging fleet between November 2007 and May 2008

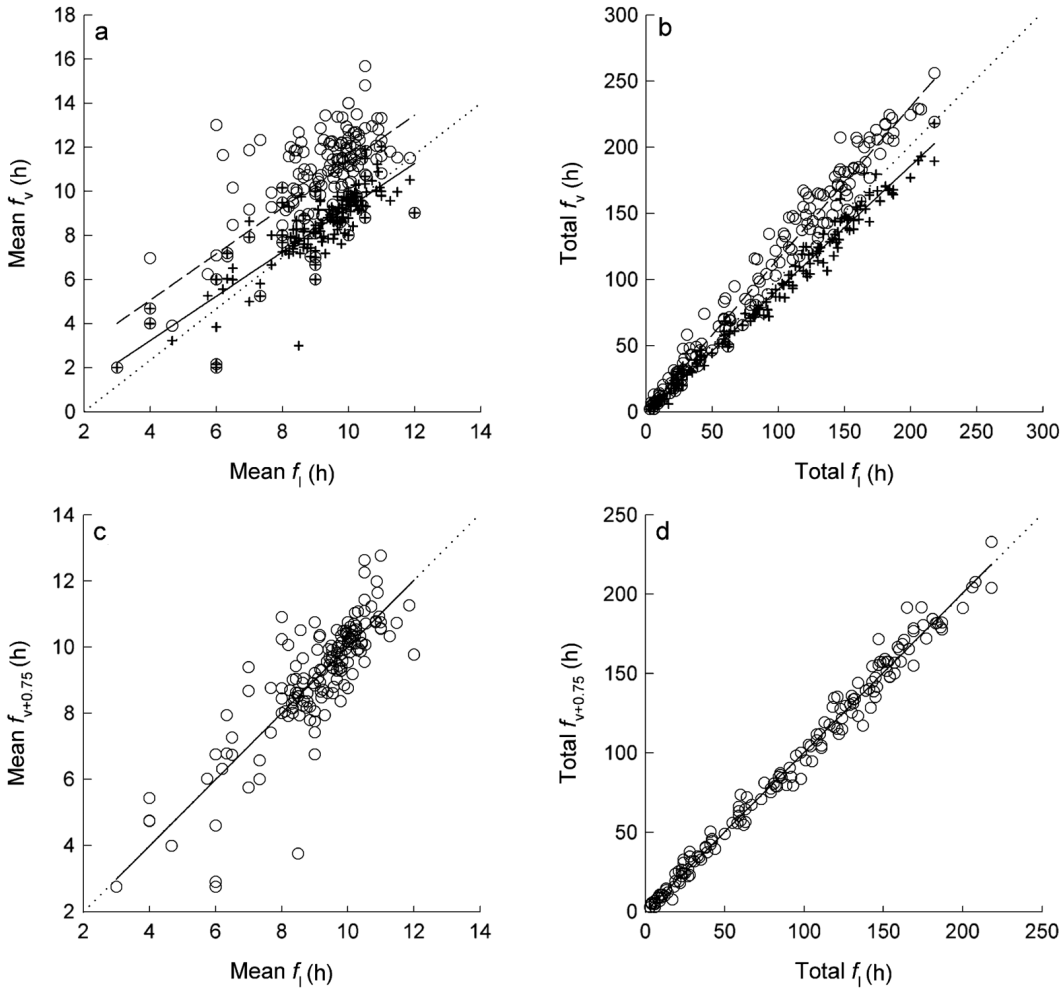

Fig. 3. Relationship between fishing time derived from vessel monitoring system data (f_v) and fishing logbooks (f_l). Dotted lines show unity between f_l and f_v; solid and dashed lines are linear regression models. (a) Mean and (b) total daily fishing time when f_v is calculated with an estimated maximum fishing speed of 3.4 knots (crosses) and 3.6 knots (circles). (c) Mean and (d) total daily fishing time when maximum fishing speed was set at 3.4 knots and increased by 0.75 h to account for underestimation due to the 2 h polling interval

Abundance

Several GAMs were fitted to CPUE data and a number of predictor variables. The final model was selected based on the AIC value. The models were fitted to individual fishing trip data using thin plate regression splines. A gamma distribution was used with a log link. Quantile-quantile plots and histograms of residuals showed the distributional assumption to be appropriate, and plots of linear predictors against residuals revealed variance to be approximately constant for all models. Models for which all parameters were significant ($p < 0.001$) are shown in Table 1. Wave height and mean monthly temperature were also included in candidate models but were not significant ($p > 0.05$), although wave height undoubtedly influenced the number of vessels fishing on a given day. Model AI2 had the lowest AIC and explained 53.1% of deviance. The model without an

Table 1. Generalised additive models fitted to individual fishing trip data with catch per unit effort (CPUE, bags km^{-2}) as the response. Only models for which all terms were significant ($p < 0.05$) are shown. Parameters are: day: time in days from 1 November 2007; x,y: position in degrees of longitude and latitude, respectively; Vessel: factor identifying each vessel. AIC: Akaike information criterion; GCV: generalised cross validation; s_i are smooth functions

Model name	Model	GCV	AIC	Deviance explained
AI1	$\log(E[CPUE]) = \text{vessel} + s_1(\text{day},x,y)$	0.140	13785.88	52.8
AI2	$\log(E[CPUE]) = \text{vessel} + s_1(\text{day}) + s_2(x,y)$	0.134	13735.53	53.1
AI3	$\log(E[CPUE]) = \text{vessel} + s_1(\text{day}) + s_2(x) + s_3(y)$	0.142	13849.36	49.2

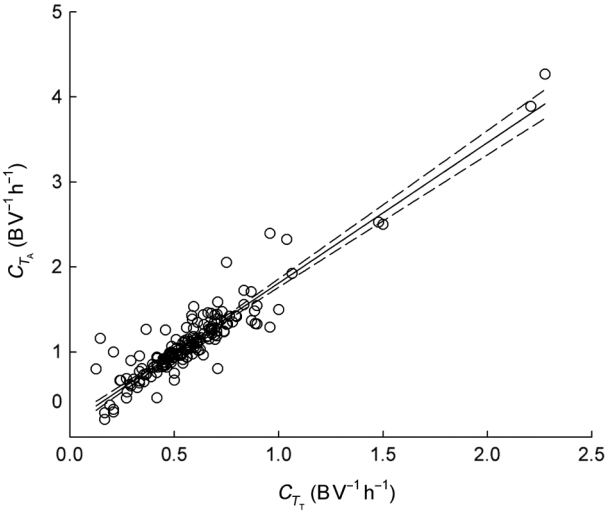

Fig. 4. Relationship between mean daily catch rate (C; bags vessel^{-1} hour^{-1} [B V^{-1} h^{-1}]) based on total available time (T_T) and total active time (T_A); $C_{TA} = 1.651 C_{TT} + 0.161$, $R^2 = 0.87$, $F_{1,168} = 1087.44$, $p < 0.0001$. Dashed lines indicate 95% confidence intervals

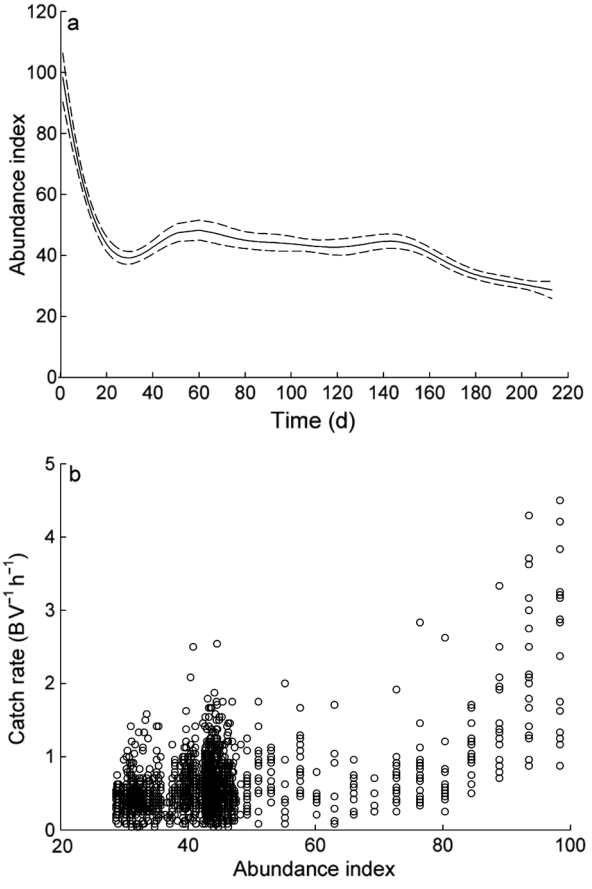

Fig. 5. *Pecten maximus.* (a) Scallop abundance index (N) over time from 1 November 2007 based on the generalised additive model smooth of the time term (model AI2; see Table 1). Dashed lines indicate ±2 SE of the smooth term. (b) N versus catch rate (C); data points are for individual fishing trips (n = 1679)

interaction term and that with a multiple interaction term resulted in higher AIC values. Therefore model AI2 was used to estimate the standardised abundance index, N, which is a relative, not absolute, measure of abundance. N declined rapidly during the first 20 d of fishing, increasing slightly between Days 30 and 50 and thereafter remaining level for 100 d, before declining to a minimum at the end of the season (Fig. 5a). When plotted against C there was a dense cluster of points between an abundance of 28 and 48 with the majority of values of C <2 B V^{-1} h^{-1} at an abundance index <40. There was clear acceleration in C above 60 and a plateau in C was not reached (Fig. 5b). Over the first 10 d of fishing, mean abundance was 82.3 while mean C was 1.24 B V^{-1} h^{-1}. During the final 10 d of the season abundance was 28.9 and C was 0.43 B V^{-1}h^{-1}.

Fishing effort

Including UK fishing effort, at least 937 km^2 were estimated to have been dredged during the fishing season, 109 km^2 of this during the first 10 d of fishing. Fishing intensity by the Manx fleet varied greatly, ranging from 0.1 km^2 dredged km^{-2} to nearly 3 km^2 dredged km^{-2} of seabed. The time spent fishing did not appear to be influenced by market demand, as the time spent fishing averaged over each 10 d of fishing ranged from 8.1 to 10.7 h d^{-1}, with a mean ± SD of 9.7 ± 0.6 h d^{-1}. However, the number of vessels fishing varied markedly from a mean (excluding days when no vessels were fishing) of 30 during the first 10 d of the season to only 3 at the end of December and beginning of January. The maximum number of UK vessels (with VMS) fishing on any one day was 25, while up to 21 Manx vessels fished on any one day.

The price paid for scallop meat varied at certain points during the season, peaking on either side of Christmas (Fig. 6a). There were only 5 main prices paid during the season. Since fishers were paid on the meat weight landed, the yield of scallops may also have had an impact on the number of bags landed. However, despite variation in the meat weight per bag, the relationship between the number of bags landed and the meat weight landed was strongly linear (Fig. 6b). Therefore, fishers did not reduce effort to land fewer bags of scallops when yields were high or land more bags to maximise profit. To examine the combined effects of variation in fishing effort and catchability on the functional response, a second suite of models was fitted to several variables with C as the response variable.

To describe the relationship between CPUE and C, the model must include the parameters used to standardise CPUE, i.e. vessel, day and position (latitude and longitude). These variables may influence the catchability of scallops; in the case of day, the smooth function is assumed to represent N. These parameters may also influence C through their effect on fishing effort. Spatio-temporal variation in fishing effort, due to the price paid for scallops or market demand for instance, may occur and thus alter C, and the fishing effort exerted by different vessels will also have varied.

Table 2. GAMs fitted to individual fishing trip data with catch rate (bags vessel^{-1} h^{-1}) as response. Only models for which all terms were significant ($p < 0.05$) are shown. Parameters are: day: time in days from 1 November 2007; x,y: position in degrees of longitude and latitude, respectively; A: total estimated area dredged per fishing trip (km^2); VCU: vessel capacity units; d: mean distance fished from departure and return ports (km); f: estimated fishing time (h); J: estimated transit time (h); w: width of dredges deployed (included as a factor with 6 levels); vessel: factor identifying each vessel. AIC: Akaike information criterion; GCV: generalised cross validation

Model name	Model	GCV	AIC	Deviance explained
FR1	$\log(E[C]) = s_1(\text{day})+s_2(x,y)+s_3(A)+\text{vessel}$	0.124	−931.53	71.4
FR2	$\log(E[C]) = s_1(\text{day})+s_2(x,y)+s_3(A)+s_4(\text{VCU})$	0.131	−818.23	68.7
FR3	$\log(E[C]) = s_1(\text{day})+s_2(d)+ s_3(A)+s_4(\text{VCU})$	0.156	−501.53	61.8
FR4	$\log(E[C]) = s_1(\text{day})+s_2(d)+ s_3(A)+s_4(\text{VCU})+s_5(x,y)$	0.130	−839.85	69.3
FR5	$\log(E[C]) = s_1(\text{day})+s_2(x,y)+ s_3(f)+s_4(J)+\text{w}$	0.116	−1026.67	72.4
FR6	$\log(E[C]) = s_1(\text{day})+s_2(x,y)+s_3(f)+s_4(J)+s_5(\text{VCU})$	0.118	−994.98	71.9

Functional response

Where fishing time is considered to be handling time, then handling time per catch unit, t, is simply a function of CPUE; that is $t = \beta/N$, where β is a constant. Substituting t in Eq. (4) and using the abundance index (N), the functional response may be described by:

$$C = \frac{aN^m}{1 + a\beta N^{m-1}} \quad (7)$$

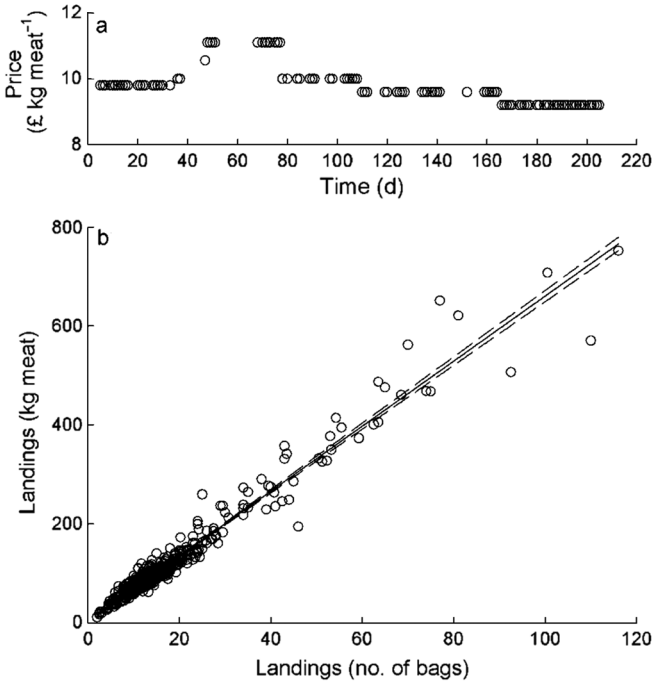

Fig. 6. *Pecten maximus.* (a) Price paid per kg of meat (gonad + adductor) during the 2007/2008 fishing season, and (b) relationship between the number of bags of scallops landed and meat weight landed. Linear regression: $R^2 = 0.957$, $p < 0.0001$, slope = 6.577, intercept = 3.012, dashed lines = ±95% confidence interval. Data derived from 416 fishing trips

Thus, t will not limit C at higher values of N, as is apparent in Fig. 5b. However, it is clear from Fig. 5b that this model is inadequate to describe the relationship between N and C. Just as CPUE may be a function of several variables other than scallop abundance, the relationship between N and C may be influenced by many variables. The width of fishing gear deployed, hold capacity, weather conditions, seabed type and transit times may all affect catches. To identify the variables affecting C, a number of models were fitted including several predictors. Quantile-quantile plots and histograms of residuals were examined to ensure that the distributional assumption was suitable, and plots of linear predictors against residuals revealed variance to be approximately constant for all models. In all cases, the value of γ used in the GCV scores was set at 1.4 to avoid over-fitting (Kim & Gu 2004, Wood 2006) and a gamma distribution was used with a log link function.

Day, position and vessel were included in model FR1 as for model AI2, with the addition of a term for the area dredged (Table 2). This model explained 71.4% of the deviance. Although the vessel term is effective in explaining deviance within the model, the generality of the model is extremely limited with this term and provides no information on why S, or C, varied between different vessels. VCU reflects the size and fishing power of vessels and therefore may explain differences in catches between vessels. When the vessel term was replaced with the VCU term, the deviance explained by the model (FR2) was reduced to 68.7% and the AIC increased to −818.2. A potentially important variable that influences the available fishing time, and therefore S, is the distance travelled to fish. However, replacing the position (x,y) term with the mean distance fished from the departure and return ports (d) substantially reduced the deviance explained (61.8%) and increased the AIC (−501.5; model FR3). Including both position and distance terms increased deviance

explained (69.3%) and reduced the AIC (–839.9). An alternative approach would be to replace distance with transit time, *J*. It is then logical to replace area dredged with fishing time, *f*, and the width of dredges deployed (included as a factor with 6 levels). This model explained 72.5% of deviance and had an AIC of –1030.97; however, the VCU term was not significant (p = 0.09). Removing the VCU term reduced the deviance explained slightly, to 72.4% and increased the AIC to –1026.67. These results suggest that VCU is a good proxy for the width of dredges deployed. Replacing the width term with the VCU term reduced the deviance explained to 71.9% and increased the AIC to –999.98. Although VCU and dredge width accounted for variability in *C*, there is also likely to be random variation in the catch rates of different vessels that is not related to a vessel's VCU or dredge width deployed. Thus, the final model adopted is a generalised additive mixed model (GAMM) that includes vessel as a random effect (*e*): $\log(E[C] = s_1(\text{day}) + s_2(x,y) + s_3(f) + s_4(VCU) + J + e$, where parameters are as defined in Table 2. The smooth terms of this model are shown in Fig. 7. The mixed model had an R^2(adj) of 0.671. Transit time was included as a parametric term, which was found to increase with *C*, showing a very slight exponential increase on the response scale. On the scale of the response variable, time exhibited the same relationship with *C* (Fig. 7a) as shown in Fig. 5a. *C* increased with fishing time up to 16 h, thereafter declining slightly (Fig. 7b), and is indicative of the effect of the curfew. It is important to note that *f* could include some fishing activity from slightly outside the territorial sea, where there is no curfew, which combined with error in the estimates can account for values >16 h. *C* increased most rapidly with VCU up to 140; thereafter, the rate of increase declined (Fig. 7c). The position term indicated peaks in *C* to the south and northwest of the Isle of Man and the minimum to the northeast

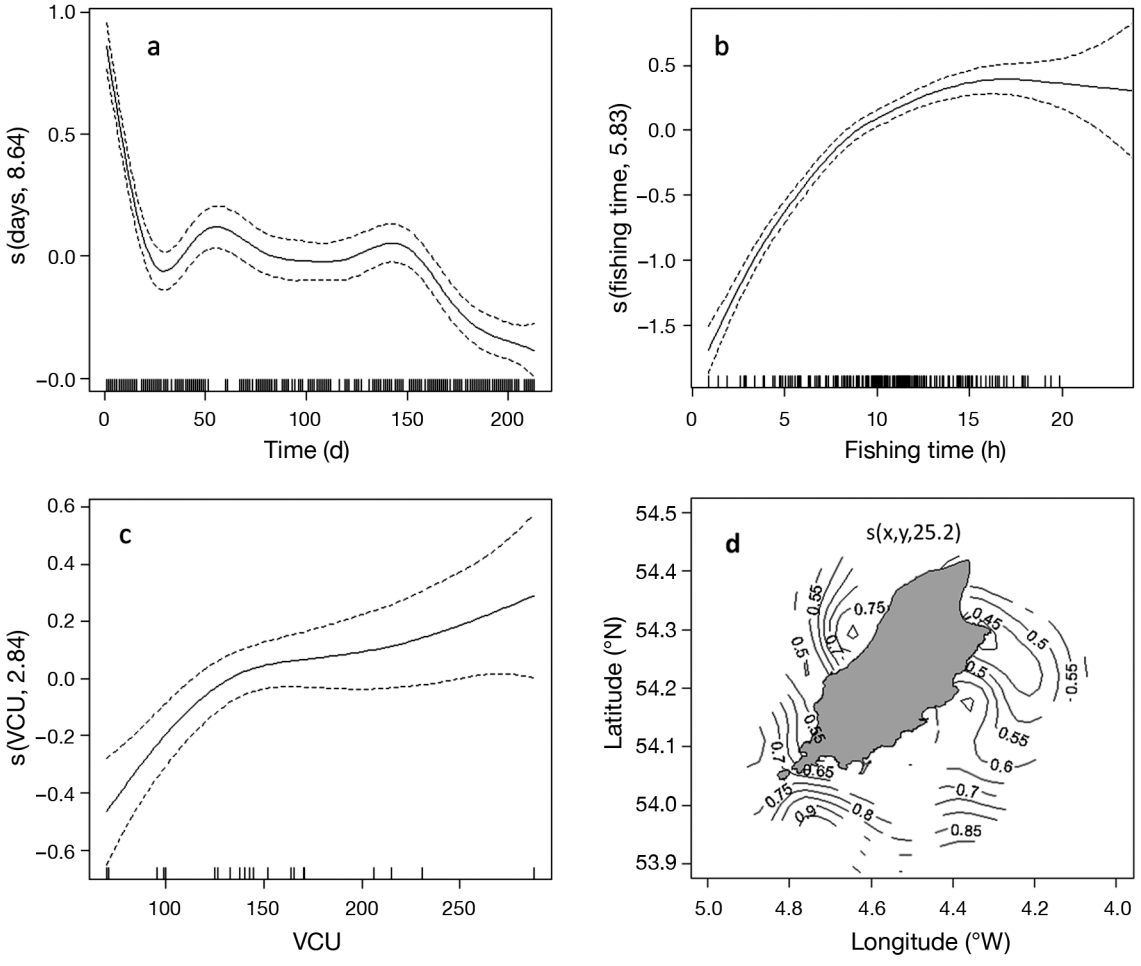

Fig. 7. Estimated smooth terms for the generalised additive mixed model: $\log(E[C]) = s_1(\text{day}) + s_2(x,y) + s_3(f) + s_4(\text{VCU}) + J + e$) for catch rate, *C*, in bags vessel^{-1} h^{-1}, fitted to individual fishing trip data (n = 1679). Vessel is included in the model as a random factor. Plots are on the scale of the linear predictor (log link). Dashed lines indicate ±2 SE and include the uncertainty about the overall mean. Estimated degrees of freedom for each term are shown in brackets. (a) Time from Day 0 (1 November 2007). (b) Time spent fishing. (c) Vessel capacity units. (d) Mean position of each fishing trip

(Fig. 7d). Whether fishing activity occurred within or outside the 3 n mile zone had no significant effect ($p > 0.05$) on catch rates. Replacing the time and VCU terms in the mixed model with area dredged reduced the R^2(adj) to 0.645 and increased the AIC from 1198.7 to 1378.2.

DISCUSSION

There are 2 fundamental questions that must be answered to understand fishing behaviour. What influences the spatial and temporal distribution of fishers, and what determines how much fishers catch? Identifying the type of functional response fishers exhibit with changing prey availability can help to answer both questions, but fishing activity is influenced by a number of parameters in addition to prey availability. In the present study, we have examined the relationship between catch rates and several variables relating to fishing effort and catchability.

An important consideration when examining the functional response of fishers is the index of prey availability used. CPUE is commonly used as an index of abundance (e.g. Beukers-Stewart et al. 2003, Maunder & Punt 2004) but can also be considered as rate of prey consumption (e.g. Johnson & Carpenter 1994). Simply using mean fleet CPUE does not provide a good index of abundance, as CPUE may become equalised over fished areas as fishers move to maintain catches (Gillis et al. 1993, Gillis 2003). Therefore, it is essential that CPUE is standardised to account for the spatial distribution of the fishing fleet (Sampson 1991). Moreover, fishery-dependent estimates of CPUE may be dependent on the fleet composition at any given time due to variability in fishing efficiency between vessels. CPUE is often standardised with reference to a standard vessel for which a long-term record of CPUE is available (Maunder & Punt 2004). However, the catches of reference vessels may also be altered by the number of competing vessels and technical advances in competing vessels (Rijnsdorp et al. 2008).

CPUE was standardised to account for vessel differences, for which VCUs were found to be a good proxy. VCUs were also found to explain 70 to 80% of the variation in earnings of Scottish trawlers (Pascoe et al. 2003). CPUE did broadly parallel the standardised abundance index. However, there were clear differences in CPUE between vessels. Moreover, other variables also influence CPUE. Temperature has a substantial influence on catches of queen scallops *Aequipecten opercularis,* due to their temperature-dependent escape response (Jenkins et al. 2003). However, Jenkins & Brand (2001) found no significant effect of season on the number of valve adductions in *Pecten maximus* following simulated fishing, and there was no clear impact of temperature in the present study. A potentially important variable not included in this study is that of the abundance of under-sized scallops. Although these are not prey to fishers, in that they are not targeted and cannot be legally landed, they may result in fewer scallops of ≥110 mm being caught. If dredges are full, then a greater percentage of undersized scallops will necessarily result in lower CPUE of scallops ≥110 mm. Scallop growth stops around December and does not resume until waters warm in March, April or May (Mason 1957). Therefore, changes in the size composition of the scallop population due to growth of scallops will have influenced their catchability at the end of the fishing season in particular. Moreover, it is at this time that the fewest scallops ≥110 mm were available. Dredges can also be filled with other bycatch such as brittlestars, or rocks. These variables may alter the functional response. In particular, the basic functional response equations assume that prey availability, N, and handling time per prey item, t, remain constant during time, T, which in most cases is not true (Hassell et al. 1976, 1977). The meat yield of scallops will also affect their value. High-grading is not thought to occur in the scallop fishery; smaller scallops over the minimum landing size could be discarded in favour of larger, more valuable scallops. However, there would be no advantage to fishers in doing so unless the vessel was nearing maximum catch capacity, which did not appear to occur.

The importance of distinguishing between the forms of functional response is that a type II or III response indicates density-dependent catchability, compared to density independence in a type I response (Eggleston et al. 2008). Furthermore, a type III response can stabilise prey populations (Hassell & Comins 1978, Nunney 1980, Nachman 2006). However, predator–prey populations do not always conform to a single response type (Jeschke et al. 2004). The results of our study indicate that the functional response of the fishing fleet did not conform to one particular type and that prey density was not high enough for prey consumption to reach a plateau. Therefore, the fishery was not at saturation, with vessels still able to exploit the highest abundance of scallops. It is important to note that almost all values of abundance >40 occurred within the first month of fishing, indicating that abundance was depleted rapidly. Fishers' knowledge of scallop distribution may have been greater after the first month of fishing, allowing them to exploit higher-density patches of scallops to maintain catches. Thereafter, prey consumption rates may have been reduced as prey became increasingly patchy (Essington et al. 2000) towards the end of the fishing season when most areas had been fished. Given that fishers cannot have

perfect knowledge of the distribution of scallop populations, the chance of a fisher encountering patches of scallops must be reduced at the end of the fishing season.

Previous catch rates are a major influence on the choice of fishing location (Hutton et al. 2004). In the scallop fishery, catches of under-sized scallops in the previous season will provide a good predictor of catches in the following season (Beukers-Stewart et al. 2003) and where fishers have knowledge of areas with high CPUE, they are likely to target them (Dreyfus-León 1999). All of the fishing activity observed in the present study corresponds with historically recognised scallop fishing grounds (Beukers-Stewart et al. 2003). Thus, fishers clearly had some knowledge of the distribution of scallop populations. However, in the scallop fishery, fishing has to be undertaken before CPUE can be determined, unlike other fisheries where sonar can be utilised (e.g. Brehmer et al. 2007, Boswell et al. 2008). Therefore, knowledge of prey distribution will necessarily be imperfect, limiting the numerical response. Moreover, interference competition may prevent fishing in optimal areas (Rijnsdorp et al. 2000a,b). Catches were not obviously suppressed by interference competition in the present study; however, high scallop abundance could mask competitive interactions. Nevertheless, both the number of vessels fishing and catches were highest at the beginning of the season. It is also possible that fishers exerted greater fishing effort when competition was greater.

The rate of deceleration in consumption rates at higher prey densities cannot be predicted; however, it is likely there would be a sharp deceleration either due to satiation (no market demand) or vessels filling their holds to capacity, and the result may be a type I/III response, as described by Jeschke et al. (2004). Type I functional responses have only been reported in filter feeders (Jeschke et al. 2004), while type III responses are often exhibited by generalist predators that can switch between prey species (Van Leeuwen et al. 2007, Kempf et al. 2008). Although scallop dredgers may take bycatch, such as *Aequipecten opercularis*, the Isle of Man scallop fleet targeted only great scallops during this open scallop fishing season, with queen scallops constituting a very small proportion of total landings, amounting to around 5 t from all vessels during the great scallop fishing season.

It may be possible to refine the analysis of the data used in this study. For example, improved estimates of fishing time may be achieved by adopting methods to reconstruct trawl tracks. Hintzen et al. (2010) used a spline interpolation technique to model fishing tracks from VMS data while Vermard et al. (2010) identified different fishing behaviour during fishing trips, using Bayesian hierarchical models. Moreover, the collection of additional data such as individual tow length and catches per tow would allow more detailed analysis of how fishers respond to changing prey availability. Nevertheless, several conclusions can be drawn from our study.

The assumptions of Holling's disc equation were not appropriate in the Isle of Man scallop fishery. Furthermore, the functional response did not conform very well to a particular type. The increasing patchiness of scallops towards the end of the season probably reduced their catchability, but fishers were able to maintain catch rates at intermediate abundance levels, suggesting knowledge of prey distribution. An important aspect of the observed functional response is that there is latent capacity in the fishing fleet. Eggleston et al. (2003, 2008) observed a type I response in a non-saturated *Panulirus argus* fishery and therefore concluded that reducing catch limits would be the most effective means of reducing landings. Similarly, setting catch limits or reducing vessels' fishing power together with a reduction in vessel numbers would result in a greater reduction in scallop landings at the highest prey densities, while limiting vessel numbers alone may have little impact. Incorporating additional variables, especially the size composition of scallop populations, into the models presented here will help to further elucidate the relationship between scallop fishers and their prey.

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Drag reduction by air release promotes fast ascent in jumping emperor penguins—a novel hypothesis

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ABSTRACT: To jump out of water onto sea ice, emperor penguins must achieve sufficient underwater speed to overcome the influence of gravity when they leave the water. The relevant combination of density and kinematic viscosity of air is much lower than for water. Injection of air into boundary layers ('air lubrication') has been used by engineers to speed movement of vehicles (ships, torpedoes) through sea water. Analysis of published and unpublished underwater film leads us to present a hypothesis that free-ranging emperor penguins employ air lubrication in achieving high, probably maximal, underwater speeds (mean ± SD: 5.3 ± 1.01 m s^{-1}), prior to jumps. Here we show evidence that penguins dive to 15 to 20 m with air in their plumage and that this compressed air is released as the birds subsequently ascend whilst maintaining depressed feathers. Fine bubbles emerge continuously from the entire plumage, forming a smooth layer over the body and generating bubbly wakes behind the penguins. In several hours of film of hundreds of penguins, none were seen to swim rapidly upwards without bubbly wakes. Penguins descend and swim horizontally at about 2 m s^{-1}; from simple physical models and calculations presented, we hypothesize that a significant proportion of the enhanced ascent speed is due to air lubrication reducing frictional and form drag, that buoyancy forces alone cannot explain the observed speeds, and that cavitation plays no part in bubble formation.

KEY WORDS: Emperor penguins · Air lubrication · Bubbly wakes · Jumping

INTRODUCTION

Emperor penguins *Aptenodytes forsteri* Gray are the largest living penguins, standing around 1.2 m high and weighing 25 to 40 kg (depending upon gender plus reproductive and nutritional states). They breed and rest on sea ice around Antarctica. As they have short hindlimbs and limited climbing ability, they have to jump from the sea onto sea ice that can vary a great deal in thickness. Their predators include leopard seals *Hydrurga leptonyx* and killer whales *Orca orcinus* and it is usually assumed that their ability to jump swiftly and without falling back into the sea is also an effective antipredator adaptation. Emperor penguins exhibit stereotypical responses when entering and leaving the water that are assumed to reflect adaptations to sustained predator presence. When entering the water they usually enter en masse, but reluctantly, with birds often pushing other penguins into the water first. Leaving the water by jumping is also usually accomplished gregariously and at high speed. To jump, an emperor penguin must achieve sufficient underwater speed to overcome the influence of gravity while the kinetic energy of entrained mass is assumed to stay with the water and contribute to splash and surface waves.

Sato et al. (2005) studied emperor penguins, instrumented to provide detailed time records of speed, flipper action and depth during dives and ascents to jump onto the ice surface through small, 1.2 m diameter

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holes in 2.3 to 2.5 m thick ice far from the open sea. The above-water heights that they achieved were small (0.2 to 0.46 m), but recorded exit speeds rose above the normal 2 m s^{-1} to between 2.5 and 3 m s^{-1} just prior to exit; this correlated well with the velocities required to overcome the effects of gravity for the given heights. Flipper action stopped some distance below the free surface, which was interpreted as implying that buoyancy played a significant role in attaining the higher exit speed (effectively reached in glide mode), a behaviour observed and modelled earlier for king and Adélie penguins (Sato et al. 2002).

The present study is based on close inspection and analysis of a widely-published film of swimming and jumping emperor penguins (BBC 2001), plus unpublished associated film provided by the BBC, which leads us to hypothesize that free-ranging emperor penguins employ drag reduction by air bubble release ('air lubrication') in achieving high speeds prior to jumping from sea water onto ice shelves. To construct a theoretical basis for future experimental testing of our air-lubrication hypothesis, we present a model and analysis of the means by which this previously unreported phenomenon could be achieved.

We propose that the air release from the plumage during ascent (as also evidenced at first glance by the pronounced wakes of air bubbles trailing ascending penguins) is believed to be similar to the process of air lubrication studied for engineering purposes. Thus early flat plate studies for turbulent flow showed that frictional drag could be reduced by up to 80% immediately downstream of microbubble injection and to 'near-100%' if plates were covered by a thin film of air (McCormick & Bhattacharryya 1973, p. 15). Increasing air flow reduces the skin friction. For example, to achieve a 60% reduction in local skin friction by injection of microbubbles in a turbulent boundary layer at a free stream velocity of 4.6 m s^{-1}, Madavan et al. (1985, Fig. 13 therein) needed a volume flow of air that was 54% of the volume flow of water in the boundary layer in the absence of bubbles. This measurement was taken at a distance of about 0.14 m downstream of the short porous section of wall where injection occurred, but drag reduction appeared to persist for as much as 60 to 70 boundary-layer thicknesses downstream (about 0.52 to 0.61 m). Measured turbulence spectra also indicated a reduction of high-frequency shear-stress fluctuations, hence a reduction of the near-wall turbulence, as one cause of drag reduction. It was found that microbubbles had to be present in the boundary layer close to the test surface, having no drag-reducing effect if they were outside the boundary layer (see also Guin et al. 1996 for discussion). A detailed recent plate study at high flow rates (6 to 18 m s^{-1}; Sanders et al. 2006) showed that a large void fraction (i.e. high ratio of bubble volume to bubble plus water volume) close to the test plate yielded the greatest reductions in drag, while bubble size was rather less important. However, although reduction in fluid density from water to air-water mixture is believed to be a major factor, this does not explain the whole of the drag reduction achieved (Sanders et al. 2006).

It should also be stressed that most plate studies have focused on the injection of bubbles into the water flow at the upstream end of the plate and been concerned with the degree to which bubbles are effective in downstream drag reduction. This follows from the principal motivation for such studies: the achievement of reduced fuel consumption in large commercial vessels such as oil tankers, in which frictional drag can make up as much as 80% of total drag (Fukuda et al. 2000), but where air injection over the whole wetted surface is impracticable. Drag reductions of 15 to 40% and speed increases of 27% have been achieved in far more modest-sized experimental vessels (though by use of macroscopic air spaces, not by injection of bubbles). A major obstacle to progress has been that propulsors (e.g. ship screws, water jets) must be protected from air bubbles (Matveev 2003).

In another approach relevant to the present study, Fukuda et al. (2000) applied air injection to plates and large ship models that had been painted with a hydrophobic paint. In this case, bubbles coalesced to form thin air films over the painted surfaces; frictional resistance was reduced by 80% in a flow of 4 m s^{-1} and by 55% at 8 m s^{-1}, which was significantly more than without paint. The reason for a significant drag reduction is readily illustrated qualitatively by considering the frictional drag F_d for the simple cases of a laminar and a turbulent boundary layer over a flat plate of length L and width B (Schlichting 1968, p. 128 and 599 therein):

$$F_{d,lam} = \tfrac{1}{2}\rho V^2 LB \times 0.664\ \mathrm{Re}^{-1/2} \propto V^{3/2}\rho\,\nu^{1/2} \quad (1)$$

$$F_{d,tur} = \tfrac{1}{2}\rho V^2 LB \times 0.074\ \mathrm{Re}^{-1/5} \propto V^{9/5}\rho\,\nu^{1/5} \quad (2)$$

where V is the free stream velocity, $\mathrm{Re} = VL/\nu$ denotes the Reynolds number, $\nu \equiv \mu/\rho$ the kinematic viscosity, μ is dynamic viscosity and ρ is density. For a given V, the ratio of frictional drag for flow of pure water and pure air at atmospheric pressure and 0°C (where the ratio of densities is 1000:1.3 and of kinematic viscosities 1.75:13.5) is about 277 for laminar flow and 511 for turbulent flow. These ratios explain qualitatively why the formation of a continuous air film along a flat plate due to coalescence of injected bubbles may give rise to 'near-100%' reduction of the skin friction, even though such a double boundary layer of air film-driven water flow does not satisfy Eqs. (1) & (2).

METHODS

The published film sequences of emperor penguins (BBC 2001) were collected at Cape Washington, Ross Sea, Antarctica under calm conditions with a flat sea surface. They total 56.04 s, consisting of 1401 fields, 0.04 s apart, and show penguins ascending rapidly and jumping out of the sea onto the ice shelf. The BBC also supplied unedited, unpublished film collected as part of the film making. This latter film, which showed that at least one leopard seal was present in the area of penguin activity, totalled about 2 h, but most footage was unusable for analysis. However, there were sufficient usable sequences to evaluate downwards and horizontal swimming near the sea surface. Selected sequences from both published and unpublished material were copied to a computer and loaded onto Motion Analysis Tools (The Ottawa Hospital Rehabilitation Centre), a software analytical program that allows frame-by-frame study, plus linear and angular measurements. Much of the material could only be considered qualitatively as the camera was either in constant motion (panning), or was directed substantially upwards or downwards, so that birds moved away from or towards the camera. Although flippers were seen to be active in both horizontal and ascent swims, no sequences permitted reliable analysis of flipper action (e.g. beat frequency, angle of incidence). However, there were several sequences that satisfied the following criteria: (1) the background (usually ice shelf) was stationary, indicating a non-moving camera; (2) the camera was close to horizontal; (3) the birds were constantly in focus; (4) if viewed from the dorsal aspect, ascending penguins were at the near-vertical phase of their ascents (so were not moving away from the camera); (5) descending birds, or horizontally-swimming birds were viewed from a completely lateral aspect (i.e. not moving away from or towards the camera); (6) distance between birds and camera was sufficient to minimize parallax problems. In these circumstances, quantitative data were extracted. Distances and speeds for any continuous sequence of fields were calibrated by assuming a standard bird length (bill tip to hindmost visible limit of feet) of 1.25 m (emperor penguins stand some 1.10 to 1.20 m high on ice with the beak at right angles to the body axis, but swim with the beak parallel to the body axis). There will inevitably be a linear error of about ±0.05 m (±4%), simply because of the variability of penguin size. The beak tip (readily discernible) was the marker position used in all such sequences.

Several near-surface sequences were available where the quantitative criteria were met, where the birds were in side view, and where the sea surface was visible. In these circumstances, it was possible to establish the angle between the body axis of the ascending/descending penguin and the horizontal sea surface.

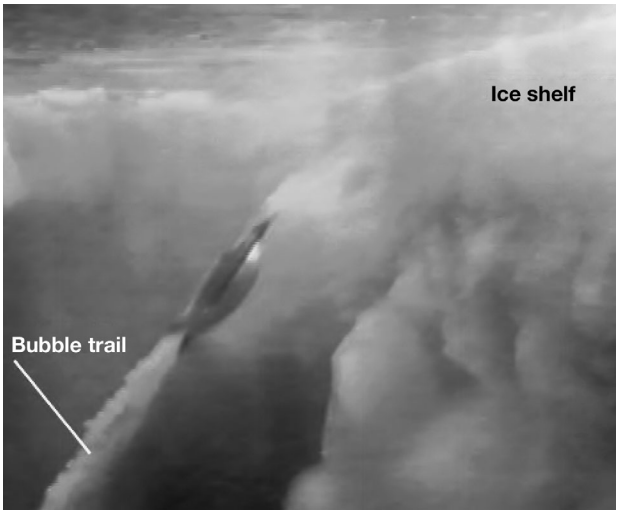

Fig. 1. *Aptenodytes forsteri*. Ascending emperor penguin approaching sea water surface close to edge of Antarctic ice shelf. Note highly visible trail of air bubbles. From BBC (2001), with permission

RESULTS

Observations

The most crucial observation of our study is that emperor penguins swimming upwards to jump out of the water trail long visible wakes of air bubbles (Fig.1). In underwater portions of the published film, 46 different penguins were seen to swim near-vertically upwards at high speed before adopting a rather shallower angle to the horizontal as they jumped through the water surface close to the ice shelf. No birds fell back and all created wakes of air bubbles throughout the ascent. The density of bubbly wakes varied amongst individual penguins, but the wakes remained constant for an individual throughout the upwards swim. There were no signs of the birds exhausting the air supply, and—as expected—wake flows followed the birds as they moved through the water surface. Also, most birds continued to use their flippers throughout the swim to the surface (i.e. there was no glide phase prior to emergence).

A priori there could be 2 possible sources of air that could generate the wakes, the respiratory system or the plumage. Vaporous cavitation could be ruled out because of insufficient speed and the fact that bubbles persisted in the wake. Antarctic fur seals exhale on ascents to avoid shallow water blackout (Hooker et al. 2005), so close-up sequences were inspected to see whether air issued from the beak/nares area; none did. It was clear that air issued from the plumage over most

of the body, forming a tight-fitting cloud of bubbles (Fig. 2). Close inspection of the bubble clouds showed that bubbles were extremely fine (visible as light blue clouds in which individual bubbles could not be discerned) at the anterior of the penguins' bodies, but became thicker and whiter towards the tail. In most close-up views the cloud was smoothly applied to the penguin body, forming a tube around the tail and hind limbs; coherent structures were visible in the early part of the wake behind the animal, but faded away as the wake bubbles dispersed and rose. Only in one image of a penguin very close to the water surface (Fig. 3) was the bubble cloud disturbed; large bubbles were also visible issuing from the breast/belly region of the individual. Bubble clouds appeared stronger on the dorsal surface of penguins ascending at angles from the vertical, presumably reflecting the tendency of air to rise in the water column (cf. Madavan et al. 1985).

In all underwater sequences, the bulk of the flippers were outside the bubble clouds, so acting against an incompressible medium. Localized signs of dorsal bubble cloud disturbance (posterior to the flippers) as the flippers beat were occasionally visible, while bubble clouds affected the base of the visible right flipper in a penguin filmed close to the sea surface (Fig. 3).

Six fast-ascending penguins trailing bubbly wakes were seen to abort ascents, their paths curving in abrupt near-vertical turns before the penguins descended again. The penguins appeared to be responding to the close proximity of other penguins or the camera operative; effectively their ascents were baulked. It was seen that air bubbly wakes continued to issue from the penguins' plumage until after they had completed the turns, but died away completely as the penguins descended. Clearly the bubbly wakes are related to ascents in the water column, not descents. Only one of the aborted ascents could be analyzed quantitatively; before slowing during the abort, the penguin concerned was travelling at 5.8 m s^{-1}. This value is within the range of swimming speeds of successfully-ascending penguins (see 'Film analysis'). This reinforces our impression that ascents are not aborted because of inadequate speed, but because of interference.

Although we inspected several hours of film in total, which recorded the movements of several hundred penguins, in no case did we see free-ranging penguins that rapidly ascended without bubble trails, or without active use of their flippers. This strongly suggests that rapid upward swimming without bubbly wakes is very rare (if it ever occurs at all). Some penguins swam upwards (without bubbly wakes) but, although these sequences were not analyzable (camera moving slightly, or birds too close to the camera), they were obviously very slow. The animals were often not even flapping the flippers, simply drifting upwards (presumably because of positive buoyancy) through the last couple of metres of the water column to the surface. Air bubble trails were seen in the case of descending penguins. In almost all cases this occurred as animals left the water surface; the bubbly trails died away within 2 to 3 flipper strokes. There were 2 exceptions, both being penguins that had clearly dived through the

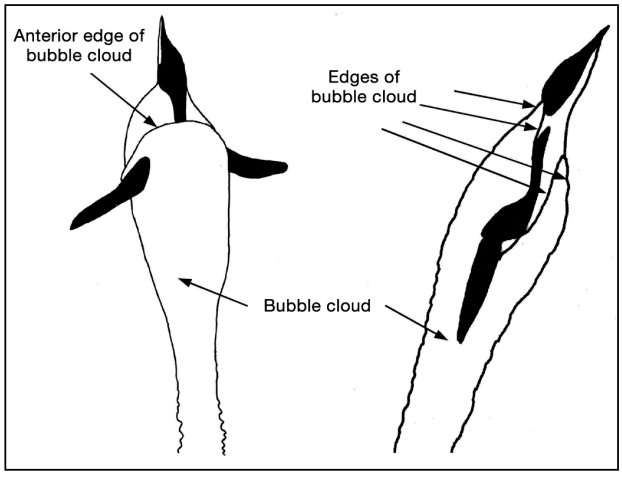

Fig. 2. *Aptenodytes forsteri*. Images of near-vertically ascending emperor penguins. Note that these drawings, drawn from sequential close-up fields (from BBC 2001) of 2 different penguins, demonstrate that the bubble clouds envelop most of the body and obscure the tail and hind limbs. Note also that the identified anterior edges of the bubble clouds correspond to areas where bright bubbles can be seen against the penguin surface. It is likely (given the close-up image shown in Fig. 3) that less visible bubbles emerge more anteriorly

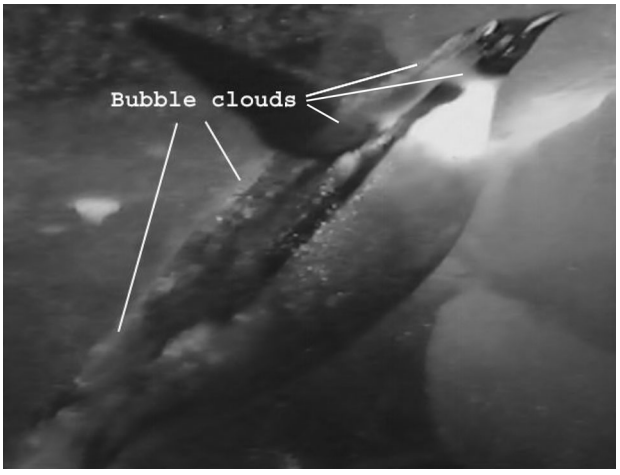

Fig. 3. *Aptenodytes forsteri*. Image of ascending emperor penguin about to break through water surface. Note fine bubbles emerging from throat plumage and waves in bubble cloud over nape. Note also the bubble cloud visible at the base of right flipper. Large bubbles are visible issuing from flank plumage. Note that no air is issuing from beak or nares. From BBC (2001), with permission

water surface from the ice shelf (of an unknown height) and trailed bubbles for several metres. One entered at a steep angle and briefly achieved 6.2 m s^{-1}, but had slowed to 1.9 m s^{-1} by the time the bubble trail ceased. The other penguin's track could not be analyzed, but the bird concerned entered the water at a shallower angle, soon converted to a very rapid horizontal movement that ended in a glide.

The only example of a long bubbly wake other than during an ascent was seen in a single example of a partially 'porpoising' penguin swimming horizontally. This was seen in the additional footage supplied by the BBC, but the cameraman panned the camera, so we could not analyze the footage (i.e. we could not estimate the speed, though it was clearly quite rapid). However, the penguin (which was not jumping entirely out of water, but following an undulating path during parts of which the dorsal section of the body was emersed), trailed bubbles throughout the sinusoidal swimming path, presumably because the plumage was loading with air each time the dorsum emerged from water. In dolphins and penguins, porpoising has been modelled as a method of intermittent locomotion whereby animals reduce their energetic expenditure at high speeds by capitalizing on short periods of unpowered movement through the air (Au & Weihs 1980). Weihs (2002) has recently revisited the topic of porpoising, but all of the emphasis has been upon reconciling the high energy cost of jumping out of water with the much reduced drag when in air. No-one has previously reported bubbly wakes during the underwater phases of porpoising in penguins, but they might conceivably be energetically beneficial.

Film analysis

Quantitative analysis of appropriate parts of the film, assuming a standard bird length (tip of beak to hindmost foot) of 1.25 m, showed swimming speeds during bubble trail ascents (n = 10 different penguins; all recorded when camera was still) as follows: range 3.8 to 6.1 m s^{-1}, mean 5.3 m s^{-1} (SD 1.01 m s^{-1}). The mean ascending speed corresponds to 4.3 body lengths s^{-1}. The mean final angle of ascent to the horizontal before jumping through the water surface (n = 6) was 60° (SD 8°). Swimming speeds of descending penguins (n = 10) were as follows: range 1.3 to 2.8 m s^{-1}, mean 1.9 m s^{-1} (SD 0.49 m s^{-1}). The mean descending speed corresponds to 1.5 body lengths s^{-1}. Mean angle of descent (n = 10) to the horizontal was 41° (SD 9°). Horizontal swimming speeds (n = 5) were: range 1.2 to 2.7 m s^{-1}, mean 1.7 m s^{-1} (SD 0.57 m s^{-1}). The mean horizontal speed corresponds to 1.4 body lengths s^{-1}. The variability of these data is similar to that observed by Kooyman et al. (1992), who used electro-mechanical data loggers to measure swimming speed. One-way analysis of variance (ANOVA) showed that there were highly significant differences amongst the ascending, descending and horizontal swimming speeds ($p < 0.0005$). Post hoc Tukey analysis showed that the descending and horizontal speeds were not significantly different from each other ($p > 0.05$), but that the penguins ascending with bubble trails travelled far more quickly, reaching a mean of 2.8 times the descending speed.

It was difficult to determine the depth at which bubble trail ascents started. No fixed camera sequences were available, nor was there a complete panned sequence from appearance of the wake to jumping through the sea surface. However, in 1 panned sequence, white wakes could be followed until the sequence ended about 3.2 m below the water surface. This sequence lasted 2.48 s, implying that the wakes started at a depth of about 16 m on the assumption that penguins moved vertically at 5.3 m s^{-1}.

In 4 jumping sequences, filmed above water at the ice edge on a different occasion and at a different location, it was possible to estimate the maximum height (of the approximate penguin centre of gravity) above the water surface achieved during jumps out of water as being 1.12 to 1.78 m. All heights substantially exceed those recorded by Sato et al. (2005) in emperor penguins jumping through ice holes. Given the mean emergence angle (β) of 60° and mean emergence velocity (V_0) of 5.3 m s^{-1} recorded in the present study, the maximal height (h_{max}) of the jumping trajectory, ignoring drag, is calculated from the equation of motion, giving $h_{max} = h_0 + V_0^2 \sin^2\alpha / 2g = 1.07$ m (where h_0 is assumed to be zero and g is the acceleration due to gravity), which agrees with observations of around 1 m for most jumping penguins. Jumps as high as 1.7 to 1.8 m agree well with a few observed high velocities, up to 8.2 m s^{-1}, just before completely leaving the water. It is likely that some acceleration occurs as the forepart of the body is in air, while the propulsive flippers are still acting against incompressible water (cf. flying fish; Davenport 1994).

Air release during ascent

Before jumping out of the water onto ice, the penguins swim at the surface and then dive on inspiration (Kooyman et al. 1971). We believe they dive with plenty of air in the plumage, with erected feathers making room for an air layer about 25 mm thick (following Du et al. 2007). Kooyman et al. (1971) described the grooming behaviour by which surface swimming emperor penguins load their plumage with air and we confirmed this by observation of parts of the un-

published BBC film. They subsequently dive to ~15 to 20 m (by which depth the air volume will have decreased by a substantial amount, see Eq. 3). During the dive, or when achieving that depth, they depress the feathers (to fix the plumage volume at the new, decreased level). When the birds swim quickly upwards, the decompressing air will flow out by virtue of the available fixed plumage volume being substantially less than the initial volume. Plumage consists of a fine, multi-layered mesh over the whole of the body surface comparable to a porous medium with an estimated pore size of <20 µm (Du et al. 2007), so the expanding air will automatically issue as small bubbles. This arrangement resembles the flat-plate experiments of Sanders et al. (2006), who used a 40 µm pore size sintered stainless steel strip for microbubble air injection. The 'active' part of the process consists solely of maintenance of depressed feathers during the near-vertical phase of the ascent in order to regulate expulsion of air driven by decompression. As bubbles continue to enter the boundary layer along the plumage, they are swept downstream and move outwards, thus increasing the void fraction in the boundary layer downstream to finally leave in the wake behind the bird; or they coalesce with other bubbles to form rather large bubbles at the outer edge of the boundary layer (see Fig. 3). It is likely that a large number of small bubbles may still remain within the boundary layer, as can be seen by calculating a typical turbulent boundary layer thickness δ in liquid flow at a distance, say x = 0.5 m downstream from the leading edge of a flat plate, estimated from Schlichting (1968, p. 599) as $\delta = 0.37(xV/\nu)^{-0.2} = 0.37 \times 0.5 \times (0.5 \times 5.3/10^{-6})^{-0.2} = 0.010$ m = 10 mm, increasing to 17 mm at x = 1.0 m. For this estimate, we have used a free stream velocity of V = 5.3 m s^{-1} and a kinematic viscosity of $\nu = 10^{-6}$ m^2 s^{-1}. Although the growth of the boundary layer on a body like that of a penguin is different from that of a flat plate, the order of magnitude of thickness is similar.

As an aid to understanding the strategy used by penguins during ascent, 2 alternative simple physical models have been examined for estimating the rate of air release during ascents. To this end, assume the volume of entrapped air can be represented by a layer of initial thickness s_0 of pure air at atmospheric pressure (i.e. an absolute pressure of ≈10 m water column). As long as there is no release, the thickness of air layer s varies with depth d below the free surface as:

$$s = s_0 (1 + d/10)^{-1} \quad (3)$$

so that at d_1 = 15 m, for example, we have 40% of the initial thickness: $s_1 = 0.4\ s_0$. Here we have used the ideal gas law, assuming isothermal conditions, so the product of absolute pressure and volume (or thickness s over a fixed area) remains constant. In reality, conditions may not be isothermal as weather-dependent Antarctic air temperatures can be significantly lower or higher than that of sea water in thermal equilibrium with sea ice (–1.9°C). However, even a 25°C difference (probably the maximum likely) will have relatively small effects on entrapped air volume, so no attempt has been made to take this into account. Also, we can safely ignore the varying static pressure associated with the change of free stream velocity along the surface of the penguin. At the front stagnation point, the pressure is higher than the local hydrostatic pressure (by 1.4 m water column at an onset flow of 5.3 m s^{-1}) while it is lower (by an estimated 0.7 m water column) near the head and (by no more than 0.3 m water column) over the rest of the body. In terms of hydrostatic pressure change with depth, the variations mentioned are comparable with the variation over length of a vertically oriented penguin. One may now consider 2 strategies: (1) the thickness of air layer in the plumage remains constant at the value s_1 during ascent while air is released due to decompression according to the isothermal volume increase of air with decreasing depth; or (2) the thickness s of the air layer decreases during ascent in a controlled way (by decompression and depression of feathers) so as to maintain a constant rate of air outflow per unit area (a velocity denoted u) at any depth. Model (1) would imply that most of the mass of entrapped air is expended at great depths, leaving little as the surface is approached, so that the bubbly wake should diminish with decreasing depth. Since observations show all bubbly wakes to be of unchanged strength during observable ascents, we favour model (2).

In this case, the air-outflow velocity u is maintained constant by the combined action of decompression and depression of feathers such that the thickness of the generated bubble layer (and ensuing drag reduction) should be unchanged during the ascent. Here the depression of feathers may help overcome the pressure drop associated with the flow of air through the fine mesh of feathers. The resulting drag reduction is assumed to depend only on the volume of air bubbles formed, not the air pressure, which varies with depth. We now calculate how the air-layer thickness s of entrapped air varies during vertical ascent with constant velocity V, starting at time t = 0 with the value s_1 at depth d_1 as before. Without air release, s varies as given by Eq. (3), where $d = d_1 - Vt$, but with constant air release u it becomes:

$$s = s_0 [1 + (d_1 - Vt)/10]^{-1} - ut \quad (4)$$

Introducing the time of ascent $t_a = d_1/V$ and $t_a u = ks_0$, where k denotes the fraction of initially entrapped air that has been used up when arriving at the sea surface, Eq. (4) becomes:

$$s/s_0 = [1 + (d_1/10)\ (1 - t/t_a)]^{-1} - k\,t/t_a \quad (5a)$$

or in terms of s/s_0 versus depth d:

$$s/s_0 = (1 + d/10)^{-1} - k\ (1 - d/d_1) \quad (5b)$$

Fig. 4 shows how the air-layer thickness decreases with depth during ascent from initial depths of d_1 = 10, 12, 15 and 20 m according to Eq. (5) for k = 1, which corresponds to all air being used up. Only positive values of s/s_0 have physical meaning, so the maximal feasible initial depth is about 10 m if air release were to continue until the free surface has been reached. Starting at a depth of 15 m, however, air release would terminate at a depth of about 5 m. It is clearly costly to start releasing air at great depths because, for a given layer thickness, relatively more mass of air is expended due to its compression. However, starting from greater depths than 10 m would be possible by the use of alternative strategies, such as intermittent release spatially along body and/or in time, or by reducing u through values of $k < 1$. Fig. 5 shows, for d_1 = 15 m, how decreasing the value of k ensures that s remains positive during full ascent, but then not all of the available air becomes useful. Considering a reference case (k = 0.9, L = 1 m, s_0 = 25 mm and d_1= 15 m), the air-outflow velocity becomes $u = ks_0V/d_1$ = 0.008 m s^{-1} and 90 % of the available air becomes useful. By comparison, it can be shown that only 60 % of air is useful in the case of model (1).

Thickness of bubble boundary layer

Next, with a few more assumptions, it is possible to estimate the thickness δ of the released air layer (evidently the air appears as bubbles, but for conservation of mass it is simpler to think in terms of a layer of pure air, which later may be interpreted as a bubble layer of some void fraction). When air is released at the rate u along a section of length L of a cylindrical body, δ would increase with distance x from the upstream point (x = 0) as given by the equation of continuity, $d(V_{BL}\delta)/dx = u$, subject to the boundary condition $\delta(0) = 0$, where V_{BL} denotes a representative velocity of the air in the boundary layer. Taking V_{BL} to be one-half of the constant swimming velocity, $V_{BL} = \frac{1}{2}\ V$, the linear increase of δ would give a mean value over length L of $\delta_{mean} = \frac{1}{2}Lu/(\frac{1}{2}V) = Lu/V$.

For our model, δ_{mean} would be constant during ascent and (using $t_a u = ks_0$ and $t_a = d_1/V$), given by $\delta_{mean} = kLs_0/d_1$. For the reference case (k = 0.9, L = 1 m, s_0 = 25 mm and d_1= 15 m) this gives δ_{mean} = 1.5 mm and a mean bubble layer (at 10 % void) of 15 mm, increasing to 30 mm at the tail end, during the whole period of ascent. Due to the body shape of a penguin, the free stream velocity will be somewhat higher than the swimming velocity, but aside from the head region (where local high velocities are incurred), not by more than 5 to 6 %, which would imply a slightly thinner bubble layer. On the other hand, bubbles probably move at velocities less than the assumed $\frac{1}{2}V$ and thus tend to lead to a thicker bubble layer.

Measurements made with Motion Analysis Tools from a close-up frame of a penguin near to the sea surface suggest fine bubble layers of thickness of ~20 mm, at locations 0.28 and 0.68 m from the tip of beak in the dorsal region (observations could not be collected from the ventral region). However, because the bird was travelling at around 60° to the horizontal at this time, it is likely that the tendency of bubbles to rise will have led to greater thicknesses of bubbles being evident in the dorsal than the ventral areas, so it is probable that 20 mm is an overestimate. This (distinctly limited) observation nevertheless shows an order of magnitude agreement with the model results.

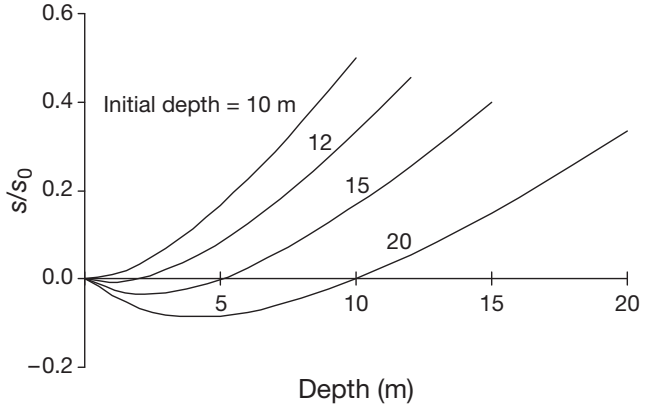

Fig. 4. *Aptenodytes forsteri.* Calculated relative air layer thickness (s/s_0) versus depth (d, in m) during ascent with constant velocity (V = 5.3 m s^{-1}) and constant air release and all entrapped air used up (k = 1), showing effect of increasing initial depth

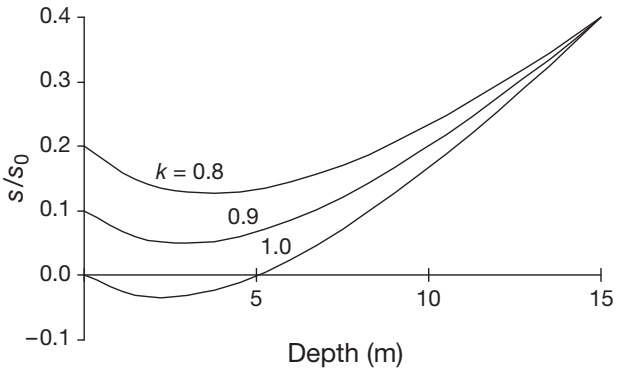

Fig. 5. *Aptenodytes forsteri.* Calculated relative air layer thickness (s/s_0) versus depth (d, in m) during ascent from d_1 = 15 m with constant velocity (V = 5.3 m s^{-1}) and constant air release, showing effect of decreasing k

Propulsive force and power

At a steady, horizontal swimming velocity V (i.e. free stream velocity), the propulsive force F_P equals the drag force:

$$F_d = C_d A \tfrac{1}{2} \rho V^2 \quad (6)$$

where C_d denotes the drag coefficient, A a characteristic area of the body and ρ the density of water, and the expended power is:

$$P = F_P V = C_d A \tfrac{1}{2} \rho V^3 \quad (7)$$

To attain a swimming velocity of about 5.3 m s^{-1}, a factor of 5.3/2 = 2.65 times the normal cruising speed of 2 m s^{-1}, would imply increases in propulsive force and power by factors $2.65^2 = 7.02$ and $2.65^3 = 18.6$, respectively, assuming an unchanged C_d. Such increases are unlikely, even for the short duration (~3 s) of ascent. However, if bubble release from the plumage causes a reduction of the product $C_d\rho$ by a factor of 18.6 (i.e. to about 5.4% of the single-phase liquid flow drag), the expended power would be unchanged from that at the normal cruising speed of 2 m s^{-1}, and the required propulsive force would be correspondingly reduced. However, it is likely that, during ascents, penguins expend more power and are aided by buoyancy, so that less drag reduction would be required to achieve the observed high speeds.

The total drag on a streamlined body such as a penguin is the sum of frictional drag in the boundary layer along the surface and form drag associated with the pressure distribution around the body. Form drag may constitute as much as 20% or more of the total drag (Schlichting 1968, Figs. 25.4 and 25.5 for a streamlined body of length to diameter ratio of 4), so even if skin friction were reduced to a negligible amount due to bubbles in the boundary layer and/or coalescence of bubbles to form patches of air film along the plumage surface, there would still remain a sizable contribution from form drag, unless this was also affected by air release.

To examine this problem we consider the classical analysis used in calculating total drag on a body from experimental data of wake measurements (e.g. Schlichting 1968, p. 166 therein). Fig. 6 shows a cylindrical control volume (dashed outline) surrounding the body subject to an incoming flow of uniform velocity V_1 and liquid density ρ over area A_1, leaving the body partly along the cylindrical side of area A_3 (to satisfy continuity) and partly downstream over area A_2 with reduced velocity and density in the wake (stippled area) due to the air release $Q_a = uA_p$ of density ρ_a. Assuming the control volume surface to be far enough from the body that pressure is uniform, the conservation of mass and balance of momentum become:

$$\rho Q_3 = \rho_a Q_a + \int \rho V_1 dA_1 - \int \rho_2 V_2 dA_2,$$

and:

$$F_d = \int \rho V_1^2 dA_1 - \int \rho_2 V_2^2 dA_2 - \rho Q_3 V_1$$

or, after elimination of Q_3 between these equations

$$F_d = \int \rho(1-\alpha_2) V_2 (V_1 - V_2) dA_2 - \rho_a Q_a V_1 \quad (8)$$

where the mixture density has been approximated by $\rho_2 = \rho_a \alpha_2 + \rho(1-\alpha_2) \approx \rho(1-\alpha_2)$, and where void fraction α_2 varies across the area A_2.

Although total drag can only be evaluated from Eq. (8) if detailed data from wake measurements are available, this equation suggests that both skin friction and form drag are affected by air release. The difference between the cases of air release with bubbly flow wake and no air release with pure liquid flow ($\alpha_2 = 0$ and $Q_a = 0$) is to be found in the distribution over A_2 of velocity V_2 and void fraction α_2 since the last term in Eq. (8) is negligible. Increasing air release will increase α_2 and increase V_2 as bubbles in the wake are being accelerated by the liquid flow, both contributions that will reduce F_d as compared to the case of no air release hence increase the drag reduction.

Required drag reduction

To determine the required drag reduction to achieve the observed high ascent speeds, we estimate the magnitude of the buoyancy force, the propulsive force and propulsive power. During normal cruising manoeuvres near the surface, penguins appear to be only slightly positively buoyant, judging by the slow rise of penguins not flapping their flippers. They are evidently quite close to neutral buoyancy.

However, prior to dive and subsequent ascent to jump, we assume the penguin fills its plumage with air

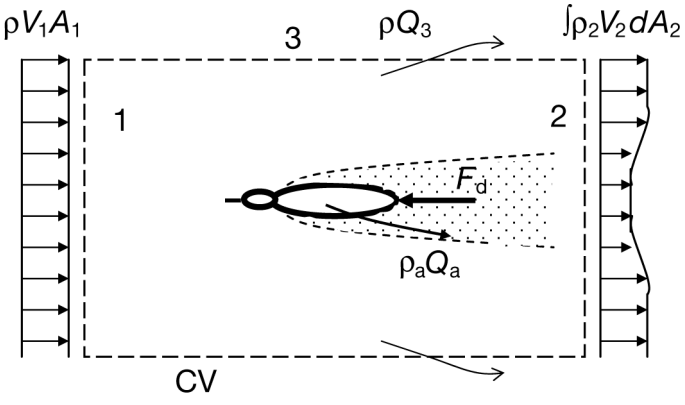

Fig. 6. *Aptenodytes forsteri.* Control volume (CV) around body to express drag force F_d in terms of momentum change of inflow (1) to outflows (2) and for (3) body with air release Q_a generating the bubbly boundary layer and wake (stippled). See 'Results: Propulsive force and power' for further explanation

at the sea surface and inspires to fill its respiratory system with air. Sato et al. (2002) state that king and Adélie penguins always dive on inspiration; there is no reason to believe that emperor penguins differ in this respect. As in the previous analysis, the air-layer thickness is taken to be s_0 = 25 mm over surface area A = 0.6 m^2, i.e. an air volume of 15 l, and the air volume of the respiratory system is taken to be at most 25 × 0.1 = 2.5 l for a 25 kg emperor penguin at an estimated 100 to 200 ml kg^{-1} according to Sato et al. (2002, Table 2 therein for king and Adélie penguins). Denoting by g the acceleration of gravity, the associated buoyancy force at the sea surface is:

$$F_b = (\rho - \rho_a) \times Vol_{air} \times g \approx 1000 \times (0.015 + 0.0025) \times 9.81 = 172 \text{ N} \quad (9)$$

This significant force corresponds to about 70% of the weight. As the penguin dives to depth d, the air is compressed so the volume of air and hence the buoyancy force F_b, decreases as s according to Eq. (3):

$$s = s_0 \, (1 + d/10)^{-1} \quad (3)$$

The air density ρ_a increases inversely with respect to s according to the ideal gas law for isothermal conditions, but the approximation $\rho \gg \rho_a$ is still reasonable for the depths in question. At depth d_1 = 15 m, for example, we have 40% of the initial thickness, $s_1 = 0.4\ s_0$ and a buoyancy force of about $F_{b,1}$ = 69 N if no air has been released.

First, assuming no air release during ascent (the hypothetical case of a fast ascending penguin not showing bubbly trails) the effect of buoyancy on attainable speed can be evaluated as follows. For steady, horizontal swimming the propulsive force F_P and propulsive power P_P may be evaluated at normal cruising speed by using the established typical values of drag coefficient of C_d = 0.02 to 0.04 (Hirata & Kawai 2001) based on surface (wetted) area for streamlined bodies of revolution. The lowest drag occurs at a length to diameter ratio of about 4.5, which is close to that of the emperor penguin (about 3.4). Hence at V_0 = 2 m s^{-1},

$$F_{P,0} = F_{d,0} = 0.02 \times 0.6 \times \tfrac{1}{2} \times 1000 \times 2^2 = 24 \text{ N} \quad (10)$$

$$P_{P,0} = F_{P,0} V_0 = 48 \text{ W} \quad (11)$$

The estimate of C_d = 0.02 is assumed to include frictional drag as well as form drag and induced drag. Due to observed flipper action, we assume the propulsive power to be at least the same during ascent as during cruising, except that there will now be an additional buoyancy-driven propulsive power, $P_b = F_b V$, where F_b is the buoyancy force (assuming a vertical ascent). For a trajectory forming the angle θ with the vertical it will be smaller by the factor cos θ.

Equating total propulsive power P to drag at the new velocity V_1 at depth d_1 gives:

$$P = P_b + P_{P,0} = F_{b,1} V_1 + P_{P,0} = C_d A \tfrac{1}{2} \rho V_1^3 \quad (12)$$

Using $F_{b,1}$ = 69 N and $P_{P,0}$ from Eq. (9) in (12) gives V_1 = 3.70 m s^{-1}at depth d_1 = 15 m. Similarly, using $F_{b,0}$ = 172 gives V_0 = 5.49 m s^{-1} at depth d_0 = 0 m.

Within the assumptions made, we conclude that buoyancy could theoretically help to increase the velocity during ascent from about 3.8 m s^{-1} at depth 15 m to about 5.5 m s^{-1} when the free surface is reached, but only if all air remained within the plumage throughout the ascent (which it clearly does not). However, this is still less than the highest observed emergence speeds (8 m s^{-1}). We may therefore again conclude that drag reduction due to the release of air bubbles must be involved in the real situation.

Second, for the actual case of air release from the plumage during ascent with an estimated constant air-outflow velocity u = 0.008 m s^{-1}, optimally the air layer thickness would then decrease from 40% of the initial thickness at depth d_1 = 15 m to about 10% as the free surface is reached (Fig. 5, case of k = 0.9). It follows that the buoyancy force would decrease from 69 N to about 39.2 N during the ascent (still assuming 2.5 l air in the respiratory system). For unchanged propulsive power, again using Eq. (12), the maximal attainable velocity would decrease rather than increase during ascent, from 3.70 to 3.03 m s^{-1}. To reach the observed average velocity of 5.3 m s^{-1} would require an increase in propulsive power from 48 W to 685 W, a factor of more than 14 times the power for the normal swimming velocity of 2 m s^{-1}. Although buoyancy plays a non-negligible role, its effects are insufficient to explain the observed velocities, and therefore there must be a substantial contribution from drag reduction due to air release during ascent to achieve the observed velocities of the order of 5.3 m s^{-1} or more.

The required drag reduction to attain the observed velocities of ascent can be evaluated as follows. For the actual case of 2.5 l air in the respiratory system and air release, leaving air layers of 40% and 10% of the initial thickness s_0, corresponding to depth d_1 = 15 m and near the surface, respectively, we set V_1 = 5.3 m s^{-1} in Eq. (12) and calculate the required reduced value of drag coefficient $C_{d,r}$ to obtain the value of required reduced drag ratio as $C_{d,r}/C_d$. Results for the estimated normal propulsive power of 48 W and twice this value are shown in Table 1. These results show that not much is gained by doubling the propulsion power and that more than 70% drag reduction is needed for the considered average velocity of 5.3 m s^{-1}, and considerably more for the higher velocities observed. Some approximate (unpublished) calculations of a bubble boundary layer on a flat plate (validated against experimental data of Madavan et al. 1985) have shown that a uniform air release of u = 0.008 m s^{-1} can provide no

Table 1. *Aptenodytes forsteri.* Drag reduction required to achieve the observed mean velocity of 5.3 m s^{-1} at depth 15 m (relative air-layer thickness, s/s_0 = 0.40) and near the surface (s/s_0 = 0.10) at (A) normal power and (B) twice normal power. $C_{d,r}$ is the required reduced drag coefficient; C_d (= 0.02) that of no drag reduction.

Propulsive power	Air-layer s/s_0 (%)	$C_{d,r}/C_d$	Drag reduction (%)
A) 48 W	40	0.463	54
	10	0.286	71
B) 96 W	40	0.517	48
	10	0.340	66

more than about 14% reduction of the frictional drag, which suggests that coalescence of bubbles to form patches of air film and/or a reduction of form drag are likely to account for the remaining reduction.

Cavitation

It might be suggested that bubble formation around the penguin is a result of cavitation. To achieve cavitation, the local static pressure should decrease to values at or below the vapour pressure of water at the prevailing temperature, $p_{sat}(T)$. For pure water, p_{sat} = 0.611 kPa at 0.01°C, but is slightly lower for seawater. Assuming normal atmospheric pressure at sea level (101.3 kPa) the static pressure at a depth of 1 m, for example, would be higher by 9.81 kPa, so the pressure lowering required to achieve cavitation is of the order of $\Delta p_c \approx 110$ kPa. For a blunt body moving through water at velocity V_S, the lowest pressure occurs near the location of greatest diameter, where the highest velocity V_C is attained. The pressure lowering at this point may be calculated from the Bernoulli equation, which is valid outside the viscous boundary layer:

$$\Delta p_c = \tfrac{1}{2}\rho V_S^2 \left[(V_C/V_S)^2 - 1\right] \quad (13)$$

We may use Eq. (13) to calculate the minimal swimming velocity V_S that gives cavitation once the pressure lowering Δp_c and the shape factor $[(V_C/V_S)^2 - 1]$ have been specified. Sample values of the latter from potential flow theory are: 3 for cylinder in cross flow, 1.25 for flow past sphere, 0.37 for flow past streamline body of revolution (18% thickness), and 0.44 for flow past a model of an approximate, axisymmetric penguin, where the 3 first values are from Schlichting (1968, p. 21–22), while the fourth value was calculated numerically from a distribution of singularities giving an axisymmetric body of approximately the shape of an emperor penguin. Using a conservative value of 0.5 gives $V_S = [2 \times 110\,000/(1000 \times 0.5)]^{1/2} = 21$ m s^{-1}.

This example shows that it is highly unlikely that cavitation could occur, given that the mean ascent swimming velocity inferred from the video records is about V_S = 5.3 m s^{-1} and that the highest values do not exceed about 8 m s^{-1}. Also, bubbles are observed at depths exceeding 1 m, and a non-gaseous cavitation bubble would quickly collapse once it had moved to positions where the pressure exceeds that of cavitation. The observation of a wake filled with bubbles far behind the penguin is proof of bubbles being filled with gas and not by water vapour.

DISCUSSION

Our recorded descent and horizontal speeds (and their variability) for emperor penguins closely agree with previously published data, giving confidence in our extracted ascent speeds. Cruising speed has been estimated at about 2 m s^{-1} (Culik et al.1994, Wilson 1995), which is similar to our observed descending and horizontal speeds (1.9 m s^{-1} and 1.7 m s^{-1} respectively). Kooyman et al. (1992) recorded 2.8 m s^{-1} from penguins swimming horizontally beneath solid ice between air holes—which constrained situation may have stimulated slightly elevated swimming speeds; Sato et al. (2005) have more recently recorded 1.7 m s^{-1}. Given the fact that none of the filmed material inspected in our study, collected from hundreds of penguins, showed animals moving upwards at high speed without bubble trails, we strongly suspect that our measured upward speeds (mean 5.3 m s^{-1}) represent the maximum speeds of which emperor penguins are capable. Our estimated speeds are certainly the highest recorded in scientific studies. Compared with a penguin cruising speed of 2 m s^{-1}, drag would be increased about 5.8-fold at the observed mean ascent speed of 5.3 m s^{-1}, given no mechanism to reduce it (Eq. 2). Clearly, drag reduction will be advantageous provided that the energetic cost of doing so is not prohibitive. Our observations and analysis unequivocally demonstrate that emperor penguins ascending rapidly in the water column to jump onto ice shelves emit bubble clouds into the turbulent boundary layer over most of the body surface throughout their ascent. Emission does not diminish as a penguin approaches the surface, but increases. Because the bubbles are produced over most of the body surface, their drag-reducing function should exceed the performance of marine engineering plate/ship models described so far, in which maintaining sufficient bubble coverage within the turbulent boundary layer is a major problem. Moreover, penguin plumage is water-repellent (due to application of preen oil), so it is feasible that thin air films may form over the feather surfaces, as shown for

water-repellent paints by Fukuda et al. (2000), promoting drag-reduction still further.

Penguin plumage can contain considerable quantities of air (Yoda & Ropert-Coudert 2004). Recent calculations suggest that as much as 96% of plumage volume is occupied by air (Du et al. 2007), and during a dive the volume of trapped air will decrease according to Eq. (3), whence shrinkage decelerates with increasing depth. At a depth of 15 m, air in the plumage will be compressed to 40% of its initial volume (and to 33% at 20 m). We believe that emperor penguins essentially 'lock' the reduced plumage air volume at a depth of 15 to 20 m. When they swim rapidly towards the surface, from about 60% (strategy 1) to 90% (strategy 2) of the initial volume is available to diffuse out through the fine plumage meshwork in the form of small bubbles that progressively coalesce along the body surface as the penguins ascend. At present we favour strategy (2) because of the observed persistence of bubbly wake formation right to the surface. Because of the characteristics of the depth:volume relationship, the expansion rate of the trapped air will be greater as the penguin approaches the sea surface (Fig. 4), thus maintaining release of air, even though the mass of trapped air is decreasing. Our model of compressed air storage is supported by observations of penguins that abort their ascent; on aborting, a penguin re-dives and bubbles soon stop issuing from the plumage, so the penguin and its 'track' become separated as the air in the plumage is repressurized. We do not know whether penguins that have aborted dives need to return to the surface to recharge the plumage with air, or still retain enough plumage air to try jumping again. Loading the plumage with air will increase penguin buoyancy, thus imposing an additional energetic cost as the birds swim downwards from the surface. The buoyancy force decreases approximately by a factor of 2.5 when diving from the free surface to a depth of 15 m, so opposing buoyancy becomes easier as the penguins dive. On the other hand, our calculations indicate that buoyancy force, though non-negligible, can play only a small part in enhancing ascent speed.

For the proposed mechanism of air lubrication to work, emperor penguins need to have considerable control over their plumage. There is good evidence that this control exists. Penguin plumage is unlike that of other birds. First, feathers are present over the entire body surface rather than being present in tracts as in most other bird species (Stettenheim 2000). Secondly, each feather has 2 parts, an anterior flattened, pennaceous part that provides the smooth, waterproof (and water-repellent) outer coating of the penguin body surface, and a posterior down-like after-feather that provides insulation (Dawson et al. 1999). Erection and depression of the pennaceous part are both under muscular control (Kooyman et al. 1976; Dawson et al. 1999). On long foraging dives, it is believed that emperor penguins compress their plumage to expel air, thereby reducing drag (Kooyman et al. 1976) and the positive buoyancy that is undesirable in diving birds (Wilson et al. 1992). Hence 'locking' of a fixed volume of air by muscle action is entirely feasible. Fast water flow will also help to flatten the pennaceous part, squashing the after-feather beneath, in turn helping to keep air volume steady during ascents. Positive control by feather depression may play a part in forcing out air during the ascent, as suggested by strategy (2).

How much does air lubrication enhance speed in fast ascents? This question cannot be answered with precision from our observations since all penguins produced bubble clouds when ascending (i.e. none was without the air lubrication, so there were no 'controls'). Though the values for ascent speeds recorded in our study considerably exceed the accepted cruising speed (~2 m s^{-1}) for all penguin species (Culik et al.1994, Wilson 1995), some of the extra swimming speed will undoubtedly be due to enhanced flipper action (by some combination of increased flipper beat frequency or increased angle of incidence of flipper to water flow direction) underpinned by anaerobic 'sprint' muscle action. Interestingly, our ascent speed values (mean 5.3 m s^{-1}, but occasionally as high as 8.2 m s^{-1}) are much higher (by about 90%) than those recorded (2.8 m s^{-1}) in a recent study of emperor penguins jumping to far more modest heights (<0.45 m) through ice holes 1.2 m in diameter (Sato et al. 2005). Sato et al. (2005) do not mention the occurrence of bubbly wakes, and 2.8 m s^{-1} is identical with the horizontal under-ice speeds reported earlier by Kooyman et al. (1992). Given the variability of the observed ascent and emergence speeds, it is clear that emperor penguins can modulate speed and emergence angle considerably, as do Adélie penguins (Yoda & Ropert-Coudert 2004).

The lack of 'controls' for the observed bubbly wake ascents means that our air-lubrication hypothesis for attainment of maximal emperor penguin speeds can only be considered as highly viable at this stage. The only method of confirming the hypothesis fully would seem to involve the construction and testing of a penguin replica that could be towed whilst emitting bubbles. This would be a technically difficult task as the complexity of penguin plumage would be difficult to replicate in a man-made porous membrane or mesh. However, this approach would appear to be more fruitful than any attempt (probably unethical) to constrain emperor penguins to ascend rapidly without air emission.

Our study only considers the emperor penguin. Since the plumage structure and control are similar in all penguin species (Dawson et al. 1999), the air lubrica-

tion ascent-adaptation may be more general amongst the family Spheniscidae. Adélie penguins in particular may repay investigation as they leap to heights of 2 to 3 m above sea level (Yoda & Ropert-Coudert 2004), yet cruise at 2 m s^{-1} (Sato et al. 2002).

Throughout our study we have assumed that the adaptive value of air lubrication lies in enhanced swimming speed and hence more effective jumps out of water. There may be additional benefits; it has recently been reported that air lubrication reduces the acoustic signal of ships (Matveev 2005). If this also applies to ascending emperor penguins, it may make them less detectable by predators that hunt by echolocation (e.g. killer whales).

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Site fidelity, homing and spawning migrations of flounder *Platichthys flesus* in the Tamar estuary, South West England

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ABSTRACT: Brand-marked flounder *Platichthys flesus* (L.) were used to study migrations, site fidelity and homing ability of individuals in the Tamar estuary, SW England, between 1976 and 1980. A total of 1308 recaptures were made, of 7401 flounder marked at 13 stations along a 29 km length of the estuary and 2 stations in Plymouth Sound. A further 1667 fish were marked on the spawning grounds in 1976, 57 being recaptured. Recaptured fish were released again, with individuals being recaptured on up to 6 occasions. In all but 118 cases, the recaptures within the estuary or Plymouth Sound were from the original capture sites, despite the displacement of 681 fish, after marking, to a variety of locations. Most flounder in the middle estuary did not move >200 m along the estuary until they left to spawn. Flounder in the upper estuary also showed high site fidelity, but were temporarily displaced by adverse conditions such as river spates. Ripe, estuary-marked flounder were recaptured at sea from 10 to 35 km west of Plymouth in water depths of 35 to 55 m. Most individuals returned to their original estuarine range after spawning. Twelve percent failed to return to the Tamar post-spawning, all migrating eastwards. A total of 200 Tamar fish were released 200 km eastwards along the coast. Many of these migrated towards Plymouth, 2 reaching the estuary, although some returned to the release site post-spawning. The results are applicable to the management of flounder stocks and of estuaries, emphasising the value of retaining intertidal mud flats in estuary development plans.

KEY WORDS: *Platichthys flesus* · Flounder · Site fidelity · Tamar estuary · Marking · Migration · English Channel

INTRODUCTION

The flounder *Platichthys flesus* (L.) is a sea-spawning flatfish that is found along European coasts from the White Sea to the Mediterranean and Black Seas and is fished commercially (Norman 1934, Wheeler 1978, Maitland & Herdson 2009). It penetrates further into fresh water in cooler northern waters (Maitland & Herdson 2009). Flounders in southern England and on the eastern side of the North Sea spend most of their lives in estuaries, penetrating into rivers where there are no barriers to migration (Wheeler 1979, Beaumont & Mann 1984, Kerstan 1991, Hutchinson & Hawkins 1993, Bos 1999). O-Group fish enter these estuaries and rivers as metamorphosing or newly metamorphosed post-larvae during April to May (Möller & Dieckwisch 1991, van der Veer et al. 1991, Hutchinson & Hawkins 1993).

In the Plymouth area, flounders typically leave the estuaries in late January to February (Dando & Ling 1980, Sims et al. 2004), returning subsequently to the estuaries. Metamorphosing young enter the estuaries in April and rapidly ascend to the freshwater zone (Dando 1984). Sims et al. (2004) found that the length of the spawning season is 28.1 ± 19.8 d (mean ± SD). Thus, most adult flounder are confined to the estuaries for ~11 mo of the year in this area.

There have been few studies on the movements of individual *Platichthys flesus* over a prolonged period. Tagging studies have shown the movement of flounder

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from the estuaries to the sea in the winter, prior to spawning, with a return to the estuaries and rivers post-spawning (Hartley 1947, Summers 1979). Acoustic tag studies on a few individuals in the River Bann, Northern Ireland, showed that the fish moved <400 m over a 12 h study period (Wirjoatmodjo & Pitcher 1984). In the Tamar estuary (Fig. 1), which enters the English Channel at Plymouth, migrations of the flounder population were first studied by Hartley (1947). He marked 1039 fish from the Tamar and Lynher estuaries with Petersen discs during the winter of 1937/1938. Of the 155 recoveries obtained, 4 were from the sea.

Early studies on the genetics of flounder in the Tamar suggested that there might be a seasonal difference in allele ratios between fish in different areas of the estuary (Marine Biological Association of the United Kingdom 1973). Although sampling in later years did not replicate this, a marking study was started in January 1976 to investigate whether there was a segregation of flounder within the 30.5 km long Tamar estuary and on the spawning grounds. The results from this marking study, to test the hypothesis that individual flounder have a limited home range within the estuary, are presented here. An account of the differences in population sizes, feeding and growth rates of flounder in different regions of the estuary will be published separately.

MATERIALS AND METHODS

Study area and fishing grounds. The Tamar estuary is 30.5 km long, measured along the mid-line of the main channel shown on 1:10 560 Ordnance Survey maps, from its mouth, at Plymouth Sound, to the weir at Gunnislake (Fig. 1c). Trawling stations along the Tamar are designated by T followed by a number representing the approximate distance in kilometres from the mouth, similarly along the Lynher, by L and a number. Stations in Plymouth Sound were similarly designated with P (Fig. 1b). Positions along the Tamar were determined from bearings to landmarks shown on the maps.

The lower 6 km of the estuary, the Hamoaze, receives water from 3 major rivers (Tamar, Lynher and Tavy). The

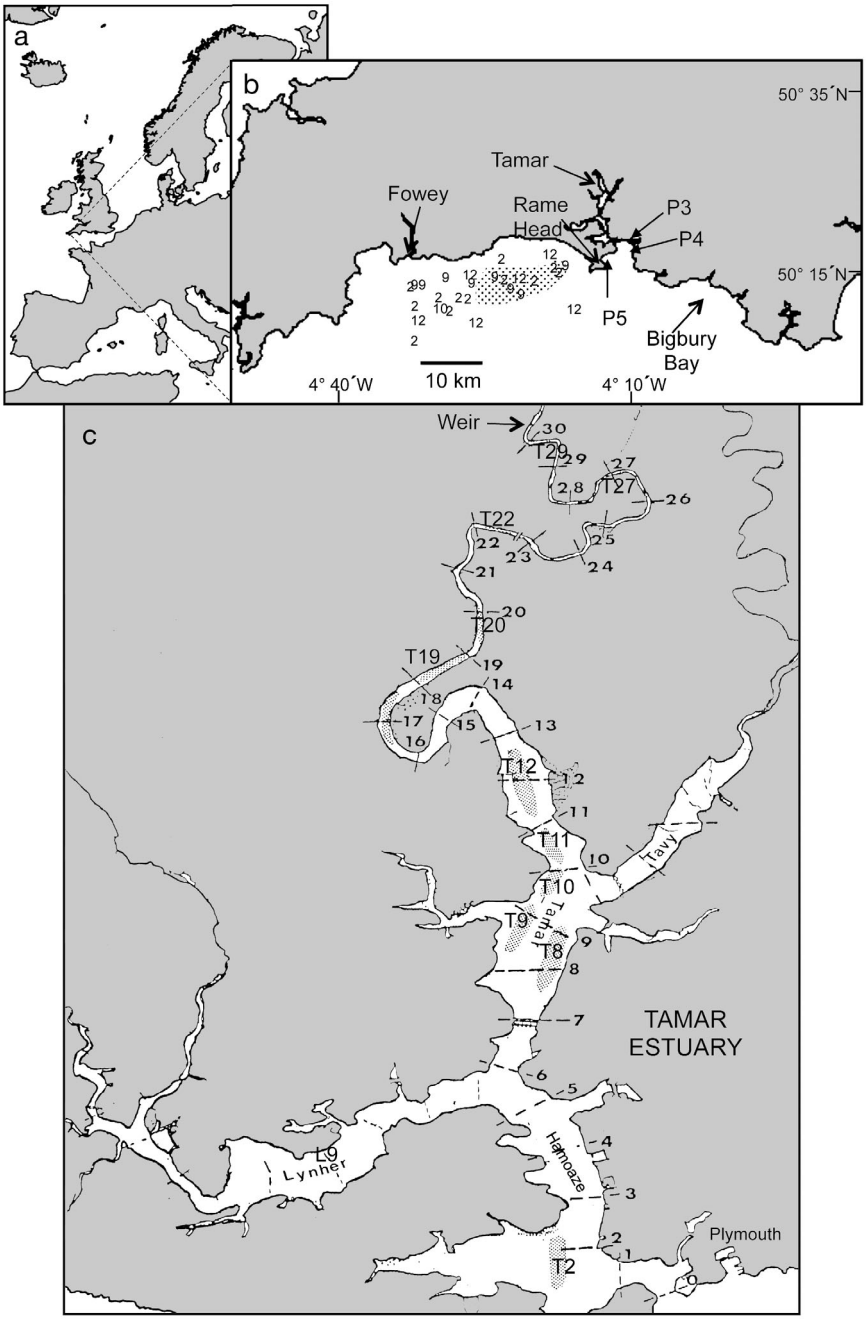

Fig. 1. Tamar estuary: (a) location in Europe; (b) positions of marking and release stations in Plymouth Sound, and the capture and release area for flounder *Platichthys flesus* marked on the spawning grounds (shaded), as well as the positions in which estuary-marked fish were caught during the spawning season; numbers refer to the estuary stations where the fish were marked; (c) the Tamar estuary system with distances in kilometres along the main channel from the mouth (marked by dashed lines). Trawling stations are indicated by T (Tamar) or L (Lynher) followed by the approximate distance from the estuary mouth in kilometres; the approximate areas trawled are shaded. This figure was reproduced in part from ordnance survey map data by permission of the Ordnance Survey (Crown copyright 2010)

Naval Dockyard and moorings made the lower 7.5 km unsuitable for trawling, and the only trawling station in this section, T2, was off a large tidal inlet, 2 km from the mouth (Fig. 1c). Detailed descriptions of the estuary and the fauna are given by Percival (1929) and Hartley & Spooner (1938). The middle reach of the estuary, 7 to 15 km from the mouth, is characterised by extensive intertidal mud flats and some long tidal creeks, or 'lakes'. On the Tamar itself, the lowest trawling stations were T8 and T9. Stn T9 was separated by a rock outcrop and a distance of 200 m from T10. There was another 200 m separating Stn T10 from T11. From Stn T12 onwards, all the trawling stations ran approximately along the mid-line of the channel. Flounder cannot penetrate above the weir. Above Stn T20, trawling was mainly undertaken on high-water spring (HWS) tides. Above 15 km, the narrow nature of the estuary means that river spates frequently wash all fish out of the upper part of the estuary and, occasionally, as on 19 January 1977, hardly any fish were present above T11 due to the high river flow.

The narrow channel and shallow depth of the Tavy prevented trawling, although flounder penetrate to the tidal limit, 15 km from Plymouth Sound. Flounder movements in the Lynher were studied extensively by Hartley (1940, 1947) and were not further investigated, beyond 1 displacement of fish into the Lynher in a study of homing ability.

Other fishing activities in the estuary were low during the present study. Salmon seines were used, in season, between 12 and 22 km along the Tamar and in the Tavy estuary, and raised nets were used from flatboats (Hartley 1940) in the upper Lynher estuary. Some angling occurred, particularly at Stns P3, T8 and T16, and there was occasional fishing by stop-net in creeks off T9 and in the Tavy.

Fishing methods and marking. Flounder were captured using an otter trawl or 3 or 3.7 m beam trawls, fitted with 55 mm mesh nylon cod-ends, using a single tickler chain and tow times of <15 min. Tows were normally made within 2 h of low water at the lower and mid-estuary stations (T2 to T12) and within 2 h of high water at stations further up the estuary, where it was both narrower and shallower. Bottom and surface temperature and salinity measurements were made with an E. I. L. Model MC5 temperature/salinity meter. Water flow rates over Gunnilake Weir (30.5 km up-estuary) were provided by the Cornwall River Authority.

The total length, sex and state of maturity of the gonad, when it could be located by feel, or by shining a strong light through the fish, were recorded before marking the fish by freeze-branding (Dando & Ling 1980). Flounder with a total length of ≥130 mm were used to provide sufficient space for the brands, although a few fish of 90 to 130 mm were marked with smaller brands. Freeze-branding had an advantage over using Petersen discs, or other external tags; there was less damage to the fish when recaptured in nets and no increased capture rate due to tags becoming caught in nets (Andersen & Bagge 1963, International Council for the Exploration of the Sea 1965).

In total, the estuarine stations were fished on >270 occasions between January 1976 and July 1982, with in excess of 1200 trawl tows being made. In addition, recaptures were made by the research launches on other occasions while fishing for different species. Most of the fishing activity occurred between January 1976 and January 1979 (Table S1, available in the supplement at www.int-res.com/articles/suppl/m430p183_supp.pdf). In general, the main stations were sampled at least monthly. Above T 20, sampling was undertaken as frequently as possible to provide information on flounder movements throughout the year, being restricted by water depth and river flow conditions (Table S1 in the supplement). Preliminary surveys showed that most flounder were found where there were extensive intertidal flats. Fishing concentrated on these areas, except where this was not possible because of moorings. Records of numbers of fish caught refer to flounder of 130 mm and upwards. Most fish were marked with an individual code, although 227 fish were marked with a brand that indicated only the date and place of capture. Within the estuary, Plymouth Sound and the approaches, 7401 flounder were marked (Table 1). A further 1667 fish were branded, using batch marks, at sea on the spawning grounds between 2 March and 2 April 1976 (Table 2). These fish were released within the shaded area shown in Fig. 1b.

Reward notices and photographs of branded fish were circulated to fishers. Recaptured flounder were re-measured and released, unless caught by other fishers. Most estuary recaptures were made by 2 research launches, while most sea recaptures were by commercial trawlers. Outside the estuary, otter trawling was carried out within Plymouth Sound, at Stns P4, P4A and P5 (Fig. 1b) and offshore at a number of stations, including grounds to the west of Plymouth towards Fowey and in Bigbury Bay to the east (Fig. 1b). Sex ratios, return rates from marking at different stations and other sites were compared using a Fisher's exact test programme (Microsoft Corporation 2010), used with permission from Microsoft.

To study long-distance homing, 200 fish were marked with Petersen discs. In the Tamar, 60 flounder from Stn T20 and 10 from T27 and T28 were released in the River Frome near East Stoke on 19 December 1977 (Fig. 2a). A further 25 flounder from T19 and T20 were released into the Frome, and 105 flounder from T9 were released into Poole Harbour, at Baiter, on 26 January 1978 (Fig. 2a). The fish, 174 to 384 mm in length, were caught between 3 November and 16 December

Table 1. *Platichthys flesus.* Totals of fish captured, marked and recaptured at each station. RS: total recaptures at station; TRMS: total recaptures anywhere of fish marked at this station; RSMS: total recaptures at station of fish marked at station; ROEPS: total recaptures of fish marked at station at other estuary and Plymouth Sound stations; RSS: recaptures at sea of fish marked at station; p represents the probability that the proportion of fish recaptured at other estuary and sound stations, of total estuary and sound recaptures from the marking station, is similar to that found in the overall totals

Stn	No. caught	No. marked	RS	TRMS	RSMS	ROEPS	RSS	p
P4	13	5	0	1	1	0	0	
P5	9	8	0	0	0	0	0	
T2	1463	926	152	172	147	8	17	0.099
T8	56	55	1	0	0	0	0	
T9	5803	2680	679	635	601	20	14	<0.001
T10	956	357	36	33	26	4	3	0.518
T11	76	71	3	4	0	4	0	<0.001
T12	3752	1634	306	302	269	25	8	0.739
T16	3	0	0	0	0	0	0	
T18	9	0	0	0	0	0	0	
T19	256	128	2	2	2	0	0	
T20	1840	543	40	38	28	10	0	0.002
T22	167	113	5	8	2	6	0	<0.001
T26	11	3	0	1	0	1	0	
T27	152	130	5	19	1	18	0	<0.001
T29	1218	748	99	93	71	22	0	<0.001
Totals	15784	7401	1314	1308	1148	118	42	

Table 2. *Platichthys flesus.* Recaptures of flounder branded on the Rame-Fowey spawning grounds between 2 March and 2 April 1976 and recaptured elsewhere. M: male; F: female

Capture and branding date	No. marked		No. recaptured						Out-of-area recaptures (date, sex, position)
			Tamar		Sea 1976		Sea 1977		
	M	F	M	F	M	F	M	F	
2 Mar 1976	91	54	0	1	0	1	0	0	24 Mar 1976, M, 50° 30.0′ N, 2° 57.0′ W
4 Mar 1976	165	73	1	3	4	0	6	0	30 Mar 1976, M, 50° 16.8′ N, 3° 57.8′ W
5 Mar 1976	122	72	0	2	5	0	0	0	
8 Mar 1976	217	97	0	1	7	2	0	0	30 Mar 1976, M, 50° 16.8′ N, 3° 57.8′ W
16 Mar 1976	81	34	2	0	1	1	0	0	
17 Mar 1976	109	58	2	2	3	1	0	0	22 Mar 1976, M, 50° 37.7′ N, 3° 26.5′ W 30 Mar 1976, M, 50° 16.8′ N, 3° 57.8′ W
18 Mar 1976	105	52	2	1	2	0	1	0	
19 Mar 1976	125	59	0	2	3	0	0	0	
1 Apr 1976	45	21	0	0	1	0	0	0	
2 Apr 1976	61	26	0	1	0	0	0	0	
Totals	1121	546	7	13	26	5	7	0	

1977 for the first release and on 23 and 24 January 1978 for the second. Fish were kept in tanks at the Marine Biological Association until they were transferred to aerated tubs of seawater or freshwater (as appropriate) for transfer by road.

RESULTS

Spawning migrations

Male flounder *Platichthys flesus* from which milt could be expressed were captured in the estuary as early as 23 November. In 1976, the first spent females were captured at Stn T2 on 27 February and in 1977, on 19 January. In contrast to the males, the female fish underwent final maturation at sea, and ripe females were caught, almost exclusively, in the Rame-Fowey area (Fig. 1). No ripe eggs could be expressed from maturing females caught within the estuary or seen in dissected gonads from estuary-caught fish, other than a few in recently spent females.

From March until the beginning of April 1976, 1121 male and 546 female flounders were caught and marked on the Rame-Fowey spawning grounds (Table 2). Males were more numerous than the females by ~2:1 ($p < 0.001$). The proportion of male flounder caught on the spawning grounds in that period was significantly

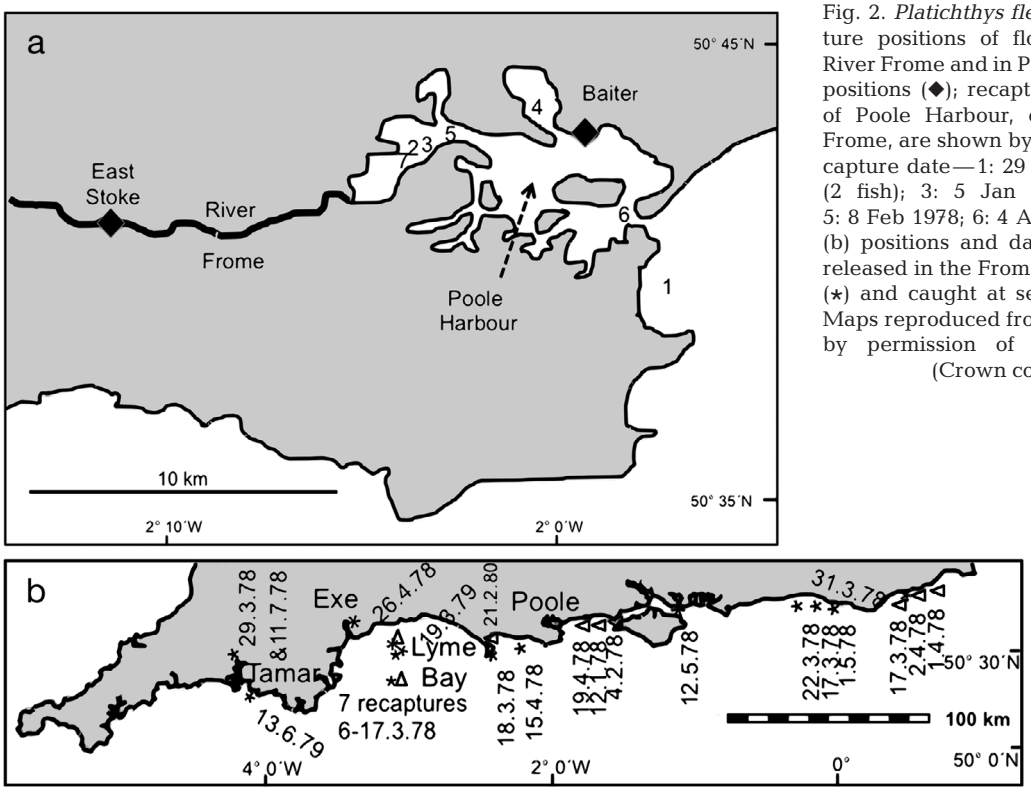

Fig. 2. *Platichthys flesus*. Release and recapture positions of flounder released in the River Frome and in Poole Harbour: (a) release positions (◆); recaptures, within the vicinity of Poole Harbour, of fish released in the Frome, are shown by numbers, in order of recapture date—1: 29 Dec 1977; 2: 4 Jan 1978 (2 fish); 3: 5 Jan 1978; 4: 19 Jan 1978; 5: 8 Feb 1978; 6: 4 Apr 1978; 7: 26 Apr 1978; (b) positions and dates of recapture of fish released in the Frome (Δ) and Poole Harbour (*) and caught at sea or in other estuaries. Maps reproduced from ordnance survey data by permission of the Ordnance Survey (Crown copyright 2010)

higher ($p < 0.003$) than that of males captured in the lower and mid-estuary in November 1976 (313 males, 210 females from Stns T2, T9 and T12). Recaptures of sea-marked flounders on the spawning grounds comprised 26 males and 5 females in 1976, and 7 males in 1977 (Table 2). Twenty-one of the sea-marked fish were recaptured within the Tamar estuary, including 11 females, 4 at T2, 14 at T9, 2 at T12 and 1 at T29. The numbers of sea-marked flounders recovered at the different stations were not significantly different ($p = 0.16$) from those expected, after considering the total number of fish captured at each station post-marking (data from Table S1 in the supplement). Significantly more sea-marked females (13 female, 7 male, 1 not sexed) were recaptured in the estuary than would have been expected by the sex ratio on marking ($p < 0.004$).

Towards the end of the spawning season, between 15 March and 12 April 1976, 45 male and 249 female flounder were caught at T2 and T9, a significantly greater proportion of females ($p < 0.001$) than that caught in the pre-spawning period or on the spawning grounds. Of the flounder caught in the estuary between 15 March and 12 April 1976, 22 were females, including 2 previously marked on the spawning grounds, only 2 marked males were re-captured. Post-spawning, between May and July 1976, the sex ratio of the flounders caught at T2 and T9 had returned to the expected 1:1 ratio, 123 males and 124 females being caught. The numbers of flounder of each sex caught at T 29 over the same period were similar, 38 males and 44 females.

Only 5 marked fish, 12% of sea-marked recaptures, were caught at sites other than the spawning grounds or in the Tamar estuary, all of these were males and recaptured east of Plymouth. Three were from Bigbury Bay, 1 from the Exe estuary and 1 from 70 km west in Lyme Bay (Table 2, Fig. 2b). Some flounder, captured at sea and branded prior to the completion of spawning in 1980, were released at P3. Two, both males, were caught in Bigbury Bay and released on 29 February 1980. One was recaptured 10 d later on the spawning grounds west of Plymouth, and the second was recaptured on 2 April 1980, also to the west on the spawning grounds. These could have been fish from small rivers east of Plymouth, caught while moving west, or Tamar fish that did not migrate with the majority of the population. Another ripe male was captured on the spawning grounds, released at P3 on 6 March 1980 and recaptured on the spawning grounds again on 9 April 1980. Similarly, a ripe female, caught west of Rame on the spawning grounds was released on 18 March 1980 and recaptured at sea in approximately the original position 13 d later.

The first flounders captured in the year at sea, west of Plymouth, were all males. In 1976, this was on 14 January and, in 1977, on 19 January. Female fish were

Table 3. *Platichthys flesus.* Recapture positions of flounder branded in the Tamar estuary and recaptured east of Plymouth

Recapture date	Marking station	Marking date	Sex	Recapture location
24 Mar 1976	T9	3 Feb 1976	F[a]	50° 24.0′ N, 3° 19.0′ W
6 Apr 1976	T9	15 Mar 1976	M	50° 16.8′ N, 3° 57.8′ W
12 Feb 1977	T10	19 Jan 1977	M	50° 27.6′ N, 3° 15.6′ W
3 Mar 1977	T10	19 Jan 1977	M	50° 27.6′ N, 3° 15.6′ W
16 Mar 1977	T9	17 Jan 1977	M	50° 24.0′ N, 3° 18.6′ W

[a]Spent

first captured on 10 February in 1976 and on 31 January in 1977. Most of the 42 recaptures at sea, of Tamar-marked flounder, were from water depths of ~35 to ~55 m between Rame Head and Fowey (Fig. 1b), i.e. from distances of up to 35 km west of Plymouth Sound. No recaptures were made further west, and only 5 recaptures (12%) were from east of Plymouth (Table 3). There was no indication of a time or spatial separation in the recaptures of fish from different estuarine stations on the spawning grounds (Fig. 1b). Comparing sea recaptures after the 1976 spawning season, since only flounder from T2 and T9 had been marked prior to this, 23 recaptures came from 4695 fish marked in the lower middle estuary (T2 to T12) and no recaptures were made of the 1666 flounder marked between T19 and T29 (data from Table S1 in the supplement). Although many of the lower to mid-estuary marked fish were marked in winter and would have included fish displaced from the upper estuary, this difference in recapture rates is significant ($p = 0.0014$).

Site fidelity of flounder in the lower and middle estuary

Details of the number of fish marked and the number of marked fish recaptured at each station are given in Table 1, together with the number of recaptures from fish marked at that station. The latter are sub-divided into the numbers recaptured at the marking station, the number caught elsewhere in the Tamar estuary and Plymouth Sound, and the number caught at sea. Details of fish marked and recaptured at each station on each day are given in Table S1 in the supplement.

Within the estuary or in Plymouth Sound, 1266 recaptures of individuals marked within the estuary were made (Table 1); 90.7% of these (1148) were from the original marking site. In the lower and middle estuary, a significantly higher recapture rate (97%) was obtained at Stn T9 (Table 1). Site fidelity at Stns T2 (96%), T10 (87%) and T12 (92%) were not significantly different from the overall mean (Table 1). Stn T11 was the exception, in that all 4 of the recaptures of fish marked at the station were from other sites.

In the middle estuary, movement of fish between adjacent trawling stations was very limited (Table 4). At T9, only 3 of 621 estuarine- and sound-marked recaptures were marked at T10. Similarly, only 2 of 30 estuarine-marked recaptures at T10 came from T9. If populations at the T9 and T10 sites freely assorted, significantly more of the flounders captured at T10 should have been marked at T9. For example, on 4 March 1977, 31 flounder were caught at T9; all 4 that were recaptures had been marked at T9. On the same day, 122 flounder were captured at T10, with 2 recaptures, both marked at T10. The numbers of T9 recaptures at the 2 sites were significantly different ($p = 0.0014$) from those expected by random distribution. Similarly, on 15 April 1977, 10 recaptures of T9 fish were made at T9, out of a catch of 45 fish, whereas, at T10, only a single T9 recapture was obtained from a catch of 48 fish; the numbers of T9 recaptures at the 2 sites were again significantly different ($p = 0.003$).

Some fishers stop-netted (at high tide) inlets that lead off T9, and several flounder marked at T9 were recovered at low water. No flounder were caught at these sites the following day and no marked fish were among the flounders the same fishers caught using the same nets in the Tavy, 2 km NE of T9.

The T8 tow lies off the opposite bank to T9 (Fig. 1c). Stn T8 was fished on 14 July 1977, when 56 flounder were caught, including 1 marked at T9. On the same day, 41 fish were caught at T9, of which 7 were previously marked at T9. The ratio of T9 recaptures at the 2 sites is significantly different from that expected by free movement between the sites ($p < 0.01$). Only 55 flounder were marked at T8. The number of fish marked on 14 July 1977 and subsequently at T9 was 374, of which 90 recaptures were later made at T9, with no T8 fish being recaptured. Had the flounder moved freely between the 2 sites then at least 13 T8 flounder should have been recaptured at T9. This difference in recaptures was again significant ($p < 0.001$).

Multiple recaptures of individuals showed they returned to the same estuarine site post-spawning. For example, a female of 328 mm total length, marked at T9 on 3 February 1976, was recaptured there again, pre-spawning on 13 February and post-spawning on 29 March and 8 October 1976. It was recaptured pre-spawning in 1977 on 18 March and later that year on 12 December. The last recapture of this individual was on 22 March 1978, post-spawning, with a length of 348 mm. All these recaptures were at T9.

At sea, 42 recaptures of estuary-marked fish were made, associated with annual spawning migration (Fig. 1b, Table 1). Only 61 (5.5%) of the estuary and sound recaptures, from flounder marked at T2 to T12,

Table 4 (this and next page). *Platichthys flesus.* Dates and positions of fish marked at one station and recaptured elsewhere in the Tamar estuary and Plymouth Sound, with notes on probable explanations for this. SM: spawning migration, PSM: post-spawning migration

Marking date	Marking station	Recapture station	Recapture date	Subsequent recaptures	Notes
13 Feb 1976	T2	P4A	17 Jun 1976		
13 Feb 1976	T2	P4A	31 Oct 1978		
27 Feb 1976[a]	T2	T9	30 Mar 1976		PSM
27 Feb 1976[a]	T2	T9	25 Oct 1977		PSM
29 Mar 1976[a]	T2	T9	26 May 1976		PSM
30 Mar 1976[a]	T2	T11	6 Jan 1977		PSM
30 Mar 1976[a]	T2	T9	27 Feb 1976		PSM
12 Apr 1976[a]	T2	T9	25 May 1976		PSM
29 Jun 1977	T2	T9	28 Sep 1977		
13 Feb 1976	T9	T12	23 Nov 1977	T9, 9 Jan 1978	Spate
30 Mar 1976[a]	T9	T13	28 Mar 1977		PSM
26 May 1976	T9	T19	7 Nov 1976		
5 Nov 1976	T9	T8	15 Jul 1977		
19 Jan 1977	T9	T10	18 Feb 1977	T10, 15 Apr 1977	
20 Jan 1977	T9	P4A	3 Sep 1979		
21 Jan 1977	T9	T2	19 Jul 1982		
18 Feb 1977	T9	T12	10 Jan 1978		
4 Mar 1977	T9	P4A[b]	7 Mar 1980		SM
18 Mar 1977	T9	T10	18 May 1977		
18 Mar 1977	T9	T2	12 May 1980		
18 Apr 1977	T9	T12	13 Jun 1977		
4 Apr 1977	T9	T10	15 Jun 1977		
4 Apr 1977	T9	T29	9 Aug 1977		Spate
18 Apr 1977	T9	T12	13 Jun 1977		
18 Apr 1977	T9	T2	24 Oct 1977		
18 Apr 1977	T9	T12	10 Jan 1978		
12 Oct 1977	T9	T20	3 Nov 1977		
11 Nov 1977	T9	T13	12 Jul 1979		
11 Nov 1977	T9	P4A	26 Jun 1978		
19 Jan 1977	T10	T11	31 Mar 1977		
4 Mar 1977	T10	T9	24 Jan 1978		
17 Mar 1977	T10	T9	12 Dec 1977		
18 May 1977	T10	T29	9 Aug 1977		
6 Jan 1977	T11	T10	18 May 1977		
6 Jan 1977	T11	T29	24 Jun 1977	T29, 8 Aug 1977	Spate
6 Jan 1977	T11	T9	9 Jan 1978		
13 Apr 1976	T12	T9	5 Nov 1976		
9 Jun 1976	T12	T2	12 May 1980		
9 Jun 1976	T12	P4A	5 May 1978		
9 Jun 1976	T12	T9	24 Nov 1977		
26 Jul 1976	T12	T20	1 Dec 1976		
26 Jul 1976	T12	T9	7 Dec 1976		SM?
26 Jul 1976	T12	T9	12 Dec 1978		
26 Jul 1976	T12	T9	25 Nov 1977		
7 Sep 1976	T12	T2	17 Mar 1977		SM?
7 Sep 1976	T12	T9	4 Apr 1977		
8 Nov 1976	T12	T10	19 Jan 1977		Spate
21 Dec 1976	T12	T20	19 Oct 1977		Spate
5 Jan 1977	T12	T20	6 Jan 1977		
5 Jan 1977	T12	T22	19 Oct 1977		
5 Jan 1977	T12	T9	21 Apr 1982		PSM
5 Jan 1977	T12	T20	28 Jul 1977		
3 Mar 1977	T12	T29	20 Oct 1977[e]		Spate
17 Mar 1977	T12	T10	15 Jun 1977		
17 Mar 1977	T12	T9	28 Sep 1977		
28 Sep 1977	T12	T9	24 Nov 1977		
28 Sep 1977	T12	T20	16 Mar 1978	T20, 6 Apr 1978	
23 Nov 1977	T12	T9	12 Dec 1977		
23 Nov 1977	T12	T9	26 Jan 1979		
24 Nov 1977	T12	T9	12 Dec 1978		

(continued on next page)

Table 4 (continued)

Marking date	Marking station	Recapture station	Recapture date	Subsequent recaptures	Notes
1 Dec 1976	T20	T29	25 Jul 1977	T29, 22 Sep 1977	Spate
1 Dec 1976	T20	T27	22 Aug 1977		Spate
1 Dec 1976	T20	T11	6 Jan 1977		SM?
1 Dec 1976	T20	P5	7 Apr 1977		SM?
13 Jan 1977	T20	T10	19 Jan 1977		Spate
11 Feb 1977	T20	T9	4 Apr 1977		
11 Feb 1977	T20	T10	30 Jun 1977		
11 Feb 1977	T20	T29	22 Aug 1977		Spate
22 Apr 1977	T20	T22	18 Nov 1977		Spate
9 May 1977	T22	T20	16 Mar 1978		Spate
9 May 1977	T22	T29	22 Aug 1977		
22 Aug 1977	T22	T12	28 Sep 1977		
24 Aug 1977	T22	T12	9 Dec 1977		Spate
22 Sep 1977	T22	T9	12 Oct 1977		Spate
22 Sep 1977	T22	T9	19 Apr 1978		
7 Jul 1977	T26	T29	22 Aug 1977		
8 Jul 1977	T27	T29	22 Aug 1977	T9, 12 Dec 1977	Spate on 12 Dec 1977
8 Jul 1977	T27	T29	25 Jul 1977[e]		
8 Jul 1977	T27	T29	21 Sep 1977[f]		
8 Jul 1977	T27	T29	20 Oct 1977		
8 Aug 1977	T27	T29	22 Aug 1977		
8 Aug 1977	T27	T29	21 Sep 1977[e]		
8 Aug 1977	T27	T29	23 Aug 1977[g]		
8 Aug 1977	T27	T29	23 Aug 1977	T29, 26 Oct 1977	
18 May 1976	T29	T9	5 Nov 1976		Spate
18 May 1976	T29	T9	7 Dec 1976		Spate
19 May 1976	T29	T21	22 Jul 1976		High temperatures, low oxygen at T29
19 May 1976	T29	T9	7 Dec 1976		Spate
7 Jul 1977	T29	T20[c]	16 Dec 1977		Spate
8 Jul 1977	T29	T9[d]	12 Dec 1977		Spate
25 Jul 1977	T29	T20	28 Jul 1977		
25 Jul 1977	T29	T27	9 Aug 1977		
8 Aug 1977	T29	T27	9 Aug 1977		
9 Aug 1977	T29	T27	26 Jul 1978		
22 Aug 1977	T29	T22	24 Aug 1977[e]		
22 Aug 1977	T29	T22	19 Oct 1977		
22 Aug 1977	T29	T20	4 Jan 1978		Spate
22 Aug 1977	T29	T9	9 Jan 1978		Spate
22 Aug 1977	T29	T20	16 Mar 1978		Spate
22 Aug 1977	T29	T20	22 Jan 1979		
22 Aug 1977	T29	T27	26 Jul 2008		
23 Aug 1977	T29	T9	20 Jun 1978		
23 Aug 1977	T29	P4A	28 Apr 1980		PSM
21 Sep 1977	T29	T20	16 Dec 1977		Spate
22 Sep 1977	T29	T20	16 Dec 1977		Spate

[a]Spent female; [bcd]prior recapture at marking station: [b]16 Jun 1978, [c]20 Sep 1977, [d]23 Aug 1977; [e]2 fish; [f]3 fish; [g]5 fish

were made at positions, in the estuary or sound, other than the original capture sites (Table 4). Of these 61, 8 were caught at sites adjacent to the marking sites, a further 11 recaptures could be explained by fish caught during migrations to, or from, the spawning grounds and 8 were probably fish displaced during high river flows, 'spates' (Table 4). The latter was demonstrated by comparing the catch rate at T12, where the main channel was fished, with the highest river flow since the previous catch (Fig. 3). Catch rates tended to peak after periods when the river flow exceeded 30 $m^3 s^{-1}$.

Five recaptures of estuary-marked flounder (2 from T2, 2 from T9 and 1 from T12) were from Plymouth Sound during the summer. Only 5 flounder were marked at P4, 4 were marked pre-spawning on 6 January 1977 and 1 of these fish was recaptured in the sound on 24 March 1977, after spawning.

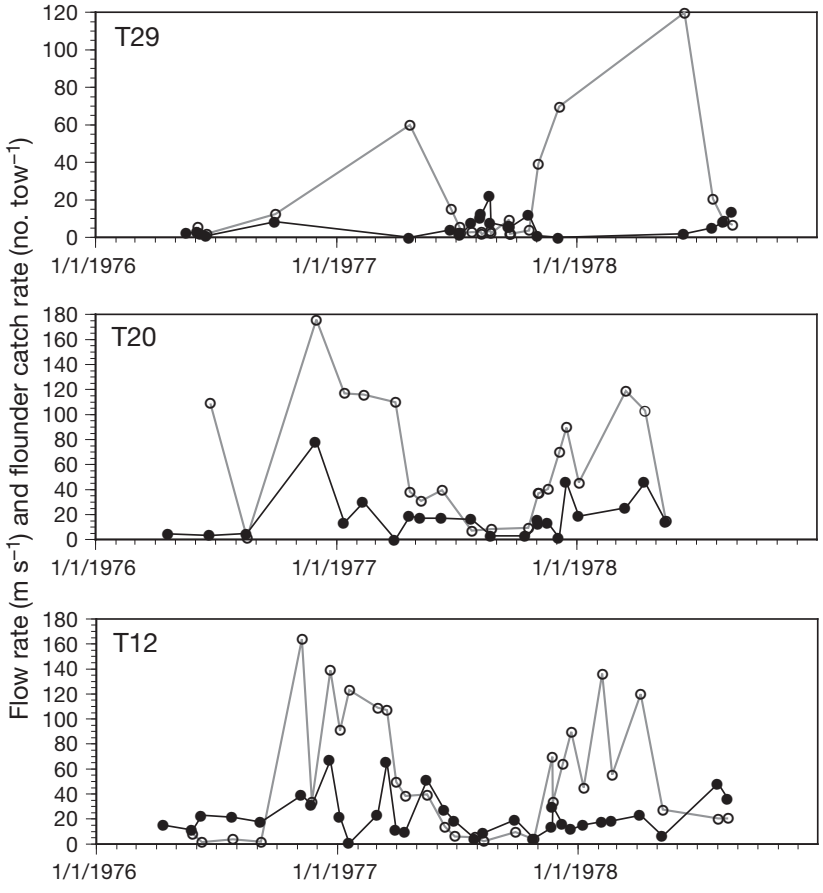

Fig. 3. *Platichthys flesus.* Variation of flounder catch with river flow: catch rates, at Stns T12, T20 and T29 (●); maximum river flow over weir, since the previous catch record at the station (○). Dates: 1 Jan 1976, 1977 and 1978

Site fidelity of flounder in the upper estuary

The upper estuary sites (Stns T20, T22, T27 and T29) showed significantly lower recapture percentages of fish that were marked at the individual sites, (74, 25, 5 and 76 % respectively) than the 87 to 96 % fidelity observed for the lower-middle estuary sites (Table 1), with 57 flounder being caught at sites in the estuary and sound other than their marking site. However, 21 of these were movements between T27 and T29, 3 could be explained by spawning or post-spawning migrations and 19 could be explained by marking or recapture at times when fish were displaced from the upper estuary during periods of high river flow.

At T29, flounder were absent, or present in low numbers, when river flows exceeded ~30 m^3 s^{-1} (Fig. 3a). Conversely, at T20, where the estuary widens to ~100 m, the catch rate increased (Fig. 3b). An example of this physical displacement of fish is shown by the recapture of a T29 marked fish at T9 on 5 November 1976, when the mean flow over the weir had been >42 m^3 s^{-1} for 4 d. Ten d later, when the flow had dropped to 16 m^3 s^{-1}, the water column from below T22, was still fresh and T20 had a bottom salinity of only 5.5 PSU at high water. Only a single flounder was captured at T22, and none above this. On 1 December 1976, the river flow at Gunnislake Weir had increased to 139 m^3 s^{-1}. The estuary above T21 was devoid of flounder. Six days later, at T9, fish marked at T13 and at T29 were recovered.

Low river flows could have similar consequences. The year 1976 was exceptionally dry in July and August (Fig. 3), with mean daily flow rates of 1.18 and 0.76 m^3 s^{-1}, respectively. The recapture of a fish, marked at T29, in salmon nets at T21 on 22 July 1976 occurred at a time when numbers of salmon were dying in the upper reaches due to low river flows, high temperatures and low oxygen levels (S. Bray pers. comm.).

Displacement and homing

The homing ability of flounder within the estuary was tested by 18 releases (681 fish in total) to sites within the estuary or Plymouth Sound that were distinct from the capture sites (Table 5). Of these fish, 128 were recaptured, 114 from the marking site and only 2 from the release site. The numbers of displaced fish recaptured at the marking sites were not significantly different ($p = 0.18$) to the numbers recaptured from those released directly at the marking site, 124 fish from 898 released (Table 5).

The return of the fish to their original site was rapid. Nine fish displaced during 29 to 30 March 1976 from Stn T9 to Stn T2 were recaptured at T9 between 13 and 15 d later. A fish from T2, released at Stn P3 on 4 January 1980, was recaptured 7 d later at T2, and a further 4 fish from this release were recaptured at T2, by 16 February 1980, prior to spawning. Similarly, 2 T2 fish released 6 d later at T2 at P 3 on 23 January 1980 were recaptured, before spawning. A further 2 T2 fish released at P3 on 1 February 1980 were recaptured at T2, before spawning, 14 d later.

All the 14 displaced fish that were recaptured away from the original marking sites could be explained by spawning migrations, or by the displacement of fish from the upper reaches of the estuary by river spates. Flounder marked at the upper stations and displaced by the autumn/winter spates, returned to the marking stations, after spawning, the following year.

Table 5. *Platichthys flesus.* Flounder marked and displaced within the Tamar estuary, together with those not displaced on the same dates, with sites of recapture. SG: Rame-Fowey spawning grounds, see Fig. 1 for other sites

Marking date	Stn	No. displaced	Release station (date)	Fish displaced		Fish not displaced		
				No. recaptured at marking station	Other recaptures	No. not displaced	No. recaptured at marking station	Other recaptures
29 Mar 1976	T2	12	T9	2	1 T9, 26 May 1976	0		
30 Mar 1976	T2	15	T9	3	1 T11, 6 Jan 1977	0		1 SG, 11 Feb 1977
26 May 1976	T2	4	P3	0	0	56	10	1 T19, 7 Nov 1976
21 Oct 1976	T2	36	P3	1	1 SG, 18 Feb 1977	2	0	
31 Dec 1979	T2	44	P3 (4 Jan 1980)	8	0	0		
31 Dec 1979	T2	8	P4	0	0	0		
15 Jan 1980	T2	8	P3 (23 Jan 1980)	3	0	0		
29 Jan 1980	T2	38	P3 (1 Feb 1980)	6	3 SG, 16 Feb 1980, 29 Feb 1980, 14 Mar 1980	0		
5 Jan 1976	T9	24	T2	4	0	0		
29 Mar 1976	T9	94	T2	33	0	0		
30 Mar 1976	T9	75	T2	30	1 T14, 28 Mar 1977 1 SG, 16 Dec 1977	0		
13 Apr 1976	T9	36	L9	2	0	0		
26 May 1976	T9	16	T12	4	1 T19, 7 Nov 1976	50	10	
9 Aug 1976	T9	21	T2	1	0	185	21	
31 Dec 1979	T9	5	P3	0	0	0		
13 Apr 1976	T12	21	T9	5	0	39	16	
26 May 1976	T12	14	T2	1	0	19	4	
9 Jun 1976	T12	10	T9	1	0	144	39	1 SG, 24 Jan 1977 1 P4A, 5 May 1978 1 T2, 12 May 1980
7 Sep 1976	T12	16	T2	0	1 T2, 17 Mar 1977	140	15	1 T9, 4 Apr 1977 1 SG, 8 Feb 1977
18 Aug 1976	T19	6	T12	0	0	49	0	1 T29, 20 Oct 1977; T9, 21 Sep 1977
1 Dec 1976	T20	81	T2	2	1 T29, 25 Jul 1977 & 22 Sep 1977	146	6	1 P5, 7 Apr 1977; 1 T11, 6 Jan 1977
22 Apr 1977	T20	67	T9	5	1 T22, 18 Nov 1977	0		
23 Aug 1977	T29	30	T22	3	1 P4A, 28 Apr 1980	68	3	1 T9, 20 Jun 1978
Totals		681		114	14	898	124	12

Displacement of flounder to Poole Harbour and the River Frome

A total of 200 Tamar flounder, tagged with Petersen discs, were released in the River Frome and Poole Harbour, ~200 km east of Plymouth (Fig. 2). The positions and dates of recapture of all the migrating fish captured at sea are shown in Fig. 2b. In total, there were 26 recaptures of fish released in the Frome and 46 of those released in Poole Harbour, i.e. 35% of the number released.

The first recapture, of a fish released in the Frome on 19 December 1977, was made 10 d later, just south of Poole Harbour (Fig. 2a). Four Frome fish were caught in the inner part of Poole Harbour in early to mid-January 1978. Sixteen of the 200 released fish had been caught at sea by the end of March (Fig. 2b). In total, 27 fish were recaptured away from Poole Harbour, 16 of these to the west and 11 to the east. Three fish were recaptured in the Plymouth area, 2 were recaptured in the Tamar. The first was recaptured at Stn T12, on 29 March following release in the Frome, a total of 100 d in which the fish had travelled a minimum distance of 240 km by river and sea. No fish were recaptured west of Plymouth.

Of the fish released into Poole Harbour, 29 were recaptured within the harbour (results not shown), 13 prior to the end of April and 16 after 1 May 1978. The latter group was assumed to have returned to the harbour after spawning. Similarly, 7 of the flounder released in the Frome were recaptured within Poole Harbour after 1 May 1978.

DISCUSSION

Although these studies were undertaken between 1976 and 1980, there is no evidence of substantial changes in the Tamar flounder *Platichthys flesus* population over the last 100 yr. The earliest records of

flounder sizes and catches were made by Holt (1897) from 1891 to 1894. A detailed study of flounder in the lower estuary was undertaken from 1937 to 1938 (Wilson 1939, Hartley 1940, 1947). Although it was not possible to study Hartley's fishing stations in the lower estuary, since wartime developments and moorings made the grounds unworkable (P. H. T. Hartley pers. comm.), the numbers and sizes of fish caught in all these studies were similar to those found in the 1970s, since Hartley gives details of the area swept by the tuck net used in the earlier studies (results not shown). McHugh et al. (2011) compared fishing records from Plymouth Sound and other inshore sites near Plymouth from 1913 to 1922 with those from 2008 to 2009. No significant differences were found between flatfish catches, including flounder, in the 2 periods. It is reasonable to assume that the results reported are applicable to the present-day flounder population.

Spawning grounds and pre- and post-spawning migrations

Although *Platichthys flesus* in the Tamar was described by Hartley (1940) as 'the estuarine fish par excellence' it is not wholly estuarine, and spawns at sea. All known flounder populations are sea-spawning; indeed few 'estuarine' fish species spawn in the Tamar (Dando 1984). The returns of Tamar flounder from offshore grounds (Fig. 1b, Table 1) showed that the main spawning grounds are between Rame Head and Fowey, 10 to 30 km west of Plymouth, over water depths of 35 to 55 m. There was no indication that flounder from different areas of the Tamar segregated on the spawning grounds (Fig. 1b). However, no fish from the upper estuary were recaptured at sea, and there is a possibility that these flounder spawn elsewhere, although recaptures of 2 fish marked in the upper estuary and recaptured in Plymouth Sound in April show that they also spawn at sea, and the recapture at Stn T29 of 2 fish marked on the spawning grounds suggest that they do spawn in the same area as the fish from the lower estuary. Flounder spawning areas north of Dieppe and in the Helgoland Bight are also offshore (van der Land 1991, Cameron et al. 1992), where water depths are between 25 and 50 m.

Peak densities on the Rame-Fowey spawning grounds usually occur in March, although earlier migration occurs with lower estuary temperatures (Sims et al. 2004). Similarly, *Platichthys olivaceus* migrate offshore when coastal water temperatures fall (Yasuda et al. 2010). Male flounder left the estuary earlier than females and tended to return later, as demonstrated by a significantly higher proportion of male fish captured at sea, as well as by an increase in the percentage of adult females captured in the estuary from mid-March to mid-April. The sex ratios of fish caught in the estuary returned to the expected 1:1 ratio later in the year.

The eastward movement along the coast of 12% of both the sea-marked and Tamar-marked founder may be an underestimate. Since reward notices were not circulated to all fishers further east, it is likely that substantially more branded fish moved east than was suggested by the recapture rate. This assumption is supported by the high percentage of recaptures (41%) of flounder, tagged with the more visible Petersen discs, that moved east from Poole; 3 as far as 170 km (Fig. 2b). Two, of 4 flounder tagged by Hartley (1940) and recovered at sea, were recovered well to the east of Plymouth, one ~360 km east.

The ability of the Tamar fish to locate their spawning grounds, or to find their way back to the estuary after spawning, is not as good as their ability to home within the estuary and Plymouth Sound. This inability of such a high percentage of flounder to return to their home estuary after spawning, if typical of other estuaries, would explain the finding of a high genetic uniformity of individuals throughout the range of the subspecies *Platichthys flesus flesus* in the Atlantic from the UK and North Sea to Spain (Galleguillos & Ward 1982, Berrebi et al. 1983, 1985, Borsa et al. 1997). Recent studies using DNA markers support the suggestion of high gene flow in this region, although proposing a genetic discontinuity in gene flow north of the Bay of Biscay (Hemmer-Hansen et al. 2007a). Although local genetic selection and/or seasonal segregation of genotypes may occur (Marine Biological Association of the United Kingdom 1973, Hemmer-Hansen et al. 2007b, Larsen et al. 2007, 2008), the high degree of mixing between populations explains why geographic clines in allele frequencies are small over this range. Marked gene clines in the Baltic flounder are believed to be due to a mixture of 2 or more subspecies (Draganik et al. 2007, Florin & Höglund 2008) and possibly 6 flounder stocks (International Baltic Seas Fisheries Commission 1998).

Seven flounder, all males, marked on the Rame-Fowey spawning grounds in 1976 were recaptured on these grounds the following year, suggesting some site fidelity to the spawning grounds. Since very few of the Tamar-marked fish were recaptured and re-released on the spawning grounds, it was not possible to estimate their fidelity to the spawning grounds. Plaice show a fidelity of at least 94% to Icelandic spawning grounds, within 30 km of the marking site, in the year after tagging, declining to 72% thereafter (Solmundsson et al. 2005). A similar fidelity to spawning grounds was found for North Sea plaice using data storage tags (Hunter et al. 2003).

Most Tamar flounder returned to the estuary and to their original marking location, as demonstrated by the multiple recaptures of individuals, before and after

successive spawning seasons. Plaice show an ability to return over much greater distances to their feeding grounds after spawning (Hunter et al. 2003, Solmundsson et al. 2005). Marine species that are estuarine spawners can also show a high fidelity for returning to their natal estuary, as shown for the weakfish *Cynoscion regalis* (Thorrold et al. 2001).

Site fidelity and homing

In the middle estuary, Stns T8 to T10 and T12, flounder exhibited a high site fidelity, 95% of the total estuary and sound recaptures from these stations were from the marking station. Few fish left their marking station until the start of spawning migration and then they returned to the same estuarine station after spawning. Flounder in some inshore populations also show relatively little movement away from their marking area (Vitinsh 1976).

Flounder living in the upper part of the estuary, above T12, were more mobile due to the hydrographic conditions in the upper estuary. Low river flow and high temperatures in summer can combine to cause de-oxygenation in the region of the estuary above Stn T22 so that few fish can survive. High river flows both flush flounder out of the upper reaches and remove the mud deposits that support many of their food organisms (Hartley 1940). Flounder are normally not caught at sea off Plymouth outside the spawning season, but are occasionally captured offshore in trawls after heavy rains (Hartley 1947). The water bailiff on the freshwater part of the estuary reported that all the mud at Stn T29 was removed within 24 h under strong flow conditions and not replaced until it was re-deposited by spring tides when river flows were low, as much as 1 m of mud then being deposited per tide (J. Adams pers. comm.). Under spate conditions, most of the upper estuary flounder probably remain in the lower estuary until low river flow and spring tides restore the habitat in the upper reaches. Of the 118 out-of-position recaptures in the estuary, 21 could be explained by upper estuary fish displaced during autumn/winter, and 8 by displaced fish being marked on the lower and mid-reaches during winter and recaptured on their normal grounds during summer (Table 4). These observations would explain the finding by Hartley (1947) that small flounders tagged at Saltash (Stn T7) during winter were often found higher up the estuaries during summer. The smaller fish are most easily displaced by strong water flows. Thus, they will preferentially accumulate in the lower reaches during winter.

The situation in the upper estuary, where there is now little intertidal mudflat following canalisation (Tamar Estuaries Consultative Forum 1999), is different to that in the middle and lower estuary, where large intertidal mudflats and inlets provide foraging areas at high tide (Hartley 1940). Comparing the ratio of adjacent stations recaptures to home station recaptures, there were 20:72 from T27 to T29, in the upper estuary, and 8:627 from T8 to T11, in the middle estuary (Table 1). A significantly higher proportion of adjacent station recaptures ($p < 0.001$) occurred in the upper estuary. The smaller intertidal feeding area probably contributes to a greater feeding movement of flounder along the estuary in this zone at high water, resulting in a greater home site longitudinal range of individual flounder in periods of low to normal river flow (Table 4). In rivers where there are no obstructions, flounder can migrate >50 km into freshwater (McCurdy 1977), and it is likely that the horizontal range occupied by individual flounder in rivers is much greater than that in tidal estuaries.

The presence of a resident population of flounder within the sound was supported by a limited amount of marking there. Recaptures of estuary-marked fish in the sound suggest that at least some of the sound population is recruited from the estuary.

The displacement studies within the estuary and Plymouth Sound showed that, of 681 flounder displaced to different sites, 114 of the recaptures were from the original marking site and only 2 were from the release site (Table 5). The latter could be due to the fish being caught at Stn T2 during their return from spawning, on the way to their home station.

Studies of other flatfish also show a high degree of site fidelity, at least during some stages of their life cycle. Juvenile plaice have a high a long-shore site fidelity, 78% remaining within 100 m of the marking site from one day to the next and returning rapidly when displaced by 100 m or more (Burrows et al. 2004). Juvenile plaice can return to their nursery ground after a displacement of 3.5 km offshore (Riley 1973). In contrast adult plaice only have a high fidelity for feeding areas covering many kilometres (Hunter et al. 2003, Solmundsson et al. 2005).

The 87 to 97% site fidelity shown by adult flounder within the lower and middle estuary (Stns T2 to T12) over the whole marking period compares with 89% shown by juvenile *Solea solea* at T9 and T10 (Coggan & Dando 1988). The majority (90 to 98%) of young *Pseudopleuronectes americanus* were found to remain within 100 m of their estuarine marking site after 1 to 3 wk (Saucerman & Deegan 1991). A very high site fidelity, in summer, was found for *Fundulus heteroclitus* in a tidal creek, with fish remaining within 36 m along one bank and returning when displaced across the creek (Lotrich 1975). Dace *Leuciscus leuciscus* show diel migrations between daylight shallow areas

and night time feeding areas, returning to the same position within a few metres in each area (Clough & Ladle 1997). Flounder show similar, albeit tidal, migrations between feeding areas on the mudflats and resting areas off the mudflats. They move onto the mudflats with the tide to feed (Hartley 1940, Wirjoatmodjo & Pitcher 1984, Raffaelli et al. 1990) and return to deep water, off the mud bank, at low tide. Since stop-netters, working in the inlet off Stn T9, captured marked fish on one day, but then captured no flounder on subsequent days, it is likely that most of the flounder have a fidelity to small foraging areas in the intertidal region.

The mechanism by which these fish species locate their home area after displacement is not known, although olfactory and magnetic cues have been suggested. Olfaction would not apply to long-range homing, such as for the fish released at Poole. If flounder can detect the earth's magnetic field, then this ability is weak. Many of the fish moved east, not towards Plymouth. The rapid deposition of sediment washed out of the upper estuary by high water flows would be expected to disrupt olfactory homing ability in the middle estuary, where site fidelity remained high throughout the year.

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Field experiments on depth selection by juvenile plaice *Pleuronectes platessa*

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ABSTRACT: After settlement on sandy beaches in spring, juvenile (0-group) plaice *Pleuronectes platessa* L. spend the summer and autumn months at depths <5 m. During this time, there is a strong length–depth relationship in which the smaller fish are most common at the shallow end of their depth range. Mark and recapture experiments with fish caught at depths of 0.5 and 2.5 m demonstrated that nearly all fish subsequently released at their depth of capture stayed at that depth, and few moved to other depths. In contrast, many fish reciprocally transplanted between these depths returned to their depth of capture within 2 d, and very few remained at the transplant depth. The results indicate that juvenile plaice have a fidelity to, and can actively select, a particular depth, although there may be some movement between depths, most of which at the time of the experiment (August/September) was directed offshore.

KEY WORDS: Plaice · *Pleuronectes platessa* · Depth distribution · Depth fidelity · Homing · Habitat selection

INTRODUCTION

Shallow coastal waters often act as nursery grounds for juvenile fishes and invertebrates because they optimise growth and minimise mortality of the vulnerable young stages. In northwest European waters, plaice *Pleuronectes platessa* L. are common and often numerically dominant members of the benthic ichthyofauna on sandy shores (Gibson et al. 1993, Beyst et al. 2001). Plaice spawn offshore early in the year, and their larvae are transported shoreward by a combination of drift and active migration (Creutzberg et al. 1978, Rijnsdorp et al. 1985, Bergman et al. 1989, Fox et al. 2006).When close to shore, the planktonic larvae metamorphose and in spring settle on the sea bed in sandy areas to take up their juvenile benthic life style. Once settlement is complete, the newly arrived plaice population is mostly concentrated at depths of <5 m, and this distribution remains essentially stable until autumn. Although the summer distribution is stable and the population is distributed between the water's edge and the outer limit of ~5 m, individuals are not uniformly distributed within this depth range. Instead, the maximum abundance occurs at 1 to 2 m. This unequal distribution is caused by an underlying length–depth relationship (Bregneballe 1961, Edwards & Steele 1968, Gibson 1973, Kuipers 1973, Gibson et al. 2002) in which mean length increases with depth. Because the length–frequency distribution is approximately normal, peak density occurs at the depth occupied by those fish close to the mean length. It is also a feature of this distribution that, although larger individuals in the population can be found at all depths within the range, the smallest individuals are absent from deeper water (Gibson et al. 2002). This general pattern of variation in length and abundance with depth is common but not universal. In the North Frisian Wadden Sea, for example, the length–frequency distribution of the population becomes bimodal with time. In this case, the larger, faster-growing fish are found in shallower water than the smaller, slower-growing ones (Berghahn 1987). The positive relationship between depth and length–abundance is not as clear in populations inhabiting very shallow water (~1 m), but even here there is usually a relationship between abundance and distance from the shoreline (Modin & Pihl 1996). This

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length–depth relationship on the nursery ground represents the innermost end of a wider distribution of all length classes initially described for plaice by Heincke (1905) and subsequently known as Heincke's law. It represents an example of a wider length–depth relationship described for many (Macpherson & Duarte 1991, Labropoulou et al. 2008) but not all (e.g. Stefanescu et al. 1992) fish species. Superimposed on this general pattern of depth distribution, however, are changes in distribution over both short and longer time scales. On beaches where there is a significant tidal range, young plaice undertake onshore/offshore tidal migrations (Gibson 1973, Kuipers 1973, van der Veer & Bergman 1986, Burrows et al. 1994, Gibson et al. 1996). Where tidal range is small, no such tidally-related movements can be detected, but in both tidal and non-tidal areas, the fish move into shallower water at night (Gibson et al. 1998). These tidal and diel movements continue throughout the summer, but in autumn there is a gradual emigration into deeper water (Hill 1971, Gibson 1973, Lockwood 1974, Poxton et al. 1983).

The observed length–depth relationship could have several, not necessarily exclusive, explanations. It may be caused by differential depth-related growth such that fish in deeper water grow faster than those in shallow water. It could also be related to predation pressure because it is known that predators are more numerous and more varied in deeper water (Ellis & Gibson 1995) and that vulnerability to predators is size related (van der Veer & Bergman 1987, Ellis & Gibson 1995, 1997, Gibson et al. 1995). Consequently, the smaller fish in that part of the population inhabiting deeper water would experience higher predation rates than larger fish. Superimposed on these 2 indirect effects is the possibility that individual fish can actively select, and maintain their position at, a specific depth. Larger fishes may be found in deeper water as a consequence of increased preferred depth with increasing size (Gibson et al. 2002). This paper describes the results of a study designed to examine this possibility. A field experiment was carried out in which marked fish were either released at their depth of capture or transplanted to another depth. A subsequent sampling programme enabled the movements of fish to be followed after marking to examine whether fish have an innate depth preference, maintain their position at the preferred depth, and return to that depth if transplanted.

MATERIALS AND METHODS

Capture methods. The experiments were carried out on the sandy Tralee beach in Ardmucknish Bay, Argyll, Scotland. The benthic ichthyofauna of this beach, which is dominated numerically by juvenile plaice, has been extensively studied in previous investigations (e.g. see Gibson et al. 1993, 1996), and the beach is described in detail in those studies. The fish used were caught with a 1.5 m beam trawl pulled by hand at a depth of 0.5 m and a 2 m beam trawl using a small boat and outboard at 2.5 m depth at 2 stations (A and B) ~360 m apart. The distance between the 2 depths was ~100 m at each station. Each trawl tow was made parallel to the beach within ± 2 h of low tide and lasted 4 min. The catch from each haul was transferred to a large shallow plastic container filled with seawater and taken to the marking station on the beach. The plaice in the hauls were carefully picked out from the plastic containers and transferred to buckets of seawater aerated with a battery-driven pump. The initial marking and release was carried out over 4 d in August 1996: at Stn A on Days 1 and 3 and at Stn B on Days 2 and 4.

Marking methods. ***Mark effect trials:*** Prior to the field experiments, 200 fish from 0.5 m and 166 from 2.5 m were returned to the laboratory and kept overnight in large tanks with running seawater. To determine whether marking and/or handling resulted in mortality, half of each sample from each depth was measured and marked as described below, and the other half measured only. The fish in the 4 treatments were then kept in separate tanks, fed minced mussel *Mytilus edulis* ad libidum and checked each morning for deaths for 4 wk.

Marking in the field: Fish were marked on a large table sited near the water's edge sufficient for 3 people to work side by side. Newly caught fish were carefully taken from the buckets and their total length measured. They were then placed eyed-side down on wet paper towels for marking. Marking consisted of a subcutaneous injection on the blind side, of acrylic paint (Rowney 'Cryla') diluted with 3 parts seawater, using 0.5 mm diameter hypodermic needles. After marking, fish were placed in separate buckets of aerated seawater before release. A fourth person recorded the length and colour of each fish marked. Each fish was marked with a colour specific to its depth of capture and subsequent treatment as described in the next section.

On the first day of capture and marking (Day 1 at Stn A, Day 2 at Stn B), the mark was positioned ventral to the lateral line. Two days after marking (Day 3 at Stn A, Day 4 at Stn B), all unmarked fish caught were given the appropriate colour mark above (dorsal to) the lateral line. Previously marked fish recaptured 2 d after release were given a second mark of a colour appropriate to their depth of capture but above the lateral line. Any marked fish recaptured >2 d after release were remarked with a previously unused colour.

Release procedure. Immediately after all fish had been marked (about 1 to 2 h after low water), approxi-

mately half of the day's catch from each depth was replaced at the depth of capture (0.5 m marked blue or 2.5 m marked yellow) and the remainder ('transplants') was released at the shallower or deeper depth. That is, half of the fish caught at 0.5 m (marked red) were released at 2.5 m and half of the fish caught at 2.5 m (marked green) were released at 0.5 m. Fish were always released at the station where they were caught; there were no transplants between stations.

Recapture methods. Fish were recaptured around the time of low water (±1.5 h) using the same method as their initial capture, and the stations were revisited at frequent intervals up to 58 d thereafter (see Table 2). After the initial 4 d marking period, once the fish had been inspected for marks, all marked and unmarked fish were released at the capture site.

The nature of the experimental treatments means that recaptured fish can be allocated to 8 categories depending on their depth of initial capture (shallow, S, or deep, D), the depth in which they were replaced or to which they were transplanted (S or D), and their depth of recapture (S or D). Each of the 8 categories was given a unique recapture code to facilitate description of the results. For example, the code SSS indicates that a fish was initially caught at 0.5 m (S) and released and recaptured at the same depth (SS) whereas the code DDS indicates that a fish was initially caught at 2.5 m (D) and released at 2.5 m (D) and subsequently recaptured at 0.5 m (S) (Table 1).

Table 1. *Pleuronectes platessa*. Codes allocated to recaptured fish. S: shallow, D: deep

Recapture code	Depth of initial capture (m)	Release depth (m)	Recapture depth (m)
SSS	0.5 (S)	0.5 (S)	0.5 (S)
SSD	0.5 (S)	0.5 (S)	2.5 (D)
SDS	0.5 (S)	2.5 (D)	0.5 (S)
SDD	0.5 (S)	2.5 (D)	2.5 (D)
DDD	2.5 (D)	2.5 (D)	2.5 (D)
DDS	2.5 (D)	2.5 (D)	0.5 (S)
DSD	2.5 (D)	0.5 (S)	2.5 (D)
DSS	2.5 (D)	0.5 (S)	0.5 (S)

RESULTS

Effects of handling and marking

Five fish died during the course of the mark effect trials, but there was no significant difference in mortality between marked and unmarked fish ($\chi^2 = 0.65$, $p > 0.25$).

Catches

The numbers of fish caught during the investigation are given in Tables 2 & 3. All 2546 fish caught between 1 and 4 August were marked and released.

The length distributions of marked fish were similar between stations at the same depth, but the mean length of fish at 0.5 m was always less than that at 2.5 m (Table 2). The difference in mean lengths at the 2 depths was caused principally by the virtual absence of fish <45 mm at 2.5 m, although the maximum lengths were similar at both depths. This difference is reflected in the consistently smaller standard errors of the deeper samples.

Recaptures

General recapture statistics

Approximately equal numbers of fish caught over the 58 d of the observations were caught and marked in deep and shallow water (Tables 2 & 3). Of these, 351 were recaptured at least once (Table 3), representing 5.2% of the total catch or 13.8% of the 2546 marked fish.

Recapture rates of marked fish were much greater in shallow water (263; Table 3) than in deep water (88). Twelve fish were recaptured twice, all at 0.5 m. Of these 12 double recaptures, 8 had been transplanted from shallow to deep water and the remaining 4 had been caught and replaced in shallow water. Overall, a much greater percentage of fish initially caught and marked in shallow water (270 of a total of 1274, 21.2%) were recaptured compared with those initially caught and marked in deep water (81 of a total of 1272, 6.4%); (Table 3). To test the hypothesis that the probability of recapture is independent of the depth of initial capture, i.e. fish initially caught in deep water have the same recapture rate as those initially caught in shallow water, a chi-squared test was applied to the contingency table given in Table 4A. The resulting $\chi^2 = 117.7$ was significant at $p \ll 0.0001$, and the null hypothesis was rejected. The depth of release rather than the depth of initial capture could also affect the probability of recapture, and so the following null hypothesis was tested: The probability of recapture is independent of the depth to which the fish were returned after tagging. The contingency table for this test is given in Table 4B. The resulting χ^2 of 0.77 was not significant ($p = 0.380$), and the null hypothesis was accepted.

Fidelity and depth selection

Nearly all of the recaptures of fish that had been caught and released at their original depth of capture (SSS, DDD) were made at that depth, and the propor-

Table 2. *Pleuronectes platessa*. Summary of total catches of fish during the experiment. The means ±SE of total length (mm) for catches made on Days 1 to 4 are shown in parentheses

Date	Days after marking	Stn A		Stn B		Total
		0.5 m	2.5 m	0.5 m	2.5 m	
1 Aug (Day 1)	0	285 (49.2 ± 0.6)	370 (60.3 ± 0.4)	–	–	655
2 Aug (Day 2)	0	–	–	394 (52.4 ± 0.5)	279 (60.7 ± 0.4)	673
Total (Days 1, 2)						1328
3 Aug (Day 3)	2	302 (52.1 ± 0.6)	315 (61.1 ± 0.4)	–	–	617
4 Aug (Day 4)	2	–	–	293 (56.1 ± 0.7)	308 (60.5 ± 0.4)	601
Total (Days 3, 4)						1218
7 Aug	4, 6	268	245	–	–	513
9 Aug	5, 7	–	–	303	242	545
Total						1058
14 Aug	11, 13	404	298	–	–	702
16 Aug	12, 14	–	–	197	264	461
Total						1163
28 Aug	25, 27	343	272	–	–	615
30 Aug	26, 28	–	–	253	257	510
Total						1125
28 Sep	56, 58	202	248	–	–	450
29 Sep	56, 58	–	–	217	195	412
Total						862
Grand total		**1804**	**1748**	**1657**	**1545**	**6754**

tions were very similar in deep and shallow water (47.0 and 43.2%, respectively; Table 5, Fig. 1). Very few had voluntarily changed their depth and were subsequently recaptured at other depths (SSD 2.6%, DDS 1.2%; Table 5, Fig. 1). Furthermore, almost all of the recaptures of fish that had been marked and transplanted to another depth were made at the original depth (SDS, DSD) and the proportions were very similar in deep and shallow water (48.5 and 50.6%, respectively; Table 5, Fig. 1). Very few were recaptured at the depth to which they had been transplanted (SDD, DSS) (Table 5, Fig. 1). These results suggest that fish have a strong depth preference, do not move readily from that depth and will return to that depth if displaced. To test this suggestion, the following null hypothesis was tested: Movement pattern after treatment is independent of the depth of initial capture, i.e. fish caught in, and returned to, shallow water (SS) are just as likely to move into deeper water as fish caught in deep water and released in shallow water (DS), and vice versa. The contingency tables for this hypothesis are given in Table 6. The resulting chi-squared tests were very highly significant ($p \ll 0.0001$) for fish from both shallow (Table 6A, $\chi^2 = 126.6$) and deep water (Table 6B, $\chi^2 = 139.6$), enabling the null hypothesis to be rejected. However, this fidelity is not absolute because supplementary sampling at depths between 0.5 and 5 m between the dates given in Table 2 caught 11 marked

Table 3. *Pleuronectes platessa*. Summary of total catches, releases and recaptures of marked fish over the 58 d of the experiment

Category	Shallow	Deep	Total	% of marked fish
Catch	3461	3293	6754	–
Marked	1274	1272	2546	–
Releases originating from				
Shallow water	613	661	1274	–
Deep water	630	642	1272	–
Recaptures originating from				
Shallow water	258	12	270	10.6
Deep water	5	76	81	3.2
Marked fish recaptured	263	88	351	13.8

Table 4. *Pleuronectes platessa*. Contingency table used to test the hypotheses that (A) the probability of recapture is independent of the depth of initial capture and (B) the probability of recapture is independent of the depth to which the fish were returned after tagging

	No. recaptured	No. not recaptured	Total
(A) Depth of origin			
Shallow	270	1004	1274
Deep	81	1191	1272
Total	351	2191	2546
(B) Depth of release			
Shallow	179	1064	1243
Deep	172	1131	1303
Total	351	2195	2546

Table 5. *Pleuronectes platessa*. Summary of recaptures of marked fish at intervals during the course of the experiment. Key to the recapture codes is given in Table 1

Days after marking	Fish initially captured in shallow water									Fish initially captured in deep water								
	SSS		SSD		SDS		SDD		Total	DDD		DDS		DSD		DSS		Total
	n	%	n	%	n	%	n	%	(n)	n	%	n	%	n	%	n	%	(n)
2	18	37	0	0	31	63	0	0	49	47	7	0	0	47	7	6.7	1	15
4–7	43	49	1	1	42	48	1	1	87	10	43	0	0	13	57	0	0	23
11–14	34	50	2	3	32	47	0	0	68	7	33	0	0	14	67	0	0	21
25–28	22	51	2	5	18	42	1	2	43	7	58	0	0	3	25	2	17	12
56, 58	10	43	2	9	8	35	3	13	23	4	40	1	10	4	40	1	10	10
Totals	127	47.0	7	2.6	131	48.5	5	1.9	270	35	43.2	1	1.2	41	50.6	4	4.9	81

fish (5 SS, 5 SD, 1 DD) at 0.9 m, 8 to 10 d after marking, and 15 (2 SD, 10 DD, 3 DS) at 1 to 2 m, 28 d after marking. Further supplementary sampling at depths of >5 to 11 m and 59 to 99 d after marking caught some marked fish at these depths; a further indication of the offshore movement at this time.

Temporal patterns of recaptures

Marked fish made up 5.3% of the total catch 2 d after marking, and this percentage rose to 10.4 during Days 4 to 7 (Table 7), after which it steadily declined, perhaps indicating a movement of marked fish into deeper water beyond the outermost sampling depth or selective mortality of marked fish. Many transplanted fish (SDS, DSD) had returned to their original depth within 2 d, but the percentage of fish returning to shallow water after transplantation (SDS) showed a decline with time. A similar trend was not obvious in the other category of transplanted (DSD) fish, although the numbers of recaptures were smaller (Table 5). However, there was a clear temporal trend in the proportions of fish initially caught in shallow water recaptured in deep water (Table 7). To test the null hypothesis that there is no change in the rates of movement of shallow water fish between depths over time, a chi-squared test was applied to the data condensed to <11 and ≥11 d to ensure that expected values were >5 (Table 8). The resulting χ^2 of 5.71 was significant at $p = 0.017$, so the null hypothesis was rejected, a further indication that there was greater movement into, and tendency to remain in, deeper water as the experiment progressed.

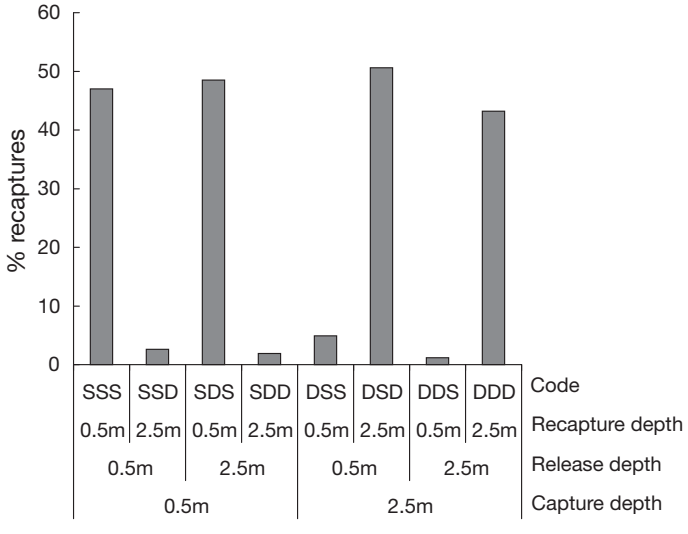

Fig. 1. *Pleuronectes platessa*. Cumulative recaptures over the course of the experiment expressed as a percentage of the 2 treatment types (S: shallow and D: deep), based on data from Table 5. Key to the recapture codes is given in Table 1

Effects of size

Because of the strong relationship between size and depth, it might be expected that there would be a greater tendency for transplanted smaller fish originating from shallow water to return to shallow water than larger fish, and vice versa for fish originating in deep water. In other words, is $L_{SDS} < L_{SDD}$ and $L_{DSD} > L_{DSS}$, where L is mean length? Unfortunately this expectation could not be tested because of the low numbers of

Table 6. *Pleuronectes platessa*. Contingency table used to test the hypothesis that movement patterns after treatment are independent of the depth of initial capture. Data from Table 5 and with the recapture codes shown. Key to the recapture codes is given in Table 1

Depth of initial capture	Recapture depth		Total
	Shallow	Deep	
(A) Fish released in shallow water			
Shallow	127 SSS	7 SSD	134
Deep	4 DSS	41 DSD	45
Total	131	48	179
(B) Fish released in deep water			
Deep	1 DDS	35 DDD	36
Shallow	131 SDS	5 SDD	136
Total	132	40	172

Table 7. *Pleuronectes platessa*. Variation in recapture rate of marked fish with time

Days after marking	Total catch (from Table 2)	No. marked fish recaptured (from Table 5)	% marked fish in catch	% shallow water fish recaptured in deep water
2	1218	64	5.3	0
4–7	1058	110	10.4	2.3
11–14	1163	89	7.6	2.9
25–28	1125	55	4.9	7.0
56, 58	862	33	3.8	21.8

fish recaptured at the earliest stages of the experiment. Fish at later stages would have grown and so their original length could not be known, thereby confounding the comparison.

DISCUSSION

Depth is an environmental factor that commonly plays an important part in determining the distribution of numerous aquatic organisms, and many species are restricted to a characteristic depth range over their lifetime. However, motile species may occupy only part of that range at different stages in their development and growth. The underlying implication of differential depth distribution between sizes, developmental or reproductive stages is that individuals can detect, select and maintain their position at particular depth or within a range of depths.

Flatfishes provide many examples of differences in depth distribution within and between species (e.g. Gibson 1973, Riley et al. 1981, Macpherson & Duarte 1991, Allen & Balz 1997, Armstrong 1997), but the studies are mainly descriptive and the extent to which such distributions are the result of external factors or of active choice has not been widely investigated. The results given in this paper provide clear evidence that individual young plaice on their nursery ground show little short-term variation in the depth occupied, indicating they can actively maintain their position at a given depth. Furthermore, the experiments demonstrated that transplanted individuals do not remain at the transplant depth and rapidly return to their original depth, thereby providing further evidence for active depth selection. This 'homing' to the depth formerly occupied can be rapid, within 2 d and probably even less, although the initial sampling frequency did not allow a more accurate measure. Return within 1 tidal cycle would certainly be possible because the return journey would have been ~100 m, a distance that the fish cover 4 times a day in the course of their intertidal migrations on the beach used for the experiments. Depth fidelity is also largely maintained as the fish undergo their intertidal movements (Gibson 1973), at least during the day, although there is a marked tendency for the population to be shallower at night (Gibson et al. 1996, Burrows 2001).

This fidelity to a particular depth can be compared with the horizontal site fidelity shown by young winter flounder *Pseudopleuronectes americanus* (Saucerman & Deegan 1991) and plaice (Riley 1973, Burrows et al. 2004), in which individuals do not stray far alongshore. Juvenile plaice also show 'homing' whereby they return to their original position when laterally displaced (Riley 1973, Burrows et al. 2004) and on a larger scale, the ability of the adults to return to spawning and feeding grounds is well documented (e.g. Hunter et al. 2003, Solmundsson et al. 2005). The clear depth fidelity of young plaice and the overall phenomenon of differential depth distribution poses the intriguing question of how it comes about.

In a review of this relationship in fishes, Macpherson & Duarte (1991) concluded that, where present, it derives from migratory or diffusive movements from shallow to deep water during ontogeny following the initial onshore drift and/or migration of larvae and the subsequent occupation of shallow water by juveniles (p. 109 in their paper). The life history of plaice (see 'Introduction') agrees well with this conclusion. Small juvenile plaice are restricted to shallow depths, whereas larger ones cover the entire juvenile depth range (Gibson et al. 2002), a 'smaller–shallower' mechanism (Middleton & Musick 1986) rather than simply 'bigger–deeper': a trend of increasing mean size with depth (Polloni et al. 1979). In relation to the importance of temperature gradients in determining distribution, Macpherson & Duarte (1991, p. 110) also commented that size–depth relationships occur over a much narrower depth range in fresh water where significant

Table 8. *Pleuronectes platessa*. Contingency table used to test the null hypothesis that there is no change in the rates of movement of shallow water fish between depths over time. Data from Table 5 with recapture codes shown. The key to the recapture codes is given in Table 1

Days after marking	Recapture depth		Total
	Shallow SSS + SDS	Deep SSD + SDD	
<11	134	2	136
≥11	124	10	134
Total	258	12	270

temperature gradients are often present within a few metres depth. A similar situation occurs in the shallow marine waters that young plaice inhabit (see e.g. van der Veer & Bergman 1986, Gibson et al. 2002). Over a wider scale to encompass the whole depth distribution of a species, Macpherson & Duarte (1991) further concluded that fish move into deeper water as they grow in order to benefit from a lower metabolism and extended life span at lower temperatures and suggest that the pattern is evolutionary in nature and has a genetic basis.

For the few months during and after settlement, the choice of depth and the length–depth relationship in young plaice undergoes several changes, implying that the preferred depth changes accordingly. In the first phase, represented by the onshore movement of metamorphosing larvae, individuals appear to settle offshore and then move inshore to congregate in the shallowest water possible, often a few centimetres deep, as though constrained only by the waterline. At this stage in the process, the first settlers have had time to grow and so are larger than later, smaller settlers still moving onshore. The length–depth relationship at this time is therefore negative. Once settlement is completed, the population goes through a brief period when there is no relationship between length and depth. The cause of this change is not known; it may be due to a movement of the larger fish in the population into slightly deeper water, i.e. a change in depth preference as size increases, or by faster growth of those fish at the deeper end of the distribution, or both. Whatever the cause, the change eventually results in a strong positive length–depth relationship that remains stable for several weeks. At the start of this period, individuals seem to have acquired a preference for (or perhaps begin to express an inherited tendency to select) a specific depth because the fish do not adjust their depth as they grow. Such a depth adjustment would be expected if there was a fixed relationship between length and depth, i.e. fish of a particular size are always found at a characteristic depth. Statistically, this phenomenon is represented by an increase in the intercept of the regression line describing the length–depth relationship whereas the slope remains constant (Gibson 1973, Gibson et al. 2002). It was towards the end of this stable period that the experiments described in this paper were done, and the results provide evidence to support the suggestion that individuals have a preference for a set depth. Following the stable period, a third change takes place and there is general movement offshore indicating a further change in preferred depth. Eventually, the great majority of fish emigrate to deeper water. This offshore movement was also detected during the later stages of the experiment.

A further question raised is how to reconcile the presence of contrasting patterns of depth choice with our current understanding of the evolutionary selective pressures influencing behaviour. Burrows (1994) constructed models of depth selection behaviour in young plaice based on the premise that fish adopt depths through tidal and diel cycles that give the best balance between finding food and avoiding predators. The strong fidelity for a specific depth that varied among individual fishes shown in the present study conflicts with this premise, and suggests that depth preference may be much less dynamic over tidal and diel cycles than previously thought.

The weak association between depth fidelity and fish size was surprising. Similar-sized and outwardly identical fishes had contrasting depth preferences, although the smallest fish were always absent from deeper water. Optimality models for habitat choice (Clark & Levy 1988, Burrows 1994) never predict 2 solutions to the same trade-off problems, making explanations for depth choice as selective advantages or trade-offs very difficult.

Varying strong preferences for depth in otherwise similar fishes suggest internal control mechanisms. Persistent differences in behaviour and physiology among individuals are sometimes termed behavioural syndromes (Sih et al. 2004) or 'personality traits' (Stamps 2007). We have no evidence as to whether observed depth preferences in young plaice have a phenotypic or genotypic basis, but it is possible that such differences may arise as a result of different experiences during early life (Davis & Stamps 2004). Post-larval plaice settling on sandy beaches may have had varying durations of planktonic development, and successful settlement of early-arriving larvae may require a faster growth rate than for late-arriving larvae. Different physical conditions through larval development may also have affected subsequent growth rate (Hovenkamp 1992, van der Veer et al. 2000).

If depth preferences have a genetic element, the different behavioural strategies adopted may represent evolutionary bet-hedging (Philippi & Seger 1989). Preference for shallow water may be a superior strategy in nursery areas where food resources are richer and predators rarer in shallow water, while deep-water preference may be a more successful strategy where predators are rarer and food is more plentiful at depth.

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Behavioural traits of competent *Concholepas concholepas* (loco) larvae

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ABSTRACT: Swimming activity of competent *Concholepas concholepas* (Bruguière, 1789) larvae, under different photoperiod, water turbulence and settlement cues were investigated in laboratory experiments. At night, larvae mainly swam to the water surface and then sank passively to the bottom. During the day, regardless of the photoperiod, larvae mainly stayed on the bottom, occasionally exhibiting upward movements. During the swimming period, larvae displayed the capacity to adhere to floating substrata through several mechanisms, including the secretion of a long and sticky mucous thread, air bubble capture or taking advantage of the water tension. The presence of *C. concholepas* prey, such as the mussel *Semimytilus algosus* and the chthamaloid barnacle *Notochthamalus scabrosus*, significantly influenced the swimming activity of competent *C. concholepas* larvae, inducing them to stay close to the bottom where prey were present. *C. concholepas* prey also triggered the initiation of larvae crawling, a characteristic of the early benthic life of *C. concholepas*. Although our laboratory experiments are proxies of nature, they are in good agreement with field observations in shallow nearshore areas (< ca. 0.5 km from shore) that recorded higher abundances of competent *C. concholepas* larvae (which are often associated with floating substrata) captured by surface planktonic tows during the night rather than day. Similarly, competent *C. concholepas* larvae abundances were higher in tows through inner nearshore foam slicks than in non-slick areas. The results suggest that the presence of endogenous swimming behaviour in these larvae may be a key factor enhancing adherence to floating substrata. The laboratory discovery of vertical buoyancy provides new information leading to a better understanding of the distribution and abundance patterns of these larvae in inner nearshore waters.

KEY WORDS: Chile · *Concholepas concholepas* · Larval distribution and transport · Swimming behaviour · Rafting · Mucous thread · Stickiness · Foam slicks

INTRODUCTION

The larvae of numerous marine benthic invertebrates spend long periods in the plankton prior to settlement (Thorson 1950, Bayne 1965, Pechenik 1986, 2000, Young & Chia 1987, Young 1995), where they must remain suspended, locate and gather food, avoid predators and unfavourable conditions, disperse to new areas, and select sites for settlement (Strathmann 1974, Palmer & Strathmann 1981, Scheltema 1986). The importance of larval behaviour has been recognised as an essential component of marine ecology (Forward 1988, Young 1995), and the role of inner nearshore oceanographic processes influencing larval dispersal and the recruitment of benthic organisms has been the focus of recent research (reviewed by Le Fèvre & Bourget 1992, Castilla et al. 2002, Largier 2002). The measurement of larval abundances and distribution in the water column are precluded in part by the absence of knowledge concerning the interactions between environmental factors and larval traits. It has been suggested that for most marine invertebrates, this

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is due to the spatio-temporal variability in larval abundance (Gaines & Bertness 1993) and unknown aspects of larval behaviour (Miron et al. 1995). Although in marine invertebrates larval transport to settlement habitats seems to be controlled mainly by hydrographic factors (Thorson 1950), larval behaviour may also influence their final destination (e.g. Butman 1987, Forward 1988, Pineda 1994, Young 1995).

The loco (Chilean abalone) *Concholepas concholepas* (Bruguière, 1789) is an economically and ecologically important component of the rocky intertidal and subtidal communities along the Chilean coast (Castilla 1988, 1999). After 1 to 2 mo of intra-capsular development, the veliger larvae of this gastropod are released into the water column (Gallardo 1973, Castilla & Cancino 1976), and then require from 3 to 12 mo of planktonic growth to reach competence (Gallardo 1973, DiSalvo 1988, Molinet et al. 2005). At competence, larvae of *C. concholepas* are mainly associated with the neustonic layer (DiSalvo 1988, Peña et al. 1994, Moreno et al. 1998, Poulin et al. 2002a,b, Manríquez et al. 2004, Molinet et al. 2005, 2006, 2008) and display pediveliger behaviour (i.e. mixed behaviour alternating between crawling and swimming, Carriker 1990, DiSalvo & Carriker 1994). Although much has been written on the biology of *C. concholepas* (see Castilla 1988, 1999, Castilla & Defeo 2001, Manríquez et al. 2009), to date little information has been published concerning the behavioural traits of competent *C. concholepas* larvae (hereafter CCL) and their role in larval transport and the establishment of the early benthic stage of this species. The use of rafting as a means of long-distance dispersal in *C. concholepas* larvae has been suggested (Castilla & Guiñez 2000), and has also been described for several other marine phyla (reviewed by Thiel & Gutow 2005a). However, the ability of CCL to adhere to floating substrata and use it as a means of transport has not yet been investigated. Thus it is possible that the production of a mucous thread by swimming CCL (DiSalvo 1988) could be a mechanism by which they adhere to floating substrata that has accumulated at surface convergence zones or slicks (i.e. floating algae, driftwood, man-made objects) and so enhance their rafting capacity.

In this study, under laboratory conditions, we investigated the swimming behaviour of CCL in response to vectors such as geotaxis, water flow and settlement cues (i.e. mussels and barnacles). Our laboratory experiments were complemented by field sampling in inner-nearshore waters (ca. 0.1 to 0.5 km from the coastline) designed to examine the relationship between diurnal and nocturnal abundances of CCL in the water column. Coastal CCL were collected to assess the relationship between their abundances and floating substrata that may serve as a complementary mechanism to enhance dispersal through rafting. Behaviours exhibited by larvae in confined containers cannot necessarily be extrapolated to those that they may display in nature; however, larval behavioural traits obtained under controlled conditions may help to determine CCL distribution in coastal environments and the establishment of the early benthic life of *Concholepas concholepas* after settlement (dispersal and recruitment niche, sensu Young et al. 2005), and might also provide useful information for future restocking and management programmes or the aquaculture of *C. concholepa*s.

MATERIALS AND METHODS

Field sampling 1. Larval abundance and floating substrata. CCL were collected intermittently between 1990 and 2009 from surface tows using a plankton net in El Quisco (32° 33′ S, 71° 41′ W) and between 2002 and 2004 in El Way (23° 42′ S, 70° 26′ W), in central and northern Chile, respectively. Samples were collected between September and January in El Quisco and between December and January in El Way, matching the months of peak abundances of CCL (Poulin et al. 2002a). Samples were collected by towing a surface plankton net with a cross section of 0.8 × 0.6 m at the open end, 1 m cloth length and 600 µm mesh (DiSalvo 1988). The acquisition of competence was verified by the presence of a distinctive premetamorphic lip at the aperture rim of the protoconch (DiSalvo 1988). Tows were made in inner-nearshore waters, between ca. 0.1 and 0.5 km from the coast, with maximum water depths of ca. 20 m. The number of CCL in each tow was expressed per unit of water sampled. Total sample volume was determined by multiplying the mouth area of the plankton net by the length of the tow which was measured by a flowmeter (General Oceanics®) set in the mouth of the net. Abundance of CCL was correlated with total wet weight of both total flotsam retained by the sieve and total amount of the red complex algae *Plocamium cartilagineum* caught in each tow. When comparisons of CCL abundance between day and night were needed, 4 or 5 tows were carried out no more than 12 h apart on specific dates (see Table 1).

The samples taken from the central Chilean coast were transported to the laboratory of the Estación Costera de Investigaciones Marinas (ECIM) in Las Cruces (33° 30′ S, 71° 38′ W), where most of the experiments were conducted. All CCL were individually assigned to 1 l plastic containers filled with UV-treated, 0.45 µm filtered seawater (FSW) and maintained on a laboratory bench at 14 ± 2°C under natural light photoperiodicity before being assigned

to the corresponding experiments. At daily intervals, FSW was changed and the microalga *Isochrysis galbana* was added with a final concentration of 30 to 50 cells μl^{-1}.

Field sampling 2. Adhesion of CCL to floating substratum as a potential rafting mechanism. To assess the potential relationship between the amount of floating debris and the abundance of CCL in inner nearshore zones, we collected additional samples in inner nearshore water areas with different abundances of debris: slick tows and non-slick tows (Shanks 1983, 1985) at El Quisco (September and December 2004) and El Way (December 2002, 2003 and January 2004). Sampling was conducted using the same methodology described in the previous section. Slicks were identified by the presence of floating debris associated with foam-covered water. In each location we employed 2 categories of non-slick tows, viz. shoreward or offshore with respect to the slick (no more than ca. 10 m away from the slick border). Moreover, to test whether CCL can reach settlement sites in the rocky intertidal zone by adhering to raft substrata (i.e. algae, debris and other), we sampled floating substrata, mainly fragments of *Plocamium cartilagineum*, from shallow rocky shore tide channels (ca. 10 m from shore and 1 m deep). Samples were collected in the vicinity of ECIM during November 2001. Samples (10 l plastic buckets) were taken from a large inner-nearshore patch of foam and floating substrata associated with foam slicks reaching the coast, and from nearby control areas of clear water without foam. For each sample (n = 7), the bucket was dragged twice through the surface of the water (ca. 20 l) to accumulate floating substrata, which was then concentrated in a 600 µm mesh sieve to assess the abundance of CCL and floating substrata.

Laboratory experiments on larval behaviour. In the laboratory, photoperiodicity, thigmokinesis (tendency to cling to objects in the water; Fraenkel & Gunn 1961) and turbulence experiments, we used flat-bottom glass test tubes, 40 cm high and 3.5 cm in diameter. Tubes were arranged vertically in transparent Plexiglas racks to minimise disturbance. In all experiments, FSW at 14 ± 2°C and with 33 was used. The CCL were acclimatised to the glass tubes for 1 h before initiating observations. All experiments using glass tubes, except those related to photoperiodicity, were conducted in darkness, and observations were performed using a dim red light. Experiments using glass test tubes lasted 72 h, and the larval behaviour was recorded at 1 h intervals. To assess larval position in the water column, tubes were divided into 4 sections (each 9.5 cm high). Larval activity was classified as swimming, hovering, crawling or resting on the bottom of the tubes.

Larval behaviour and measurements of swimming speeds. Observations of swimming behaviour were made by eye or assisted by a stereomicroscope. To assess larval upward and downward speeds, a video camera (SONY Handycam CCD-TVR608 Hi8) was placed in front of the tubes. The video images were processed and speeds calculated as the time required for an individual larva to traverse 1 or 2 cm in the water column.

Expt 1. CCL survivorship on rafting algae. To assess whether CCL can successfully survive grazing on the rafting algae, we deployed 8 CCL inside individual 0.3 l glass beakers filled with FSW. In each beaker, we placed a small fragment of *Plocamium cartilagineum* as a source of food. In a control group, 8 CCL were placed in beakers filled with FSW without fragments of *P. cartilagineum*. The experiment was run for 14 d, and the water was changed every second day. After 14 d, settlement and metamorphosis were induced by exposing the surviving CCL to small rocks with recently settled aggregations of the barnacle *Notochthamalus scabrosus* (Manríquez et al. 2009).

Sixty individual larval trajectories, oriented parallel to the walls of the tubes, were considered for the speed calculations. To assess gravitational fall speeds, 11 CCL were anaesthetised in a solution of menthol and seawater (10 g l^{-1}) until total withdrawal of the velar lobes. Larvae were placed in a glass cylinder (diameter 6.5 cm) containing 2 l of FSW at 14°C. Gravitational fall speeds were timed over a distance of 20 cm, and observations were made by eye. Active downward larval speeds were measured using a stopwatch from the surface down to the point where they entered the second section of the tube.

Expt 2. CCL photoperiodicity and circadian rhythm. The influence of different photoperiods on larval behaviour was evaluated (September to October 1991) by assigning groups of 6 CCL to each of the following treatments: (1) natural light:dark cycle (Control, 12 h light:12 h dark); (2) inverted light:dark cycle (Treatment 1, 12 h dark:12 h light); (3) constant light (Treatment 2); and (4) constant darkness (Treatment 3). Circadian behavioural rhythms can persist for several weeks in the laboratory in the absence of any environmental cues (Naylor 1988). Therefore, in this experiment, CCL were acclimatised in the laboratory for the altered photoperiod for 48 h. During daylight hours, the experiments were illuminated with white overhead fluorescent lighting, from which the measured light intensity on the bench-top varied slightly around a mean value of 15 $\mu E\ m^{-2}\ s^{-1}$, an intensity not designed to investigate phototaxis or mimic angular light distribution and intensity in the sea.

Expt 3. CCL and thigmotaxis. We conducted laboratory experiments to determine whether CCL display the ability to swim and to cling to floating objects. Moreover, we tested whether this behaviour is affected

by different degrees of water movement. The observations were made during September and October 1991, in groups of 6 CCL in individual tubes and applying the following treatments. Treatment 1: tubes filled with FSW only (Control); Treatment 2: FSW with small pieces of wood (0.3 cm long × 0.02 cm wide × 0.02 cm high) floating in the water column); Treatment 3: FSW with small fragments (ca. 0.9 cm^2) of *Plocamium cartilagineum* (suspended in the centre of the tubes with the aid of transparent monofilament line). Three separate experiments were conducted under 3 different water movement regimes: in the first, tubes were maintained immobile in the support rack. In the second and third, the rack bearing the tubes was placed on top of an adjustable orbital platform shaker set at 50 and 100 rpm, respectively. In an additional experiment (October 2006), we tested whether CCL adhere to floating substrata, such as fragments of *P. cartilagineum*, by active upward swimming followed by climbing, or as a consequence of a common surface convergence of CCL and substrata moved upward by the water current. Two groups of 12 CCL were assigned individually to 2 Plexiglas aquaria (33 l, 30 cm high × 30 cm wide × 45 cm length) with the following treatment conditions: (1) floating substrata and still FSW and (2) floating substrata with an upward current. To achieve an upward current flow (ca. 0.2 l s^{-1}), the aquarium was equipped with an electric water pump which generated a circular water current from the bottom of one side of the aquarium to the water surface on the other side. To avoid dragging larvae and fragments of algae through the pump, we placed a 600 µm mesh across the side of the aquarium where the pump was placed. We deployed 20 fragments of *P. cartilagineum* as floating potential substrata for CCL in each aquarium. To ensure a similar surface area of *P. cartilagineum* in each treatment, we cut the algae to provide a surface area of approximately 2.5 cm^2. The CCL were placed in the aquarium, acclimatised for 10 min, after which the fragments of algae were introduced. The experiment was conducted overnight (00:00 to 03:00 h), and after 20 min exposure to the experimental conditions, we recorded whether each larva had adhered to the floating algae. In this experiment, we used CCL and fragments of *P. cartilagineum* collected at El Quisco. However, due to logistical problems, the experiment was conducted at the Laboratorio Costero de Recursos Acuáticos de Calfuco in Valdivia (39° 46′ S, 73° 23′ W).

Expt 4. Mucous thread and CCL adhesion under different water movement and settlement cues. Two series of experiments (September to October 2009) were conducted at the Laboratorio Costero de Recursos Acuáticos de Calfuco in Valdivia with CCL collected in Antofagasta and transported to Valdivia within 24 h of collection. In the first experimental series, individual CCL (n = 12) were randomly assigned to separate 25 cm high 2 l glass beakers in which a clean air stone was placed near the bottom. Intense water movement was achieved by setting a high bubbling regime (8 l h^{-1}) in half of the bottles. In the control (n = 12), no bubbling was used. Larvae were maintained under those conditions for 8 h, after which time the observations were conducted. Each larva was gently touched with a soft artist's brush to determine whether the production of the mucous thread assisted adhesion to the brush. In the second experimental series, we tested the combined effect of water movement and settlement cues in triggering adhesion of CCL to the water tension. Water movement was achieved by using a rack system of swinging paddles in which the Plexiglas paddles pivoted inside 1 l glass beakers. An electric rotary motor allowed us to control the speed of the pivoting frequency, which was set at 0 (control), 0.1 (Treatment 1) and 0.4 (Treatment 2) sweeps s^{-1}. For each water movement regime, we used small rocks bearing ca. 100 recently settled barnacle aggregations of *Notochthamalus scabrosus* and bare rocks of an equivalent size which served as controls. For each water condition, we used 8 CCL assigned to individual 1 l glass beakers filled with 0.45 µm seawater. Larvae were maintained there for 6 h, and then the electric motor was switched off, allowing us to conduct the observations of larval behaviour during nighttime hours. From direct observations, we noted 5 different behavioural patterns: crawling on the rocks, crawling on the surface of the rearing beakers, swimming, drifting and taking advantage of the water surface tension to remain at the surface. Drifting larvae were considered to be those moving with the water current with or without the velum extended from the protoconch.

Expt 5. Settlement cues and swimming of CCL. This experiment was conducted to determine whether swimming of CCL is modulated by the presence of settlement cues (September to October 2006). Larvae were assigned individually to glass beakers filled with 2 l of 0.45 µm FSW with the following treatments in which prey were offered at the bottom of the aquaria: ca. 200 g of live specimens of the mussel *Semimytilus algosus* of ca. 0.2 mm in length (Treatment 1, n = 10) and small rocks bearing ca. 150 recently settled barnacles *Notochthamalus scabrosus* (Treatment 2, n = 10). As a control group, we used beakers without prey (n = 10). *S. algosus* were used in Treatment 1 because they induce settlement in *Concholepas concholepas* (Manríquez et al. 2004, 2008, 2009) and are the preferred prey for early post-metamorphic *C. concholepas* under laboratory and field conditions (Dye 1991, Méndez & Cancino 1990). Rocks with *N. scabrosus* were

used because small settlers of *C. concholepas* (ca. 0.2 to 0.5 of peristomal length) commonly occur in the microhabitat of the rocky intertidal zone (Manríquez et al. 2009). In the beakers, prey were allowed to acclimatise for 2 h before CCL were added. Larval activity was monitored continuously for periods of 3 h during the night (00:00 to 03:00 h) and day (10:00 to 13:00 h), using a dim red light to make nocturnal observations. During the observations, larval activity was classified as swimming or crawling, and a standardised number of swimming displacements towards the water surface per hour were used to compare the treatment and control conditions. Larvae taking advantage of the water surface tension to remain at the surface were gently disturbed with a soft artist's brush to induce them to sink.

Statistical analysis. Natural abundances of CCL and the number of swimming displacements were analysed by 1-way ANOVA. When data deviated from normality and/or did not fit a normal distribution after transformation, we used non-parametric statistics. A 2-way ANOVA was used to determine whether the number of larvae swimming or adhering to substrate differed between intensities of water movement. Rhythmicity in the photoperiodicity experiment was determined by plotting autocorrelation coefficients calculated at 1 h lag intervals (= sampling intervals) as a function of lag intervals (Broom 1979). Peaks in the autocorrelation plots exceeding the 95% confidence intervals were considered to indicate statistically significant rhythmicity at $p < 0.05$. To detect possible cyclical behaviour, period lengths were calculated with spectral Fourier analysis, which aims to identify the dominant frequency of an apparently periodic signal (Dowse 2007). The proportions of CCL adhering to the experimental substrata with and without upward seawater current were compared by a 2-proportion *Z*-test (Zar 1999).

RESULTS

Field sampling 1. CCL larval abundance and floating substrata

In the shallow inner nearshore collection sites, CCL were found in diurnal and nocturnal surface tows. When both diurnal and nocturnal inner nearshore tows were made, no more than 12 h apart, significantly more larvae were found in nocturnal tows (Table 1). In diurnal tows, a positive linear correlation was found between the amount of flotsam and both the amount of *Plocamium cartilagineum* and the number of CCL (Fig. 1a,b). Similarly, in diurnal tows, a positive linear correlation was found between the amount of floating *P. cartilagineum* and the number of CCL larvae (Fig. 1c). In nocturnal tows, the only significant correlation found was that between the amount of flotsam and the amount of *P. cartilagineum* (Fig. 1d). No correlation was found between the amount of flotsam and the number of CCL (Fig. 1e) or between the amount of *P. cartilagineum* and the number of CCL (Fig. 1f). During the analyses of the plankton samples in the laboratory, CCL were frequently found adhering to fragments of *P. cartilagineum* and empty tubes of Chaetopteridae polychaetes (Fig. 2e,f) and other floating biotic and abiotic substrata, such as wood, fragments of fronds and holdfasts, plastic, candy wrappers and fragments of polystyrene packing, amongst others.

Table 1. *Concholepas concholepas.* Abundance of competent larvae in nearshore diurnal and nocturnal tows. Data are mean number of larvae (± SE; number of tows) per 100 m^3 and were compared using 1-way ANOVA. Statistically significant ($p < 0.05$) differences are indicated with an asterisk (*)

Date	Diurnal tows	Nocturnal tows	*F*	p
26–27 Nov 1990	1.68 (0.64; 6)	6.70 (2.18; 6)	7.05	0.0240*
28–29 Nov 1990	0.48 (0.05; 5)	2.19 (0.38; 5)	19.66	0.0022*
09–10 Sep 1994	8.24 (3.12; 5)	7.05 (1.27; 5)	0.11	0.7408
27–28 Oct 1994	1.90 (1.90; 5)	6.77 (0.62; 5)	5.94	0.0407*
31 Nov–1 Dec 1994	0.42 (0.24; 4)	3.12 (1.04; 4)	6.43	0.0443*
02–03 Dec 1994	0	8.34 (2.1; 4)		
23–24 Nov 1995	0	4.67 (0.36; 4)		
27–28 Nov 1995	0.23 (0.04)	7.03 (0.52; 4)	172.58	<0.001*

Field sampling 2. CCL adhesion to floating substratum as potential rafting mechanisms

In Antofagasta, significantly more CCL were found within tows through the foam slicks than in the shoreward or offshore tows (Table 2). On average, ca. 35 and 110 times fewer CCL were found in the offshore and shoreward tows, respectively, than in the slick tows. In El Quisco, CCL were only found in tows through the foam slicks and on the shoreward side. However, average numbers of CCL were significantly higher in the foam slicks. No CCL were found in the offshore tows (Table 2). In samples collected from shallow intertidal tide channels (using buckets), CCL were only found in those samples that contained floating *Plocamium cartilagineum*. Average (± SE) numbers of CCL collected per quantity of *P. cartilagineum* were: 0.067 ± 0.021 larvae g^{-1} in September (n = 7); 0.051 ± 0.027 larvae g^{-1} in October (n = 7); and 0.024 ± 0.011 larvae g^{-1} in November (n = 7). No CCL were found in samples from clear shallow waters.

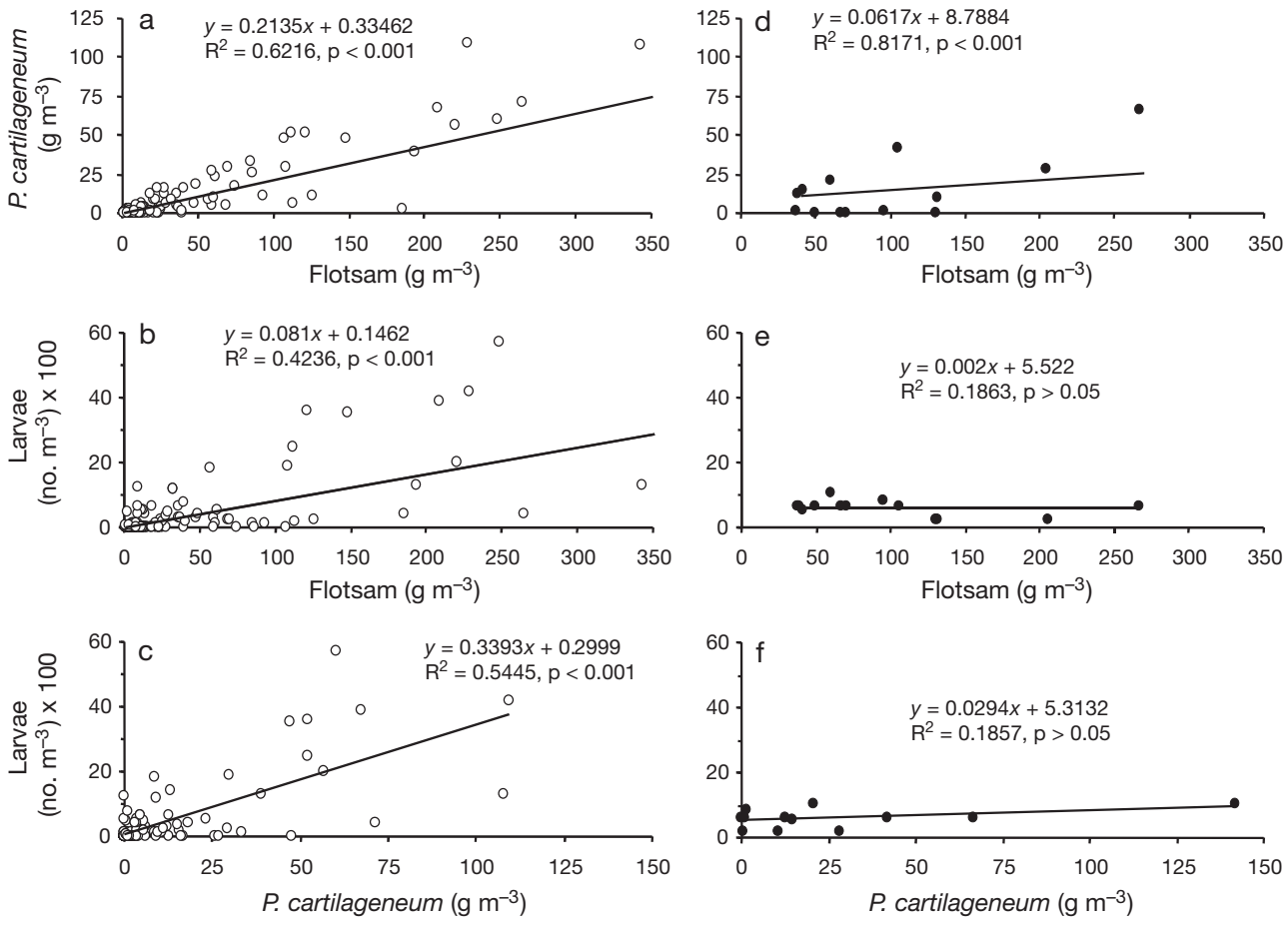

Fig. 1. *Concholepas concholepas*. Relationships between the amount of flotsam, *Plocamium cartilagineum* and the number of competent larvae in diurnal and nocturnal tows. White (left panels) and black (right panels) circles are diurnal and nocturnal tows, respectively

Larval behaviour and measurements of swimming speeds

During swimming in the laboratory, CCL retained the 4 large ciliated velar lobes (ca. 4 mm long, Fig. 2b) expanded and oriented upward lifting and dragging the protoconch (ca. 1.7 mm long, Fig. 2a,b) with the umbo pointing downward. Larvae displayed the 'hop and sink' method of locomotion in which they swam alternatively upwards and downwards (Bainbridge 1961). The upward swimming speed ranged from 0.17 to 0.60 cm s^{-1} (average ± SD speed = 0.41 ± 0.11 cm s^{-1}; n = 60). Once the velar lobes made contact with the water surface, they were flapped and the larvae swam downward maintaining the velar arms expanded. However, occasionally after the velar lobes made contact with the water surface, total withdrawal of the velar lobes was observed and the larvae sank downwards. Downward swimming speed ranged from 0.32 to 0.69 cm s^{-1} (average = 0.48 ± 0.09 cm s^{-1}; n = 60). Maximum speeds were obtained just after larvae made contact with the water surface (average = 0.75 ± 0.12 cm s^{-1}; n = 60). Larvae also attached to the surface water tension using their foot (Fig. 2c) or by hanging down from the water surface by a mucous thread (also see DiSalvo 1988) secreted from a small hole in the base of the foot (Fig. 2g). Larvae were also observed to float using air bubbles captured with their foot (Fig. 2d), a method described by DiSalvo (1988) for CCL. While floating, the larvae fully withdrew their velar lobes inside the protoconch in a resting attitude (Fig. 2c). Gravitational fall velocities of the anaesthetised larvae ranged between 1.45 and 2.89 cm s^{-1} (average = 2.08 ± 0.38 cm s^{-1}; n = 11).

Expt 1. CCL survivorship on rafting algae

In the laboratory, all CCL (n = 8) maintained in FSW for 2 wk with fragments of *Plocamium cartilagineum* survived, successfully settled and metamorphosed when exposed to rocks covered with barnacles. However, no CCL (n = 8) survived in the control condition without fragments of *P. cartilagineum*.

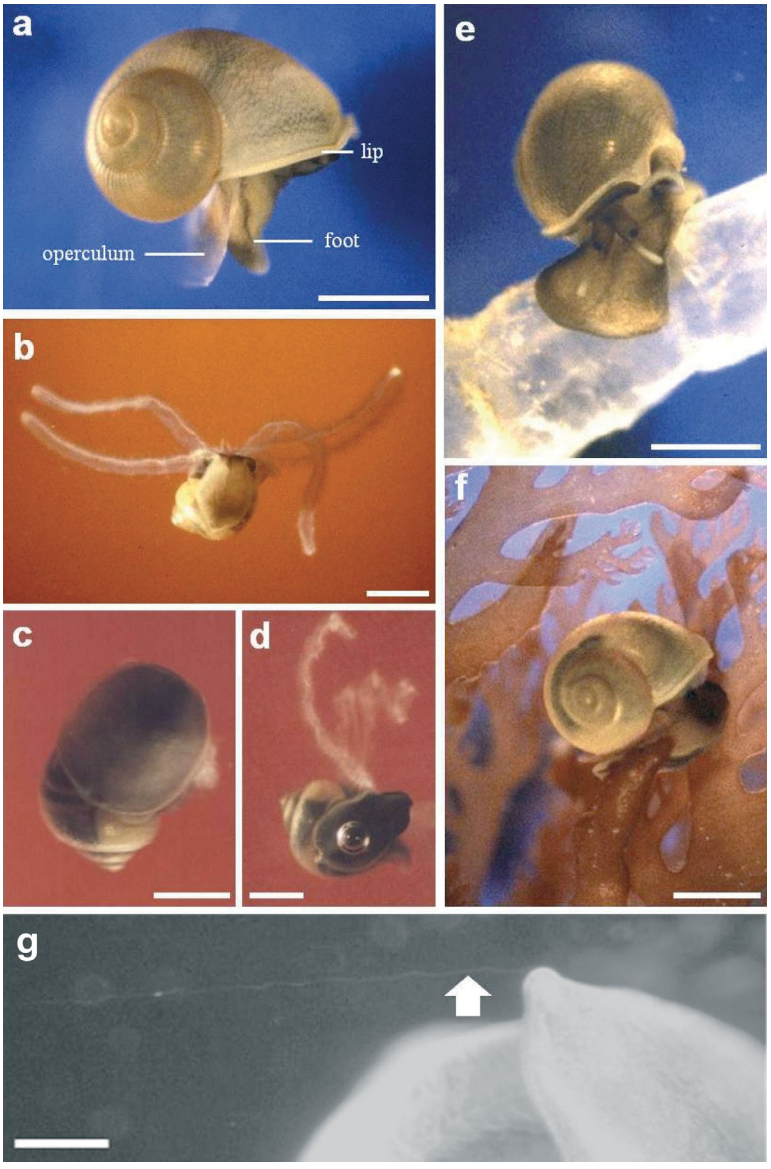

Fig. 2. *Concholepas concholepas*. Photographs of competent larvae. (a) Lateral view showing the upturned lip on the edge of the shell aperture denoting competence, the foot and the larval operculum; (b) free-swimming competent larvae showing expanded velar lobes lifting and dragging the protoconch; (c) larva attached to the water surface using its foot; (d) larva floating using bubbles captured with its foot; larva adhering to (e) empty tubes of Chaetopteridae polychaetes and to (f) fragments of the red algae *Plocamium cartilagineum*; (g) a hyaline mucous thread emerging from the posterior end of the larval foot. The white arrow in (g) depicts the mucous thread. All scale bars = 1000 µm

Expt 2. CCL photoperiodicity and circadian rhythm

Regardless of the experimental photoperiod, the maximum numbers of swimming CCL under still water laboratory conditions were observed during the hours coinciding with natural darkness (Fig. 3). Larvae were observed swimming vertically throughout the entire water column in the tubes, swimming rapidly to the surface and sinking passively to the bottom. However, during the day, larvae stayed at the bottom of the tubes, crawling or making small excursions upwards that seldom allowed them to reach the upper part of the water column. Regardless of observation time, larval displacement from the water surface to the bottom was achieved by either swimming or passive sinking. In all 4 experimental photoperiods used, the Fourier analysis detected a swimming period length of ca. 24 h. These analyses revealed a significant negative autocorrelation (i.e. above the 95% confidence limit) only between 9 and 11 h (time spent swimming). Beyond the peak of activity at 11 h, autocorrelation coefficients never reached significance in the explored range of hours.

Expt 3. CCL and thigmotaxis

The swimming activity of CCL was concentrated mainly at night (Fig. 4), and 100% of larvae were recorded swimming for 2 to 12 h periods. When floating substrata were made available at the surface of the experimental tubes, between 20 to 30% of the larvae adhered to the substrata: larvae remained adhered to wooden substrata for a maximum of 3 h and adhered continuously to fragments of *Plocamium cartilagineum* for up to 36 h. At night, in tubes agitated at 50 rpm, 100% of larvae were observed actively swimming for a period of between 2 and 12 h. When substrata were made available at the surface, up to 30% of larvae adhered to the wooden substrata and up to 50% to *P. cartilagineum*: they remained adhered to pieces of wood for up to 6 h and adhered continuously to fragments of *P. cartilagineum* for up to 40 h. Water movement had a negative effect on nocturnal swimming activity when the tubes were agitated at 100 rpm. At this speed, swimming activity was reduced to 66%. No larvae were observed adhering to pieces of wood; however, they adhered to fragments of *P. cartilagineum* for up to 12 h. A 2-way ANOVA on the average number of adhered CCL larvae indicated a significant effect of floating substrata, water movement and the interaction between them. A similar analysis of the average number of swimming CCL detected a significant effect of water movement. However, the presence of floating substrata

Table 2. *Concholepas concholepas.* Abundance of competent larvae in diurnal tows made in (slick tows), and adjacent to, nearshore frontal foam slicks. Waters adjacent to the foam slicks were sampled for offshore and shoreward tows (non-slick tows). Data are mean number of larvae (± SE; number of tows) per 100 m^3 and were compared using Kruskal-Wallis analyses of variance

Date	Slick tows	Offshore tows	Shoreward tows	df	χ^2	p
Antofagasta, Dec 2002	45.67 (7.32; 4)	0.39 (0.39; 4)	1.31 (0.62; 4)	2	8.46	0.0145
Antofagasta, Dec 2003	35.33 (8.86; 4)	0	0.67 (0.38; 4)	2	7.33	0.0256
Antofagasta, Jan 2004	73.33 (24.66; 4)	0	0	2	10.46	0.0054
El Quisco, Sep 2004	5.37 (0.13; 4)	0	0.30 (0.30; 4)	2	9.37	0.0092
El Quisco, Dec 2004	10.03 (2.33; 4)	0	1.17 (0.44; 4)	2	9.42	0.0090

and their interaction effect with water movement was not significant (Table 3). In agitated seawater, we observed a transparent mucous thread originating from the posterior end of the larval foot (Fig. 2g). In the second experiment, significantly more CCL ended up adhered to *P. cartilagineum* with an upward seawater current (85%, n = 20) than in the still seawater treatment (30%, n = 20; *Z*-test for 2 proportions, $Z = 3.19$, $p < 0.05$).

Expt 4. CCL mucous thread and larval adhesion under different water movement and settlement cues

Mucous threads that allowed CCL to hang from floating substrates or from the water tension were up to 5 cm long. Contact between the soft brush and the larvae resulted mostly in the larva attaching to the brush by a mucous thread. In the agitated water treatment, 92% of larvae formed a mucous thread (n = 12). However, in still conditions, only 8% CCL displayed the same response (n = 12). On occasion, once the brush was shaken and lifted, mucous threads as long as 30 to 40 cm were observed. In the second experimental series, regardless of the water movement treatment and not taking into account the least represented behaviour (glass crawling, GC), the behavioural responses were significantly different in the presence or absence of barnacles (χ^2: 39.83, df = 3, $p < 0.0001$, Fig. 5). On the other hand, regardless of the presence of barnacles and not considering the least represented

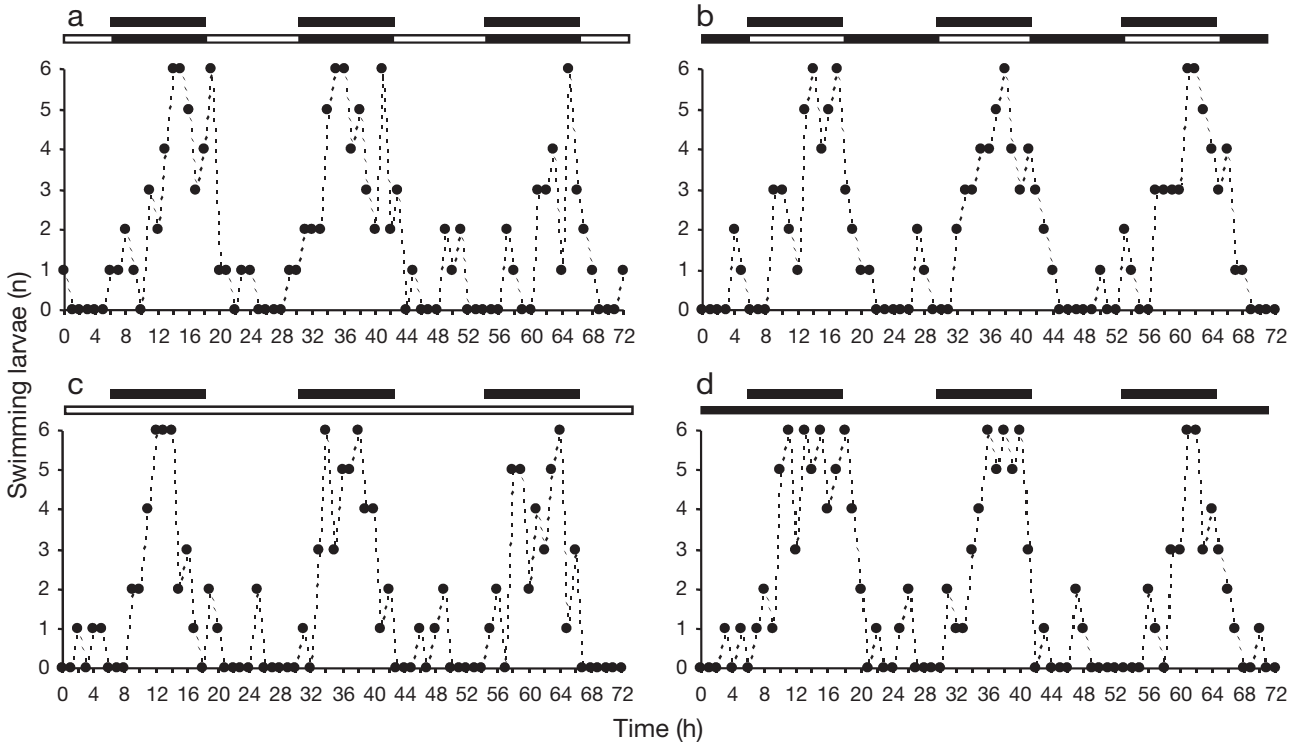

Fig. 3. *Concholepas concholepas.* Seventy-two hour records of swimming behaviour in competent larvae during 4 different photoperiods: (a) natural light:dark cycle; (b) inverted light:dark cycle; (c) constant light; and (d) constant darkness. Observations were for 72 h at 1 h intervals using 6 newly captured competent larvae in each treatment, with 1 larva assigned to each test tube. The horizontal white and black bars immediately above the graphs represent hours of light and darkness, respectively, according to the treatment. The 3 horizontal and black bars at the top of the figure represent the natural nighttime hours

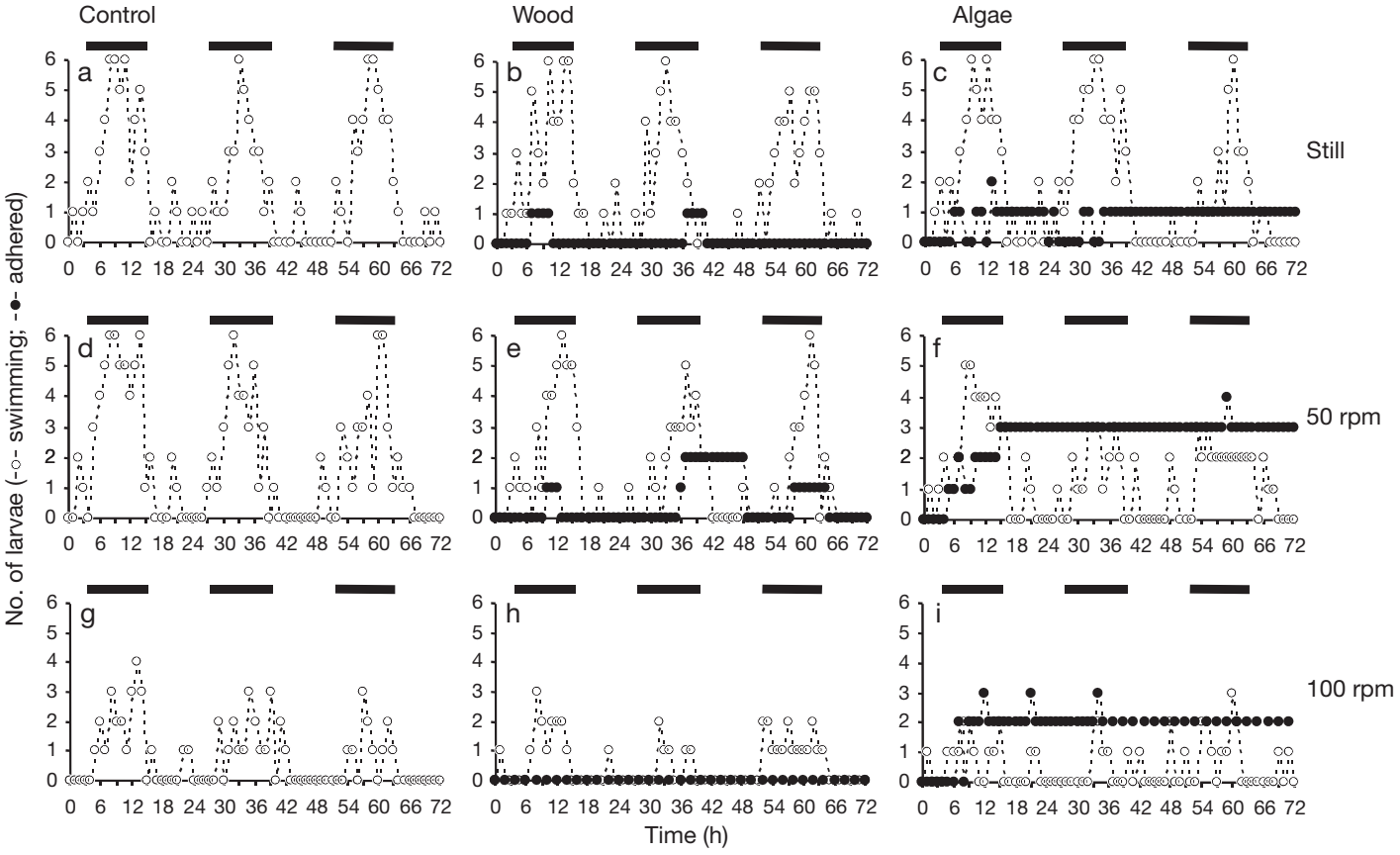

Fig. 4. *Concholepas concholepas.* Expt 3: effect of water movement and the presence of floating objects on the swimming behaviour of competent *C. concholepas* larvae (CCL). Behaviour was assessed by placing CCL, 1 individual randomly assigned to each tube, in the water columns of test tubes. Water movement was simulated by placing the test tubes on an orbital shaker: (a–c) still; (d–f) 50 rpm; and (g–i) 100 rpm. The presence/absence of floating objects was as follows: (a,d,g) 0.45 µm filtered seawater only (Control); (b,e,h) small pieces of floating wood (Treatment 1); and (c,f,i) small fragments of *Plocamium cartilagineum* suspended in the centre of the tubes with the aid of a transparent monofilament line (Treatment 2). White circles are swimming larvae and black circles are larvae attached to floating objects. The 3 horizontal and black bars at the top of the figure represent the natural nighttime hours

behaviour (GC), the behavioural responses were significantly different between water movement treatments (χ^2: 21.634, df = 6, $p < 0.0001$, Fig. 5). In general, in the absence of barnacles, swimming was the most recorded behaviour. In the presence of barnacles, however, crawling on the surface of the rocks was more frequently observed. Direct observations during the experiments detected the active participation of the CCL during the initial phase of mucus drifting, indicated by the secretion of a hyaline mucous thread through the pedal pore before the CCL were carried away by the water currents.

Expt 5. Settlement cues and swimming of CCL

Overnight, significantly more swimming displacement events by CCL were recorded per hour in the control than in the treatments with mussels or barnacles ($F_{2, 29} = 97.95$, $p < 0.001$, Fig. 6). On average, ca. 65 times more upward displacements were observed in the control. When not swimming at night and close to

Table 3. *Concholepas concholepas*. Expt 3: results of 2-way ANOVAs on the effect of presence of floating substrata and water movement on the number of adhered and swimming competent larvae. Numbers of larvae were averaged across the 72 h of observations in each treatment (see 'Materials and methods' for details)

	df	SS	*F*	p
Adhered larvae				
Substrate	2	401.86	918.38	<0.0001
Water movement	2	64.97	148.47	<0.0001
Substrate × Water movement	4	76.54	87.46	<0.0001
Swimming larvae				
Substrate	2	14.73	2.83	0.0597
Water movement	2	190.45	36.60	<0.0001
Substrate × Water movement	4	8.06	0.78	0.5418

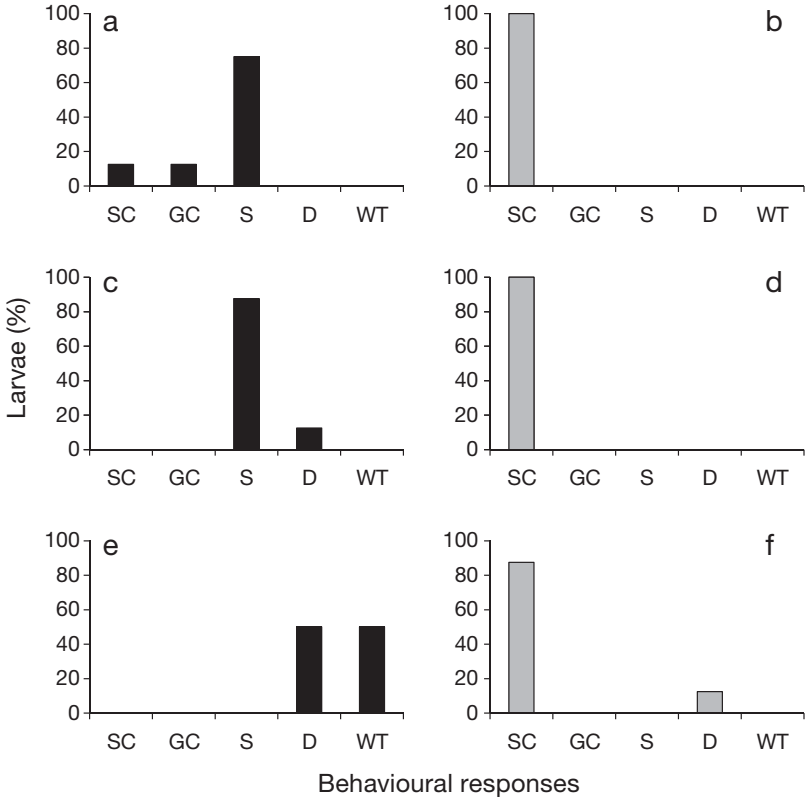

Fig. 5. *Concholepas concholepas*. Percentage of competent *C. concholepas* larvae (CCL) in the absence (black, left panels) and presence (grey, right panels) of settlement cues displaying different behaviours (SC: stone crawling, GC: glass crawling, S: swimming, D: drifting, WT: taking advantage of the water tension) under 3 water movement regimes (pivoting frequencies): (a,b) still, (c,d) low and (e,f) high. See Expt 4 in 'Materials and methods' for further details

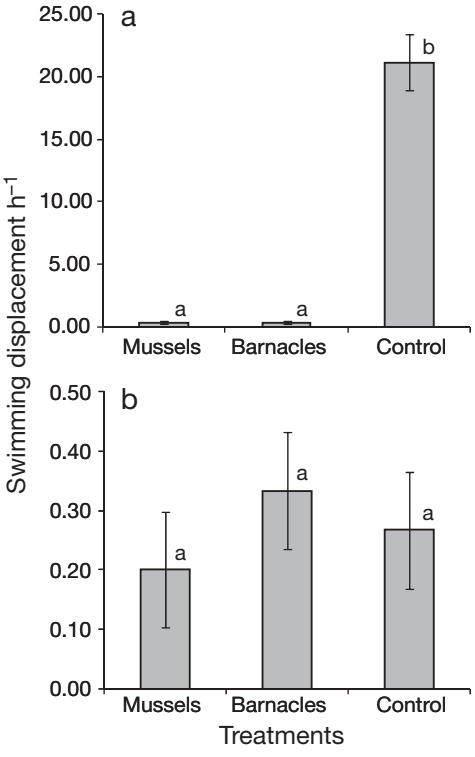

Fig. 6. *Concholepas concholepas*. Effect of the presence of the mussel *Semimytilus algosus* and the chthamaloid barnacle *Notochthamalus scabrosus* on the swimming displacements of competent larvae. Larval displacements were conducted during 3 h periods of (a) nighttime and (b) daytime. In the control treatment, no prey were included in the experimental arena. Bars with the same letters are not statistically different from one another

the water surface, CCL were observed crawling, resting on the surface of the presented prey, or swimming close to them on the bottom. During the daylight hours, swimming displacement was rarely observed, and no significant differences between the treatments and the control were found ($F_{2, 29} = 0.45$, $p > 0.05$, Fig. 6).

DISCUSSION

Natural abundance of CCL

Presence of CCL in the water column during the sampling months agrees with previous reports in the same geographic area (Poulin et al. 2002a,b). Similarly, the present study is in good agreement with the presence of egg capsules (Manríquez & Castilla 2001) and recruits (Martínez & Navarrete 2002) of the species in the same area before and after the *Concholepas concholepas* larval season. The high abundances of CCL found in inner-nearshore shallow waters, within ca. 0.5 km of the shore, during nocturnal tows suggest that our laboratory results are in agreement with observed nocturnal vertical migration. However, there is evidence that in deeper waters (ca. 40 to 100 m) and further offshore (ca. 1 to 5 km from the coast), this pattern of vertical migration and CCL abundance may be reversed, with higher abundances during diurnal hours (Poulin et al. 2002b).

CCL adhesion to floating substratum as potential rafting mechanisms

The ability of CCL to adhere to floating substrata and raft is supported by the following results: the positive correlation between the abundance of CCL and floating substrata in diurnal tows, higher abundances of CCL in inner nearshore foam slicks compared to non-slicks, the higher abundances of CCL on coastally

derived algal debris compared to clean water samples, and the capacity of CCL to adhere to buoyant substrata in laboratory conditions. In the literature, rafting generally involves passive transport with a low energy expenditure by post-larval stages, juveniles and small adults (Anderson 1971, Levinton 1979, Highsmith 1985, Martel 1988, Martel & Chia 1991a,b, Thiel & Gutow 2005a, Cañete et al. 2007). However, our results suggest that rafting may also be used by late larval stages such as CCL in nearshore environments to assist them in reaching the coast. Rafting by *Proclamium cartilagineum* is consistent with the capacity for rafting described for other species of algae (Edgar 1987, van der Merwe & McLachlan 1987, Thiel & Gutow 2005b, Vandendriessche et al. 2007). Under laboratory conditions, fresh fragments of *P. cartilagineum* maintained in running seawater and exposed to natural light regimes with seawater temperature ranging between 10 and 25°C began the degradation process after ca. 15 d, and total degradation occurred by ca. 30 d (P. H. Manríquez pers. obs.). This suggests that the time that CCL can spend rafting on fragments of *P. cartilagineum* will depend in part on the integrity of this substratum.

The ability of early post-settlers of *Concholepas concholepas* to feed on biofilm has been reported in the literature, even after active predation on live mussels has started (DiSalvo 1988). Therefore, it is possible that rafting CCL may switch from filter feeding (Vargas et al. 2006a) to feeding on small prey associated with the raft substrata or herbivory, as suggested by DiSalvo (1988). This is supported by the presence of a fully developed radula in CCL (P. H. Manríquez pers. obs.) and their high survivorship when maintained in FSW with fragments of *Proclamium cartilagineum* (present study). However, the potential for early herbivory in this carnivorous species needs further investigation. *P. cartilagineum* is a cosmopolitan species (Dixon & Irvine 1977), which occurs from the low intertidal down to a depth of 30 m (Lancellotti & Trucco 1993), and whose detached foliaceous branches are commonly found in inner nearshore coastal foam slicks or forming part of massive aggregations of coastally derived algae found off the coast of central Chile (P. H. Manríquez pers. obs.). Therefore, this substratum is widely available as potential raft substrate for other early stages of invertebrates. However, our study demonstrates that CCL adhere to biotic and abiotic substrata, suggesting that adhesion is not selective.

Off central Chile, it has been suggested that internal tidal bore warm fronts could play an important role in the onshore transport of neustonic invertebrate larvae (Vargas et al. 2004), a mechanism also described for other inshore environments (Pineda 1991, Largier 2002). Between 10:00 and 23:00 h and during the season when CCL were collected in central Chile, the predominant upwelling-favourable winds were primarily from the southwest–west; these push water both onshore and along-shore (Narváez et al. 2004). Therefore, surface currents with the potential for transporting floating substrata and the associated rafting organism to the coast may be an important physical transport mechanism. In the same vein, the wind-driven shoreward transport of CCL by Langmuir lines (Kingsford 1990) has also been suggested (Moreno et al. 1998). Additionally, we suggest that CCL may also be carried shoreward in surface foam slicks generated by tidally forced internal waves (Shanks 1983, 1985, 1986, Pineda 1991), adhered to buoyant substrata accumulated on frontal foam lines (Shanks et al. 2000). Similarly, high abundances of barnacle nauplii found at river plume fronts in central Chile suggest that in nearshore environments, transport of flume-aggregated larvae to onshore habitats can be enhanced by local factors (Vargas et al. 2006b).

The information available concerning other mucous-thread drifting gastropods (Lane et al. 1985, Martel & Chia 1991a,b) indicated the active participation of the foot-raising behaviour in the initiation of the drifting behaviour during post-metamorphic stages. However, our results expand this behaviour to larval stages. Foot-raising, mucous thread production and drifting behaviour has also been recorded under laboratory conditions in post-metamorphic stages of *Concholepas concholepas* associated with self-righting behaviour (P. H. Manríquez pers. obs.), suggesting that this behaviour might play an important role during the ontogenetic plankton-benthic transition, allowing a rapid relocation from unfavourable microhabitats or reducing the time spent by CCL in a vulnerable upside-down position after dislodgement.

Photoperiodicity, thigmotaxis and turbulence

Despite the limited evidence of endogenous rhythms in zooplankton (Forward 1988), our results showed a marked endogenous nocturnal rhythm in the swimming activity of CCL under several different laboratory conditions of photoperiod and water movement. This suggests the existence of an internal rhythm as has been described for other veligers that swim up into the plankton at night to feed, and passively sink below the photic zone during daytime. Such behaviour of reverse diel migration may be advantageous in reducing predation and exposure to harmful UV rays, and perhaps enables larvae to take advantage of lower metabolic costs at cooler depths. Withdrawal of the velar lobes and passive sinking, similar to that observed with anaesthetised CCL, was frequently observed in our

study. Gravitational fall speeds of the anaesthetised CCL with a withdrawn velum were several times greater than larval upward and downward swimming speeds. These differences have been interpreted as a mechanism that may allow veliger larvae to accumulate close to the bottom (Bayne 1964). By using this mechanism, CCL may be transported both passively and rapidly near the seabed and avoid offshore transport. According to our measurements of vertical swimming velocities, and despite interactions between small-scale turbulence and swimming, in shallow waters of ca. 20 m, CCL would need about 1 h to reach the water surface by exclusively swimming upwards. Therefore, in inner-nearshore environments, CCL would stay at the surface at night, reducing the risk of predation and enhancing wind or surface current dispersion towards the coastal habitats. In contrast, in offshore deeper water (ca. 1 to 5 km from the coastline), depending on the depth, CCL would theoretically need ca. 3 to 7 h to reach the water surface. Under such conditions, it is highly probable that CCL could not reach the surface by exclusively swimming upwards. Therefore, as suggested, upwelling events could occasionally move the CCL to the surface (Poulin et al. 2002b). In the laboratory, CCL kept in still and agitated seawater alternately swam up and sank, as has been described for other veligers (e.g. Cragg & Gruffydd 1975, Hidu & Haskin 1978). This highlights the fact that gravity is an important vector cue for the orientation of CCL.

Although laboratory conditions used in our experiments are highly artificial (i.e. water agitated with air bubbles), our results clearly show that under such conditions CCL are able to cling onto floating substratum both in still water and under moderate water movement. The negative effect of high water movement on CCL swimming mirrors the sinking behaviour found in competent larvae of other coastal gastropods, such as *Ilyanassa obsoleta,* exposed under laboratory conditions to water turbulence (Fuchs et al. 2004, 2007). This suggests that through sinking, CCL reaching turbulent and coastal shallow waters may improve their chances of settling by descending closer to the benthic substrata favourable for settlement and metamorphosis.

CCL settlement cues and larval swimming

Our study also showed that the pattern of swimming activity described above was not evident when settlement cues were present. The effect of mussels and barnacles on swimming and triggering settlement confirms the observations of previous studies (DiSalvo & Carriker 1994, Manríquez et al. 2004, 2009). However, the effect of depth on larval displacement in *Concholepas concholepas* should be investigated further. Therefore, if CCL behave in nature as in the laboratory, we suggest that larval behaviour might differ depending on the presence or absence of settlement cues and the water depth.

CCL mucous thread and water movement

Our observations in the upward current experiment (Expt 3) allowed us to observe the production of mucous threads by CCL and their attachment to fragments of floating alga. This ability of CCL to produce a mucous thread and entrap air bubbles confirms the observations of previous studies with CCL (DiSalvo 1988). Similar records have been described for bivalves and other gastropods regarding production of mucous thread and upward transport by turbulence and determining the secondary drifting of an infaunal bivalve (Verwey 1966, Lane et al. 1985, Beukema & de Vlas 1989, Martel & Chia 1991a,b, Nozais et al. 1997, Wang & Xu 1997). Therefore, it appears that the use of a mucous thread is a common phenomenon in veliger larvae and may be important for onshore larval transport. In CCL this ability seems to be important up to the point where settlement-inducing cues trigger their persistence near the bottom. This information may be useful in the design of collecting devices, which could contain natural settlement cues, such as nearshore floating cages that would collect CCL travelling in the water column or cages deployed in the subtidal or low rocky intertidal to collect CCL which reach the benthic environment. This highlights the role of behavioural traits during the ontogenetic niche-shift from planktonic to benthic existence in marine invertebrates with complex life cycles, such as *Concholepas concholepas*. Finally, we suggest that these traits should be incorporated into the methods used to capture CCL whether for research purposes, i.e. in order to understand their recruitment variability, or for the initiation of aquaculture or natural restocking.

Note that while this manuscript was being edited for resubmission, ca. 2800 CCL were collected during 3 days in late September of 2010 at 2 locations in Antofagasta Bay associated with nearshore foam slicks. Since our surface plankton net was broken, larval sampling was made using a heavy-duty deep swimming pool leaf net (800 µm mesh) connected to a plastic pole to collect the floating material on top of the water (ca. 20 cm), while the boat was immobile alongside the foam slick. Since no CCL were collected in nearby water without debris and foam, this evidence reinforces our conclusions regarding the importance of nearshore mechanisms of transport and larval behaviour in *Concholepas concholepas*.

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Interactive effects of losing key grazers and ecosystem engineers vary with environmental context

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ABSTRACT: Loss of biodiversity may cause significant changes to ecosystem structure and functioning. Evidence from long-term *in situ* removal experiments is rare but important in determining the effects of biodiversity loss against a background of environmental variation. Limpets and mussels are thought to be important in controlling community structure on wave-exposed shores in the UK: limpets as key grazers, mussels as ecosystem engineers. A long-term factorial removal experiment revealed interactive effects that varied between 2 shores in SW England. At one site (Harlyn), removing limpets caused a significant shift in community structure, but where limpets were lost, the presence or absence of mussels made little difference. Where limpets were present, however, the removal of mussels changed the structure and variability of the community. At the other site (Polzeath), the loss of mussels caused significant changes in community structure, and limpets played a less important role. At Harlyn, fucoid algae were abundant throughout the year. There were fewer algae at Polzeath, and cover was dominated by the summer bloom of ephemerals. At Harlyn, the limpets played a major role in controlling algae, but their effects were mediated by the presence of mussels. Other grazers were not able to fulfil their role. At Polzeath, mussels were far more important, and ephemeral algae grew on them regardless of the presence or loss of limpets. These findings emphasise the need to assess spatial and temporal variation in the effects of biodiversity loss and the importance of interactive effects of loss of multiple species from different functional groups.

KEY WORDS: Biodiversity · Functional groups · Spatial variation · Long term · Removal experiment · Limpets · Mussels · Interactive effects

INTRODUCTION

Extensive changes in biodiversity have been widely documented (Millennium Ecosystem Assessment 2005). The effects of changing biodiversity on ecosystems remain unclear despite extensive research into biodiversity–ecosystem functioning (BEF) relationships (Millennium Ecosystem Assessment 2005, Naeem et al. 2009). Concerns have recently been raised that many BEF experiments to date have been too short to properly characterise the effects of diversity loss, particularly in marine ecosystems (Stachowicz et al. 2008a,b). Laboratory- or mesocosm-based manipulations need to be supplemented by field-based removal experiments to ensure that general models derived from such studies are realistic (Díaz et al. 2003, Stachowicz et al. 2008a,b, Crowe & Russell 2009). The extent of spatial and temporal variation in consequences of biodiversity loss is also unclear and must be more fully characterised if an effective predictive framework is to be developed (Cardinale et al. 2000).

In seeking to improve prediction of effects of diversity loss, there has been a recent shift in emphasis from species-level diversity to functional diversity (Crowe & Russell 2009), such that the functional traits of taxa (e.g. feeding modes, habitat provision) are explicitly taken

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into account when considering impacts of their loss (Stachowicz et al. 2007, Mouchet et al. 2008, Griffin et al. 2009, O'Gorman et al. 2010). The loss of key taxa or strong interactors (Hurlbert 1997) within functional groups is likely to have a disproportionate effect on ecosystem structure and functioning. Although the functional role of key taxa can sometimes be replaced by other members of the group, longer-term studies have demonstrated their importance in the face of changing environmental circumstances (O'Connor & Crowe 2005). Combined effects of losing multiple key taxa from different functional groups are likely to be substantial and also interactive, such that the effects of losing 1 species depend on the presence or absence of others. BEF experiments have rarely been designed to test for the presence of such interactions. The widespread occurrence of interactive effects would have significant consequences for the predictability of effects of loss of diversity on ecosystem function, particularly if the nature of interactions varies with spatial and temporal environmental heterogeneity (Stachowicz et al. 2008a).

Although documented global extinctions are rare in the marine environment, local extinctions and dramatic changes in abundance are widespread (e.g. Airoldi & Beck 2007, Stachowicz et al. 2007). Rocky shore ecosystems are highly productive and can be a significant source of detrital material underpinning coastal food webs (Whittaker 1975, Raffaelli & Hawkins 1996). Many are characterised by the presence of strongly interacting species (Allison et al. 1996; sensu Hurlbert 1997). They also lend themselves to long-term experimental manipulation (Connell 1974, Paine 1977), particularly removal experiments (Díaz et al. 2003), and have already contributed significantly to the BEF debate (Allison et al. 1996, Stachowicz et al. 2007). On rocky shores in the Northeast Atlantic, both limpets (Southward 1964, Hawkins 1981, Hawkins & Hartnoll 1983, Jenkins et al. 1999a, 2008, Coleman et al. 2006) and mussels (Seed 1996) are thought to have key roles in driving ecosystem structure and functioning, although the relative importance of their respective roles is yet to be established (Hawkins et al. 1992).

Limpets regulate algal recruitment by grazing the early stages of macroalgae contained within epilithic microbial films (Hill & Hawkins 1991) and in some cases direct consumption of mature algae (Davies et al. 2007). This has been demonstrated by the establishment of opportunistic and fucoid algae where limpets have been removed or excluded (Jones 1948, Southward 1964, Hawkins 1981, Jenkins et al. 1999a, 2008). In the absence of key species, availability of food for other grazers (such as littorinids and trochids) may increase (Cubit 1984, Dye & White 1991, Mak & Williams 1999), perhaps leading to increases in their abundance. Even if their numbers increase, however, they may or may not be capable of controlling algal growth as effectively as limpets (O'Connor & Crowe 2005). The effect of herbivory on diversity of primary producers remains controversial (Olff & Ritchie 1998). High grazing pressure seems to reduce algal diversity while moderate grazing pressure can increase it (Paine & Vadas 1969, Lubchenco 1978, Anderson & Underwood 1997, Aguilera & Navarrete 2007). There is also evidence that plant diversity may depend more on spatial heterogeneity and variance in grazing pressure than its mean intensity (Olff & Ritchie 1998, Benedetti-Cecchi 2000, Sommer 2000).

Mussels have been described as foundation species or ecosystem engineers (Jones et al. 1994, Lawton 1994) because they modify their environment, changing its suitability for other organisms. Intertidal mussel populations provide a biogenic structure for a diverse array of species including annelids, crustacea and other molluscs (Lohse 1993, Seed 1996, Crowe et al. 2004). The architectural complexity of mussel shells decreases the influence of wave action, temperature and sunlight while increasing relative humidity and sedimentation (Sebens 1991). The biological activities of living mussels, such as filter feeding and biodeposition, can also affect biota (Crooks & Khim 1999).

There is also considerable potential for mussels and limpets to interact in their effects on community structure, but the nature of such interactions may not be easy to predict. By providing habitat for other grazing gastropods (Lohse 1993), mussels could reduce the effects of loss of limpets by increasing the likelihood that other grazers will colonise the area. On the other hand, by providing refuges for algal propagules from both physical stress (Hruby & Norton 1979, Vadas et al. 1990, Brawley & Johnson 1991) and grazing pressure, they could enhance algal cover and reduce the effects of gastropod grazing (Hawkins & Hartnoll 1982, Lubchenco 1983, Witman 1985, Chapman 2000, Wahl & Hoppe 2002). Nevertheless, grazing marks found on and amongst mussels imply that at least some grazing does occur on this substratum (Lohse 1993, O'Connor & Crowe 2008).

Here we report a long-term field-based removal experiment designed to test the following hypotheses:

(1) Losses of key species from different functional groups (limpets as key grazers, mussels as ecosystem engineers) will each cause changes in ecosystem structure. Effects of loss of combinations of these species may be interactive. In particular:

(a) Other grazers will increase in abundance in response to loss of limpets, an effect which may be mediated by changes in habitat availability caused by loss of mussels.

(b) Cover and composition of macroalgal assemblages will change in response to changes in grazing

pressure and availability of refuges provided by mussels.

(2) Individual and interactive effects of loss of key species will vary spatially and through time.

MATERIALS AND METHODS

Study sites. The research was done at Harlyn Bay and Polzeath, 2 sites on the north coast of Cornwall in SW England separated by 6.5 km (50° 34′ 50″ N, 4° 55′ 28″ W and 50° 32′ 36″ N, 4° 59′ 33″ W, respectively). They are typical of the wave-exposed rocky shores on this coast. The shore at Polzeath consists mostly of gently sloping, slate bedrock platforms with only a few boulders and loose rocks. Despite the exposure of this site, its shallow gradient causes a reduction in wave energy. Harlyn Bay has a similar bedrock to that of Polzeath, with a higher degree of wave exposure. At each site, mid-tidal levels were covered by a mosaic of mussels, fucoid algae and barnacles. In the experimental area, mussels (a mixture of *Mytilus edulis, M. galloprovincialis* and hybrids) dominated the primary space, covering on average 79 ± 1.98% (SE, n = 24) and 64 ± 2.0% (n = 24) of plots at Polzeath and Harlyn, respectively. Overall cover of algae was greater on average at Harlyn than at Polzeath (38 ± 4.2%, n = 24 versus 18 ± 4.5%, n = 24) at the start of the experiment (Fig. 1). The main foliose algae were *Fucus vesiculosus* var. *linearis*, *F. spiralis, F. serratus*, *Porphyra* spp., *Ulva lactuca* and *U.* (formerly *Enteromorpha*) *intestinalis*, and there were also some turf-forming and encrusting species, such as *Corallina officinalis*, *Gelidium* spp., *Lithothamnion* spp. and *Ralfsia* spp. A similar suite of algal species occurred at each site, but their relative proportions varied (see 'Results'). Densities of grazing gastropods were similar at each shore. The assemblage included limpets (*Patella vulgata*, *P. depressa* and *P. ulyssiponensis*), littorinids (*Littorina littorea*, *L. mariae*, *L. obtusata*, *L. saxatilis*) and trochids (particularly *Gibbula umbilicalis* and some *Osilinus lineatus*).

Experimental design. To investigate the individual and combined effects of losing limpets and mussels, a factorial design was used. The treatments were as follows: + limpets, + mussels (+L+M); + limpets, – mussels (+L–M); – limpets, + mussels (–L+M); – limpets, – mussels (–L–M). There were 4 replicate plots per treatment. As part of a complementary study, 4 additional plots were initially set up for each treatment. These were destructively sampled after 11 mo. Eight replicate plots were therefore available for the first 11 mo of the experiment and 4 were available thereafter.

Procedures. In October 1998, plots measuring 0.5 × 0.5 m were chosen at each site, marked and randomly assigned to each of the 4 treatments. All but 5 of the plots had >60% cover of mussels, and all were separated from each other by a minimum of 1.5 m. Mussels were removed from the appropriate plots by chiselling. Care was taken to avoid damage to other organisms attached to the rock surface itself. Limpets were removed manually from the relevant plots and from a surrounding buffer zone 0.25 m wide to reduce reinvasion. They were prised from the substratum using screwdrivers every month for the duration of the experiment. Any mussels reinvading plots were also removed, but such reinvasions were rare.

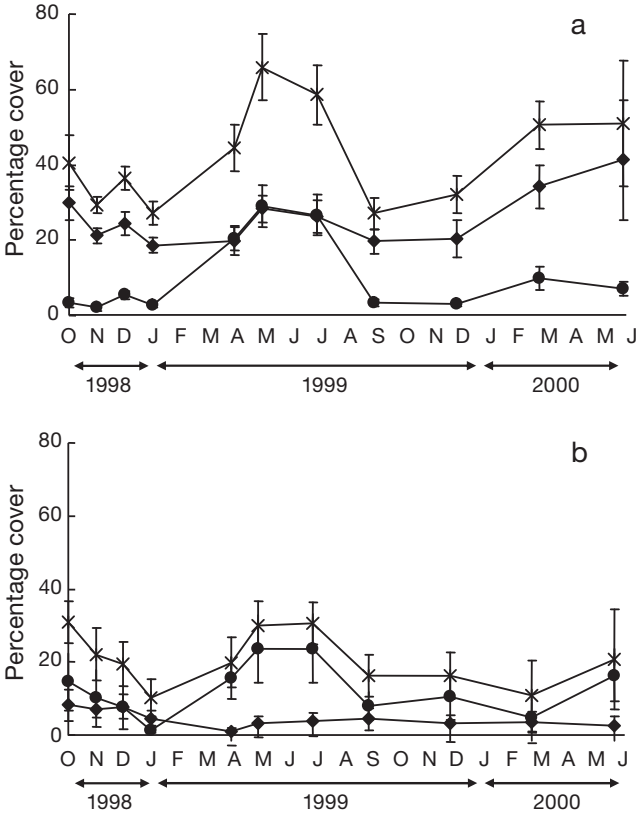

Fig. 1. Percentage cover of fucoid algae (◆), ephemeral algae (●) and all algae combined (×) in control plots (+ limpets, – mussels) throughout the experiment at (a) Harlyn and (b) Polzeath. (shown are means ± SE; n = 8 until September 1999, n = 4 thereafter)

The experiment was monitored monthly for the first 3 mo after initiation and every 3 mo after that for a total duration of 20 mo. Plots were monitored using a 0.5 × 0.5 m quadrat, strung to provide 49 intersection points for estimates of percentage cover. The following variables were recorded: (1) overall percentage cover of fucoid algae, (2) after moving fucoids aside, percentage cover of sessile organisms (particularly mussels and barnacles), other algal species and *Fucus* germlings (defined as *Fucus* plants <2 cm long). The point of attachment (barnacle/rock or mussel shell) of algae and sessile fauna was noted in each case. Sessile spe-

cies that did not occur under an intersection were recorded as present and assigned 0.5% cover in the analyses; (3) abundances of mobile species (mainly gastropods). Again, associations with mussels or bare rock were noted. Individuals within 0.5 cm of a mussel shell were considered to be associated with mussels. Littorinids on algae were considered associated with mussels if the alga they were on was attached to a mussel shell. Limpets <1.5 cm long were recorded as 'juvenile limpets'. In October 1999, to provide a more detailed analysis of the role of mussels as a refuge for *Fucus* germlings, the numbers of germlings growing on mussels and on bare rock in each plot were counted.

Analyses. To guide formal analyses, changes in algal cover in unmanipulated controls (+L+M) were examined over the 20 mo duration of the experiment. There was a distinct summer peak in total algal cover at each of the sites (Fig. 1). This was driven largely by variations in cover of ephemeral/green algae (*Porphyra* spp., *Ulva lactuca* and *U. intestinalis*). At each site, cover of ephemeral algae rose from <10% in winter and spring to >20% in summer (May to July). At Harlyn, there was also a moderate cover (~30%) of fucoid algae (*Fucus vesiculosus evesiculosus* and *F. spiralis*) throughout the year (Fig. 1). At Polzeath, there was very little fucoid algal cover (mean <10%) at any time (Fig. 1). Any influence of treatments on algal cover during the summer peak was therefore most likely to exert a strong influence over the export of macroalgal detritus from the shores. Therefore, the main analyses focussed on representative times from the summer peak in each of July 1999 and June 2000.

Non-metric multidimensional scaling (nMDS) was used to assess the impact of the treatments on overall community structure (excluding limpets and mussels). nMDS is an ordination technique based on rank dissimilarity, in this case measured with the Bray-Curtis index on square-root transformed data (Clarke 1993). SIMPER analysis in the PRIMER package was used to assess which species were most influential in causing similarity among plots within treatments and dissimilarity among different treatments (Clarke & Warwick 1994). Permutational multivariate analysis of variance (PERMANOVA, McArdle & Anderson 2001, Anderson 2005) was used to test hypotheses of differences in community structure. Analyses of data from each of the 2 selected dates were based on Bray-Curtis similarities of square-root transformed data. Factors were Site (2 levels, random), Limpet (2 levels, fixed, orthogonal) and Mussel (2 levels, fixed, orthogonal). Separate analyses were done for each sampling occasion.

To test hypotheses about the responses of grazers other than limpets to the experimental removals, a series of analyses of variance were done on data derived by combining abundance of all grazers other than limpets (*Littorina littorea*, *L. saxatilis*, *L nigrolineata*, *L. obtusata/mariae*, *Gibbula umbilicalis*, *Osilinus lineatus*, *Melaraphe neritoides*). The count data were $\ln(x+1)$ transformed prior to analysis. Factors were Site (2 levels, random), Limpet (2 levels, fixed, orthogonal) and Mussel (2 levels, fixed, orthogonal). Separate analyses were done for each of the 10 sampling occasions after manipulation to avoid non-independence. Homogeneity of variance was tested with Cochran's test. Post-hoc pooling was used as appropriate to maximise the power of tests of relevant terms (Underwood 1997). The Student-Newman-Keuls (SNK) procedure was used for post hoc multiple comparisons (Underwood 1997).

Analysis of variance was used to test effects of the treatments on cover of fucoid algae (*Fucus vesiculosus evesiculosus*, *F. spiralis* and *F. serratus*), cover of ephemeral algae (*Porphyra* spp., *Ulva lactuca* and *U. intestinalis*) and total algal cover (including turf-forming and encrusting species). The model and procedures used were the same as those used for grazers (described above). The same analysis was applied to counts of *Fucus* germlings made in October 1999.

Associations of *Fucus* germlings with mussels and bare rock in the presence and loss of limpets were tested using chi-squared analysis based on counts made in October 1999. For each plot, the number of germlings on mussels and the number of germlings on bare rock were counted. Percentage covers of these substrata were used to generate expected distributions based on random chance. Separate analyses of observed versus expected frequencies were completed for combined data from all plots assigned to (1) +L+M and (2) –L+M.

RESULTS

Manual removals of limpets were effective in maintaining considerably reduced densities and hence grazing pressure. Averaged across the experiment as a whole, sampled 10 times, limpet removals at Harlyn reduced limpet density from 34.0 ± 1.40 m^{-2} (SE, n = 165) to 7.6 ± 0.65 m^{-2}, and at Polzeath, the reduction was from 43.7 ± 2.20 m^{-2} to 9.0 ± 0.98 m^{-2}. At each site, densities were reduced by approximately 80%.

Multivariate community structure

At Harlyn, the loss of mussels and the loss of limpets had significant effects on community structure on both sampling occasions (Fig. 2, Tables 1 & 2). The effect of limpets was more marked than that of mussels, partic-

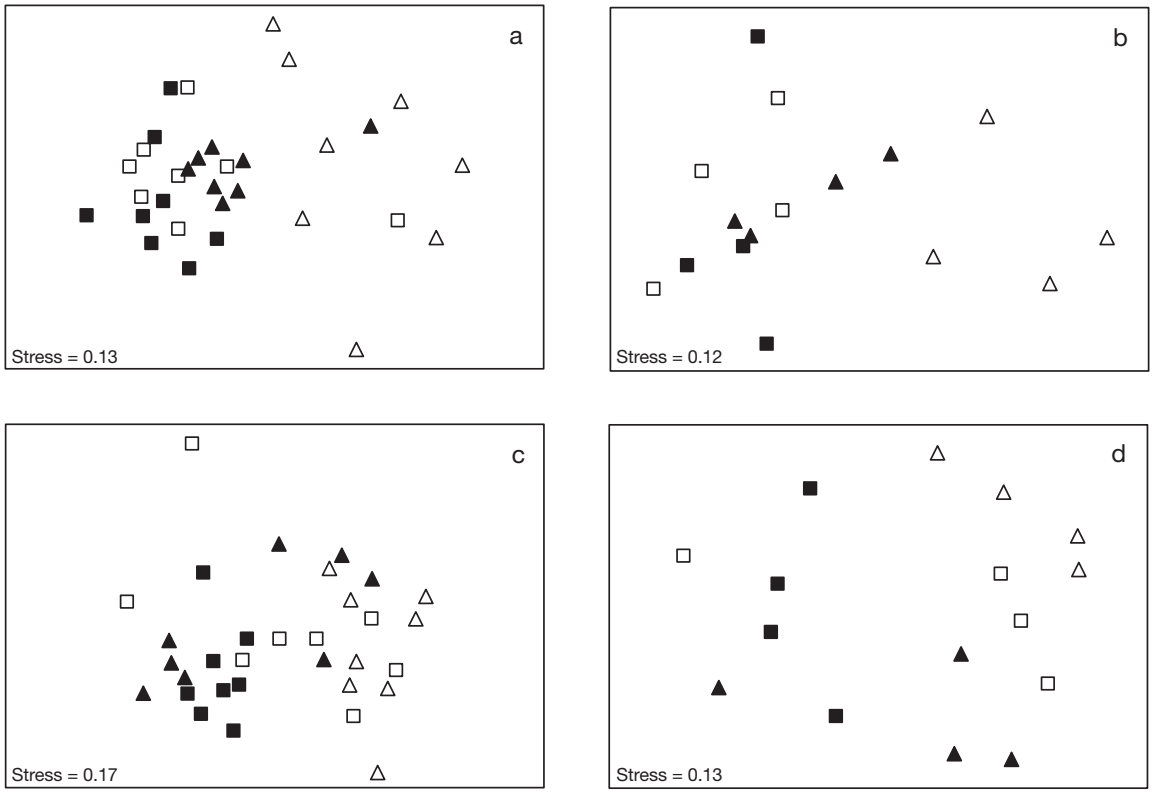

Fig. 2. Non-metric multi-dimensional scaling (nMDS) representations of community compositions in the experimental plots in (a) July 1999 at Harlyn, (b) June 2000 at Harlyn, (c) July 1999 at Polzeath and (d) June 2000 at Polzeath. ▲: + limpets, + mussels; △: + limpets, – mussels; ■: – limpets, + mussels; □: – limpets, –mussels. For (a) and (c), n = 8, for (b) and (d), n = 4

ularly in July 1999, after 9 mo, but also in June 2000, after 20 mo (Fig. 2). The most dramatic shift in community structure was caused by the removal of mussels from plots at which limpets were left in place (Fig. 2a). These plots also became considerably more variable than those in other treatments (see widely spread points, Fig. 2a). Mussels and limpets did not have interactive effects on community structure (Table 1). At the end of the experiment, plots without limpets were distinct from plots with limpets due to increases in fucoid algae and *Ulva intestinalis* and reductions in barnacle cover (Table 2). Those species also contributed most to the dissimilarity of plots with and without mussels, with presence of mussels causing increased cover of algae and reduced cover of barnacles (Table 2).

At Polzeath, the loss of mussels had a more consistent effect on community structure than did the loss of limpets (Fig. 2, Tables 1 & 2). In June 2000, the presence or loss of limpets had no discernible effect (Fig. 2d, Table 1, pairwise post hoc comparisons). As at Harlyn, barnacles were again important in contributing to dissimilarity among treatments and again tended to have greater cover where limpets were absent or mussels were present (Table 2). Other taxa influencing multivariate patterns included *Ulva intestinalis* and *Fucus vesiculosus* var. *linearis*, which, although rare, were strongly associated with mussels and the loss of limpets and *Porphyra*, which was exclusively associated with mussels (Table 2).

Table 1. Results of PERMANOVA analyses for July 1999 and June 2000. Analyses were based on Bray-Curtis similarities of square-root transformed data. $^{*}p < 0.05$, $^{**}p < 0.01$ (based on Monte Carlo simulations)

Source	df	July 1999		June 2000	
		MS	Pseudo-*F*	MS	Pseudo-*F*
Site (S)	1	16977.0	13.41**	9450.7	8.58**
Limpet (L)	1	10992.0	7.02*	4809.9	1.85
Mussel (M)	1	9678.8	2.08	6823.4	6.14*
S × L	1	1566.9	1.24	2593	2.35*
S × M	1	4651.4	3.68**	1110.7	1.01
L × M	1	2335.4	1.41	2042.2	0.92
S × L × M	1	1658.2	1.31	2213.9	2.01
Residual	56/24[a]	1265.5			

[a]There were 56 df for the residual in July 1999 and 24 in June 2000 (see 'Materials and methods: Experimental design')

Table 2. SIMPER analyses for June 2000 corresponding to significant PERMANOVA results. Listed are the 5 species in each case that contributed most to dissimilarity between groups of treatments. Abundance data shown were untransformed so that abundances/percentage covers were interpretable. The SIMPER analyses presented were based on square-root transformed data to correspond with the PERMANOVA presented in Table 1. Avg. abund: average abundance or cover (untransformed); Avg. diss: average dissimilarity among pairs of samples in terms of the species in question; Diss/SD: a measure of variation in the contribution of the species to dissimilarities between pairs of samples; Contrib%: percentage contribution of the species to the average overall dissimilarity between groups of treatments; Cum.%: cumulative contribution of the listed species

Species	Avg. abund With	Avg. abund Without	Avg. diss	Diss/SD	Contrib%	Cum%
With versus without limpets at Harlyn (average dissimilarity 65.73)						
Fucus vesiculosus var. *linearis*	11.73	41.58	12.52	1.22	23.47	23.47
Fucus spiralis	10.20	26.02	12.03	1.34	22.56	46.03
Barnacles	21.43	7.53	5.74	1.11	10.77	56.80
Ulva intestinalis	3.57	7.78	5.69	1.05	10.66	67.46
Gibbula	1.88	1.50	3.02	1.25	5.65	73.11
With versus without limpets at Polzeath (average dissimilarity 63.51)						
Barnacles	34.18	20.15	30.23	1.27	47.59	47.59
Ulva intestinalis	2.42	8.04	10.02	1.11	15.77	63.36
Unidentified alga	3.06	0.64	5.33	0.67	8.39	71.76
Fucus vesiculosus var. *linearis*	1.28	4.34	5.17	0.80	8.14	79.90
Porphyra spp.	2.81	2.04	4.67	0.78	7.35	87.25
With versus without mussels, both sites combined (average dissimiliarity 60.73)						
Fucus vesiculosus var. *linearis*	16.96	12.50	11.40	1.40	18.76	18.76
Barnacles	10.91	30.74	9.64	1.16	15.88	34.64
Ulva intestinalis	9.63	1.28	8.13	1.30	13.38	48.02
Fucus spiralis	11.86	6.25	7.63	0.75	12.56	60.58
Porphyra spp.	3.25	0.00	4.27	0.77	7.04	67.62

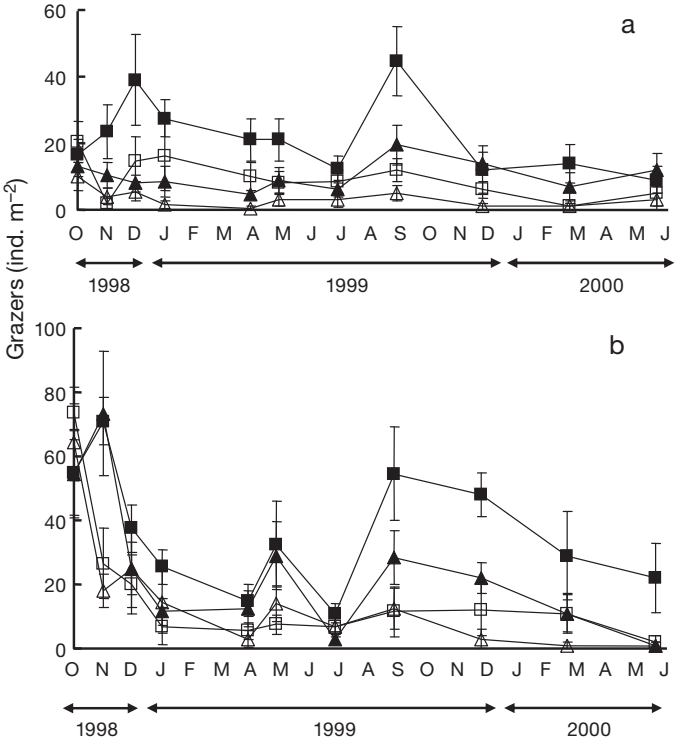

Fig. 3. Number of grazers other than limpets in plots throughout the experiment at (a) Harlyn and (b) Polzeath. ▲: + limpets, + mussels; △: + limpets, – mussels; ■: – limpets, + mussels; □: – limpets, –mussels; shown are means + SE; n = 8 until September 1999, n = 4 thereafter

Other grazers

Over the course of the experiment, more than 90% of the grazers other than limpets were topshells *Gibbula umbilicalis*, but littorinids, such as *Littorina littorea*, *L. obtusata* and *L. mariae*, were also found. Their combined numbers varied considerably through time (Fig. 3). Nevertheless, they were consistently more abundant when mussels were present than when they were absent (Table 3). On several occasions, there were trends for increases in abundance of other grazers in response to loss of limpets (Fig. 3). July 1999 was the only occasion, however, on which loss of limpets caused detectable increases in abundance of other grazers (Table 3).

Macroalgae

At each of the sites, there were no clear differences in total algal cover in the different treatments for the first few months of the experiment (Fig. 4). The treatments began to diverge in spring 1999, about 6 to 7 mo after initiation. After that time, cover at Harlyn ranked consistently greatest in plots from which limpets had been removed, i.e. greatest in –L+M plots, second greatest in –L–M plots, third greatest in +L+M plots and least in +L–M plots (Fig. 4a). At Polzeath, cover tended to be greater in treatments with mussels than in treatments without mussels; the influence of limpets was secondary (Fig. 4b). The greatest separation between treatments corresponded to the summer peak of algal cover, particularly at Polzeath (Fig. 4).

In July 1999, the removal of mussels and of limpets had significant effects on cover of fucoids at Harlyn, but not at Polzeath (Table 4a.i: S × M); at Harlyn, cover of fucoids was greater in the presence of mussels and where limpets had been lost (Fig. 5a; SNK procedure, $p < 0.01$). There were very few fucoids at Polzeath, and cover was not affected by the treatments (Fig 5c; SNK procedure, $p > 0.05$). Mussels and limpets had interactive effects on ephemeral algae that varied from site to site (Table 4a.ii:

Table 3. Analyses of variance of abundance of grazers other than limpets in December 1998, January 1999, April 1999, May 1999, July 1999, September 1999. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Source	df	Dec 1998		Jan 1999		Apr 1999		May 1999		July 1999		Sept 1999	
		MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Site (S)	1	5.14	6.12*	0.35	0.44	0.00	0.00	2.47	3.17	0.10	0.20	0.32	0.35
Limpet (L)	1	3.12	3.72	4.51	2.64	5.04	3.47	0.62	0.30	1.27	637.78*	1.56	1.70
Mussel (M)	1	6.70	8.18**	9.39	11.82**	6.58	12.14***	7.19	9.24**	0.41	203.19*	16.94	18.42***
S × L	1	0.62	0.74	1.71	2.15	1.45	2.68	2.10	2.70	0.00	0.00	0.59	0.64
S × M	1	0.04	0.05	0.00	0.00	0.02	0.04	0.00	0.00	0.00	0.00	0.29	0.31
L × M	1	1.66	1.98	2.66	3.34	0.00	0.00	0.36	0.46	0.93	0.87	0.25	0.28
S × L × M	1	0.03	0.04	0.45	0.56	0.03	0.05	0.01	0.02	1.07	2.09	0.44	0.48
Residual	56	0.87		0.81		0.56		0.81		0.15		0.95	

S × L × M): at Harlyn, ephemerals grew wherever mussels were present or limpets were absent, but did not grow where mussels had been removed and limpets left in place (Fig. 5a, SNK procedure, $p < 0.01$). Ephemerals grew most prolifically (mean cover 81%) where mussels were present and limpets had been removed (Fig. 5a); at Polzeath, ephemerals only grew abundantly where mussels were present, and were more abundant on mussels when limpets had been removed than when limpets had been left in place (Fig. 5c, SNK procedure, $p < 0.01$). The total cover of algae was affected by loss of limpets and by loss of mussels at each site (Table 4a.iii: S × L, M). The effect of loss of limpets was more pronounced at Harlyn (S × L, SNK procedure, $p < 0.01$) than at Polzeath (S × L, SNK procedure, $p < 0.05$).

The pattern of results was similar in June 2000, although cover of fucoids at Harlyn appeared greater than in 1999 and cover of ephemerals was reduced relative to the previous year at each site (Fig. 5). Again, fucoid algae were rare at Polzeath and unaffected by the treatments (Fig. 5d, Table 4b.i: S × L; SNK procedure, $p > 0.05$). At Harlyn, fucoids were affected by limpets, regardless of the presence of mussels (Fig. 5b, Table 4b.i: S × L; SNK procedure, $p < 0.01$). Cover of ephemerals depended entirely on the presence or absence of mussels at each site (Fig. 5b,d, Table 4b.ii: M). Total cover of algae at Harlyn depended on the combination of limpets and mussels present (Table 4b.iii: M, S × L; SNK procedure, $p < 0.01$). At Polzeath, which was dominated by ephemerals, only the loss of mussels had a significant effect on total algal cover (Table 4b.iii: M, S × L; SNK procedure, $p > 0.05$).

Role of mussels as refuges for *Fucus* germlings

Limpets and mussels had a strongly interactive effect on recruitment of *Fucus* germlings. In samples taken in October 1999, after 12 mo, germlings only occurred in plots with limpets when mussels were present, but were also found in plots from which mussels had been removed if limpets were absent (Fig. 6, Table 5). When limpets were present at Harlyn, disproportionately larger numbers of germlings were found on mussels within each plot than on bare rock ($\chi^2 = 140.4$, $p < 0.01$). Where limpets had been lost, however, patterns were more variable among plots, but a disproportionate number of germlings were generally found on rock ($\chi^2 = 46.9$, $p < 0.01$). At Polzeath, there were too few germlings for chi-squared analysis (Fig. 6).

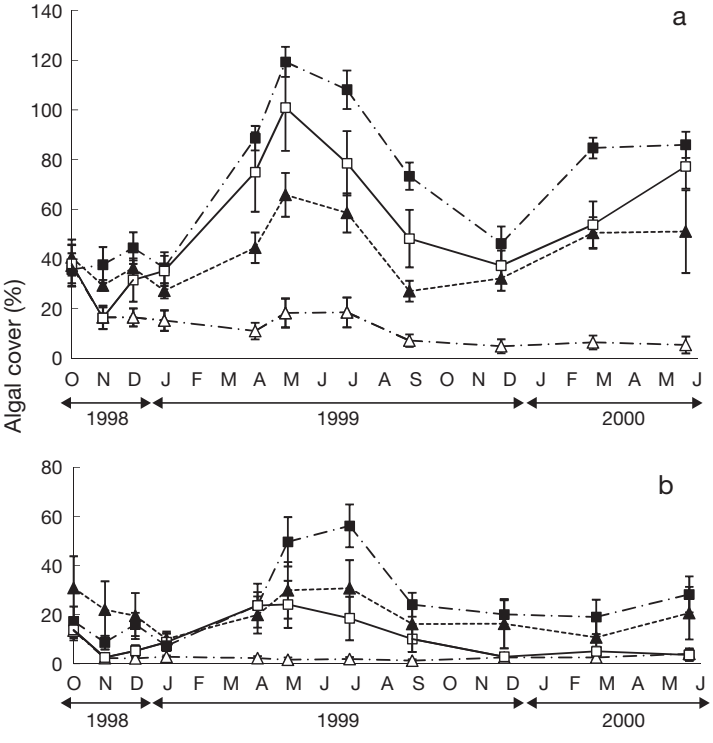

Fig. 4. Total percentage cover of algae in each treatment throughout the experiment at (a) Harlyn and (b) Polzeath. ▲: + limpets, + mussels; △: + limpets, – mussels; ■: – limpets, + mussels; □: – limpets, –mussels; shown are means + SE; n = 8 until September 1999, n = 4 thereafter

Table 4. Analyses of variance of algal cover in July 1999 and June 2000 for fucoid algae, ephemeral algae and all algae combined. *p < 0.05, **p < 0.01, *** p < 0.001

Source	df	(i) Fucoid algae		(ii) Ephemeral algae		(iii) All algae combined	
		MS	*F*	MS	*F*	MS	*F*
(a) July 1999							
Site (S)	1	11180.95	89.83***	5646.34	11.28**	24454.70	39.76***
Limpets (L)	1	1697.44	1.40	12559.09	14.0	23038.69	5.04
Mussels (M)	1	1914.28	2.09	34447.38	123.6	18557.93	1981.92*
S × L	1	1212.61	9.74**	896.91	1.79	4569.42	7.43**
S × M	1	913.85	7.34**	278.71	0.56	9.36	0.02
L × M	1	134.68	1.05	349.69	0.14	2.34	0.01
S × L × M	1	128.77	1.03	2414.07	4.82*	376.26	0.61
Residual	56	124.46		500.57		615.05	
Cochran		*C* = 0.2790		*C* = 0.2981		*C* = 0.2729	
(b) June 2000							
S	1	14087.23	50.18***	65.90	0.71	13286.95	46.14***
L	1	4748.01	16.91***	137.53	1.48	6501.42	22.58***
M	1	812.15	2.89	1394.18	15.02***	4575.90	15.89***
S × L	1	3629.73	12.93**	31.28	0.34	4974.53	17.28***
S × M	1	287.46	1.02	84.63	0.91	84.63	0.29
L × M	1	896.66	3.19	39.92	0.43	415.44	1.44
S × L × M	1	1174.79	4.18	9.42	0.10	1019.26	3.54
Residual	24	280.72		92.82		287.95	
Cochran		*C* = 0.4517*		*C* = 0.5987**		*C* = 0.4824*	

DISCUSSION

Our study comprised a comparatively simple experiment, focussed on 2 taxa noted for their strong, but different roles in rocky shore ecosystems. Its factorial design, however, enabled characterisation of interactive effects. By replicating it in space and extending it through time, valuable insights were gained into variation in effects of loss of key species. The sites selected were very similar to one another in physical terms and were not far apart (only 6.5 km), yet the effects of species loss varied considerably between them. Harlyn had greater algal cover, particularly of fucoids, and was more strongly affected by loss of limpets, whose influence was substantially modified by the presence of mussels. Polzeath's algal assemblage was

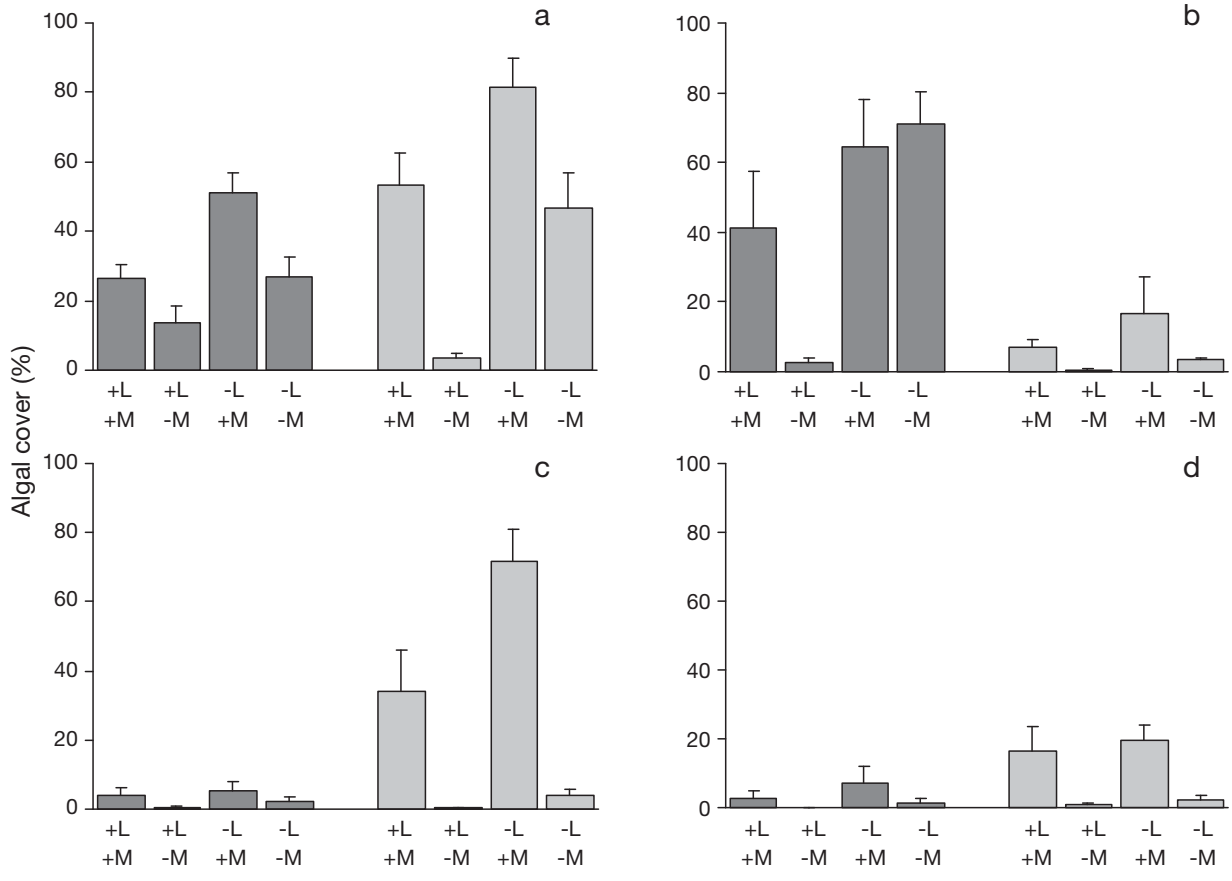

Fig. 5. Percentage cover of fucoid algae (dark shading) and ephemeral algae (pale shading) in plots at (a) Harlyn in July 1999, (b) Harlyn in June 2000, (c) Polzeath in July 1999 and (d) Polzeath in June 2000. Shown are means + SE. For (a) and (c), n = 8, for (b) and (d), n = 4. L: limpet; M: mussel; +: left in place; –: removed

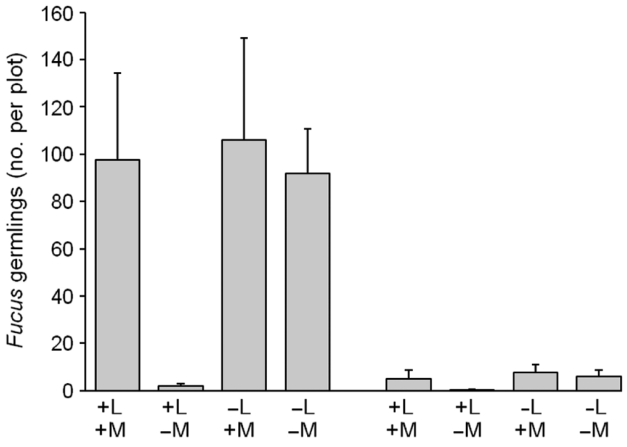

Fig. 6. *Fucus* spp. Abundance of germlings in plots at Harlyn and Polzeath in October 1999. Shown are means + SE; n = 4. L: limpets; M: mussels; +: left in place; –: removed

more dominated by ephemeral algae (particularly *Porphyra*) and was more strongly affected by loss of mussels than of limpets.

By sampling repeatedly over a 2 yr period, we were also able to recognise patterns of temporal variation in the natural and manipulated communities. Certainly, no effects of diversity loss were apparent during the first 6 to 7 mo of the experiment, and no significant effects would have been detected until 8 to 9 mo. This finding lends further weight to recent calls by Stachowicz et al. (2008a,b) for longer-term BEF experiments to accurately capture the responses of real ecosystems to changes in biodiversity. There was also some variation between Year 1 and Year 2, both in terms of natural algal cover and the influence of loss of limpets and mussels.

Limpets have here been shown again to be a dominant force within the grazer functional group on rocky shores (Lodge 1948, Branch 1981, Hawkins 1981, Jenkins et al. 1999a, O'Connor & Crowe 2005, Coleman et al. 2006). By using well-replicated manual removals in open plots and monitoring responses of all grazers, the current study was able to discriminate unequivocally between the roles of limpets and other grazers. Other grazers rarely increased in abundance in response to loss of limpets and were not able to compensate for their loss. It should be noted, however, that at Polzeath the influence of limpets was limited. Assemblages and cover of algae there were more strongly affected by the presence or absence of mussels. This may be explained in part by the low levels of natural cover of fucoid algae at Polzeath and lack of recruitment of canopy species (see also Coleman et al. 2006).

Where mussels were removed, the cover of barnacles (mainly *Chthamalus* spp.) increased. However, these alternative ecosystem engineers were unable to compensate in functional terms for the absence of mussels as they failed to prevent changes in algal cover and assemblage structure, despite their documented capacity to do so in the NE Pacific (Farrell 1991 and see Maggi et al. 2009 for a Mediterranean example of variation in influence of ecosystem engineers). Our study has shown that mussels can indeed play a key role on NE Atlantic shores, apparently providing a specialised habitat for some species of algae (e.g. *Porphyra* only occurred on mussels regardless of the presence or loss of limpets) and offering a refuge for algae from limpet grazing, with a consistent trend for increased algal cover in the presence of mussels. Mussels also modify physical conditions and offer a large surface area for attachment, and the value of complex microhabitats for algal recruitment has been documented by a number of authors (e.g. Norton 1983, Brawley & Johnson 1991). It is notable, however, that even within plots containing mussels, there was a significant increase in algal cover when limpets were removed, suggesting that limpets do indeed graze among mussels to some extent on these shores (see also Witman 1985, Lohse 1993, O'Connor & Crowe 2008). Although recruitment of fucoid germlings varied substantially between sites, the experiment provided clear evidence for the consistent roles of limpets in controlling their survival and mussels in providing a refuge for them. In the presence of limpets, germlings grew only on mussels (see also Jenkins et al. 1999a); in their absence, they were disproportionately abundant on rock. Changes in overall diversity and community structure caused by loss of mussels are underestimated in the current study because it did not include consideration of the interstitial fauna (Lohse 1993, Seed 1996).

It is not unusual to observe differences in interaction strength between species at different locations. Such discrepancies are a reflection of the differing abiotic and biotic conditions and the relative species abundances at each site (e.g. Farrell 1991, Kim 1997). In different habitats, grazers may have different effects

Table 5. *Fucus* spp. Analysis of variance of abundance of germlings counted in October 1999. Data were square-root transformed. Cochran's $C = 0.33$ (not significant). $^{*}p < 0.05$, $^{**}p < 0.01$

Source	df	MS	*F*
Site (S)[a]	1	142.63	12.55**
Limpet (L)	1	20.56	0.94
Mussel (M)	1	14.48	2.14
S × L[a]	1	21.98	1.93
S × M[a]	1	6.76	0.59
L × M[a]	1	61.48	5.41*
S × L × M[b]	1	13.06	1.15
Residual[c]	56	11.30	

[a]Tested over pooled $MS_{b+c} = 11.36$, 25 df

(Lubchenco 1983). Johnson et al. (1997) showed that limpets had an impact on algal recruitment at some sites on the Isle of Man but not all. Similarly, manipulations of grazer density and *Ascophyllum* on sheltered shores suggested that limpets played a very limited role in structuring the mid-shore community of sheltered shores (Jenkins et al. 1999b). This is in sharp contrast to the situation on more exposed barnacle-dominated shores of northwest Europe, where the ability of limpets to limit algal recruitment means that they are often the dominant structuring organism (Southward 1964, Southward & Southward 1978, Hawkins 1981, Hawkins et al. 1992).

While the interaction examined here was the role of limpet grazing and mussel refuges in determining algal abundance, on other coastlines, different interactions between grazers and refuges are apparent. On NW Atlantic coasts, where limpets are absent, mussels are thought to competitively exclude *Fucus* from mid-shore assemblages when biotic and physical disturbances are lacking (Menge & Sutherland 1976, Petraitis 1987, Chapman & Johnson 1990). In contrast, McCook & Chapman (1991) found that *Fucus* was competitively dominant to mussels on shores in Canada during primary succession after ice scour, when grazers were rare. The impact of grazing intensity on fucoids at the different locations may have caused the differences in these results (see also Jenkins et al. 2008). In the Mediterranean, Benedetti-Cecchi et al. (1996) found no evidence of interactions between limpets and mussels in determining algal abundance, and each species had independent effects that were consistent among locations.

The magnitude of change in algal cover caused by loss of grazers and/or mussels was substantial and would undoubtedly have a significant effect on ecosystem functions such as nutrient sequestration and export of detrital material from these shores to other coastal habitats. Although more sophisticated methodologies are now available for assessing ecosystem functioning *in situ* on rocky shores (e.g. Nielsen 2001, Martins et al. 2007, Noël et al. 2010), changes in macroalgal cover provide a meaningful indication of functional impacts with potential to affect provision of goods and services by the coastal environment. Sampling a range of functional variables, however, may yield different outcomes, which themselves vary in space and time (Duffy 2009).

Characterising species according to their functional roles and interaction strength has considerable potential to improve the generality of BEF models by accounting for apparently idiosyncratic variation due to so-called selection effects (Allison et al. 1996, Petchey 2004, Crowe & Russell 2009, O'Gorman & Emmerson 2009). Nevertheless, where combinations of strong interactors are lost, comparatively unpredictable interactive effects many have a major influence, as in the current study. To improve prediction of the effects of biodiversity loss, it is also necessary to characterise the factors underpinning variation in its consequences. In this case, initial variation in algal cover and assemblage structure prior to treatment appears to have been important, but these patterns may themselves have been driven by variation in the physical environment. Although theoretical models are valuable in stimulating new research pathways and synthesising existing findings, long-term experiments replicated in a wide range of environmental contexts are needed as an empirical basis for them (Stachowicz et al. 2008a,b, Boyer et al. 2009, Duffy 2009, Naeem et al. 2009).

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Population demographics of native and newly invasive populations of the green crab *Carcinus maenas*

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ABSTRACT: Green crabs *Carcinus maenas* (L.) are native to north-western Europe, but have been spread globally by humans during the last 200 yr. Reproductively viable populations have been present for <10 yr in British Columbia, Canada. In the present study, *C. maenas* were collected from 2 geographically separated locations, Anglesey (UK) and British Columbia (Canada), to compare body-size and colour distributions between native and newly invasive populations. Crabs were captured using baited traps and collected by hand at both intertidal and shallow subtidal elevations. Crabs from British Columbia were significantly larger than those from Europe. The largest male, 101.1 mm, and the largest female, of 85.4 mm carapace width, were both captured in British Columbia. The native populations showed a higher frequency of red-coloured crabs than the introduced population, which consisted predominately of green-coloured male crabs. Green-coloured integuments are typical of individuals in the early stages of intermoult. Accordingly, the high frequency of large, green-coloured *C. maenas* in British Columbia suggests that individuals in this population have an atypically high growth rate and achieve a larger body size and, hence, potentially greater fecundity. Moreover, the scarcity of small *C. maenas* in British Columbia may indicate that the existing population comprises only the first or second generation of recruits. The observed differences in body size and colour distribution are perhaps indicative of release from an as yet undetermined growth-limiting factor (possibly parasites) and provide a unique opportunity to study the dynamics of a newly invasive population as it recruits and matures.

KEY WORDS: *Carcinus maenas* · Crab · Distribution · Colour form · Moult stage

INTRODUCTION

The European green crab *Carcinus maenas* (L.) is native to northwest Europe and the western Mediterranean (Crothers 1968). Because of its tolerance of a wide range of environmental conditions, it has become a wide-spread invasive species, with the potential to outcompete and displace native crab species (Broekhuysen 1936, Grosholz & Ruiz 1995, Grosholz et al. 2000). *C. maenas* is also an important predator of molluscs, with the potential to affect the population size and structure of both natural populations (Edgell & Rochette 2008) and commercial farming operations (Jensen & Jensen 1985), for which it is considered a pest species.

With the increase in international trade during the last century, *Carcinus maenas* has been distributed around the globe, most probably via transfers of aquaculture species, such as oysters, and ship ballast water. Reproductively viable populations have been reported in Australia, South Africa and North America, while isolated specimens have been discovered in

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South America, SE Asia and Japan (Carlton & Cohen 2003). Specimens of *C. maenas* were first found on the west coast of North America in 1989, when a number of individuals were captured in San Francisco Bay (Cohen et al. 1995). In 1997, populations were discovered along the coast of Oregon and Washington (Behrens-Yamada & Hunt 2000). The first specimens in Canada were reported from Barkley Sound, British Columbia, in 1999; these 5 individuals were assumed to be stray recruits, and there was no evidence of an established population (Behrens-Yamada & Gillespie 2008). However, in 2006, several reproductive populations were discovered on the west coast of Vancouver Island (Gillespie et al. 2007). These individuals probably represented a cohort of crabs that settled in 2005, and evidence suggests that these populations may now persist in this area (Behrens-Yamada & Gillespie 2008).

In its native range, *Carcinus maenas* typically reaches a maximum size of 86 mm carapace width (CW) (Crothers 1968), although a single specimen of 100 mm has been reported from western Sweden (Behrens-Yamada 2001). The colour of its integument varies from a pale yellow-green through orange to a deep red-brown, which is most apparent on the underside and legs (Crothers 1968). The red-brown colour accumulates during a prolonged intermoult period or possibly at terminal anecdysis (Kaiser et al. 1990, McGaw et al. 1992, Reid et al. 1997). These red-coloured individuals are thought to represent the reproductive phase, whereas green-coloured individuals are actively moulting and resources are diverted toward growth (Reid et al. 1997, Wolf 1998, Styrishave et al. 2004).

Given that *Carcinus maenas* has only been established in Barkley Sound for a few years, we had a unique opportunity to study the demographics (size, sex and colour) of this population. While a number of studies have characterized the population spread of *C. maenas* in the Pacific Northwest (reviewed in Behrens-Yamada 2001, Behrens-Yamada & Gillespie 2008), they have not compared the size and colour distributions of a newly invasive cohort with those of native populations from Europe.

MATERIALS AND METHODS

Carcinus maenas were collected between April and September, primarily using baited net traps; however, because trapping can select for larger-sized individuals (Miller 1990, Ihde et al. 2006), we also supplemented trapping with hand collection (turning rocks in the intertidal and snorkeling) methods. Crabs were collected from their native range in 1989 and 1990 at 3 different sites in the Menai Strait, Anglesey, UK (53° 13′ 36″ N, 4° 09′ 24″ W; 53° 13′ 20″ N, 4° 10′ 17″ W; 53° 07′ 45″ N, 4° 18′ 31″ W), and at 3 sites in Barkley Sound, British Columbia, Canada, during 2008 and 2009 (49° 01′ 43″ N, 125° 18′ 16″ W; 49° 02′ 13″ N, 125° 19′ 54′ W; 49° 02′ 36″ N, 125° 09′ 36″ W). Because the trapping activities at the 2 locations were separated by a significant interval, with the potential for long-term effects associated with changes in local seawater temperature, additional trapping was carried out in the Menai Strait from December to April, 2009 to 2010, in order to determine whether any significant change in population demography had occurred over the 20 yr period. The collection methods and site topography were replicated as closely as possible between the UK and Canadian sites. Collections were made on 2 days each month, using 2 different sizes of trap at each site. In the UK, small cylindrical netlon traps of 0.5 cm mesh size were 60 cm in length and 30 cm diameter, with 2 entrances of 7 cm each. The larger traps constructed of 2 cm mesh were 120 cm in length and 60 cm diameter, with 2 entrances of 15 cm each. Folding oval fish traps were used in Canada, the smaller traps (0.9 cm mesh) were 60 cm in length, 45 cm wide and 30 cm in height, with 2 entrances of 7 cm each. The larger traps (2.5 cm mesh) were 90 cm in length, 60 cm wide and 60 cm in height, with 2 entrances of 15 cm each. Because low salinity can affect both size- and colour-distribution patterns in this species, all the sites chosen were situated away from the influence of freshwater (salinity > 22; McGaw & Naylor 1992a,b) and consisted of a similar habitat of large boulders on gravel with fucoid and kelp seaweeds. Collections were made from mean mid-water level to approximately 2 m below mean low water.

Crabs were measured to the nearest 0.1 mm across the widest part of the carapace (from the outside of the first spine), and the sex and colour (green, orange, or red) was noted. Detailed measurements of the larger specimens were also made. In order to avoid recapture of the same individuals, the crabs were not returned to the collection sites.

We tested for factors that affected carapace size using a Kruskal-Wallis test on ranked data. Fixed-effect tests included crab origin (UK vs. Canada), sex (male vs. female) and integument colour (green vs. red). To balance the analysis, we randomly selected 208 individuals from each origin × sex × colour group — n = 208 was the smallest sample size in any one of these groups. Because of a significant origin × sex × colour interaction term ($p = 0.006$), the analysis was broken down to test for: (1) the effect of origin on carapace size, between UK and Canadian populations, and (2) the effects of sex and colour on carapace width within each region separately.

RESULTS

A total of 6890 crabs were collected; 5096 crabs were collected in the Menai Strait, and 1794 from Barkley Sound. Initially, crabs were divided into 3 colour groupings: green, orange, or red. Statistical analysis showed, for the most part, that size distributions for orange and red crabs were similar, suggesting they were of the same cohort. Data for orange and red coloured crabs were, therefore, pooled and termed red. Colour distributions differed between the 2 areas. In the Menai Strait, 55% of males were green and 45% were red, while 36% of females were green and 64% were red. In Barkley Sound, 84% of male crabs were green and 16% red, while for females 47% were green and 53% were red (Table 1).

Red crabs were significantly larger than green crabs at both locations (Table 1). In Barkley Sound, the average red male was 3% larger than the average green male (2% difference between median values) and the average red female was 7% larger than green females (5% difference between median values). In the Menai Strait, the average red male was 13% larger than the average green male (11% difference between medians) and the average red female was 21% larger than green females (20% difference between median values). Moreover, crabs from Barkley Sound were 39% larger than those from the UK ($p < 0.0001$) (Table 1). In Barkley Sound, 71% of crabs were male, this was higher than the percentage of males (60%) found in the Menai Strait. The latter may contribute to the greater proportion of large crabs in Canada, because male crabs were significantly larger than females in both regions (Fig. 1).

Since the collections at the 2 sites were separated by a significant time period, additional collections were carried out in the Menai Strait between December and April, 2009 to 2010, and compared with the earlier samples collected during the same months. Differences in size were apparent between green-coloured males and red-coloured females; however, these differences were not substantial. The mean (±SD) size of green-coloured males collected from 1989 to 1990 was 48.7 ± 8.6 mm compared with the 50 ± 13.2 mm of green males measured from 2009 to 2010 (*t*-test, $p = 0.044$). Red females collected from 1989 to 1990 were larger than red females collected 20 yr later (50.3 ± 9.8 vs. 46.8 ± 7.1 mm; *t*-test, $p = 0.008$). There was no significant difference in the mean sizes of red-coloured males (54.9 ± 11.3 vs. 53.4 ± 7.7 mm; *t*-test, $p = 0.46$) or green-coloured females (40.7 ± 13.9 vs. 42.4 ± 5.6 mm; *t*-test, $p = 0.18$).

The largest male and female crabs in our samples were both captured in Barkley Sound. The male crab had a green integument, a CW of 101.1 mm, a carapace length (rostrum to 1st abdominal segment) of 74.8 mm, and weighed 247.8 g. Its crusher claw height was 40.2 mm and its length was 45.2 mm. The cutter claw was 29.2 mm high and 36.3 mm in length.

The largest female crab also had a green integument, 85.4 mm CW, a carapace length of 60.6 mm and weighed 124.3 g. The right chela was 23 mm in height and 31.3 mm in length. The left chela was 18.9 mm in height and 27.8 mm in length. The pereiopods on both specimens were intact, and there was no evidence of damage or epibiont fouling on the carapace.

Table 1. *Carcinus maenas*. Descriptive data for 2 populations of *C. maenas*. Mean size (±SD) and minimum, maximum and median sizes (in mm), and the percentage of green- versus red-coloured individuals in a given population

Population	Size (mm)				Percentage
	Mean ± SD	Minimum	Maximum	Median	
United Kingdom					
Green males	51.3 ± 13.6	8.2	83.1	53.4	55
Red males	58.2 ± 9.8	17.0	79.3	59.2	45
Green females	43.1 ± 12.7	8.0	71.0	45.1	36
Red females	52.2 ± 9.0	22.4	74.1	54.1	64
Western Canada					
Green males	76.1 ± 11.2	24.0	101.1	78.3	84
Red males	78.2 ± 8.6	44.1	94.1	80.1	16
Green females	60.1 ± 11.1	29.9	85.4	62.1	47
Red females	64.4 ± 8.9	39.9	82.5	65.4	53

DISCUSSION

To date, the maximum size attained by green crabs *Carcinus maenas* appears highly consistent, both in its native range and in locations where it has become established (Australia, east coast of North America, Patagonia, South Africa) and is classified as an invasive species (Ropes 1968, Le Roux et al. 1990, Hidalgo et al. 2005, Audet et al. 2008). There are reports of an individual green crab from British Columbia that attained 106 mm CW (T. Therriault & C. DiBacco, DFO Canada, pers. comm.); maximum sizes of 110 to 115 mm CW may, therefore, be attainable (Grosholz & Ruiz 1996). In Barkley Sound, this species reached unusually large sizes; >30% of the 1794 crabs collected were 80 mm or larger, whereas in the UK only 3 of the 5096 crabs collected (0.06%) had carapace widths exceeding 80 mm. The largest male collected in Barkley Sound was 101.1 mm CW; while not the largest reported specimen, it is within the upper range of

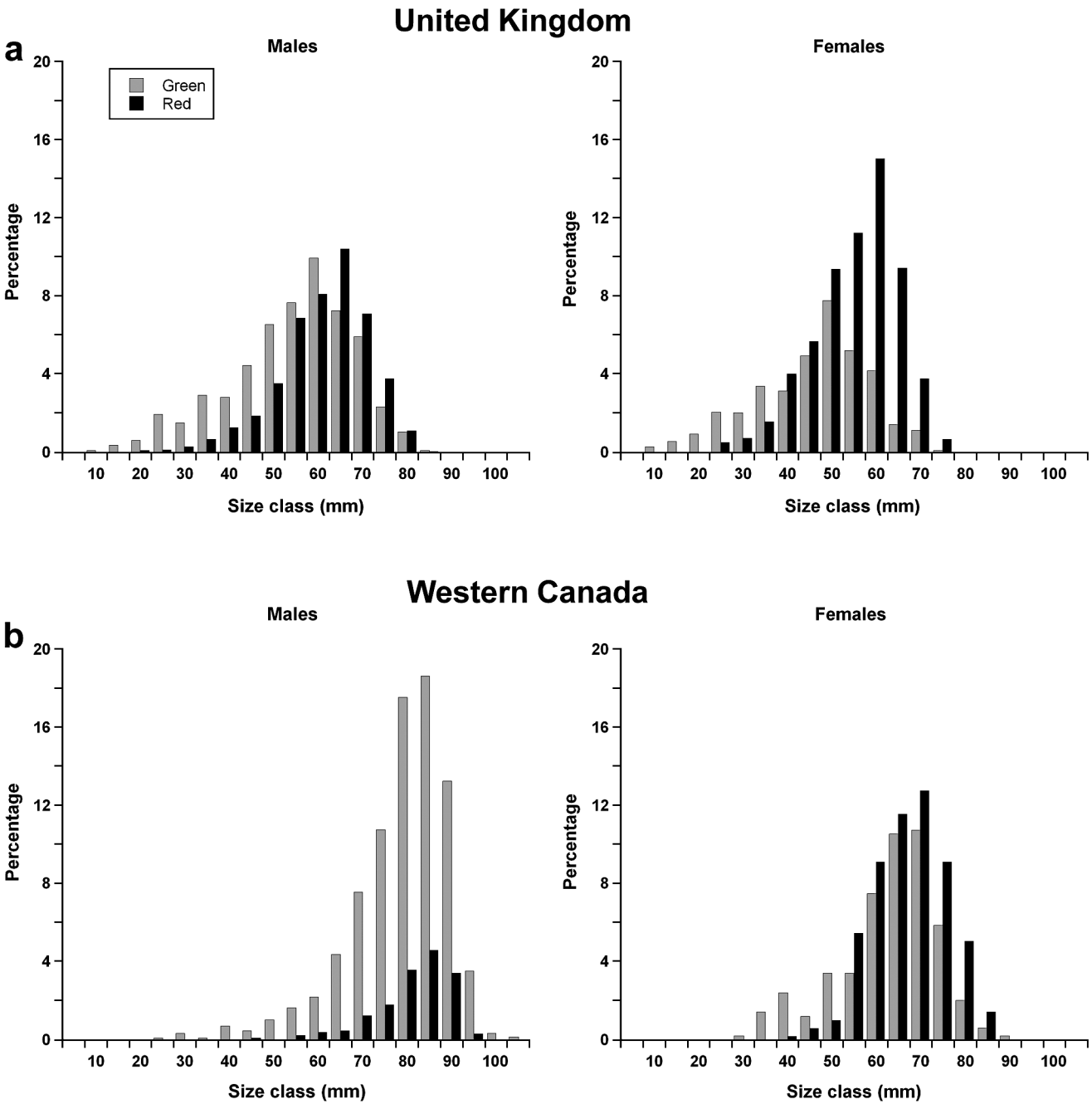

Fig. 1. *Carcinus maenas*. Percentage of green- and red-coloured males and females in 5 mm size classes. Crabs were collected in traps and by hand in (a, upper panels) Menai Straits, Gwynedd, UK (n = 5096), and (b, lower panels) Barkley Sound, British Columbia, Canada (n = 1792)

reported maximum sizes. In general, females were smaller than males, and, although they may reach >90 mm CW (J. Drewery, FRS Marine Labs, pers. comm.), the 85.4 mm CW female specimen also appears to be one of the largest published records for this species.

The larger size of crabs on the west coast of North America has been noted previously, but, to date, no definitive conclusions for this observation have been drawn (Grosholz & Ruiz 2003). The west coast populations are descended from crabs from the east coast of North America, but, at present, there is no evidence to suggest that these were genetically distinct populations (Bagley & Geller 2000). Low winter sea temperatures (<5°C) limit the growth and reproductive season (Behrens-Yamada 2001) and can stop recruitment and increase mortality of older, larger individuals (Berrill

1982). However, seawater temperatures in the Menai Strait (Harvey 1972) and Barkley Sound (Bamfield Marine Sciences Centre, Ocean News Records) are similar, and in South Africa, where water temperatures are warmer and the growing season is longer, the crabs are no larger than their native counterparts (Le Roux et al. 1990). Prey items are also plentiful in both areas, and there is little evidence to suggest that growth in crabs from Europe is limited by food availability (Klein Breteler 1975, Berrill 1982). It has been postulated that a lack of predators on the North American west coast may allow *Carcinus maenas* to attain larger sizes (Grosholz & Ruiz 2003). However, most animals that prey upon crabs are opportunistic feeders (Torchin et al. 2001), and there are a large number of fish species (Hart 1973) and >20 species of decapod crustaceans that are likely to eat *C. maenas* (Hunt & Behrens-Yamada 2003). The most plausible explanation for the size differences is that a release from parasites, which slow growth, allows crabs on the west coast to grow more rapidly and attain a larger size (Torchin et al. 2001). This phenomenon is not only limited to *C. maenas*, but appears to apply to a number of other invasive phyla (Torchin et al. 2001).

The underside of *Carcinus maenas* varies in colour from a pale green, through orange, to a deep red-brown (Kaiser et al. 1990, McGaw et al. 1992). The red colouration builds-up during a prolonged intermoult period, as the pigments in the carapace denature (Reid et al. 1997, Taylor et al. 2009). However, all crabs, irrespective of initial colour, turn green immediately after moult. In general, red crabs tend to be larger than their green counterparts and are thought to direct energy towards reproduction rather than growth (Reid et al. 1997). In the Menai Strait, the green-coloured crabs were 17.3% smaller than red-coloured crabs, a significantly greater difference than the 5% size variation observed for Barkley Sound populations. The high proportion of these large green-coloured crabs in Barkley Sound suggests that the population is in an actively growing rather than a reproductive phase (Reid et al. 1997, Wolf, 1998). The fact that only 5 pre-copula pairs and 4 berried females were found during specimen collections further substantiates this assertion.

The reason why crabs in Barkley Sound appear to exhibit short intermoult periods and, thus, rapid growth is unclear. Conspecifics feed on small crabs, and the presence of large individuals can, thus, cause the small crabs to delay moulting, because they can be more easily preyed upon when the shell is soft (Klein Breteler 1975). When *Carcinus maenas* settles in a previously unoccupied zone, they are larger than subsequent cohorts, because they are not moulting-limited by larger individuals (Klein Breteler 1975). This appears to be a plausible explanation for the predominance of large green-coloured crabs in Barkley Sound. We also hypothesize that, because of the large number of potential crustacean predators/competitors in Barkley Sound, *C. maenas* diverts energy towards growth to gain a size-based predation refuge. This would allow them to avoid predation and possibly outcompete native crabs, such as *Cancer gracilis* (Dana, 1852) and the smaller *Cancer productus* (Randall, 1839), which occur sympatrically (Jensen & Jensen 1985, T. Therriault & C. DiBacco pers. comm.). Our preliminary experiments support this idea: when 2 size groups of *C. maenas* (45 to 60 mm and 80 to 95 mm) were introduced into a tank with *C. productus* of 120 to 160 mm, the smaller individuals suffered 50% mortality within 24 h, whereas none of the crabs >80 mm were injured (I. J. McGaw unpubl. obs.).

It could be argued that differences in carapace colour between the regions were a result of other factors, such as consumption of different food types; however, the diet of *Carcinus maenas* tends to be fairly conserved when comparing native and invasive populations (Grosholz & Ruiz 1996). In addition, the red colour is not the only characteristic of a prolonged intermoult; thicker or worn carapaces with heavier epibiont loads (McGaw et al. 1992) and discoloured gills (Legeay & Massabuau 2000) are also indicative of a prolonged intermoult. These were not evident in the large green-coloured crabs in Barkley Sound. Observation of the setae of the exopodites (O'Halloran & O'Dor 1988, Kaiser et al. 1990) of 6 red- and 8 green-coloured crabs from Barkley Sound confirmed that red crabs were in mid- to late 'C' stage, whereas the green crabs were in early 'C' stage, which again suggests that individuals in the population moulted more frequently, channelling energy towards growth rather than reproduction.

The demographics of the western Canadian cohort have already changed. In 2006 the population was much smaller, and only 6% of individuals were red in colour (Gillespie et al. 2007). The increase in the percentage of red-coloured individuals can be partially explained because the red colouration accumulates as the crabs age (Gillespie et al. 2007). Nevertheless, the high number of green-coloured male crabs in the large size classes (>80 mm) and the fact that the red crabs had very few epibionts on the carapace suggests that they are still moulting relatively frequently (McGaw et al. 1992). The lack of small green-coloured individuals shows that recruitment was low after 2007, and the population is primarily composed of a single ageing cohort. If this species persists and recruits further in this area, it will be of interest to see how the population characteristics change over time with respect to those of more established and native populations.

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Effects of demersal trawling along the west coast of southern Africa: multivariate analysis of benthic assemblages

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ABSTRACT: This study is the first to examine the benthic impacts of the otter-trawl fishery on hake in the southern Benguela upwelling region. Infauna were sampled at 4 sites, from southern Namibia to Cape Town by means of 5 replicate grab samples at each of 2 trawling treatments (heavily and lightly trawled areas), paired at each site. The large invertebrate epifauna was also sampled at 2 of these sites using a fine-meshed otter trawl. Sites ranged in depth from 350 to 450 m. Environmental attributes (sediment particle size, total organic carbon, depth, salinity, temperature and dissolved O_2 concentration) were examined along with faunal assemblage composition. Vertical profiles of water mass characteristics showed little long-shore variation, apart from slightly lower O_2 concentrations in the north. Difficulties of pseudo-replication in benthic impact studies are discussed, and methods for circumventing these suggested. There were significant differences in sediment characteristics among the 4 sites, but only 2 sites showed different sediment characteristics between trawling treatments. Studies of species richness, evenness and numbers of infaunal individuals showed little difference between trawling treatments at 3 sites and species diversity was similar between treatments at all 4 sites. Multivariate analyses show marked differences in both infaunal and epifaunal assemblages among the sites and between trawling treatments at all sites. The analyses suggest that differences in trawling intensity are at least partially responsible for significant variation in benthic assemblage composition between heavily and lightly trawled areas. These findings contrast to those in shallower waters in the northern hemisphere, where infauna are more sensitive to trawling than epifauna. This study shows that epifaunal abundances, number of species and species diversity decrease with increasing trawling intensity, and that there are also considerable changes in epifaunal assemblages in more heavily trawled sites.

KEY WORDS: Demersal trawl fishing · Fishing impacts · Infauna · Epifauna · Pseudo-replication · Benthic assemblages · Benthic biota · Benguela

INTRODUCTION

Mobile demersal fishing gears are deployed on every continental shelf in the world (Collie et al. 2000) with nearly 20 million km^2 (75% of the continental shelf) subjected to trawl and/or dredge activities (Kaiser et al. 2002). Such fishing activities have a profound effect on the ecosystem (Dayton et al. 1995, Jennings & Kaiser 1998, Watling & Norse 1998, Auster & Langton 1999, Hall 1999, Kaiser & de Groot 2000), and are one of the greatest sources of anthropogenic disturbance to marine benthic assemblages (Kaiser et al. 2000, 2006). The challenge now lies in quantifying the extent of direct and indirect impacts at local, regional and ecosystem scales, and finding practical ways to mitigate the impacts.

Two approaches to quantifying trawl impacts are generally adopted. Chronic impact studies compare conditions between heavily fished, lightly fished and unfished areas, by assigning otherwise similar areas to treatments on the basis of historical estimates of trawling effort. This approach accurately represents

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trawling disturbance in terms of real effort and spatial dimensions (McConnaughey et al. 2000). Acute impact studies involve experimental fishing activity to contrast before and after effects between fished and unfished conditions. Such manipulative experimental approaches provide a more structured investigation but seldom accurately represent the effort and spatial scale of a commercial fishery. Both approaches are often used to investigate the effects of disturbance on invertebrate macrofauna, which plays important ecological roles in both structuring the habitat and as prey (Gray 1974).

Collie et al. (2000) and Kaiser et al. (2006) conducted global meta-analyses investigating the effects of different types of fishing impacts on benthic assemblages on various substrates, ranging from gravel to mud, and revealed the greatest impacts to be from scallop dredging activities in biogenic habitats. Kaiser et al. (2006) further identified soft-sediment habitats to be vulnerable to trawling with recovery times measured in years. These studies have provided useful information on the immediate effects, severity of impact and recovery times in a wide range of habitat types and environmental conditions. However, the fishing impact research assessed in these meta-analyses was typically of small-scale experimental studies at spatial scales measured in km^2 (Collie et al. 2000, Kaiser et al. 2006). Furthermore, only one of the studies analysed (Engel & Kvitek 1998) was conducted in an upwelling ecosystem (central California), whilst no studies analysed were conducted on sediments at outer shelf depths greater than 200 m, i.e. conditions typical of the trawling grounds of the Benguela ecosystem.

Several studies conducted in the Irish and North Seas have examined the response of benthic biota to quantified gradients of fishing intensity at the scale of the fishery, thus assessing the effects of trawl disturbance under realistic conditions (Jennings et al. 2001, Hiddink et al. 2006, Queirós et al. 2006, Hinz et al. 2009). Results from these studies showed that impacts of chronic trawl disturbance are cumulative and can lead to profound changes in benthic assemblage composition, with both infauna and epifauna showing marked effects on sand and muddy substrates. Studies conducted at the scale of the fishery are essential to test predictions generated from smaller-scale experimental studies and provide applicable information to fishery managers and policy makers (Hinz et al. 2009).

Quantification of fishing impacts on the environment is frequently confounded by natural perturbations, a history of fishing activities and small-scale patchiness (Dayton et al. 1995, Jennings & Kaiser 1998, Auster & Langton 1999, Gordon et al. 2005). Interactions resulting from natural variability and trawling activities are illustrated in Fig. 1. Ideally, one would apportion these effects and compare fished areas to the same areas before fishing commenced. This is usually not possible when investigating large-scale ecosystems and one is confronted by issues relating to unreplicated sites and pseudo-replication, which Hurlbert (1984) defines as the use of inferential statistics to test for treatment effects where treatments are either not replicated or replicates are not statistically independent. Replication is often impossible or undesirable when investigating large-scale systems and when large effects of treatments are expected or when the cost of replication is great, experiments involving unreplicated treatment may be the best option (Hurlbert 1984, Oksanen 2001). Hurlbert (1984), however, cautions that conclusions derived from unreplicated treatments should not be extrapolated to broader application.

The most dramatic changes in the assemblage composition occur at the onset of fishing in a pristine area or when the fishing pressure increases dramatically from a very low level. Once fishing at a commercial scale has begun, the system enters a 'fished' state and any changes as a result of increased fishing intensity are often smaller and more difficult to detect (Jennings & Kaiser 1998). Many of the world's demersal trawl grounds were transformed to a 'fished' state more than a century ago, well before the scientific value of pristine habitats was understood. The lack of suitable control sites is one of many reasons for establishing marine protected areas in ecosystems that are most heavily fished (Jennings & Kaiser 1998).

The South African demersal otter-trawl fishery has existed for over 100 years and spans the southern Benguela upwelling region between depths of 200 to 800 m (Payne & Punt 1995). It is South Africa's most valuable fishery and worth ~R2.5 billion or US$ 365 million (FIH 2009). The Namibian demersal hake fishery started in the late 1950s (Boyer & Hampton 2001) and is that country's largest, most lucrative fishery (Bianchi

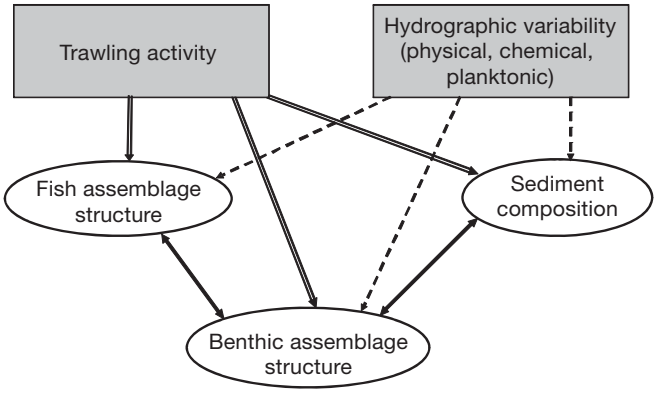

Fig. 1. Illustration of the complexity of interactions between trawling impacts and natural variability on benthic community structure. Solid lines = direct effects; broken lines = natural variability; double arrows = interactions

et al. 2001). Low oxygen water (<2 ml l^{-1}), frequently associated with inshore areas (<200 m) in this upwelling region (Chapman & Shannon 1985, Monteiro & van der Plas 2006), is unlikely to directly impact biotic assemblages in deeper waters. However, changes in fish behaviour during such events (e.g. greater concentrations in deeper water and increased juvenile predation) and impacts on recruitment success (Hamukuaya et al. 1998) may indirectly influence deep-water benthic assemblages. Technological advances in fishing and vessel equipment since the 1950s have allowed the fishery to expand into deeper waters, down to 1000 m at times (Griffiths et al. 2004, Fairweather et al. 2006). The fishery operating in the southern Benguela region is comprised mostly of stern trawlers ~50 m length, which use otter-trawl configurations with trawl doors ~1.8 t each (Wilkinson & Japp 2005). The physical impacts of demersal fisheries and their concomitant ecosystem effects have not previously been studied in an upwelling region or in southern Africa.

This study aims to quantify the effects of demersal trawling on benthic biota in the southern Benguela upwelling region. As with other similar studies (Engel & Kvitek 1998, Thrush et al. 1998, Kaiser et al. 2000, Queirós et al. 2006, Hinz et al. 2009), there were no suitable, unfished control sites in the region. Comparisons of benthic biota between areas that are intensively fished with those of similar habitat type, but which are lightly fished, are the best alternative. Benthic invertebrate assemblages were sampled at 4 sites spanning the study region and compared between areas with different levels of trawling disturbance. This research aims to address the following questions:

(1) Does intense trawling result in significantly different abundance, biomass and/or diversity of benthic infaunal and/or epifaunal assemblages?

(2) To what extent can environmental effects be disentangled from anthropogenic effects in terms of impacts on benthic assemblages? (see Fig. 1)

(3) How do the impacts of trawling as detected by studies conducted in shallow areas at the scale of the fishery in Western Europe compare with those observed in deeper waters in upwelling regions?

MATERIALS AND METHODS

Study sites and sampling design. The spatial distribution of commercial fishing activity from 2003 to 2007 in South Africa was plotted using a geographic information system (GIS) with start to end points of actual trawl positions reported from vessel monitoring systems (Wilkinson & Japp 2005), incorporating more recent unpublished data for this study. Namibian

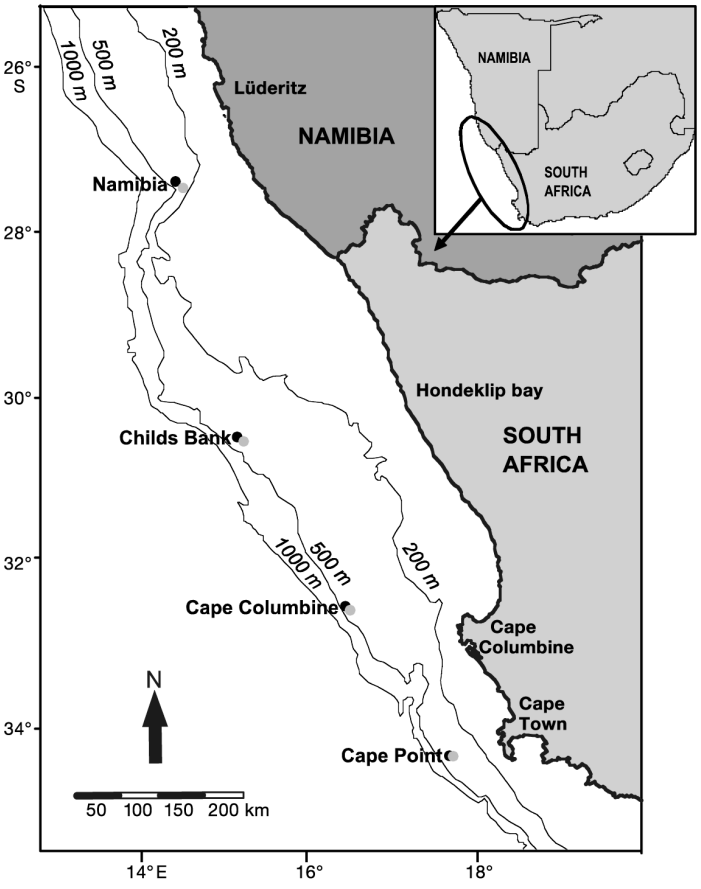

Fig. 2. Location of 4 selected sample sites. Black circles represent heavily trawled (HT) areas, grey circles represent lightly trawled (LT) areas

Catch Per Unit Effort (CPUE) data from 2004 to 2005 (MFMR 2005) were translated into hours fished. Based on this, 4 sites were identified (Fig. 2) as areas of intense commercial trawling (HT areas) with adjacent areas, in a similar habitat type, where trawling was considerably lighter (LT areas). Every effort was made to minimize differences in depth and sediment type between paired 'trawling treatment' areas. The 4 sites are named from north to south, Namibia, Childs Bank, Cape Columbine and Cape Point, respectively (Fig. 2) and ranged from 346 to 459 m depth (Table 1). The trawl intensity at each South African site, Childs Bank, Cape Columbine and Cape Point, was defined by calculating the number of trawl passes within a 0.5 n mile radius of each site over a 5 year period (2003 to 2007) using unpublished commercial data obtained from the former Department of Environmental Affairs and Tourism, now Department of Agriculture, Forestry and Fisheries (DAFF). HT vs. LT areas sampled at the Namibian site were defined by the total number of hours fished in each area during 2004 and 2005 (MFMR 2005). To compare trawl intensity

Table 1. Co-ordinates of benthic grabs and trawls, depths and trawl intensities at the sites sampled. Swept area gives an estimate of the proportion of the sampled area trawled per yr, based on the width of the net and number of trawl tracks. HT: heavily trawled; LT: lightly trawled

	Depth (m)	Trawl intensity (no. yr^{-1})	Original data[a]	Proportion swept area	Depth (m)[b]
Namibia					
HT	405	1.8 to 2.7	18.3 to 27.7 h	4.156	406 to 409
LT	435	0.02 to 1.07	0.17 to 11 h	1.011	446 to 453
Childs Bank					
HT	400	1.6	285 tracks	2.939	399 to 400
LT	350	0.17	30 tracks	0.309	346 to 349
Cape Columbine					
HT	436	1.5	271 tracks	2.795	–
LT	412	0.6	112 tracks	1.155	–
Cape Point					
HT	349	1.5	270 tracks	2.784	–
LT	348	1.04	187 tracks	1.928	–

[a]Duration or no. of tracks; [b]maximum depth during trawl replicates

among sites and with other studies, trawl tracks from South African sites and hours fished at the Namibian site were converted to estimated times trawled per year (shown in Table 1) using the equation (vessel speed in knots) × (gear width in m) × (effort in h per yr) (see Hinz et al. 2009). The average width of the trawl net opening (30 m) and the average towing speed of offshore trawl vessels (3.5 knots) (Wilkinson & Japp 2005) were also used to calculate the proportion of sampled area trawled per year (Table 1) at each site and area. These values provide comparable estimated fishing effort values based on the best currently available data.

Biological sampling. To sample the infauna, a 0.2 m^2 van Veen grab was used to collect 5 replicate samples at each HT and LT area from all 4 sites from the RV 'Dr Fridtjof Nansen' in April 2007 and FRS 'Ellen Kuzwayo' in February 2008 (Cape Point, Table 1). Sediment volume for each grab was measured and 250 ml sediment sub-samples collected for organic and sediment particle size analysis. The remaining sediment was washed over 2 stacked sieves with mesh sizes of 10 and 1 mm and with a 1 mm mesh covering the seawater hose, preventing large planktonic organisms from washing into the samples. All infauna >1 mm in size retained by the sieves was placed into sample bottles and preserved in 96% ethanol, then replaced 24 to 48 h after initial preservation to ensure adequate preservation. Infauna was sorted and identified to the lowest possible taxonomic level, usually species. The abundance and biomass were recorded for each taxon.

Epifauna were sampled with 3 replicate trawls in HT and LT areas at the Namibian and Childs Bank sites during April 2007 (Fig. 2, Table 1). The trawl gear consisted of 47 m footrope with 12 cm roller disks, 18 to 22 m mouth opening, 32 mm cod end mesh, lined with 25 mm mesh (defined as Gesund Super). All epibenthic invertebrate fauna retained by the net were sorted by taxon, identified, counted and weighed. All unknown specimens were preserved for further identification. Epifaunal abundance and biomass values were scaled to a uniform trawl duration of 30 min. For the purposes of this study, infauna are defined as those invertebrates sampled by grab, whereas epifauna are defined as those sampled by trawl net. Due to the semi-quantitative nature of sampling epifauna by trawl net, the epifaunal abundance and biomass data were categorized on an approximately log-scale for further analyses (Table 2).

Environmental variables. At each sampling site, water column profiles of depth, salinity, O_2 and temperature were measured using a CTD meter.

Sediments were dry sieved to separate gravel (>2 mm) and sand (<2 mm and >63 µm) while mud (<63 µm) was wet sieved and quantified using a calculated pipetting factor according to standard conventions (Folk 1968). The mass of each component was converted into percentages and the gravel-sand-mud texture category of each replicate sample determined using Folk's classification triangle (Folk 1968).

The inorganic carbon component was removed from sediments by adding 50% hydrochloric acid. Sediment was dried and washed with 1 M ammonium formate that removed any acid residue. The organic carbon contents were measured with a Thermo Flash 1112 elemental CHN analyzer.

Statistical and numerical analyses. ***Univariate analyses:*** Diversity indices (number of species, abundance, Pielou's species evenness, Shannon-Wiener diversity and average biomass per 0.2 m^2) of infauna and epifauna (excluding biomass) were computed (Clarke & Warwick 2001). The absolute values of epifaunal biomass were not appropriate for further uni-

Table 2. Categories allocated to abundance and biomass measures of epifauna collected from demersal trawls

Category	Abundance (ind.)	Biomass (kg)
1	1–10	0.001–0.010
2	11–100	0.011–0.100
3	101–1000	0.101–1.000
4	1001–10 000	1.001–10.000
5	10 001–100 000	10.001–100.000

variate analysis as a result of prior log categorization. The response of univariate indices to trawling intensity per year were explored using least square regression analyses. Prior to analysis, abundance and biomass data were $\log_{10}$ transformed.

Environmental analyses: Proportions of sand and mud were strongly positively correlated (Pearson ρ = 0.99), thus only one of these variables (% sand) was used for further analysis. Gravel contributed very small proportions to the overall sediment composition (<5%) and was excluded from further statistical analyses. Following arcsine transformation (Sokal & Rohlf 1969, Zar 1999), replicate sand values and total organic carbon (TOC) were analysed separately. Statistical differences among sites and between treatments were analysed using ANOVA, with p-values determined by permutation rather than by reference to *F* distribution tables, thus giving robustness to normality assumptions using permutational multivariate ANOVA (PERMANOVA) (Anderson et al. 2008). Factor trawling Treatment (fixed) was crossed with Sites (fixed factor) in a 2-way crossed ANOVA design.

Multivariate analyses: To test for differences in assemblage composition between trawl treatments, sample by species matrices were calculated for both abundance and biomass measures of infauna and categorized epifauna at all sites. The infaunal abundance and biomass matrices were 4th root transformed (Field et al. 1982, Clarke & Warwick 2001) while the epifauna data had effectively been transformed by log-scale categorization (Table 2). We compared samples on the basis of species abundances or biomasses using the Bray-Curtis measure of (dis)similarity on both infaunal and epifaunal data. These were summarized in cluster dendrograms and MDS plots. PERMANOVA (Anderson et al. 2008) was used to test for significant differences in assemblage structure among sites (Namibia, Childs Bank, Cape Columbine and Cape Point) and between trawling treatments HT vs. LT (Anderson et al. 2008). PERMANOVA was also used to test epifaunal abundance and biomass data between HT and LT areas at Namibia and Childs Bank. Treatment (fixed factor) was crossed with Site (fixed factor). PERMANOVA tests the dissimilarity values generated by the resemblance matrix on which permutations are based, generating a test statistic value of pseudo-*F* and pseudo-*t* for pair-wise tests (Anderson et al. 2008). However, these tests were compromised by pseudo-replication and the probabilities calculated are too low. The tests were therefore repeated using the interaction term as the denominator in the *F*-ratio, giving an ultra-conservative probability value. The true probability lies somewhere between the 2 calculated values, depending upon the degree of pseudo-replication (K. R. Clarke pers. comm.). To assess which species contribute most to differences between groups, SIMPER analyses were conducted (Clarke 1993).

The relationships between biotic (abundance and biomass) assemblage data and all measured environmental variables were investigated using a Distance Based Linear Model (DISTLM) (Anderson et al. 2008). Trawl intensity was used to quantify fishing pressure in the DISTLM analysis as a continuous variable (times trawled per yr). The contribution of environmental variation and fishing pressure in influencing the biotic data distribution was simultaneously assessed. DISTLM partitions the variation in data distribution according to a multiple regression model (based on predictor variables), as selected by the user, e.g. forward, stepwise, best fit. The 'forward' procedure and Adjusted R^2 criteria options (Anderson et al. 2008) were used in this study. The environmental variables selected for the model were Sand, TOC (arcsine transformed), Depth and Times trawled per year. All multivariate and diversity analyses were performed using PRIMER v.6 and its add-on package PERMANOVA+ (Clarke & Warwick 2001, Clarke & Gorley 2006, Anderson et al. 2008).

RESULTS

Environmental variables

Table 3 summarises the results of sediment and water column characteristics measured at the areas sampled. There is little variation in water temperatures or salinities at the 8 sites. The entire sampling region was thus uniform with respect to water mass characteristics during the sampling period, although it is noted that the Namibian areas had lower near-bottom O_2 levels than the other 3 sites. Sites sampled in this study were deeper than the coastal hypoxic region where the biota are likely to be influenced seasonally by reduced O_2 levels (Decker 1970, Monteiro & van der Plas 2006),

The sediments were classified according to the Folk Classification triangle (Folk 1968). A conventional main effects PERMANOVA test shows significant differences in Sediments among Sites, between Treatments and the interaction between Sites and Treatments (Table 4). However, these tests are compromised by pseudo-replication and non-independence; thus ultra-conservative tests are also presented (right hand columns, Table 4) using the interaction term as the denominator in calculating the *F*-value (K. R. Clarke pers. comm.). Here only TOC was found to differ significantly among sites, mainly due to the high values at Childs Bank (see Table 3). The true significance levels lie somewhere between the conventional and ultra-conservative tests.

Table 3. Sediment and water column characteristics measured at each heavily trawled (HT) and lightly trawled (LT) sample area. Sand and total organic carbon (TOC) data are mean ± SE (n = 5). Temperature was 7 to 8°C and salinity was 34.6 to 34.8 at all sites

	Sediment	Sand (%)	TOC (%)	Oxygen (ml l^{-1})
Namibia				
HT	Muddy sand	83.16 ± 0.45	0.64 ± 0.08	2
LT	Sand–muddy sand	89.18 ± 1.37	1.48 ± 0.24	2
Childs Bank				
HT	Muddy sand	77.04 ± 2.23	14.23 ± 0.41	4
LT	Muddy sand	72.61 ± 0.68	11.37 ± 0.35	4
Cape Columbine				
HT	Muddy sand–sandy mud	52.56 ± 2.56	2.85 ± 0.24	4
LT	Sandy mud	21.22 ± 0.79	5.55 ± 0.48	4
Cape Point				
HT	Muddy sand	84.35 ± 1.26	0.89 ± 0.06	4
LT	Muddy sand	78.04 ± 3.06	1.25 ± 0.19	4

Univariate measures of biotic diversity

Fig. 3 depicts the trends and least-square regression analysis results in various measures of diversity plotted against trawling intensity (times trawled per yr) measured at all 4 sites and both HT and LT areas. For the infauna, there is little trend and generally a wide scatter for evenness, biomass and species diversity with no significant regression analyses. The fits are slightly better for overall abundance and species richness (number of species), but the regression analyses remain non-significant. The results are rather different for the epifauna, although the data are only from 2 sites. The fits for species richness, overall abundance, and diversity are better ($R^2 > 87\%$), with negative slopes for all 4 measures, suggesting negative impacts of trawling on the epifauna. Only the abundance measures show a significant decline between HT and LT areas ($F = 19.13$, df = 1, 2, p = 0.048). The species richness and diversity follow identical trends, although these are not significant. Owing to the semi-quantitative logarithmic categorization of the epifauna data (Table 2), the diversity and evenness values in Fig. 3 should not be compared with other studies.

Benthic infauna

A species accumulation curve (not shown) of all 40 infaunal grab samples approaches an asymptote, suggesting that the 256 species obtained approximate those occurring in this Benguela ecosystem habitat. Fig. 4 shows a MDS plot based on infaunal abundance data from all sites, depicting 4 main site groups, and also shows at Namibia and Childs Bank clear separation of HT and LT areas. At the other 2 sites, HT and LT areas are not as well separated. Analysis of biomass data from all sites showed a similar MDS grouping pattern (not shown). This suggests that the biogeographic variation with latitude accounts for greater variation than between heavily and lightly trawled areas at each site.

Main effects PERMANOVA of the same abundance data shows significant differences among Sites, Treatments and the interaction between Sites and Treatments (Table 5). However, just as for the environmental data, these results are compromised by pseudo-replication and the significance levels are exaggerated. Again, very conservative pseudo-*F* tests, using the interaction mean square as a denominator, are presented in the right hand columns, but these are not significant for Site or Treatment and add little information to the analysis.

Table 4. Test statistics for main effects PERMANOVA of percentage of sand and total organic carbon (TOC, arcsine transformed) among 4 sites with heavily trawled and lightly trawled areas. The number of unique permutations possible exceeded 9000 in all cases. Significant values at p < 0.05 are indicated in **bold**. Conservative *F*-ratio has interaction mean squares value as denominator

	df	SS	MS	Pseudo-*F* (conventional)	p	Pseudo-*F* (conservative)	p
Sand							
Site	3	32.103	10.701	264.45	**0.0001**	7.5	>0.05
Treatment	1	1.324	1.324	32.72	**0.0001**	0.93	>0.05
Site × Treatment	3	4.278	1.426	35.239	**0.0001**	–	–
TOC							
Site	3	36.410	12.137	444.81	**0.0001**	21.2	**<0.05**
Treatment	1	3.879×10^{-4}	3.879×10^{-4}	1.42×10^{-2}	0.9091	6.78×10^{-4}	>0.05
Site × Treatment	3	1.717	0.572	20.97	**0.0001**	–	–

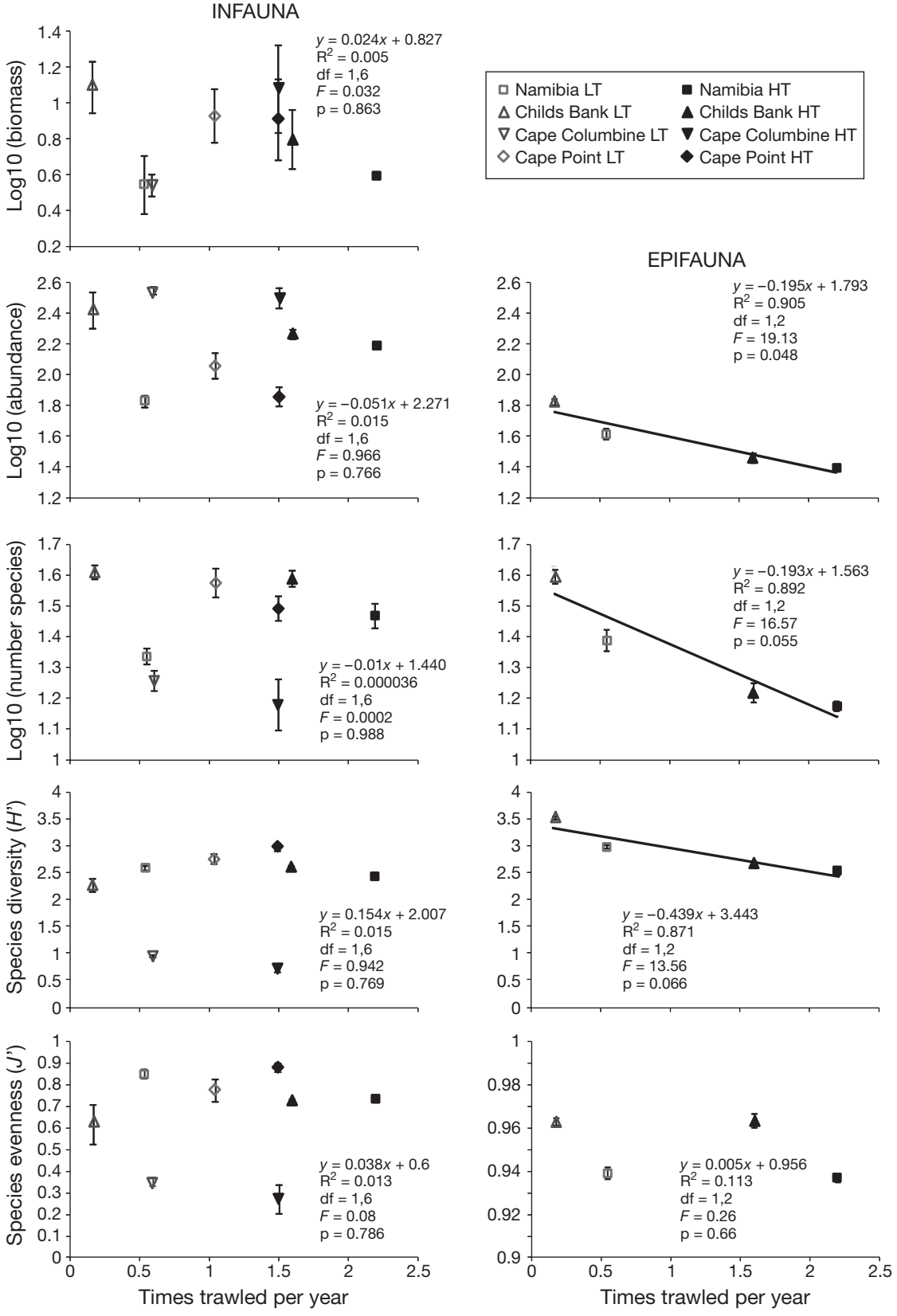

Fig. 3. Infaunal and epifaunal diversity indices with least square regression analysis results included. Regression lines included only if $R^2 > 0.85$. HT: heavily trawled; LT: lightly trawled

Infaunal indicator species

The main species responsible for the Bray-Curtis MDS clusters are revealed by a SIMPER analysis (Clarke 1993) comparing trawling treatments (Fig. 5). A few species showed consistent trends among sites where the echinoderm *Ophiura* sp. are more abundant at HT areas and the polychaete *Chloeia inermis* had higher biomass at LT areas, while others, e.g. the echinoderms *Amphiura* sp. and *Brissopsis lyrifera capensis*, showed irregular occurrence among sites and trawling treatments. Several species were unique to specific sites and occurred either exclusively, or in greater biomass, at LT areas. These include the tanaid crustaceans *Apseudes cooperi* at Namibia and *Tanais philetaerus* at Cape Columbine, an unidentified ascidian, sipunculid *Phascolosoma* sp. and chiton *Leptochiton sykesi* at Cape Point.

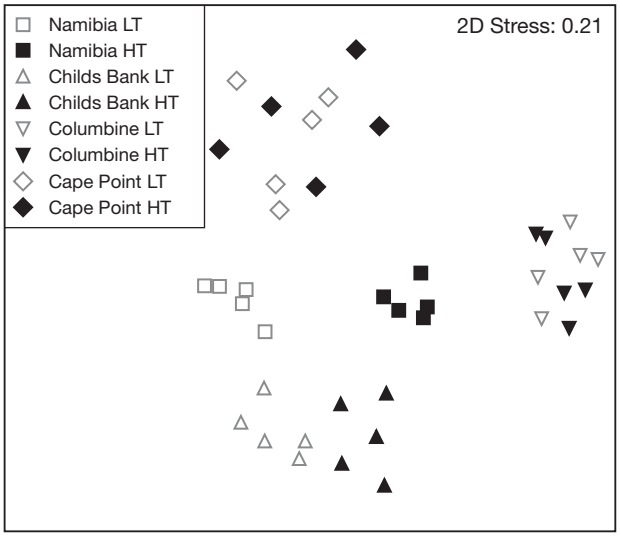

Fig. 4. Infaunal abundance: multi-dimensional scaling plot of all study sites and treatments after 4th root transformation, based on Bray-Curtis similarity. HT: heavily trawled; LT: lightly trawled

Relation of infauna to environment and trawling treatment

The relationship of infaunal assemblage composition (based on abundance data) to both natural and trawling influences was investigated using a linear modelling approach, DISTLM. The results are given in Table 6. The pseudo-*F* tests are again potentially compromised by pseudo-replication, but it can be seen that all 4 variables contribute considerably to the variation observed (marginal tests). To disentangle trawling effects from natural variability, sequential tests were also applied that showed the cumulative effects of each variable once the previous variable(s) had been accounted for. The sequence specified started with natural variables (sediment properties) with trawling intensity being fitted last. Sand and TOC, together with depth, account for 37% of infaunal variability, whilst trawling intensity accounts for only 5.3% direct effect on infauna. These results do not take into account the possibility that trawling may influence sediment composition made up of sand and TOC and thereby indirectly influence the assemblage composition. Similar results were obtained using biomass data but are not presented here.

Epifauna

A total of 81 epifaunal species was sampled at the Childs Bank and Namibian sites. Fig. 6 shows 4 groups of 3 samples each, with the 2 sites well separated, and Namibian trawl treatments closer to each other than the Child's Bank treatments. The low stress value (0.05) indicates the validity of the 2-D presentation of this small data set.

Epifaunal biomass SIMPER analyses (Fig. 7) reveal that several species of urchins such as the *Spatangus capensis*, *Echinus gilchristi* and *Brissopsis lyrifera capensis* and the crab *Exodromidia spinosa* occur at LT areas, while other species such as the bristle worm *Euphrosine* sp. show great variability between trawling treatments at

Table 5. Infauna: test statistics for main effects PERMANOVA of infaunal abundance and biomass. The number of unique permutations possible exceeded 9000 in all cases. Conservative *F*-ratio has interaction mean squares value as denominator

	df	SS	MS	Pseudo-*F* (conventional)	p	Pseudo-*F* (conservative)	p
Abundance							
Site	3	44125	14708	10.72	0.0001	3.3	>0.05
Treatment	1	4071.5	4071.5	2.97	0.0001	0.93	>0.05
Site × Treatment	3	13183	4394.4	3.20	0.0001	–	–
Biomass							
Site	3	44503	14834	9.43	0.0001	3.3	>0.05
Treatment	1	4474.8	4474.8	2.85	0.0001	2.93	>0.05
Site × Treatment	3	15164	5054.6	3.21	0.0001	–	–

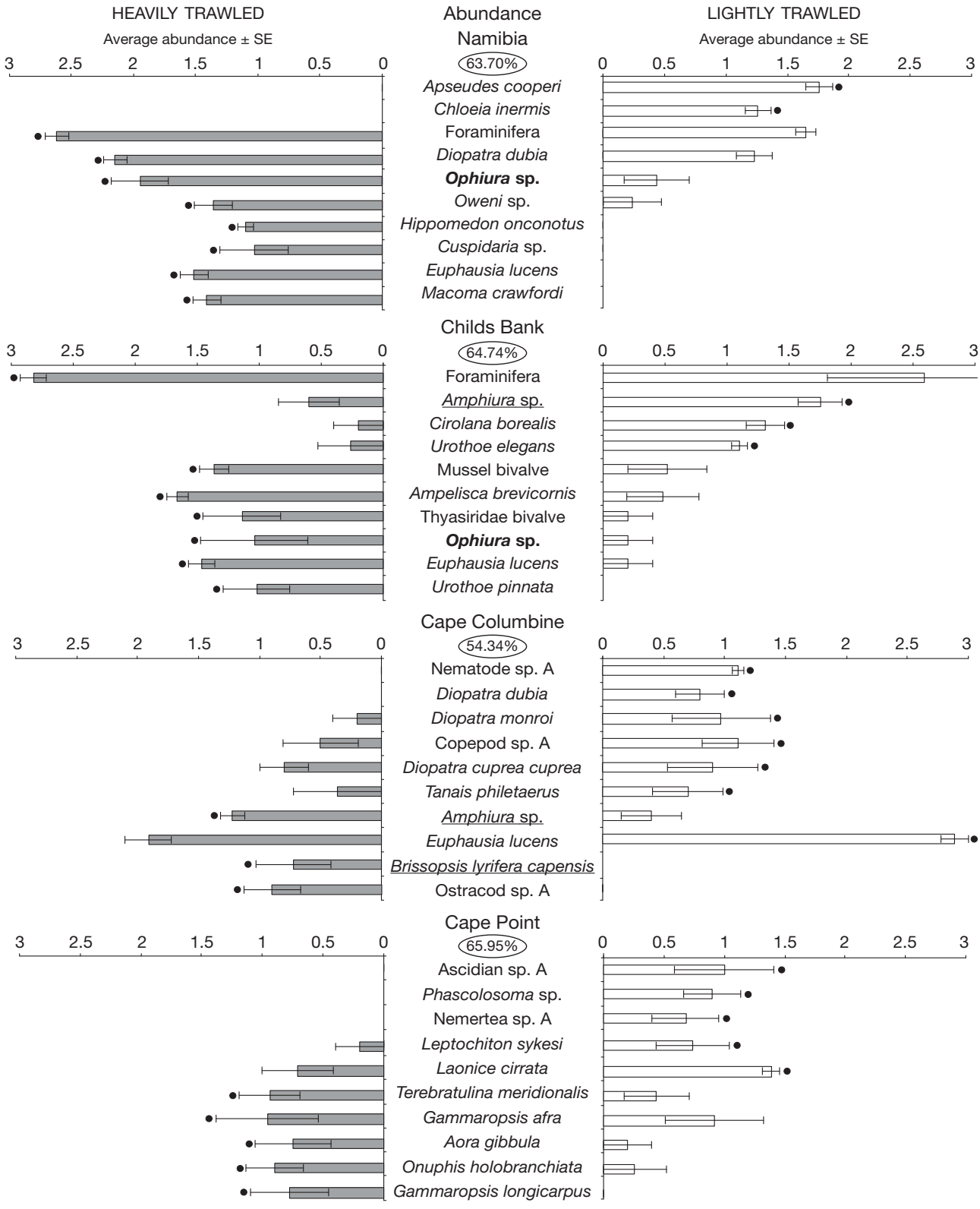

Fig 5. Infaunal SIMPER analysis of abundance data (top 9 species contributing to differences) between lightly and heavily trawled areas at each site (± SE) after 4th root transformation and Bray-Curtis dissimilarity. Species in bold show consistent trends between sites. Species underlined show opposite trends between sites. Percentage dissimilarity between treatments at each site is indicated in ellipse. A black circle indicates the area of greater abundance per species

Table 6. Test statistics for Distance-based Linear Model (DISTLM) analyses marginal and sequential tests based on 'Forward' procedure and Adjusted R^2 criteria of infaunal abundance at the 4 sites sampled. Marginal tests show how much variation each variable explains when considered alone, ignoring other variables. Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account

Variable	Adjusted R^2	SS (trace)	Pseudo-*F*	p	Proportion	Cumulative	Residual df
Marginal tests							
Sand	–	16399	7.0106	0.0001	0.15575	–	–
TOC	–	11355	4.5935	0.0001	0.10785	–	–
Depth	–	13887	5.7737	0.0001	0.1319	–	–
Times trawled	–	4617.9	1.7431	0.459	0.04386	–	–
Sequential tests							
+Sand	0.13354	16399	7.0106	0.0001	0.15575	0.15575	38
+TOC	0.2347	12444	6.023	0.0001	0.11819	0.27395	37
+Depth	0.32098	10452	5.7015	0.0001	0.09926	0.37321	36
+Times trawled	0.36121	5634.1	3.267	0.0001	0.05351	0.42672	35

the 2 sites. The burrowing anemone *Actinauge richardii* consistently occurs in greater abundance and biomass in heavily trawled areas at both sites.

DISCUSSION

Sampling design

In studying the effects of trawling, it is difficult in practice to find areas of habitat suitable for trawling that are not trawled at all. The best alternative under these circumstances is to study areas that are heavily trawled for comparison with lightly trawled areas of comparable habitat (McConnaughey et al. 2000). We approached this study using a 2-way crossed design to separate variation due to trawling (treatment) and to natural environmental variability using both conventional ANOVA and multivariate PERMANOVA in which the *F*-ratio is calculated using the remainder (error) MS term as the denominator. In doing this it is almost impossible to avoid issues of pseudo-replication. The conventional *F*-ratios calculated give unrealistically low probabilities under the null hypothesis of no effect. The large values of the interaction MS term evident in this study (Tables 4 & 5) suggest that pseudo-replication may indeed be a problem (K. R. Clarke pers. comm.). To account for this we recalculated the *F*-ratios using the interaction term in the denominator. This gives an ultra-conservative result, which if significant, indicates that the effect is certainly real. In the present study we found that the sedimentary organic carbon differed significantly among the 4 sites (Table 4), but there were no other significant effects on the sediments (Table 4) or infaunal assemblages (Table 5) among sites or between trawling treatments based on the ultra-conservative tests. To mitigate potential pseudo-replication problems, future studies of this nature should do one of the following:

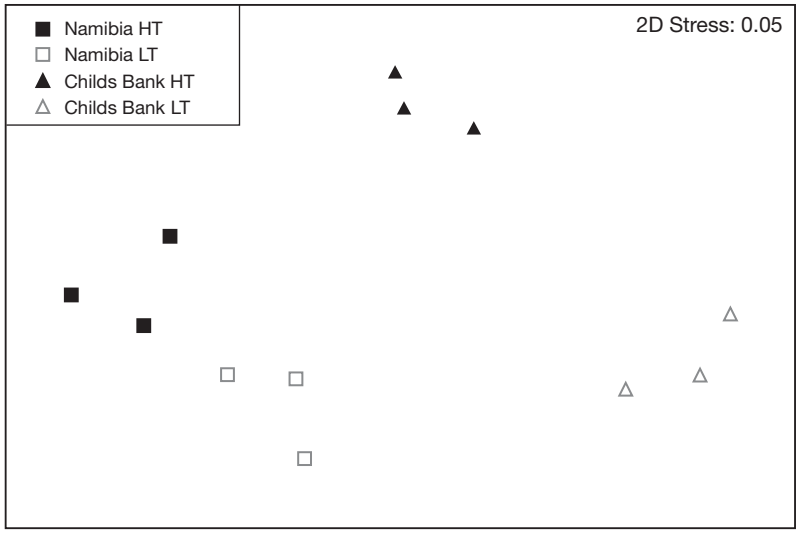

Fig. 6. Epifaunal abundance: multi-dimensional scaling plot of categorized data at Namibia and Childs Bank with paired heavily trawled (HT) and lightly trawled (LT) areas

(1) Sample more areas within each site, so that there are multiple areas which have been lightly trawled interspersed with multiple areas that have been more heavily impacted, the likelihood being that this sampling would be at the expense of reducing the number of wide-scale locations visited. The possibility then exists of differentiating natural variability from trawling impacts, at the geographic scale of that site.

(2) Sample more wide-scale sites whilst retaining the pairwise matching of a heavily and lightly trawled area at

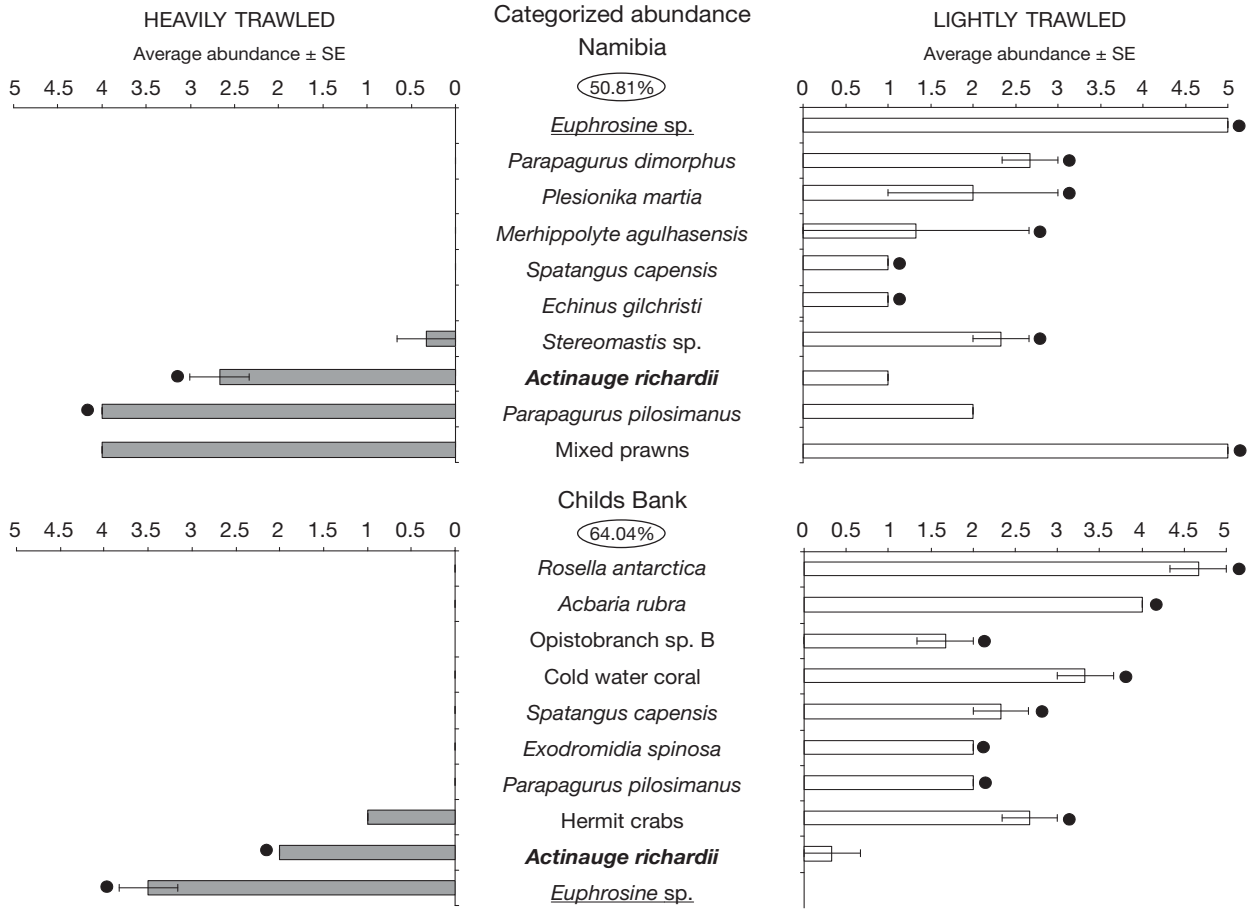

Fig. 7. Epifaunal SIMPER analysis of categorized abundance data (top 10 species contributing to differences) between lightly and heavily trawled areas at each site (±SE) with Bray-Curtis dissimilarity. Species in bold show consistent trends between sites. Species underlined show opposite trends between sites. Percentage dissimilarity between treatments at each site is indicated in ellipse. A black circle indicates the area of greater abundance per species

each site. This would allow inference about differences in trawling impact at much wider spatial scales to be drawn. No information is then available for a particular site about whether observed differences between heavily and lightly trawled areas is really due to trawling levels or other environmental conditions, but such findings may result from the set of wide-scale sites as a whole. In the ANOVA terminology of the above results, the number of degrees of freedom for the interaction mean square may be sufficiently large so that a consistent trawling effect at all sites manifests itself as a significant main effect of trawling in relation to the interaction mean square. The appropriate model here is that of a 2-way crossed design in which the trawling effect is fixed but the site-to-site variation is a random effect.

Which of these 2 strategies is preferable will therefore depend on a host of factors, including the aims of the study. It is, however, important to note that there can only be limited gains from increasing the number of replicate grabs or trawls in an area, by comparison with an increase in effort at a higher level of the design. More incisive analyses would come from using BACI designs, which allow comparisons of before and after implementing no-fishing control measures in the same areas.

Diversity indices versus multivariate methods

This study found no relationship between trawling intensity and the infaunal univariate measures of total abundance, biomass, species richness, diversity and evenness over all 4 sites. This contrasts with results from studies conducted in the Irish and North Seas (Jennings et al. 2001, Hiddink et al. 2006, Queirós et al. 2006, Hinz et al. 2009) where significant effects of trawling intensity were found in univariate measures of infaunal abundance, biomass, species richness and/or productivity in considerably shallower (<75 m)

mud or sandy sediment environments. Furthermore maximum trawling intensities investigated in the Irish and North Sea studies ranged from 3.5 to 18.2 times per yr compared to only 2.3 times per yr in the present study. Epifauna sampled in the Benguela region showed a significant decline in total abundance with increasing trawling intensity, while species richness and diversity also declined, but not significantly (Fig. 3). This contrasts in some respects with the results of Hiddink et al. (2006) and Hinz et al. (2009), who found that univariate measures of epifaunal biomass, production, abundance and/or species richness differed significantly with trawling intensity, but that overall infauna were more sensitive than epifauna. A study conducted by Engel & Kvitek (1998) in the upwelling, deeper >180 m, region of central California, however, report greater changes in epifaunal species than infauna when comparing heavily and lightly fished areas, findings similar to the present study.

Multivariate results are slightly more conclusive, in that the multi-dimensional scaling graphs depict differences among the sites for both infauna (Fig. 4) and epifauna (Fig. 6), with distinct differences between trawling effects for 2 of the 4 infaunal sites (Namibia and Childs Bank) and both epifaunal sites (also Namibia and Childs Bank). These results are similar to those of Hinz et al. (2009). Our attempt to confirm these graphical multivariate results statistically was confounded by pseudo-replication. Multivariate graphical results have however proved to be robust and generally give clearer results in this situation than the univariate diversity indices. Similar findings are reported by Kaiser et al. (1998) and Thrush et al. (1998) from studies conducted to detect changes due to trawling. Multivariate analyses are considered to incorporate a greater amount of assemblage information and have been suggested to be more sensitive at detecting changes in assemblages than univariate measures (Gray et al. 1990, Warwick & Clarke 1991).

Separating trawling from environmental effects

Multivariate graphical results show larger inter-site differences than within-site trawling treatment differences for infauna (Fig. 4) and marginally for epifauna (Fig. 6). However, these results are complicated by the large sedimentary differences between areas and sites. This is particularly so at Cape Columbine where the percentage of sand ranged from 52% at the HT area to 21% at the LT area, compared to 72 to 89% sand at all other sites and areas (Table 3). Was the area lightly trawled because the fish assemblage was different due to the different sediment, or was the difference in sediment due to less trawling? Evidence from the other 3 sites suggests that trawling may have little effect on the sediments. Similarly, percentage of TOC was significantly higher at Childs Bank than at all other sites (Table 4). With such large variability among sites in the benthic environment, even at similar depths, it is very difficult to show significant differences between trawling treatments and to distinguish trawling effects from environmental ones. Thus the conservative form of the PERMANOVA showed no significant differences among sites or treatments (Table 5), although the conventional analyses were all significant for both infaunal abundance and biomass data. The DISTLM marginal analyses were all significant for environmental and treatment variables in explaining infaunal assemblage variation (Table 6) but the various 'explanatory' variables are not independent of each other and need to be considered in combination. Sequential tests that eliminate the contributions of previous variables, and specify sedimentary variables fitted first, show that environmental variables contribute a cumulative 37% to variability with trawling contributing a further 5.3% to the overall assemblage variation, with the remaining 57.4% unexplained (Table 6). The relative percentage variation accounted for when fitting environmental variables first, indicates the potentially small size of any direct trawling effects in relation to that of the differing environmental conditions. In view of the complex processes involved in influencing benthic assemblage structure (see Fig. 1), and taking into account the difficulties of replicate sampling and obtaining a strong gradient of trawling intensities, it was impossible to obtain statistically rigorous results to separate trawling effects from environmental ones in this study. A similar difficulty was reported by Queirós et al. (2006), where the impact of trawling could not be disentangled from that of sediment composition.

In comparing infaunal and epifaunal species indicative of trawling effects in studies in the Benguela and California upwelling regions and Irish and North Seas, it is evident that different animal groups are likely to serve as potential indicators of the effects of trawling (Table 7). In the Benguela and Californian infaunal analysis, an ophiuroid brittle star increased with heavier trawling, while the polychaete *Chloeia* sp. decreased in the Benguela but increased in California. In the Irish Sea *Prionospio* spp. polychaetes increased, while *Phoronis* sp., molluscs and nemertea decreased. In the case of the Benguela epifauna, a burrowing anemone *Actinauge richardii* increased while the echinoid urchins *Spatangus*, *Echinus* and *Brissopsis* decreased. In California, no epifaunal species were reported to increase with heavier trawling while species of sea pen, sea star, anemone and sea slug all decreased. There were no significant epifaunal species

changes in the Irish Sea study (Hinz et al. 2009). Thus it appears that different groups and components of the benthos are affected differently in different environments, bearing out the general conclusions of Kaiser et al. (2006), who studied different sediment types and different types of fishing.

Comparison of shallow versus deep trawling

Assessing trawl impacts at 4 sites spread over 800 km at about 400 m depth on the outer continental shelf of the Benguela upwelling ecosystem show some similarities to a study conducted in the central California upwelling region (Engel & Kvitek 1998) and some differences from studies conducted in sheltered, shallower waters in western Europe (Jennings et al. 2001, Hiddink et al. 2006, Queirós et al. 2006, Hinz et al. 2009). Despite the greater depth, the sediments of the Benguela outer shelf otter-trawl grounds are generally classified as muddy sand (Table 3), being coarser than the muds of the shallow (26 to 75 m depth) North and Irish Seas environments. The intensity of heavy trawling was much greater in western Europe (up to 18.2 times per yr) and California (4 times per yr) compared to only 2.3 times per yr in the Benguela system. Where multivariate analyses were used (Hinz et al. 2009, present study), graphical separation of infaunal and epifaunal assemblages between heavily and lightly trawled areas was evident, with slightly clearer separation for epifauna. Results from western European studies showed greater change in infaunal than epifaunal univariate indices while results from the Benguela upwelling ecosystem showed greater epifaunal change (Fig. 3), similar to findings from the central California upwelling region. Trawling disturbance in deeper, upwelling regions indicates a more dramatic change in epifaunal assemblages than infauna (Engel & Kvitek 1998, present study), while infaunal assemblages show greater change in shallow, sheltered areas (Jennings et al. 2001, Hiddink et al. 2006, Queirós et al. 2006, Hinz et al. 2009). Meta-analyses of trawl impact by Collie et al. (2000) and Kaiser et al. (2006) reveal variable responses of benthic biota with different sediment types, habitat types and fishing gear. Differences in biotic response would thus be expected from habitats as diverse as shallow, sheltered seas and outer shelf upwelling systems. A study conducted by Kaiser et al. (2000) in the Irish Sea concludes that chronic fishing has resulted in a shift from relatively sessile, emergent, high biomass species to benthic assemblages dominated by smaller-bodied infauna, and that these assemblages represent an alternative stable state. Detection of epifaunal changes with increasing trawl intensity in upwelling regions of southern Benguela and central California indicate less impacted or more resistant environments and assemblages than the shallow, sheltered North and Irish Seas where greater changes in infaunal assemblages are consistently detected.

Implications for management

Benthic assemblages of each environment and sediment type respond to trawling differently, as found in the meta-analyses of Collie et al. (2000) and Kaiser et al. (2006). Jennings et al. (2001) noted that there were different effects of trawling in 2 areas depending upon the intensity of trawling and the sediment type, with more effect on the infauna than epifauna on the more heavily trawled, muddy ground. The less heavily trawled area showed no significant effect on biomass or production. In the present study, on sandy mud substrates at 400 m depth, where heaviest trawling was less intense than in similar studies, epifauna provided the best indicators in response to trawling, both in

Table 7. Indicator taxa of trawling effects reported from several studies. +: increase with heavier trawling; –: decrease with heavier trawling; Echino: echinoderm; Poly: polychaete; Phoro: phoronid; Crust: crustacean. Sources: Benguela = this study, California = Engel & Kvitek (1998) Irish and North Seas = Jennings et al. (2001), Queiros et al. (2006), Hiddink et al. (2006), Hinz et al. (2009)

		Benguela	California	Irish and North Seas
Infauna	Increase	*Ophiura* (Echino)	*Ophiura* (Echino) *Chloeia* (Poly)	*Prionospio* (Poly)
	Decrease	*Chloeia* (Poly)		*Phoronis* (Phoro) Mollusca Nemertea *Amphiura* (Echino)
Epifauna	Increase	*Actinauge* (Anthozoa)		*Prionospio* (Poly)
	Decrease	*Spatangus* (Echino) *Echinus* (Echino) *Brissopsis* (Echino) *Exodromidia* (Crust)	*Ptilosarcus* (Pennatulacea) *Mediaster* (Echino) *Urticina* (Anthozoa) *Pleurobranchea* (Mollusca)	*Phoronis* (Phoro) Mollusca Starfish (Echino, not significant)

terms of univariate indices and at the species level. Thus it appears that for this region and depth range, at current trawling intensities, benthic monitoring should include epifaunal assemblages; however, any changes in infaunal assemblages detected may indicate an altered stable state, providing useful early warning information. It is desirable to have a more rigorous sampling design with a greater number of areas and trawl intensities than was possible in the present study. The inclusion of a BACI sample design, where some areas are closed to fishing for a lengthy period, is most likely to allow better discrimination between fishing and environmental effects.

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REVIEW

Physiological and ecological responses of crustaceans to ocean acidification

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ABSTRACT: The sensitivity of marine crustaceans to ocean acidification is poorly understood, but can be assessed by combining data from physiological and ecological studies. The species most at risk are exclusively marine and have limited physiological capacities to adjust to environmental change. They are poor iono- and osmoregulators and have limited abilities to compensate for acid–base disturbances. The problems are compounded in slow-moving, relatively inactive species because they have low circulating protein levels and low buffering capacities. Species living in low-energy environments, such as deep-sea and polar habitats, are particularly vulnerable, because they are metabolically limited with respect to environmental change. Elevated pCO_2 levels in seawater, such as those predicted for the year 2300, are known to have diverse effects on calcification rate, little effect on egg production and a negative effect on growth rate and moulting frequency in marine crustacean species. At these levels, embryonic development is negatively impacted, but larval and juvenile stages do not appear to be affected, unless the changes in pCO_2 are accompanied by rising temperatures. Overall, marine crustaceans are broadly tolerant to the seawater pCO_2 levels expected by 2100 and 2300, but only in the medium-term (weeks) and only in the more adaptable species. The reductions in growth rate are of concern, as these changes could affect species survival, distribution and abundance. Studies are urgently needed to evaluate whether the patterns of vulnerability identified here in crustaceans will still be relevant after long-term (months) exposure to the relevant pCO_2 levels, in combination with changes in other environmental factors.

KEY WORDS: Climate change · Crustacean · Calcification · Development · Growth · Marine · Ocean acidification · Reproduction

INTRODUCTION

Acidification of the world's oceans by the absorption of anthropogenic CO_2 is causing so much concern that it is gaining recognition alongside climate change as 'the other CO_2 problem' (Doney et al. 2009). Global atmospheric pCO_2 levels have increased from 0.03 to 0.04 kPa since pre-industrial times and are predicted to reach ~0.08 kPa by 2100 ('business-as-usual' CO_2 emission scenario, Houghton et al. 2001). More than a third of the atmospheric CO_2 emitted into the atmosphere since the beginning of the industrial revolution has been absorbed by the oceans, resulting in an alteration in the seawater carbonate system to give a 30% increase in H^+ concentrations (0.1 pH unit) and a 16% reduction in carbonate ion concentrations (Feely et al. 2004, Fabry et al. 2008). As ocean acidification is happening at a rate that outstrips the neutralising action of sedimentary antacids, it is predicted that the continued release of fossil-fuel CO_2 into the atmosphere will reduce ocean pH levels from present day levels of 8.1 to 7.8–7.7 by the end of the century (Orr et al. 2005), and to pH 7.4 by 2300 if atmospheric CO_2 reaches 0.20 kPa (Caldeira & Wickett 2003). Critically, pH levels will be lower than those experienced for the past 25 million yr (Royal Society Report 2005, Widdicombe & Spicer 2008).

The biological effects of ocean acidification are still far from clear, although interest in this area has intensified considerably over the past 7 yr (Pörtner et al. 2004, Fabry et al. 2008, Pörtner 2008, Przeslawski et al. 2008, Doney et al. 2009). Over this time period, there

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has been a tendency to concentrate on marine taxa considered to be the most vulnerable to ocean acidification, such as cnidarians, echinoderms and molluscs. These taxonomic groups have received the most attention because calcification of the external shells and skeletons is influenced by the changes in seawater pCO_2, pH and $[CO_3^{2-}]$ associated with ocean acidification. In extreme cases, for instance, elevated seawater CO_2 can cause dissolution of the calcified skeleton and reduce calcification rates (e.g. Gattuso et al. 1998, Langdon et al. 2000, Kleypas et al. 2006, Gazeau et al. 2007). Physiological studies have also revealed that echinoderms and bivalve molluscs are likely to be the most vulnerable to ocean acidification because they are poor iono-regulators and show little ability to buffer the acidifying effects of elevated CO_2 in their body compartments (Fabry et al. 2008, Widdicombe & Spicer 2008, Doney et al. 2009, Melzner et al. 2009, Dupont et al. 2010). The resulting consequences can be far reaching as acidification of body compartments can lead to metabolic depression (Michaelidis et al. 2005, Miles et al. 2007, Rosa & Seibel 2008), a reduction in energy stores (Langenbuch & Pörtner 2002, 2003) and a reduction in growth rate (Michaelidis et al. 2005, Beniash et al. 2010). Physiological studies can therefore be used to explain species-related differences in sensitivity, which, in turn, can be used to predict changes in individual performance and survival. Consequently, physiological changes have been used in the recent past to inform on the ecological effects of ocean acidification (Fabry et al. 2008, Guinotte & Fabry 2008, Widdicombe & Spicer 2008, Dupont et al. 2010). Over the past 7 yr there has been a concerted effort to switch attention from short-term acute exposures (hours to days) to extremely high pCO_2 levels (hypercapnia) to more relevant pCO_2 over longer time intervals, such as medium-term (weeks) to long-term exposure (months) (Fabry et al. 2008, Widdicombe & Spicer 2008, Doney et al. 2009). There has also been a move towards studies based on community mesocosms in order to examine changes in biodiversity and community structure (Widdicombe et al. 2009, Hale et al. 2011). In addition, there is a growing realisation that concomitant changes in other environmental variables, such as temperature, salinity and oxygen, may also modify responses to ocean acidification and further decrease chances of survival (Fabry et al. 2008, Widdicombe & Spicer 2008, Findlay et al. 2010a,b). Finally, there has been an increasing interest in the survival of early developmental and reproductive stages, which are likely to be the most vulnerable to ocean acidification (Dupont et al. 2008, 2010, Kurihara 2008).

Collectively, these approaches have demonstrated that the ability to tolerate ocean acidification is species specific and varies within phyla and between closely related species (Doney et al. 2009, Melzner et al. 2009, Hale et al. 2011). As we learn more about the long-term effects of ocean acidification on the physiology and ecology of marine invertebrates, it is becoming apparent that even those species generally tolerant of ocean acidification are under threat. Medium- to long-term compensation for projected ocean acidification conditions could prove to be energetically costly. Examples already exist in the literature to indicate that energy can be diverted away from key biological processes such as growth and reproduction towards compensatory responses (e.g. Wood et al. 2008, Beniash et al. 2010). On the other hand, certain species may be more resilient than once thought because they can acclimatise or adapt to the changes. Clearly, we need to examine the effects of ocean acidification on a wider range of species from different taxa to get a better idea of the possible effects of the projected climate change conditions on marine species, communities and ecosystems. Valuable lessons could be learned from taxa that have been largely overlooked, especially those that are considered to be tolerant of ocean acidification, such as crustaceans.

The effects of oceanic acidification on marine crustaceans have received some attention, however, the studies are disparate and have been conducted on widely divergent species for varying lengths of time at different pCO_2 levels. Our general lack of knowledge on the potential effects of ocean acidification on marine crustaceans is surprising because most crustaceans are characterised by a mineralised chitinous exoskeleton, which could be affected by changes in seawater carbonate chemistry. Crustaceans are also ecologically and economically important. In addition, there is a wealth of background physiological information that can be used to explain differing sensitivities to ocean acidification. If crustacean species are adversely affected by ocean acidification, then this could have far-reaching ecological consequences, as crustaceans are primary and secondary consumers and an important food source for higher trophic levels. For instance, crustacean species form the bulk of the zooplankton and can be present in vast numbers, either as pelagic larvae or as adults. Total biomass can reach impressive levels, as shown in the Southern Ocean where Antarctic krill *Euphausia superba* reach a total biomass of 133 million tonnes at any one time (Atkinson et al. 2009). Any adverse effects could also have an impact on the shellfish industry, as several decapod species (lobsters, crabs, prawns and shrimps) can be cultured or harvested for food or bait. Shellfish culture, which includes both crustaceans and bivalves, has increased in importance in recent years, reaching 20% of the global seafood production (T. Pickerell, Shellfish Association of Great Britain, pers. comm.).

Most of the 68 000 extant species of Crustacea described to date are marine (Martin & Davis 2001, 2006). While some groups are exclusively marine (e.g. cirripeds, euphausiids, stomapods) and occupy every available niche in the ocean, others are primarily marine, but have brackish, freshwater and semi-terrestrial/terrestrial representatives (e.g. ostracods, copepods, isopods, amphipods, decapods). Subsequently, crustaceans occupy a range of aquatic habitats that experience differing degrees of environmental variability. Those occupying deep oceans and high latitudes come from relatively stable environments where physical factors show little variation over temporal and spatial scales. Other environments, such as the intertidal zone and estuaries, can experience wide and rapidly changing fluctuations in physical factors in response to diurnal changes in tidal height. In estuarine environments, seasonal changes in physical variables are affected by changes in the inputs of freshwater and nutrients. Consequently, crustaceans are unusual when compared with other marine taxa. This is because they show a wide variety of responses to salinity change, from those that can regulate against external changes to those that simply conform. Studies on crustaceans can therefore provide researchers with an ideal opportunity to examine the relationship between environmental variability and the capacity to tolerate ocean acidification, which has recently been debated in the literature (Fabry et al. 2008, Widdicombe & Spicer 2008).

The purpose of the current review is to bring together, for the first time, all of the ocean acidification studies that have been carried out on crustaceans to date. The review will follow the development of the field from early physiological studies on the effects of hypercapnia to the effects of long-term exposure to more relevant pCO_2 levels on individual performance and fitness. The physiological data will be used to investigate the presence of any emerging patterns or trends that may explain why certain groups of crustaceans are more vulnerable to ocean acidification than others. The subsequent ecological repercussions will be reviewed by summarising our current understanding of the following: the possible energetic implications of medium-term exposure to relevant pCO_2 levels, the potential impacts on calcification rates and growth in crustaceans, as well as a summary of the latest observations on the effects of ocean acidification on development rates and larval survival. As such, the current review will use physiological and ecologically relevant responses to give an overall view on the biological effects of ocean acidification on crustaceans. This information will be used to identify areas for future research so that we can make a more informed assessment on the future prospects for marine crustaceans in a high CO_2 world.

PHYSIOLOGICAL RESPONSES TO OCEAN ACIDIFICATION

The most immediate responses to ocean acidification in marine crustaceans are best described at the individual level by physiological adjustments to changes in seawater carbonate chemistry. As the majority of crustaceans are committed water-breathers, they are in close contact with their external environment via the gills or equivalent structures, which are specialised for respiratory gas and ion exchange (Taylor & Taylor 1992). When carbonate chemistry of the seawater changes during ocean acidification, CO_2 excretion across the gills is compromised, causing an increase in CO_2 in the haemolymph (extracellular compartment). Subsequent changes in haemolymph pH are buffered to various extents by the mechanisms described in the following subsection. Such adjustments are important because they maintain the acid–base equilibria of the body fluids within the limits needed for protein function. This is particularly true for the intracellular compartment, where changes in pH are tightly controlled. A rise in intracellular $[H^+]$ can disrupt key biological processes such as metabolism, protein synthesis, iono-regulation and cell volume control (Gaillard & Malan 1983, Wheatly & Henry 1992, Whiteley 1999). Although pH disruptions can be tolerated in the haemolymph or extracellular compartment to some extent for short periods (hours), haemolymph pH regulation is important to maintain oxygen supply. Increasing $[H^+]$ will decrease the oxygen affinity of the respiratory pigment, reducing oxygen delivery to the tissues (Taylor & Whiteley 1989, Whiteley & Taylor 1992). Disruptions to extra- and intracellular acid–base balance can, therefore, have far-reaching consequences by compromising survival and adversely effecting ecologically relevant factors such as metabolism and growth.

Short-term acute exposure to hypercapnia

Most of what we currently understand about the physiological mechanisms involved in the compensation of acid–base imbalances comes from laboratory-based studies on decapod crustaceans (prawns, lobsters, portunid and xanthid crabs) exposed to acute elevations in pCO_2 (hypercapnia). Although the CO_2 levels investigated during short-term hypercapnia are much higher then the levels projected for future climate change scenarios, these studies are invaluable because they provide a mechanistic basis for understanding differences in the sensitivity of marine invertebrate taxa to ocean acidification (Fabry et al. 2008, Pörtner 2008, Widdicombe & Spicer 2008, Melzner et al. 2009, Hale et al. 2011). Most importantly, the short-

term exposure of crabs to either hypercapnia or external changes in salinity has demonstrated that acid–base balance is closely associated with iono-regulation because both homeostatic processes share the same mechanisms (Truchot 1975, 1981, 1992, Cameron 1978, Henry & Cameron 1982, Cameron & Iwama 1987, Whiteley 1999, Whiteley et al. 2001). Closer inspection of the mechanisms involved has revealed that pH adjustments in the haemolymph are buffered by haemolymph proteins (mainly haemocyanin) and bicarbonate ions. However, pH adjustments are dominated by electroneutral ion exchange across the gill epithelia, as the majority of buffer HCO_3^- comes from the external seawater (93%) and the remainder (7%) comes from internal stores (Cameron 1985). Moreover, crustacean species that are more tolerant to hypercapnia maintain a higher haemolymph HCO_3^- (Pörtner et al. 2004, Melzner et al. 2009), although HCO_3^- levels do not generally exceed values >50 mmol l^{-1} (Cameron & Iwama 1987). During electroneutral ion exchange, inward HCO_3^- from the seawater is exchanged for Cl^- after the catalysed hydration of CO_2 by carbonic anhydrase, and outward H^+ is exchanged for Na^+ (Taylor & Taylor 1992, Wheatly & Henry 1992, Whiteley 1999). These ion exchanges are driven by a basolateral Na^+/K^+-ATPase (Towle & Kays 1986, Taylor & Taylor 1992) and, possibly, an apical H^+-ATPase (Onken & Putzenlechner 1995, Freire et al. 2008). Consequently, environmental disruption of haemolymph acid–base status is more likely to be compensated in strong iono- and osmoregulators, where ion exchange mechanisms are well developed. This relationship could well explain why freshwater crustaceans, which are strong iono- and osmoregulators, can survive considerable acidification of their freshwater habits (Abrahamsson 1972, McMahon & Stuart 1989, Felten et al. 2008, Weber & Pirow 2009). Likewise, strong iono- and osmoregulators are likely to be less vulnerable to ocean acidification, because they possess the mechanisms that enable them to compensate for haemolymph acid–base disturbances, at least in the shorter term.

Medium-term exposure to relevant CO_2 levels

Exposure to smaller increases in seawater CO_2 (i.e. 0.10 to 0.20 kPa) over longer time intervals of weeks to months is more relevant to the potential changes that could occur as a result of ocean acidification. To date medium-term laboratory-based physiological studies in adult crustaceans have concentrated on alterations in compensatory capacities over time. The information available, however, is limited and can be traced back to a handful of studies that have either examined acid–base adjustments or calcification rates. Overall, it appears that medium-term exposure to pCO_2 levels more representative of ocean acidification has the potential to adversely affect growth and reproduction by diverting energy towards the maintenance of effective compensatory responses.

Acid–base compensation and energetic repercussions

Only 3 studies have examined the ability of crustaceans to adjust internal acid–base imbalances during medium-term exposure to projected pCO_2 levels. In the strong iono-regulating prawn species *Palaemon elegans* and *P. serratus*, complete compensation for a pCO_2 of 0.30 kPa was observed after 30 d of exposure (Dissanayake et al. 2010). However, ion homeostasis was maintained at the expense of acid–base balance. Two species of crabs, *Necora puber* and *Cancer magister*, which are relatively poor iono-regulators, were also able to compensate haemolymph acid–base disturbances within 24 h when exposed to CO_2 at 0.10 to 0.20 kPa (Pane & Barry 2007, Spicer et al. 2007). Compensation in all 4 species was achieved by an elevation in haemolymph [HCO_3^-]. Continued exposure to the same pCO_2 level in *N. puber* had a detrimental effect, as bicarbonate buffering started to fail after 16 d when [HCO_3^-] reached 27 mmol l^{-1} (Spicer et al. 2007). However, haemolymph [HCO_3^-] was found to be much lower after 30 d at the same pCO_2 in a separate study (Small et al. 2010). Exposure to an even higher pCO_2 level of 2 kPa (pH of 6.05) limited survival to between 4 and 5 d, because haemolymph pH fell despite a huge increase in haemolymph buffer base up to 55 mmol l^{-1} (Spicer et al. 2007). This bicarbonate value is similar to the maximum value obtained by Cameron & Iwama (1987) for the blue crab *Callinectes sapidus* during hypercapnia. Both observations support the existence of a threshold [HCO_3^-] in the haemolymph of approximately 50 mmol l^{-1}. The inability to increase [HCO_3^-] beyond this level is thought to be a compromise between acid–base balance and iono-regulation, although it is also possible that the medium-term adjustments are metabolically expensive as suggested by Pörtner et al. (2004) for other invertebrate species.

Acid–base adjustments made by crustaceans are likely to be metabolically expensive over weeks to months, due to the dependence on HCO_3^- uptake from the seawater via electroneutral ion exchange. Electroneutral exchange of HCO_3^- for Cl^- and H^+ for Na^+ is, in turn, dependent on the presence of ion gradients across transport epithelia that are maintained by active ion-transporting pumps, Na^+/K^+- and H^+-ATPases (Cameron & Iwama 1987, Pörtner et al. 2004, Santos et al. 2007). The actual costs associated with active ion

transport are unclear, but estimates of Na^+/K^+-ATPase activity range from 2.8 to 40% of total energy expenditure, indicating a considerable cost to the individual (Pannevis & Houlihan 1992, Leong & Manahan 1997). If the costs associated with the acid–base balance are indeed significant, then crustaceans that are good compensators could be adversely affected during ocean acidification. Either the costs will be limiting and restrict homeostatic processes or energy will be diverted away from other energy-demanding processes. In both situations, individual performance will be affected. Even though the energetic consequences of ocean acidification are unknown, some indication of the possible effects on performance can be obtained from experiments in which crustaceans are acclimated to various salinities. For instance, it is well known that the maintenance of ion gradients between the extracellular fluid and the external medium is energetically costly, especially during hypo- and hyper-osmoregulation (Gilles 1983, Moreira et al. 1983, McNamara & Moreira 1987, Péqueux 1995, Freire et al. 2008). The increase in energetic costs associated with iono-regulation has recently been used to explain differences in protein synthesis rates in the tropical prawn *Macrobrachium rosenbergii* (Intanai et al. 2009). In *M. rosenbergii* whole animal fractional rates of protein synthesis were highest at an iso-osmotic salinity of 14 psu, when the prawns were expending the minimal amount of energy on iono- and osmoregulation (Wang et al. 2004, Intanai et al. 2009). As protein synthesis rates are a major determinant of growth, these observations suggest that growth was compromised during hypo- and hyper-osmoregulation. Whether ocean acidification would have a similar effect on protein synthesis rates is not yet known.

Given that the energetic costs of acid–base regulation could be fairly substantial, it is also possible that the associated costs themselves could decrease during ocean acidification to reduce ATP demand. Such a response has been observed in the musculature of the intertidal polychaete *Sipunculus nudus* during hypercapnia (Pörtner et al. 2000). In this species, intracellular pH is protected during an extracellular acidosis by an increase in the importance of Na^+-dependent Cl^-/HCO_3^- exchange for H^+ transport over Na^+/H^+, Na^+/K^+-ATPase, and possibly H^+-ATPase activity. The benefit here is the shift in ion-transporting mechanisms from those with higher to lower ATP demands. An extracellular acidosis in *S. nudus* was accompanied by a decrease in metabolic rate, suggesting that a decrease in the energetic demands of acid–base regulation has an effective energy-saving role (Pörtner et al. 1998, 2000). Whether this strategy exists in crustaceans exposed to more moderate increases in pCO_2 is not known.

Calcification rates

Currently it is relatively unclear whether the net calcification rate (balance between rates of calcification and dissolution) of the chitinous-mineralised crustacean exoskeleton will be adversely affected by ocean acidification. Calcification processes in crustaceans are likely to be less vulnerable to ocean acidification than those present in echinoderms or molluscs, because exoskeletal $CaCO_3$ is mostly in the more stable form of calcite rather than the more soluble aragonite (Boßelmann et al. 2007, Neues et al. 2007). In addition, calcification processes are well removed from external changes in seawater carbonate chemistry and are known to depend on HCO_3^- rather than on CO_3^{2-} (Cameron 1985). The crustacean exoskeleton also contains amorphous calcium carbonate, which is highly soluble and acts as a transient source of Ca^{2+} (Boßelmann et al. 2007, Neues et al. 2007). It is tempting to speculate that amorphous $CaCO_3$ may also act as a source of HCO_3^- for acid–base homeostasis. Interestingly, the proportion of amorphous calcium salts in the exoskeleton varies between species and depends on lifestyle (Neues et al. 2007). It may therefore influence compensatory capacities by providing a labile source of HCO_3^-. Currently, it is not known how these various forms of $CaCO_3$ are affected by ocean acidification. However, the formation of $CaCO_3$ in the crustacean exoskeleton is thought to depend on the maintenance of an alkaline pH in the exoskeletal compartment, which is reported to be 0.3 pH units higher than that in the haemolymph (Wood & Cameron 1985).

Despite the lack of information on calcification processes in crustaceans, ocean acidification has the potential to influence calcification rates in 2 ways. First, ocean acidification could influence precipitation of $CaCO_3$ in the exoskeleton by reducing the alkaline pH in the exoskeletal compartment (Wood & Cameron 1985). Second, ocean acidification could interfere with post-moult calcification of the new exoskeleton, which is dependent on a large uptake of Ca^{2+} and HCO_3^- across the gills from the surrounding seawater (Neufield & Cameron 1992, Wheatly 1997). The influx of Ca^{2+} and HCO_3^- is particularly sensitive to an increase in external $[H^+]$ as it reduces branchial HCO_3^- uptake (Cameron 1985, Cameron & Wood 1985). This suggests that the reductions in seawater pH associated with ocean acidification could potentially interfere with post-moult calcification. A similar response has been observed in the blue crab *Callinectes sapidus* during hypercapnia. In this species, post-moult calcification, which normally takes 14 d, took twice as long, as the HCO_3^- necessary for calcification was obtained from metabolic CO_2 (Cameron 1985). Any delay in the post-moult calcification process could be fatal, as crus-

taceans are particularly vulnerable to predation during this period. Their exoskeletons are soft, and the newly moulted crustaceans are unable to move or defend themselves. As a consequence, ocean acidification has the potential to increase mortality rates indirectly by delaying the calcification process during moulting.

Despite the potential for adverse effects on calcification rates, medium-term exposure to moderate elevations in seawater CO_2 indicates that the calcified structures in crustaceans (exoskeleton and the barnacle shell wall plates) are well protected from ocean acidification. In all crustacean species studied to date, calcification rates either remain the same or increase after a period of CO_2 exposure (Wickins 1984, Findlay et al. 2009, McDonald et al. 2009, Ries et al. 2009). An increase in calcium content was first observed in the exoskeleton of *Penaeus monodon* after 36 d of exposure to a decrease in seawater pH of 7.9 to 6.4 pH units (Wickins 1984). A similar response was observed in the blue crab *Callinectes sapidus*, the king prawn *Penaeus plebejus* and the lobster *Homarus americanus* (Ries et al. 2009). All 3 species were exposed to seawater equilibrated with pCO_2 levels that were 2, 3 and 10 times higher than pre-industrial levels (0.06 ± 0.01, 0.09 ± 0.01 and 0.29 ± 0.05 kPa, respectively) for 60 d, which is nearly twice the exposure period experienced by *P. monodon* in the earlier study by Wickins (1984). Such a response may reflect the ability to effectively maintain elevated pH levels at the site of calcification. It may also demonstrate that the outer organic layer, or epicuticle, acts as an effective barrier between the mineralised exoskeleton and the seawater (Ries et al. 2009). In contrast, long-term exposure to a pCO_2 of 0.01 kPa for 30 wk resulted in morphological damage in the marine shrimp *P. pacificus*, due to shortening of the second antennae (Kurihara et al. 2008). The authors attributed this damage to the dissolution of $CaCO_3$ stores by the ensuing disruptions to acid–base homeostasis, which are more likely to occur in the long term.

Recent studies on 2 species of intertidal barnacles have revealed differences in net calcification rates during elevated pCO_2. In the tropical barnacle *Amphibalanus amphitrite*, an increase in the calcification rate was implied by the observed increase in basal shell diameter after 11 wk at pH 7.4, which required greater force to cause shell breakage (McDonald et al. 2009). Compensatory responses, however, were localised, as the central wall plates succumbed to dissolution at pH 7.4 and were weaker than individuals held at pH 8.1. This observation suggested that individuals held at pH 7.4 would be more vulnerable to predation. In contrast, the maintenance of mineral content in the shells of the cold-temperate/boreal barnacle species *Semibalanus balanoides* after 20 d at pH 7.3 indicated an ability to compensate for shell dissolution in seawater saturated with aragonite and calcite, but an inability to enhance calcification rates (Findlay et al. 2010b). Given that the growth rates of *S. balanoides* were slower at pH 7.3 than at pH 8.1, Findlay et al. (2010b) concluded that calcification of the shell under acidifying conditions was energetically demanding, resulting in the reallocation of resources, which compromised individual fitness. Clearly, crustaceans show some ability to compensate net calcification rates for medium-term exposure at relevant pCO_2. However, a few detrimental effects were observed due to reductions in the strength of calcified protective plates and reductions in growth rates.

EMERGING PATTERNS OF VULNERABILITY: THE PHYSIOLOGICAL EVIDENCE

Some generalisations about the crustacean groups most likely to be affected by ocean acidification can be made by combining physiological responses from earlier studies on hypercapnia with those from recent experiments on long-term exposures to more moderate levels of pCO_2 (Pane & Barry 2007, Spicer et al. 2007, Widdicombe & Spicer 2008). To date, it appears that vulnerability to ocean acidification may be related to differences in lifestyle and to differences in the ability to compensate for environmental change. As stated previously, it is predicted that strong iono- and osmoregulating species are likely to be the most tolerant to ocean acidification, simply because they have the compensatory mechanisms to respond to acid–base disruptions. These species tend to inhabit shallow coastal environments under freshwater influence, where they experience natural variations in seawater pCO_2, pO_2, salinity and temperature. For instance, when left behind in rock pools during the night, crabs can experience increased pCO_2 and decreased pH levels and pO_2 in the seawater (Truchot & Duhamel-Jouve 1980, Morris & Taylor 1983). Marine crustaceans can also be exposed to increased pCO_2 in deep-sea vent systems and in the surface waters of the open ocean where they also experience vertical gradients in pH and pO_2 (Fabry et al. 2008). Early physiological studies have demonstrated that the ability to compensate acid–base disturbances in the face of environmental change is highly variable among species. This is true among those species that have a subtidal distribution and experience stable conditions in their natural environment. For instance, aerial exposure and subsequent elevation of haemolymph CO_2 is fully compensated by the European lobster *Homarus gammarus*, is partially compensated by the edible crab *Cancer pagurus* and remains uncompensated in the swimming crab *Necora puber* and the spider crab *Maja squinado* (Taylor &

Whiteley 1989, Whiteley 1999). Moreover, physiological studies have shown that some species of intertidal crabs do not compensate for the effects of aerial exposure when exposed at low tide (Burnett & McMahon 1987). Instead they undergo metabolic depression and wait until the tide returns. Despite these differences in compensatory capacities, *N. puber* and *C. magister* are able to survive exposure to pCO_2 levels more relevant to ocean acidification, at least in the medium-term, i.e. up to 60 d (Pane & Barry 2007, Spicer et al. 2007, Small et al. 2010). Consequently, it remains unclear whether the ability of crustaceans to compensate for highly variable environments increases their tolerance to ocean acidification. However, this may have more to do with the limited data set collected to date and less to do with existing patterns of vulnerability. Clearly, there is a need to investigate the physiological responses in crustacean species from a broader range of marine habitats during exposure to relevant ocean acidification conditions for longer periods of time.

The ability to compensate for the effects of ocean acidification can also vary with lifestyle. Decapod crustaceans with high rates of activity have a greater capacity for passive compensation of haemolymph acid–base disturbances (i.e. buffering by non-bicarbonate buffers) than slow-moving, relatively inactive species due to species-related differences in respiratory variables. Relatively fast-moving species, such as the swimming crab *Necora puber*, have higher circulating levels of haemocyanin than slow-moving, relatively inactive crabs, such as *Maja squinado* (Watt et al. 1999). Higher haemocyanin levels lead to higher oxygen-carrying and non-bicarbonate-buffering capacities, in keeping with the higher aerobic requirements and higher rates of metabolic CO_2 production. The lower haemocyanin levels characteristic of slow-moving species are associated with relatively low rates of oxygen uptake and relatively high levels of circulating lactate levels, showing some reliance on anaerobic metabolism (Watt et al. 1999). Similar characteristics may contribute to the inability of the deep-sea tanner crab *Chionoecetes tanneri* to buffer an accumulating haemolymph acidosis when exposed to short-term hypercapnia (1% CO_2, ~1.28 kPa, for 24 h) (Pane & Barry 2007). For example, haemolymph protein levels were significantly lower in *C. tanneri* than those determined in a shallow-water species, *Cancer magister*, under the same conditions. The reduction in buffering capacity in *C. tanneri* was compounded by a failure to raise HCO_3^- levels beyond 3 mmol l^{-1} (Pane & Barry 2007). The lack of compensatory ability could be explained by the low temperatures at which the measurements were taken (3°C) or by the fact that deep-sea crabs have low metabolic rates, in keeping with their habitation of a stable, harsh and resource-limited environment.

By inference, these observations suggest that other species living in similarly low-energy environments will be susceptible to ocean acidification. This is particularly pertinent at polar latitudes, where marine invertebrates are stenothermal, have poor thermal tolerances and are characterised by relatively low metabolic rates (Peck 2002, Pörtner et al. 2007). Acid–base characteristics have only been determined in 1 species of polar marine crustaceans, the giant Antarctic isopod *Glyptonotus antarcticus*. This species has relatively low circulating levels of protein, resulting in low haemocyanin oxygen-carrying and protein-buffering capacities (Whiteley et al. 1997). The latter is 2.5- to 7.5-fold lower than the range of values estimated in other aquatic crustaceans (Taylor & Taylor 1992). Not only is the lower buffering capacity a problem in terms of compensating for the effects of ocean acidification, the oxygen affinity of *G. antarcticus* haemocyanin is highly sensitive to a reduction in pH (Jokumsen et al. 1981). Both characteristics decrease the involvement of the respiratory pigment in the transport of oxygen from the gills to the tissues. It appears that *G. antarcticus*, just like the deep-sea crab *Chionoecetes tanneri*, will be unable to compensate for the effects of ocean acidification. As a result, both species will be more vulnerable to the associated changes in seawater chemistry.

POTENTIAL ECOLOGICAL EFFECTS OF OCEAN ACIDIFICATION

Very little information is available on the potential impacts of ocean acidification on the ecology of crustaceans. There is some evidence to show that ocean acidification may affect crustacean species at the population level by influencing the growth or reproductive performance of adults. In addition, there is a growing interest in the potential effects of ocean acidification on early life-cycle stages in benthic and pelagic crustacean species. Collectively, it appears that sensitivities to ocean acidification vary among species and with ontogeny. However, the lack of data makes it difficult to observe any emerging trends, and it is impossible to discuss the available information without resorting to individual studies.

Effects of ocean acidification on growth rate

The only evidence to date of the effects of elevated pCO_2/reduced pH on growth rates in adult crustaceans comes from 1 species of marine shrimp and 2 species of penaeid prawns (Wickins 1984, Kurihara et al. 2008). Growth rates in all 3 species were affected by elevated CO_2, but the marine shrimp *Palaemon pacificus* was

more sensitive than *Penaeus occidentalis* or *P. monodon* (Table 1). Not surprisingly, CO_2 had more of an effect when levels were increased and seawater pH was reduced to 7.6 pH units or lower. For example, when adult *P. pacificus* were held at a pCO_2 of 0.10 kPa (pH = 7.89 ± 0.05) there was no change in growth rate for 30 wk and then only in females (Kurihara et al. 2008). At the higher pCO_2 level of 0.20 kPa (pH = 7.64 ± 0.09), both growth rate and moult frequency decreased after 7 wk, and no animals survived beyond 15 wk. In penaeids, the growth rate declined when seawater pH fell below 7.4 due to a decrease in moulting frequency and an increase in intermoult period from 5 to 6–9 d (Wickins 1984).

Effects of ocean acidification on reproduction and development

Our understanding of the reproductive effects of ocean acidification in crustaceans is restricted to a small number of observations on egg production, and rates of embryonic and larval development (Table 1). Changes in egg production were observed in *Palaemon pacificus* held at a pCO_2 of 0.10 kPa (Kurihara et al. 2008). Higher levels of pCO_2 (0.20 kPa), however, had no effect on egg production in the copepods *Acartia tsuensis* and *A. steueri* after 27 d of exposure (Kurihara et al. 2004a,b, Kurihara & Ishimatsu 2008) or in the barnacle *Amphibalanus amphitrite* held at pH 7.4 (McDonald et al. 2009). The influence of elevated CO_2 on embryonic development has only been investigated in 1 barnacle species, *Semibalanus balanoides*. In this intertidal species, a pCO_2 of 0.09 kPa reduced rates of embryonic development in isolated egg masses and delayed time to hatching by 19 d (Findlay et al. 2009).

In contrast, there is little evidence to show that ocean acidification is detrimental to larval and juvenile stages (Table 1). Currently, data on larval development under relevant levels of pCO_2 are available for 4 crustacean species: the copepod *Acartia tsuensis* (Kurihara & Ishimatsu 2008), the barnacle *Amphibalanus amphitrite* (McDonald et al. 2009), the lobster *Homarus gammarus* (Arnold et al. 2009) and the spider crab *Hyas*

Table 1. Effects of elevated seawater CO_2 on indices of growth and reproductive capacity in a variety of crustacean species. pCO_2: partial pressure of CO_2 calculated from values published as ppm (mole fraction) assuming a barometric pressure of 101.35 kPa. Dashes represent absence of available data

Species	pCO_2 (kPa)	pH	Time	Effect	Source
Adults					
Acartia tsuensis	0.20	7.4	27 d	No effect on survival, body size, development rate, or egg production	Kurihara & Ishimatsu (2008)
Calanus finmarchicus	0.8	6.85	72 h	No effect on adult growth, decrease in egg production	Mayor et al. (2007)
Acartia steueri	0.20–1.0	7.4–6.8	8 d	Decreased egg production at <pH 6.8	Kurihara et al. (2004a,b)
Acartia erythraea	0.51–1.0	7.0–6.8	8 d	Decreased egg production at <pH 6.8	Kurihara et al. (2004a,b)
Amphibalanus amphitrite	–	7.4	8–11 wk	No effect on growth or egg production	McDonald et al. (2009)
Semibalanus balanoides	0.09	7.7	104 d	Decreased survival	Findlay et al. (2009)
Penaeus occidentalis	–	7.6 & 7.3	56 d	Decreased growth rates	Wickins (1984)
Penaeus monodon	–	7.9–6.4	36 d	Decreased growth rates	Wickins (1984)
Palaemon pacificus	1.0	7.9	30 wk	No effect on growth	Kurihara et al. (2008)
	0.20	7.6	15 wk	Decreased growth and egg production	Kurihara et al. (2008)
Eggs/larvae					
Acartia erythraea	0.20–1.0	7.4–6.8	2d	Increase in nauplius mortality rates and hatching rate	Kurihara et al. (2004a,b)
Acartia tsuensis	0.20	7.4	27 d	No effect on development rate or hatching success	Kurihara & Ishimatsu (2008)
Calanus finmarchicus	0.81	6.95	72 h	Decreased hatching success	Mayor et al. (2007)
Euphausia superba	1.0–2.0	7.7/7.4	26 d	Decreased hatching success	Kurihara et al. (2008)
Amphibalanus amphitrite	–	7.4	8–11 wk	No effect on larval condition, cyprid size and attachment, or metamorphosis	McDonald et al. (2009)
Semibalanus balanoides	0.09	7.7	104 d	Decreased rates of embryonic development, hatching and post-larval growth	Findlay et al. (2009, 2010b)
Echinogammarus marinus	0.20	7.5	18–20 d	No effect on rates of embryonic development or hatchling number	Egilsdottir et al. (2009)
Gammarus locusta	0.10	7.6	–	No effect on growth rates to maturity	Hauton et al. (2009)
Palaemon pacificus	0.20	7.6	–	Decreased body size in settling juveniles	Kurihara et al. (2008)
Homarus gammarus	0.12	–	–	No effect on hatchling number or rate of development	Arnold et al. (2009)

araneus (Walther et al. 2010). In all 4 species, elevation in pCO_2 to <0.02 kPa had no effect on rates of larval survival or development (Table 1). In addition, elevated pCO_2 had no effect on larval condition and cyprid size, attachment, or metamorphosis in *A. amphitrite* (McDonald et al. 2009). In *H. gammarus*, this may be due to the fact that the exoskeletons of planktonic decapod larvae (zoeae) are unmineralised, while those of megalopae and benthic juveniles are only partially calcified (Anger 2001). This is likely to reduce the potential negative effects of ocean acidification on calcification rates during larval moults. Hatching success in the copepods *Acartia erythraea* and *Calanus finmarchicus* was negatively affected, but at pCO_2 levels of 0.50 to 0.80 kPa, which far exceed the values predicted for the year 2300 (Kurihara et al. 2004a,b, Mayor et al. 2007). In addition, the growth rates of early life stages of *Semibalanus balanoides* from the metamorphosing cyprids to early juveniles were significantly reduced by a decrease in seawater pH from 8.1 to 7.3 (~0.04 to 0.30 kPa) (Findlay et al. 2010b). A similar drop in pH (8.2 to 7.4), however, had no effect on juvenile to adult growth rates in the tropical barnacle *A. amphitrite* (McDonald et al. 2009). In addition, reductions in pH down to 7.8 (0.06 kPa) and 7.6 (0.10 kPa) had no effect on the growth rates of juveniles to adolescence or to sexual maturity in the amphipod *Gammarus locusta* (Hauton et al. 2009).

COMBINED EFFECTS OF OCEAN ACIDIFICATION AND OTHER ENVIRONMENTAL VARIABLES

Apart from some early work, the interactive effects of multiple stressors on the survival of marine crustaceans has been poorly studied, despite the fact that ocean acidification is occurring simultaneously with changes in temperature, salinity and oxygen. Early physiological studies on the effects of diurnal changes in temperature, pCO_2 and pO_2 that occur naturally in rock pools demonstrated that acid–base changes in the haemolymph of the shore crab *Carcinus maenas* were less than those induced by exposure to a single factor (Truchot 1986). The combined effects of hypoxia and hypercapnia at night and the reverse situation during the day had opposing effects on acid–base balance and acted to minimise physiological disturbances. Moreover, the environmental changes happened so rapidly that HCO_3^- did not have time to accumulate in the haemolymph. Instead, an increase in haemolymph pH was brought about by hypoxia-induced hyperventilation. In contrast, the simultaneous exposure of decapod crustaceans to hypoxia and hypercapnia in the laboratory resulted in the accumulation of haemolymph HCO_3^-, which fully compensated the haemolymph acidosis (Truchot 1984, Burnett 1997). In *Callinectes sapidus*, for example, haemolymph pH remained unchanged during hypoxia at a pCO_2 of 0.35 kPa, and even increased at a pCO_2 of 0.49 kPa (Burnett 1997). The increase in haemolymph pH or alkalosis was beneficial to the crabs, especially during hypoxia, as it served to increase haemocyanin oxygen affinity and hence oxygen loading at the gills (Burnett 1997). A small increase in L-lactate levels in the haemolymph during hypoxia would have had the same effect (Truchot 1980, Burnett 1997). In contrast to *C. sapidus*, the deep-sea crab *Chionoecetes tanneri*, which has a relatively poor capacity for acid–base compensation, was unable to buffer the haemolymph acidosis induced by exposure to both hypoxia and hypercapnia (Pane & Barry 2007).

Of the few studies that have been carried out to date, most have focused on the combined effects of ocean acidification and temperature. Attention has either been given to the physiological responses of adults or to the survival rates of larvae. In adult crustaceans, the physiological consequences of ocean acidification and temperature have been restricted to 2 species of subtidal crabs, *Cancer pagurus* and *Hyas araneus* (Metzger et al. 2007, Walther et al. 2009), and to a species of nektonic shallow-water prawn, *Metapenaeus joyneri* (Dissanayake & Ishimatsu 2011). In *C. pagurus*, exposure to 1% CO_2 (~1.0 kPa) and either a progressive decrease or increase in temperature reduced upper thermal limits and increased mortality rates (Metzger et al. 2007). A similar response was observed in the spider crab *H. araneus*, when exposed to more relevant pCO_2 levels of 0.07 and 0.30 kPa (Walther et al. 2009). An elevation in pCO_2 in *H. araneus* not only lowered the upper thermal tolerance limit, it also increased the heart rate and reduced haemolymph pO_2 levels when temperatures rose above 10°C. Collectively, these data suggest that thermal tolerances are reduced in crabs under high CO_2 conditions, due to a limitation in oxygen supply as described in teleost fishes by Pörtner & Farrell (2008). Elevated pCO_2 and temperature can also affect swimming performance, as exposure of *M. joyneri* to a pCO_2 of 1.0 kPa at 3 acclimation temperatures (10, 15 and 25°C) for 10 d significantly reduced critical swimming speeds (Dissanayake & Ishimatsu 2011). However, elevated pCO_2 had more of an effect on swimming performance than temperature, even though acclimation to the highest temperature (25°C) decreased aerobic scope (difference between standard and active metabolic rates). The authors attributed this observation to the fact that the prawns were held at temperatures outside their normal thermal optima (Dissanayake & Ishimatsu 2011). As a consequence, oxygen supply was restricted and aerobic performance was reduced. Given that reductions in

thermal tolerance windows have been linked to reductions in growth performance and reproductive activity, as well as reductions in biogeographical ranges and shifts in community composition, the combined effects of ocean acidification and temperature could have wide-ranging ecological implications (Pörtner 2002, 2010, Somero 2002, Pörtner & Farrell 2008).

Ecological studies have concentrated on the effects of elevated pCO_2 and temperature on the growth and survival of post-larvae from 2 species of barnacles (Findlay et al. 2010a,b) and from the spider crab *Hyas araneus* (Walther et al. 2010). Even though both barnacle species were collected from similar intertidal habits on the southwestern coast of England, differences in growth and shell development were observed between the cold-water species *Semibalanus balanoides* and the warm-water species *Elminius modestus* (Findlay et al. 2010a). Exposure to pCO_2 levels of 0.04 and 0.10 kPa at 2 temperatures (14 and 18°C) had no effect on post-larval growth rates in *S. balanoides*, but the higher pCO_2 and temperature treatment significantly reduced growth rates in *E. modestus.* In contrast, the shell calcium content in *S. balanoides* was reduced by CO_2 and by temperature, but neither factor had any effect on the calcification rates in *E. modestus*. In summary, it appears that *S. balanoides* post-larvae are able to maintain growth, but at the expense of shell calcification. On the other hand, *E. modestus* post-larvae are able to maintain the integrity of their calcified shells, but at the expense of growth. The ability to maintain mineralised shell plates during elevated pCO_2 and temperature exposure was attributed to differences in thermal tolerance brought about by sampling populations from different parts of their geographic distribution (Findlay et al. 2010a). Interestingly, a sub-arctic population of the cold-water species *S. balanoides* was observed to be more sensitive to CO_2 than the population in southwestern England, at the southern limit of its distribution range (Findlay et al. 2010a,b). Growth and development of post-larval *S. balanoides* from Kongsfjorden, Svalbard, at 79°N, was negatively impacted by elevated CO_2, but surprisingly an increase in temperature of +4°C had no effect (Findlay et al. 2010b). In contrast to the southern population, the northern population of *S. balanoides* also managed to maintain net calcification of their shells during elevated CO_2, suggesting that resources were reallocated from 1 energy-demanding process to another as discussed in greater detail by Findlay et al. (2010b). Comparisons between populations of *H. araneus* from similar latitudes (temperate and sub-arctic) revealed that development time was slower in the northern compared with the southern population under present day pCO_2 conditions (0.04 kPa) (Walther et al. 2010). An elevation in pCO_2 to 0.30 kPa delayed rates of development and reduced growth rates and overall fitness of larvae from both populations. An increase in pCO_2 to 0.07 kPa, however, had no effect. The megalopa emerged as the most vulnerable stage of development in *H. araneus*, as it was the most sensitive to temperature in the north and the most sensitive to CO_2 levels in the south. The authors attributed the increase in sensitivity in the megalopa to reductions in thermal tolerance (Walther et al. 2010). They also predicted that both ocean acidification and global warming would affect the recruitment of the benthic juvenile stages in this species. A decrease in the abundance of *H. araneus* has already been observed in the North Sea around Helgoland, where temperatures have increased by 1.1°C over the last 40 yr (Walther et al. 2010).

Finally, the specific effects of elevated pCO_2 and temperature on marine community diversity and structure have recently been addressed by using artificial substrate units planted on the shore at extreme low tide (Hale et al. 2011). After the establishment of marine invertebrate communities, the artificial substrate units were removed and exposed to 8 different treatments (4 pH levels at 2 different temperatures). After 60 d of exposure, the combination of low pH (7.3 and 6.7) and elevated temperature (16°C) significantly changed community structure and lowered diversity. However, at the higher pH levels (8.0 and 7.7) and elevated temperature, species abundance and diversity increased. Relevant to the present review was the fact that while molluscs and echinoderms were the most affected, and annelids the least, crustaceans showed an intermediate response. More specifically, gammaridean amphipods showed a marked decrease in abundance at low pH and elevated temperatures, but increased in abundance along with an isopod species at pH 7.7 and 7.3. Furthermore, the loss of the skeleton shrimp *Caprella acanthifera* from the higher temperature treatments contributed to changes in species richness. Overall this community-based mesocosm study revealed that the ecological impacts of the 2 environmental variables were greater than either factor in isolation. In addition, the study concluded that the changes in community structure were due to species-specific differences in tolerances (Hale et al. 2011). However, the authors stipulated that ecosystem-level responses to ocean acidification and global warming could not simply be explained by a reduction in individual performances. They also attributed the observed responses in species diversity to changes in community interactions, their argument being that the loss of the more vulnerable species provided opportunities for more tolerant species.

The dependence of community-led changes in marine ecosystems during ocean acidification on species-specific physiological tolerances has parallels to the

selective survivorship associated with the Permo-Triassic mass extinction, which occurred around 250 million yr ago (Pörtner et al. 2005, Knoll et al. 2007). This extinction event resulted in the loss of up to 54% of late Permian marine families, 68% of the genera and 92% of the species, resulting in a major re-organisation of the marine ecosystem. It has been argued that these ancient extinctions can be explained in terms of the physiological responses of marine invertebrates to the combined effects of environmental hypoxia, hypercapnia, sulphide toxicity and rising temperatures that prevailed at the time (Pörtner et al. 2005, Knoll et al. 2007). Moreover, the ability to compensate for hypercapnia is thought to be a key to survival. Interestingly, those groups that were more vulnerable to hypercapnia experienced significantly higher rates of extinction, although survival rates were also related to the presence or absence of a calcified exoskeleton and its relative proportion to soft tissues (Knoll et al. 2007). Arthropods were described by Knoll et al. (2007) as possessing a calcium carbonate skeleton of moderate mass with respect to supportive tissue and body fluids that were relatively well buffered. Although not the most vulnerable grouping, this group lost around 54% of its genera during the end-Permian mass extinction.

In summary, it appears that, in adult crustaceans, an increase in pCO_2 to 1.0 kPa during an increase in temperature causes physiological disruption and has a synergistic effect on an individual's performance. An increase in mortality rates was also observed in the subtidal crab *Cancer pagurus* (Metzger et al. 2007) and in the shallow-water prawn *Metapenaeus joyneri* during moulting (Dissanayake & Ishimatsu 2011). However, the sensitivity to multiple stressors varies among species, with differential effects on individual fitness and survival leading to changes in community structure and interactions in an intertidal marine community (Hale et al. 2011). Such differences could also explain selective survival during the end-Permian mass extinction when there was a diversity collapse in the marine environment (Knoll et al. 2007). Overall the combination of CO_2 and temperature levels relevant to ocean acidification and global warming have little effect on the performance of post-larvae, at least in 2 species of barnacles (Findlay et al. 2010a,b). However, sensitivity does appear to change with life-cycle stage, as shown in *Hyas araneus*, where one particular stage of development was identified as being the most vulnerable (Walther et al. 2010). Moreover, sensitivity of early life stages to a single environmental variable can change within species according to geographical distribution (Findlay et al. 2010b, Walther et al. 2010). One population can be more sensitive to pCO_2, while the other is more sensitive to temperature (Walther et al. 2010). Although investigations into the combined effects of elevated pCO_2 and temperature in crustaceans are few and far between, there has only been 1 study on the effects of elevated pCO_2 and reduced salinity. This is surprising given the role of ion and acid–base homeostasis in the determination of a species' sensitivity to ocean acidification. In this particular study, the exposure of the intertidal amphipod *Echinogammarus marinus* to elevated CO_2 (0.20 kPa) at 3 salinities (10, 22 and 35 psu) had little effect on hatching success and developmental rate (Egilsdottir et al. 2009). Overall, reductions in salinity were found to be more important than elevations in CO_2.

CONCLUSIONS AND FUTURE DIRECTIONS

The main purpose of the present review was to summarise our current understanding of the potential biological effects of ocean acidification on marine crustaceans and to identify and characterise those species or groups most at risk. The study of marine crustaceans can make a valuable contribution to ocean acidification research because crustaceans occupy a wide variety of aquatic habitats and show a range of tolerances to environmental change. As a result they demonstrate a range of responses that can be used to increase our understanding of the mechanisms that determine tolerances to ocean acidification, as well as clarify the subsequent long-term effects on performance and survival. The physiological studies carried out to date suggest that the most vulnerable groups are those that are unable to compensate for the changes imposed by elevated pCO_2 and reduced pH levels. These species tend to be poor iono- and osmoregulators, living in low-energy environments with low metabolic rates and low routine levels of activity, such as deep-sea and polar environments. From the limited data set, it appears that these species are characterised by low buffering capacities and a general inability to mobilise HCO_3^- ions from the seawater or from the exoskeleton to buffer the acid–base disturbances caused by ocean acidification. Moreover, they are highly specialised for living at low and stable temperatures and may be metabolically limited with respect to further change. Consequently the more vulnerable species are less likely to succeed in overcoming the combined effects of ocean acidification and increasing temperature or reduced salinity and pO_2 levels resulting from climate change. In addition, they are less likely to be able to compete with warm-water invasive species that will be more adaptable and better able to exploit available resources. Crustacean species likely to be more tolerant of ocean acidification are those currently inhabiting fluctuating environments, such as estuaries and shallow coastal regions. These species are less likely to

suffer long-term reductions in fitness because they have the capacity to compensate acid–base disturbances via ion exchange mechanisms. The exceptions are slow-moving crabs with poor haemolymph-buffering capacities. The latter may be more vulnerable to ocean acidification due to their limited capacity to adjust their acid–base physiology. Overall, we still have little idea of how these various species will cope during prolonged exposure to elevated pCO_2 on a scale of months to years, or how multiple stressors will affect individual fitness. However, the indication is that concomitant changes in temperature, salinity and oxygen can have important synergistic effects.

Given the close association between physiological capacities and the ability to cope with ocean acidification, there is a continuing need to examine the mechanisms responsible for these compensatory responses. The relationship between ion regulation and acid–base balance is still far from clear. Even less is known about the mechanisms underlying calcification processes in crustaceans. All 3 physiological processes, i.e. iono-regulation, acid–base balance and calcification, could be linked via the mobilisation of Ca^{2+} and HCO_3^- from the exoskeleton (Whiteley 1999). In addition, it is unclear whether those species that can tolerate ocean acidification will be able to maintain compensatory responses over time, and whether less tolerant species will be able to acclimatise or even adapt to the changes in seawater carbonate chemistry. Future studies are needed to examine physiological and ecological responses to ocean acidification in crustacean species with differing tolerances to environmental change over longer time intervals at relevant pCO_2 levels and in combination with changes in temperature, salinity, or oxygen levels. The resulting data can then be used to inform on the groups of crustaceans most likely to be adversely affected by ocean acidification and climate change. It can also be used to explain patterns of vulnerability in other marine taxa. In addition, it is vital that we increase our understanding of the capacity of marine crustaceans to adapt to the effects of ocean acidification. Such information will help towards forecasting the potential long-term effects of ocean acidification and climate change on marine ecosystems (Kurihara 2008). Even though a few multi-generation experiments have been conducted to date (Kurihara & Ishimatsu 2008), many more are needed in order to examine the potential for adaption under future ocean acidification scenarios.

Currently, there is little evidence to suggest that early life stages are more vulnerable to ocean acidification than adults, but the data set is extremely limited. Recent work suggests that survival rates are affected and subtle changes in the ability to calcify the exoskeleton during growth by moulting may have long-term repercussions for survival and recruitment. From the 2 barnacle species studied to date, it appears that ocean acidification and climate change will not affect post-larval survival (Findlay et al. 2010a,b), but sensitivities can vary with stage of development (Walther et al. 2010). In addition, it has been shown that ocean acidification can affect growth rates and moulting frequencies in crustaceans (Kurihara et al. 2008, Dissanayake & Ishimatsu 2011). Further work is needed to determine whether this is a general effect or whether it is species specific. If moulting frequencies and mortality rates in crustaceans are more generally affected by ocean acidification, this could have a profound effect on species survival, distribution and abundance. Overall, future studies are needed to identify any potential bottlenecks during development and to examine the combined effects of ocean acidification and other environmental variables on the survival of early life stages from crustacean species with differing tolerances to environmental change. Although marine crustaceans are currently considered to be broadly tolerant of ocean acidification, closer examination reveals that certain species and developmental stages could be adversely affected. It is important that the scientific community considers the impacts of ocean acidification and climate change on representatives from all marine invertebrate phyla in order to truly appreciate the resulting effects on species richness, community structure and function, and ecosystem processes.

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Where's the 'reef'? A five year study of serpulid tube bioerosion in a Scottish sea loch

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ABSTRACT: In the British Isles, aggregations of the tubicolous polychaete *Serpula vermicularis* L. occur only in a few localities in western Scotland and Ireland. In Loch Creran, Argyll, where written observations extend back to the 1880s, build-ups of skeletal debris constituting a true reef framework are notably absent. To investigate the taphonomic processes affecting the residence time of relict tube material, cleaned and weighed skeletal fragments were deployed on panels amongst living worm aggregations. Fragments were either open to the environment or enclosed in mesh cylinders to exclude grazing urchins. Panels were recovered and fragments re-weighed after 1, 2, 3 and 5 yr *in situ*. Caged fragments typically increased in dry weight over time, while most open fragments remained at steady-state or showed small weight decreases. Change in weight was correlated with the number of living tubeworms which had settled onto the skeletal fragments. Open fragments showed no consistent temporal trend in weight change, suggesting that urchin grazing was not a major bioerosive process over the 5 yr experimental timescale. Data from another locality where serpulid aggregations suffered mass mortality between 1984 and 1994 show that tube debris can persist for at least 15 yr in sea loch environments. *S. vermicularis* aggregations in sea lochs may be relatively transient features, appearing and disappearing over decadal timescales.

KEY WORDS: *Serpula vermicularis* · Bioerosion · Reef · Sea loch · Taphonomy

INTRODUCTION

Mass occurrences of tubicolous serpulid polychaetes have been described for ~10% of species in the subfamily Serpulinae and are known from many areas of the world (ten Hove 1979, ten Hove & van den Hurk 1993). In the British Isles, serpulid aggregations occur only in a few localities on the west coasts of Scotland (Fig. 1) and Ireland (Minchin 1987, Moore et al. 1998). The most extensive examples are found in Loch Creran, a fjordic sea loch in Argyll, west Scotland, where aggregations of *Serpula vermicularis* L. occupy a narrow depth band (1 to 13 m) fringing the loch periphery and covering a total area of ~108 ha (see Fig. 2 in Moore et al. 2009 for distribution as of July 2005). The *S. vermicularis* aggregations in Loch Creran are regarded as biogenic 'reefs' (Holt et al. 1998), and ongoing scientific studies aim to promote effective conservation management of this rare biotope (Moore et al. 1998, 2003, 2009, Poloczanska et al. 2004, Chapman et al. 2007, Hughes et al. 2008).

Differing concepts of what constitutes a biogenic 'reef' have been discussed at length in both the geological (Heckel & Yablonsky 1979, Riding 2002) and biological (Hendrick & Foster-Smith 2006, Rabaut et al. 2009) literature. A feature common to most definitions is the creation of elevated seabed topography by the accumulation of dead skeletal material, providing substratum for continued growth of the 'reef'-building organisms. In Loch Creran, *Serpula vermicularis* 'reefs' consist of masses of densely-intertwined tubes standing >50 cm clear of the seabed and up to 60 cm in lateral extent (Moore et al. 1998). Mature worms extend their tubes at a mean rate of 33 mm yr^{-1} (Hughes et al. 2008). Collapse and subsequent upward regrowth of clusters of tubes create 'ring-reefs' up to 2 m in diameter (Moore et al. 2009), with total coverage in some areas exceeding 10% of the seabed (Moore et al.

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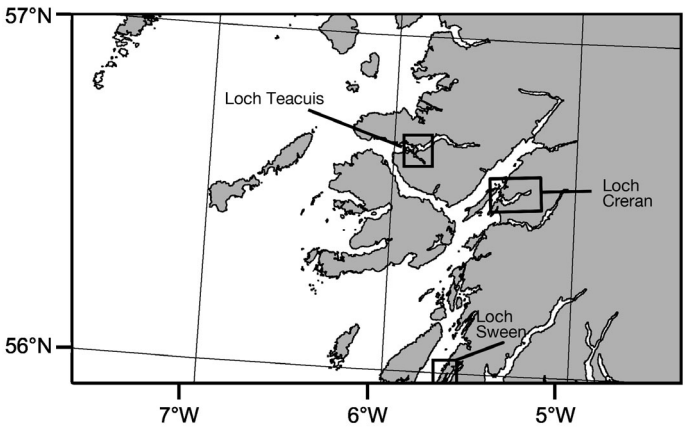

Fig. 1. Scottish west coast, showing locations of the 3 sea lochs with current or former records of *Serpula vermicularis* aggregations

1998). The *S. vermicularis* aggregations originate on small stones or bivalve shells on a gently-sloping muddy sand substratum (Moore et al. 1998). However, although tube fragments are clearly visible on the seabed in the 'reef' zone, thick deposits of skeletal debris are notably absent (author's pers. obs.). There are no population-level estimates for carbonate production by *S. vermicularis* in Loch Creran, but an observation in 1882 (Anderson Smith 1887) suggests that tubeworm aggregations may have been present in the loch for well over a century. This raises questions concerning the post mortem fate of serpulid tubes in Loch Creran, and the factor(s) which prevent build-up of relict material on the seabed.

In the marine environment, carbonate skeletons can be broken down by a combination of physical abrasion, chemical dissolution and bioerosion (Smith & Nelson 2003, Wisshak & Tapanila 2008). Loch Creran is highly sheltered from wave action, and strong tidal currents occur locally only in the entrance channel and at the Creagan Narrows sill (Gage 1972, Moore et al. 1998). In this low-energy setting the rate of physical abrasion is likely to be very low, with chemical dissolution and bioerosion being potentially more significant taphonomic factors. Bioerosion on high-latitude shelves takes place through the action of endolithic algae, fungi, boring sponges and other invertebrates, combined with the grazing activity of macroinvertebrates such as urchins (Wisshak 2006). The urchin *Psammechinus miliaris* is abundant in Loch Creran (Kelly 2000) and occurs in association with the 'reefs' (Poloczanska et al. 2004). It has been recorded feeding on *Serpula vermicularis* tubes in Ireland (Bosence 1979) and is therefore potentially an important bioeroder in this serpulid 'reef' habitat.

This paper presents the results of a 5 yr field experiment designed to measure the residence time of *Serpula vermicularis* tube debris in Loch Creran and investigate the factors affecting skeletal carbonate balance in the 'reef' zone. The work will contribute towards a greater understanding of biogenic 'reef' dynamics in a high-latitude temperate coastal environment.

MATERIALS AND METHODS

Fieldwork was carried out on the southern shore of Loch Creran at a site (56° 30.98′ N, 05° 22.05′ W) used in an earlier study of survivorship and tube growth (Hughes et al. 2008). Small clusters of tubes containing living *Serpula vermicularis* were collected by scuba divers in July 2004 and returned to the laboratory. The worms were then killed by overnight immersion in fresh water and extracted from their tubes using forceps. Attached algae and soft-bodied epifauna were also removed. The empty skeletons were then cleaned by 24 h immersion in dilute sodium hypochlorite solution, washed in running fresh water and dried under low heat in a drying cabinet. Thirty-two skeletal fragments were then individually weighed on an electronic top-pan balance and labelled with a numbered plastic tag held on by a cable tie. The dried fragments were ~15 to 20 cm in length with a mean (±SD) dry weight of 43.25 ± 13.52 g.

Weighed and labelled skeletal fragments were mounted in pairs on square (30 × 30 cm) plastic mesh panels and held in place with cable ties. On 8 panels, the skeletal fragments were fully exposed to the environment ('Open' panels, Fig. 2a). On the remaining 8 ('Caged' panels, Fig. 2b) each fragment was enclosed within a plastic mesh cylinder (length 21 cm, diameter 5.5 cm, mesh apertures 5 × 5 mm) designed to exclude urchins. On 15 September 2004, the panels and attached skeletal fragments were returned to the collection site in Loch Creran and deployed on the sea bed by divers among living *Serpula vermicularis* aggregations. Water depth at the deployment site was ~10 m, with a substratum of gently sloping muddy sand. At 10 m depth, water temperature in Loch Creran has an annual range of approximately 6 to 12°C, with a salinity of 32 to 33 psu (Gage 1972). Loch Creran has a relatively high tidal flushing rate (Edwards & Sharples 1986) and in consequence is always well oxygenated (Gage 1972). The associated fauna of the Loch Creran serpulid 'reef' zone was decribed by Poloczanska et al. (2004).

The panels were deployed in a line roughly parallel to the shore, with a spacing of ~1 m and Open and Caged panels alternating. Panels were pinned to the sea bed by a ~20 cm long aluminium rod at each corner, with an upright numbered flag planted next to each one to facilitate relocation on later dives. Recov-

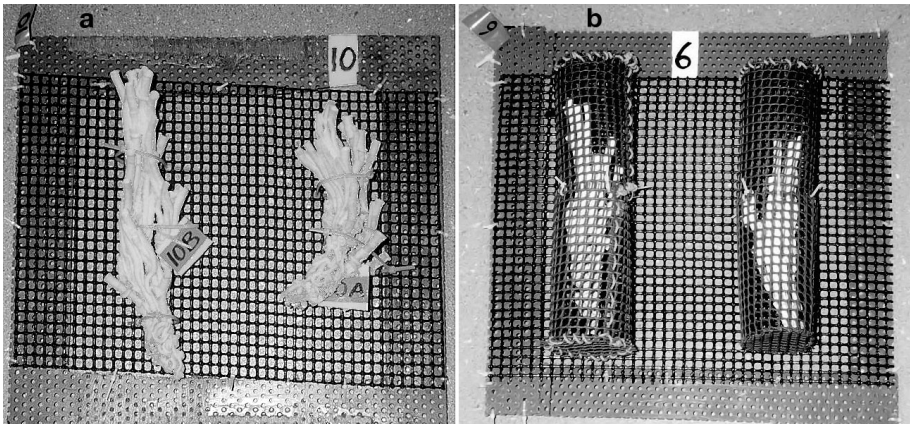

Fig. 2. (a) Open and (b) Caged panels (30 × 30 cm) with attached weighed fragments of *Serpula vermicularis* tube material, photographed in September 2004 prior to deployment on the seabed in Loch Creran

ery dives were made on 29 October 2005 (~1 yr deployment), 24 August 2006 (~2 yr), 2 September 2007 (~3 yr) and 12 September 2009 (~5 yr). On each occasion, 2 Open and 2 Caged panels were removed by a diver and returned to the surface, taking care to avoid any damage or loss of material from the panels. Additional dives were made between the recovery dates to check the condition of the remaining panels and re-erect any fallen marker flags, but the skeletal fragments were not touched or interfered with in any way.

In the laboratory, the recovered panels were immersed overnight in fresh water, and the skeletal fragments were then carefully cleaned of soft-bodied fouling organisms using forceps. The mesh cylinders were cut open to allow access to the Caged fragments. Mobile invertebrates found inside the cylinders were identified and counted. Calcareous tubes, shells and colonies (serpulids, barnacles, bryozoans) recruited to the skeletal fragments and contributing to the carbonate balance were left in place. Living serpulids removed from the recovered skeletons were counted.

After removal of fouling biomass, the skeletal fragments were cleaned in running fresh water, with gentle 'hosing' into the tube apertures to wash out accumulated sediment. Any calcareous fragments breaking loose were retained for inclusion in the final weighing. The cleaned skeletons were then soaked in dilute sodium hypochlorite solution for 24 h, washed again in fresh water, dried and re-weighed. Dry weight change was expressed as percentage increase or decrease relative to the original weight of each fragment. Tubes containing living *Serpula vermicularis*, representing animals recruited since panel deployment, were present on almost all recovered fragments. Distal portions of tubes recruited to Caged fragments often extended through the cylinder mesh. Projecting tube sections were included in the dry weight measurement if they clearly originated on the enclosed skeletal fragment, but tubes recruited onto the cylinder mesh were excluded.

RESULTS

Weight change of serpulid skeletal fragments

All panels and skeletal fragments deployed in 2004 were recovered successfully. Mean dry weight of Caged fragments was greater than original values after all deployment periods, with 15 out of 16 fragments showing a percentage increase (Fig. 3). The largest recorded increase was a near-doubling in weight (~97%) after 3 yr *in situ*, but there was a wide range of variation at each time point. Mean weights for Open fragments showed relatively small percentage decreases at 1, 3 and 5 yr, with a small increase in mean weight at 2 yr (Fig. 3). The largest individual weight loss recorded was ~49% for a fragment after 5 yr *in situ.*

After confirmation of data normality, percentage weight change was analysed in a 3-factor nested

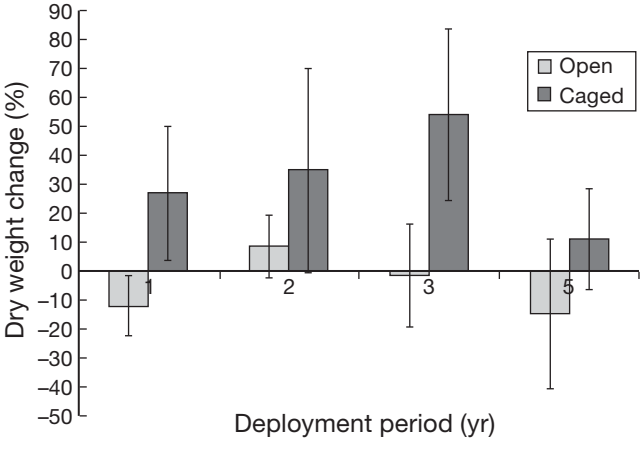

Fig. 3. Percentage change in dry weight of Open and Caged skeletal fragments after 1, 2, 3 or 5 yr deployment on the seabed in Loch Creran. Each bar represents the mean (±SD) value of 4 fragments in each category

Table 1. Summary for 3-factor nested analysis of variance for percentage weight change of serpulid skeletal fragments deployed in Loch Creran. Main effects are Treatment (n = 2; Open, Caged), Time (n = 4; 1, 2, 3 and 5 yr) and Panel (n = 8, nested within Treatment and Time). Adj.: adjusted

Source	df	Adj. SS	Adj. MS	*F*	p
Treatment	1	10772.9	10772.9	14.73	0.005
Time	3	4041.6	1347.2	1.84	0.218
Treatment × Time	3	1178.2	392.7	0.54	0.670
Panel	8	5851.6	731.5	1.74	0.165
Error	16	6726.6	420.4		
Total	31	28571.0			

analysis of variance (Zar 1984) with main effects Treatment (n = 2, fixed factor), Time (n = 4, fixed factor) and Panel (n = 8, random factor nested within Treatment and Time). Only the Treatment main effect was statistically significant (p = 0.005), with Time, Treatment × Time and Panel all non-significant (Table 1). These results confirm that percentage weight change showed no consistent trend over time, as illustrated by the plot of mean values in Fig. 3. The lack of a significant Panel effect reflects the wide differences sometimes recorded between adjacent fragments, especially in the Open treatment (for example, at 1 yr, weight change on Panel 15 was +1.17% for Fragment A, versus –24.23% for Fragment B).

Role of serpulid recruitment in skeletal carbonate balance

At the time of recovery, Open skeletal fragments and mesh cylinders were often fouled by foliose and filamentous red algae (Fig. 4). Open fragments were usually heavily silted and had few attached sessile invertebrates. In contrast, the mesh cylinders enclosing the Caged fragments always contained a variety of sessile and mobile invertebrates, of which the most common (often several individuals per cylinder) were the crab *Pisidia longicornis*, the brittlestar *Ophiothrix fragilis* and the terebellid polychaete *Eupolymnia nebulosa*. Other taxa included ascidians (*Pyura microcosmus* and *Ascidia* spp.), squat lobsters *Galathea squamifera* and several species of errant polychaetes and small scallops (*Chlamys* spp.). Five mesh cylinders contained 1 or 2 small specimens (test diameter 10 to 15 mm) of the urchin *Psammechinus miliaris.* These urchins, and other invertebrates of adult size, must have entered as larvae or juveniles, taken up residence and eventually grown too large to exit through the mesh apertures.

Tubes containing living *Serpula vermicularis* were present on 14/16 Open and 15/16 Caged fragments (Fig. 5). Numbers were higher on Caged (maximum recorded = 40, mean ± SD = 11.1 ± 11.8 tubes fragment^{-1}) than on Open fragments (maximum = 11, mean = 3.6 ± 3.5). The number of living *S. vermicularis* was significantly positively correlated with percentage weight change in both treatments (Caged, Pearson correlation = 0.646, p = 0.007; Open, Pearson correlation = 0.735, p = 0.001). Open fragments with 3 or more living *S. vermicularis* remained at steady-state or showed a relatively small (<24%) weight increase, while weight loss was recorded from fragments carrying 2 or fewer occupied tubes (Fig. 6a). In the Caged treatment (Fig. 6b), the wide scatter of data points partly reflects the fact that the counts of living serpulids include tube size as an additional source of variation (recent recruits in small tubes and older recruits in larger tubes contribute equally). Empty tubes of serpulids that had settled on the fragments but died before panel recovery are also not represented in the analysis. These could not be reliably distinguished from the older tubes owing to the structural complexity of the skeletal fragments.

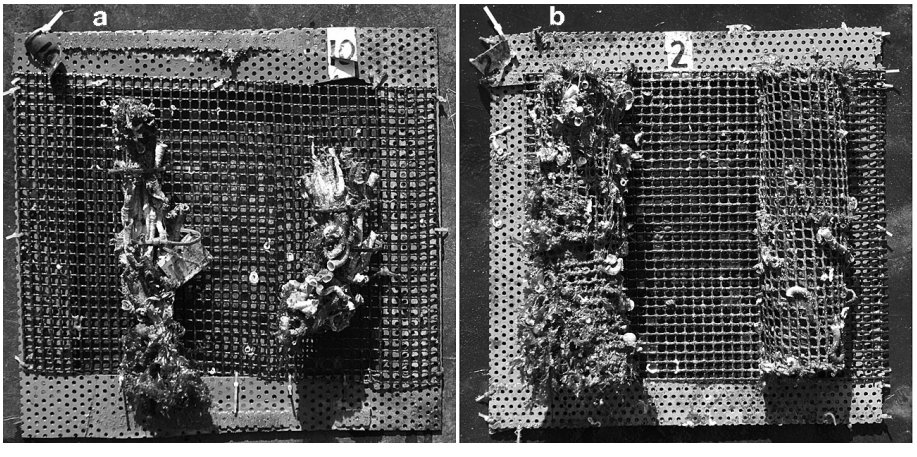

Fig. 4. Panels photographed on 12 September 2009 approximately 1 h after recovery from Loch Creran, following 5 yr *in situ*. (a) The Open panel is the same one shown pre-deployment in Fig. 2a. The skeletal fragments on the Open panel are colonized by foliose red algae. (b) The left-hand mesh cylinder of the Caged panel is also partly obscured by red algae. Tubes of *Serpula vermicularis* recruited during the deployment period can be seen projecting through the mesh of both cylinders

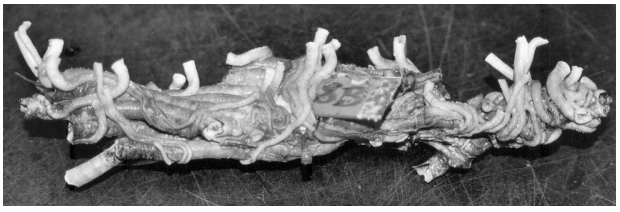

Fig. 5. Caged skeletal fragment (Fragment 8B), recovered on 29 October 2005 after just over 1 yr *in situ*. The cleaned fragment carries >20 *Serpula vermicularis* tubes recruited during the deployment period, 19 of which were occupied by living worms at the time of recovery. The new tubes are mostly whiter than the underlying relict skeleton and follow an upwards growth trajectory away from the substratum. Dry weight of this fragment increased by +18.59% over the 1 yr deployment period. Length of fragment: 18 cm

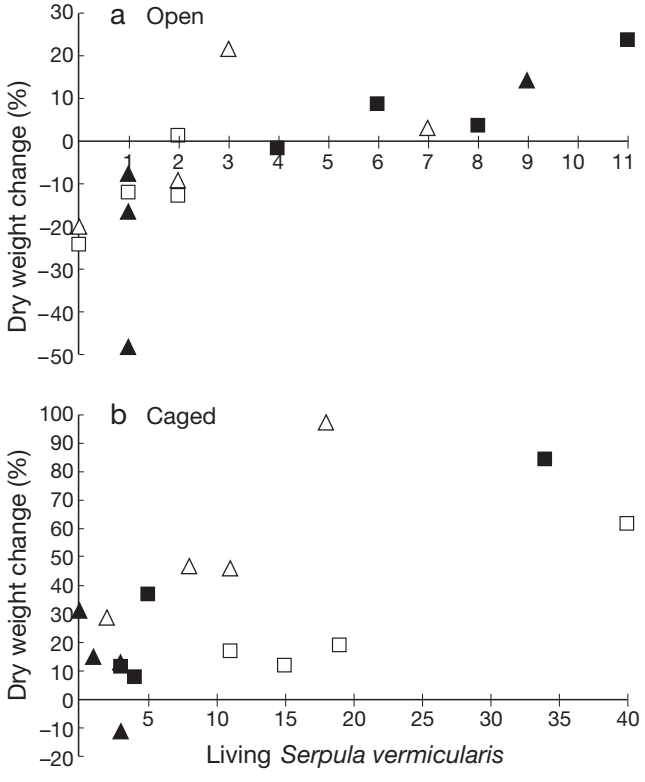

Fig. 6. Percentage dry weight change of (a) Open and (b) Caged skeletal fragments as a function of the number of living serpulid worms present on the fragments at the time of recovery. Symbols indicate the *in situ* deployment time of each fragment: 1 yr (□), 2 yr (■), 3 yr (△), 5 yr (▲)

DISCUSSION

Taphonomic factors influencing tube residence time

The main objective of this study was to investigate factors that might explain the lack of accumulated tube debris on the Loch Creran seafloor. The absence, over 5 yr, of a consistent trend towards weight loss on Open panels might suggest that relict tubes are highly persistent, but we must be cautious about extrapolating this result beyond the timescale of the experiment. Carbonate skeletons on the seafloor can show non-linear patterns of weight change, with initial stability or increase resulting from epifaunal settlement, followed by weight loss and fragmentation brought on by the cumulative effects of bioerosion (Smith & Nelson 2003). The experiment illustrates the challenge of quantifying processes operating over timescales much longer than the 2 to 3 yr extent of a typical research project. At 5 yr duration, this is the longest bioerosion study yet reported from a high-latitude shelf sea (previous studies tabulated by Wisshak 2006), but is still insufficient to provide a definitive answer to the original question.

At the outset, the urchin *Psammechinus miliaris* was identified as a potential macroinvertebrate bioeroder of serpulid tube debris in Loch Creran. The caging treatment was designed to exclude this species, but the relatively small weight loss of Open fragments over 5 yr suggests that urchins did not significantly erode the dead serpulid tubes. The growth of foliose algae on the panels and cages (Fig. 4) may also be an indication of relatively low grazing pressure. On Open panels, the presence of 2 to 3 living serpulids per fragment marked an apparent threshold in mass balance, such that over a 5 yr timescale, tube growth of new recruits could outweigh the combined effects of bioerosion and chemical dissolution. The higher densities of living serpulids on Caged fragments may be explained by preferential settlement on the enclosed fragments, and/or a macroinvertebrate grazing effect exerted by the removal of new recruits rather than by erosion of the underlying dead skeleton.

Bioerosion by urchins and other large grazers may be more important in the tropics than at high latitudes (Wisshak 2006). In the Firth of Lorne, just outside Loch Creran, Akpan & Farrow (1985) found that endolithic algae were major bioeroders of mollusc shells, but urchin grazing traces were recorded on only 9% of shells sampled. Urchin grazing was also of minor importance in the 2 yr Kosterfjord (Sweden) experiment, the most detailed study of high-latitude bioerosion undertaken to date (Wisshak et al. 2005). In laboratory experiments, *Psammechinus miliaris* fed on oyster shells heavily infested with boring sponges and polychaetes but did not attack unbored shells (Hancock 1957). The state of endolithic community development in serpulid tube debris may therefore determine the timing and extent of later bioerosion by urchins. Future studies should quantify the extent of endolithic bioerosion, identify the organisms responsible and estimate the frequency of urchin grazing traces on open and caged fragments using scanning electron

microscopy, a method employed successfully in other carbonate bioerosion studies (Beuck & Freiwald 2005, Wisshak 2006)

Chemical dissolution of carbonates in temperate shelf settings was formerly considered negligible owing to calcite and aragonite supersaturation of the overlying seawater (Smith & Nelson 2003). However, it is now known that microbial respiration in organic-rich sediments can acidify porewaters to levels sufficient to erode at least the less stable carbonate phases (aragonite, high-Mg calcite). The process takes place at or near the sediment–water interface and is most rapid in oxic sediments irrigated by bioturbation (Aller 1982). The high calcite content of *Serpula vermicularis* tubes (93.6%, Mg content not recorded, Vinn et al. 2008) should confer a relatively high durability but may be counteracted by the very high surface-to-volume ratio, which is probably more important than carbonate mineralogy in determining dissolution potential (Smith & Nelson 2003). Observations from Linne Mhuirich, a small, sheltered inlet of Loch Sween in mid-Argyll (Fig. 1), provide an insight into longer-term tube persistence. *Serpula vermicularis* 'reefs' were recorded here from 1975 (R. Mitchell pers. comm. cited by Moore et al. 1998) to 1984 (Lumb 1986). A September 1994 survey (O. Paisley & D. J. Hughes unpubl.) found that the 'reefs' had died out completely, leaving only dead skeletons, and no recovery has taken place since (Hughes et al. 2008 and subsequent pers. obs.). Relict tube material is still locally abundant and conspicuous at the sediment surface in Linne Mhuirich (Fig. 7a). Large fragments collected in July 2009 (Fig. 7b) must have persisted for at least 15 yr, and since the last record of living 'reefs' was in 1984, this material could be >20 yr old. Serpulid debris in Linne Mhuirich has therefore persisted for much longer than the 5 yr duration of the Loch Creran experiment, despite the presence of abundant *Psammechinus miliaris* in both localities (author's pers. obs.).

Fjordic sea lochs receive large inputs of terrigenous organic matter (Ansell 1974, Loh et al. 2002) and are sites of rapid sediment accumulation (Cage & Austin 2010). Deposition rate in the 'reef' zone around the periphery of Loch Creran has not been measured, but 5 yr *in situ* was insufficient to bury the panels. Experimental burial of skeletal fragments below the sediment–water interface would therefore be necessary to measure chemical dissolution rate in the absence of other factors leading to carbonate loss or accretion.

Dynamics of *Serpula vermicularis* 'reefs' in Scottish sea lochs

The taphonomic processes discussed above, which determine the residence time of tube debris, operate independently of the biological factors affecting population dynamics of *Serpula vermicularis* in Loch Creran. With respect to the lack of accumulated tube debris, the focus of attention to this point has been on processes leading to post mortem degradation of carbonate skeletons. However, an alternative hypothesis is that *S. vermicularis* 'reefs' have not existed in Loch Creran for long enough to generate thick deposits of skeletal debris. Anderson Smith (1887) described tubeworm aggregations in the loch in 1882, but the next published record comes from a diving survey in June 1989 (Connor 1990), and there is no published evidence confirming the presence of serpulid 'reefs' in Loch Creran between those dates. In November 1967,

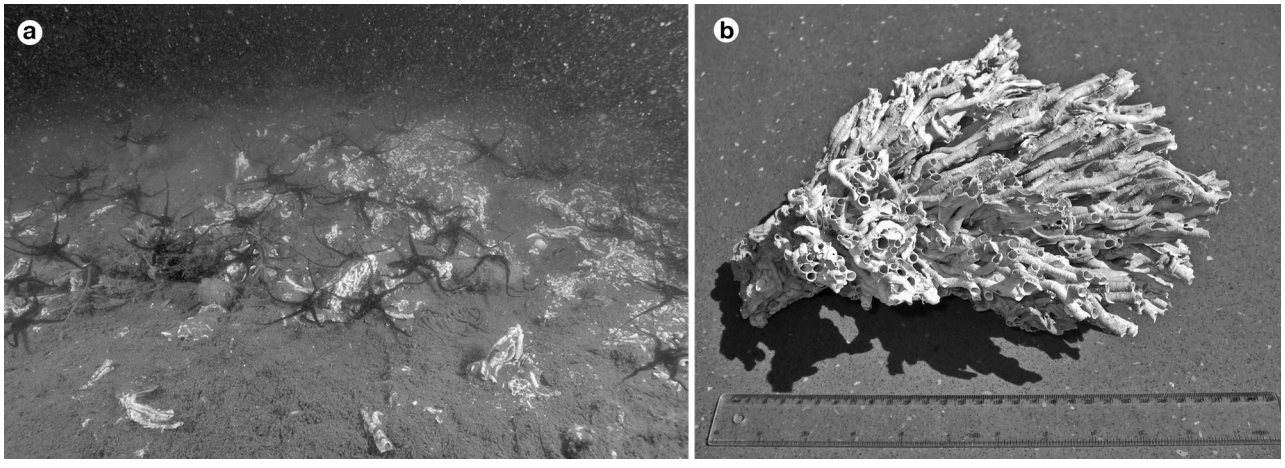

Fig. 7. (a) Seabed photograph taken in Linne Mhuirich, Loch Sween, on 17 May 2005, showing abundant relict *Serpula vermicularis* tube debris at the sediment surface. Black brittlestars are *Ophiocomina nigra.* Site location 55° 59.90′ N, 05° 38.92′ W; water depth ~4 m. (b) Cleaned and dried mass of relict *S. vermicularis* tubes collected in Linne Mhuirich, Loch Sween, on 11 July 2009. Plastic ruler (30 cm) indicates scale. Collection site location 56° 00.16′ N, 05° 38.47′ W; water depth ~2 m

Gage (1972) towed an Agassiz trawl through South Shian Bay, an area which now supports some of the best-developed serpulid 'reefs' in Loch Creran (Moore et al. 1998, 2009). *S. vermicularis* was recorded in the sample species list, but Gage did not mention it in the text. Since the tow formed part of a survey of the loch's benthic macrofauna, large masses of aggregated tubes would almost certainly have been noted if present in the trawl. In May 1969, benthic zonation was surveyed by scuba divers along a transect out from the loch's northern shore (to 15 m depth). The survey results (Gage 1974) again do not mention *S. vermicularis*, although the station falls within the current zone of 'reef' occurrence (Moore et al. 1998, 2009). Negative evidence of this kind must be treated with caution, but it raises the possibility that serpulid 'reefs' in Loch Creran have developed to their current state only since the late 1960s. In July 2006, small *S. vermicularis* aggregations were found at several stations in the upper basin of Loch Teacuis, a sea loch in Morvern, western Scotland (Fig. 1). None were recorded on a 1996 survey of the loch, suggesting that 'reef' formation here has begun very recently (Dodd et al. 2009). Alongside the apparent mass mortality in Linne Mhuirich, the Loch Teacuis discovery provides evidence that *S. vermicularis* 'reefs' in Scottish sea lochs may be relatively transient features, forming and disappearing over decadal timescales. If this hypothesis is correct, there may be no direct continuity between the existing 'reefs' in Loch Creran and those observed by Anderson Smith (1887) over a century ago. Coring of nearshore sediments, followed by sclerochronology or radiometric dating of any buried tube fragments could be investigated as a technique to clarify the long-term history of *S. vermicularis* 'reefs' in Scottish sea lochs.

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An application of the theory of island biogeography to fish speciation on seamounts

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ABSTRACT: Seamounts can be considered as islands in the deep. For many species, depth is just as much a barrier to dispersal as is the water between oceanic islands. This leads to the hypothesis that seamounts could be places where speciation readily occurs. Recent advances in the theory of island biogeography have allowed some detailed predictions about the degree of endemism and the diversity of species on oceanic islands. We have adapted this theory to seamounts, as underwater equivalents of islands. Three elements of this theory were tested as an illustration of what could be done, using published data on the diversity of reef-dwelling and benthic fish species found along the Hawaiian-Emperor seamount chain in the Pacific. Poor sampling makes it impossible at present to test the hypothesis that endemism is a humped function of seamount age. The data agrees with a further prediction that the total number of species is a humped function of seamount age. Finally, the prediction that fish diversity should be a function of seamount age and area is unsupported. We propose that the theory we have attempted to test could serve as a guide to what fish diversity might be expected from further sampling work.

KEY WORDS: Seamount · Island biogeography · Speciation · Hawaiian-Emperor Chain · Benthic and reef fish

INTRODUCTION

Documenting the existence and origin of biodiversity in the world's oceans is made difficult by the nature of the habitat. Despite this, the task cannot be neglected given that the oceans cover two-thirds of the world's surface and are the source of a significant amount of food produced for human consumption. We need to know what is in the sea and how diversity arises if management and conservation are to be effective. Steps in the right direction have been taken by the recently completed Census of Marine Life (http://www.coml.org/), which has contributed to a significant increase in our knowledge of oceanic biodiversity, and seamounts were included in this decade long effort (Pitcher et al. 2007; http://censeam.niwa.co.nz/).

Seamounts are numerous, with perhaps 100 000 existing throughout the world's oceans (Kinchingman et al. 2007). We argue in this paper for seamounts to be thought of as the underwater equivalent of oceanic islands, thus opening up the study of their biodiversity to the powerful theoretical developments in island biogeography. The patterns of abundances and the special adaptations of the fauna and flora of oceanic islands are of great interest to those who want to understand the origin of biodiversity and in particular endemism as a characteristic of the fauna of many of these islands (Whittaker & Fernández-Palacios 2007, Grant & Grant 2008, Price 2008). As seamounts share many characteristics such as in their volcanic origin and their degree of isolation with oceanic islands, they could also be sites for high levels of endemism, as was first proposed by Hubbs (1959). In this paper, we develop this hypothesis and make some preliminary test of it using data on benthic and reef based fish found along the Hawaiian-Emperor (H-E) seamount chain. Our analysis is preliminary and illustrative, as so little is known about the fish communities on seamounts, making definitive tests of ideas out of reach at present. The principle aim of the paper is to demon-

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strate and develop the rudiments of an approach to the study of seamount fish diversity, which could be a fruitful guide to future work.

SEAMOUNTS AS UNDERWATER ISLANDS

Many oceanic islands and seamounts share their origin in volcanic activity (Wessel 2007, Staudigel & Clague 2010). In the case of many seamounts the volcano was just not big enough to push material above sea level. Other seamounts are the remnants of volcanic oceanic islands that have been destroyed by erosion (Price & Clague 2002). Volcanoes that do not break the sea surface can still achieve a great height above the sea floor. The extrusion of all the extra material forming the seamount can lead to a period immediately after the eruption when the seamount sinks as the weight of the structure depresses the earth's crust (Price & Clague 2002, Wessel 2007). The rapid period of sinking can last for about 1 Ma (million yr before present) and can reduce the height of the seamount by 1000 to 1500 m (Price & Clague, 2002). This gradual sinking is likely to be as important for speciation on seamounts as it is on islands. This sinking will often mean that there is a positive correlation between seamount peak depth and age.

Although seamounts extend from the seabed upwards, they do not offer a uniform habitat from top to bottom. This is of course also true of terrestrial islands, but their lower regions are sharply demarcated by the division between the terrestrial and marine habitats, each requiring very different adaptations for survival. This has obvious consequences for the biota in each habitat, setting sharp boundaries to distributions. Does a seamount have similarly sharp limits between any of its habitat zones? Marine organisms are just as limited by the physical conditions they can tolerate as are terrestrial plants and animals, and depth is one of the most overbearing abiotic factors affecting marine organisms, with physical conditions changing rapidly with depth (Mann & Lazier 2006, Longhurst 2007). Do these physical conditions all change gradually with depth, or is there an equivalent to the land/sea boundary at some clearly demarcated depth? As Longhurst (2007) writes:

> As biogeographers have long been aware, the most significant environmental gradient and discontinuity in the ocean is horizontal, between shallow and deep layers, rather than in the vertical plane […]. This gradient lies at the seasonal or tropical pycnocline and is globally associated with the change from epipelagic to deeper ecosystems. (p. 43–44)

The depth of the pycnocline varies with latitude and in relation to landmasses (Longhurst 2007). Its depth is also dynamic through the year, being strongly influenced by varying insolation and wind-induced mixing. This sharp boundary at the pycnocline means that, for the purposes of this paper, we only consider seamounts with peaks that are <500 m from the surface. At the bottom of this range, conditions will be closer to those in the deep ocean, but at shallower depths peaks will be in the euphotic zone and projecting through the pycnocline. These relatively shallow seamounts are most likely to have biodiversity that is high and dynamic.

Spatially the biota on these subsurface islands will be subject to the same factors that influence the biota on terrestrial islands in the sea. They will be separate from other islands and from continental shelves in the euphotic zone, and they will be exposed to immigration of organisms from these other locations. The degree of immigration will be a function of an organism's life history, and as many fish have pelagic eggs and larvae, they can be expected to have the potential to be dispersed widely. This needs to be taken into account when considering opportunities for the evolution of endemics. This problem is no different to birds and insects that can fly. As with degrading terrestrial islands, some of these seamounts will be expected to gradually sink as time passes so that eventually their tops pass below 500 m and out of the direct influence of surface events.

THEORIES OF ISLAND BIOGEOGRAPHY AND THE STUDY OF SEAMOUNT BIODIVERSITY

The first, and still the most cited, theory which attempts to explain the number of species found on islands is by MacArthur & Wilson (1967). Their hypothesis was that the number of species on an island is determined by the balance between species arriving by immigration and species becoming extinct. Much research has been based on this theory, but Hubbell (2001) and, most recently, Whittaker et al. (2008) have modified and expanded the theory to account for processes and observations not included in the original. This paper is not the place for a full explanation of this new work, but a brief outline of Whittaker et al.'s (2008) theory is required to make the rest of the paper understandable.

The original theory of island biogeography (MacArthur & Wilson 1967) did not develop in detail the evolution of species once they had arrived on an island. Evolution is part of Hubbell's (2001) theoretical development as well as of the theory proposed by Whittaker et al. (2008). The latter is tailored to the particular histories that will be experienced by an oceanic island which will exist for a limited period of time. As we are assuming that seamount islands will also last for a limited period of time, it might be expected that they too will be the scenes of specia-

tion. Whittaker et al.'s (2008) theory of island biogeography is depicted in Fig. 1. This theory is called the General Dynamic Model, or GDM. As Fig. 1 shows, immigration and extinction are still the important inputs and outputs to and from the ecosystem, but as the island ages speciation rises to a peak and then decreases as biodiversity in general increases and there are fewer opportunities for new forms to evolve. This is because there are fewer available niches as previous adaptations have filled those niches most readily exploited. The GDM predicts that large remote islands will be dominated by evolutionary change more than those islands closer to a landmass source. Note that the carrying capacity of the island, labelled as *K*, increases to a peak and then falls as the island is reduced in height and area by erosion and subsidence. The same process is hypothesised to happen in seamounts, with those having peaks starting above 500 m gradually dropping out of the euphotic zone.

The GDM has random immigration and extinction events providing basic inputs to an island but then assumes that competition for resources can drive speciation. Animals or plants found as endemics on only one island are taken as a measure of evolutionary dynamics within an archipelago. It has been argued by Emerson & Kolm (2005a,b) that there is an association between high species numbers on an island and a high proportion of single island endemics. These authors argue that high diversity leads to increased competition for resources and to greater rates of speciation.

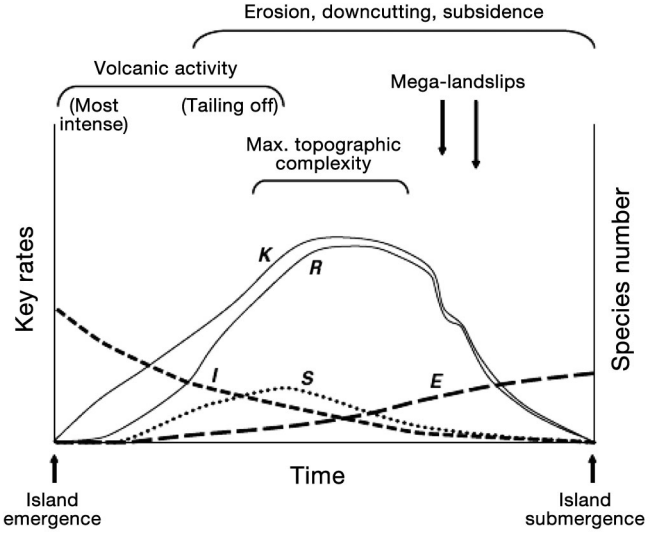

Fig. 1. General dynamic model as proposed by Whittaker et al. (2008). *I* and *E* represent the immigration and extinction rates of species. *S* is the rate of speciation, *K* is the carrying capacity, and *R* is the realised species richness resulting from a combination of immigration, speciation, and extinction. Unlike a terrestrial island, underwater seamount islands would reduce in height mainly through subsidence with little erosion. Undersea landslips could still be possible and there is evidence for these. (Figure from Whittaker et al. 2008)

A number of predictions can be derived from the GDM (Whittaker et al. 2008), but several of them will be hard to test as the required data is difficult to obtain. We focus on just 3 predictions that are relatively easy to test. These are that: (1) The number of single seamount endemics (SSE) should be a humped function of seamount age. (2) Seamount (SM) species number should be a humped function of SM age. (3) This relationship between diversity and age will be improved by adding seamount area so that

$$\text{Diversity} = a + b(\text{Age}) + c(\text{Age}^2) + d(\log \text{Area})$$

where *a*, *b*, *c*, and *d* are fitted parameters.

The predictive power of the equation in prediction 3 is tested by Whittaker et al. (2008) against data sets on oceanic island fauna from the Canary Islands, Hawaii, USA, the Galápagos, the Marquesas and the Azores. Generally, diversity in the data sets used is well described by the regression.

Using data on benthic and reef-based fish from the H-E seamount chain, we show how the GDM might be used to analyse seamount biodiversity. Data from benthic and reef-based fish were used because fish in these groups are more likely to be dependent on a seamount and more likely to be non-migratory. Seamounts are attractive to many far-ranging species such as tuna and sharks, which gather at the good feeding opportunities offered by a seamount, but these are excluded from our analysis as they are unlikely to be seamount specific (Holland & Grubbs 2007, Litvinov 2007, Morato et al. 2010). First we discuss briefly our current knowledge of diversity and endemism of seamount fish.

DIVERSITY AND LEVELS OF ENDEMISM IN THE FISH COMMUNITIES OF SEAMOUNTS

Levels of diversity and rates of endemism on seamounts have been reviewed by Stocks & Hart (2007) and Shank (2010). At present, estimates of species diversity for any group are beset by problems derived from sampling. When attempting to compare seamount and continental margin diversity, the latter habitat will usually have been sampled with greater intensity than will the seamounts. Also, the gear used for data collection is more likely to sample the relatively smooth bottom of the shelf margin more effectively than it will the rough and rocky seamount. Seamounts are also under-sampled, as shown by plots of numbers of species collected against number of samples for 180 seamounts (Stocks & Hart 2007). Such plots always

produce a straight line for all seamounts sampled so far with no sign of the number of species reaching an asymptote. This implies that we still do not have good estimates of total species for any seamount.

The data on fish is not yet good enough to examine whether continental margins have more or less species that do seamounts. Equally, species evenness, which provides an indication of whether or not the community is dominated by a few species, cannot be estimated properly. The only indication we have that evenness might be low is the evidence from commercial fisheries on seamounts, which tend to be dominated by one or a few species.

Stocks & Hart (2007) defined endemism as a species that is found on one seamount or a group of seamounts close together and nowhere else in the ocean. It is never possible to be sure that a species is endemic, especially as seamount sampling is so poor. There is always the chance that the apparently endemic species exists elsewhere but has just not yet been discovered. A check on FishBase (www.fishbase.org, last accessed July 5, 2010) will sometimes show that a species found on just one seamount has also been recorded elsewhere in the relevant ocean basin. In addition, because databases of seamount fish are compiled from a range of sources varying in age and the taxonomic precision of the providers, the problem of synonymy also arises. A species record from a seamount, which appears to be unique may be thought to be so because the data gatherers used an old name for a species. Again, a check on FishBase will first point to the synonym and then show that the species has been found in many other parts of the same ocean.

An early survey by Wilson & Kaufmann (1987) found that 11.6% of 449 species of fish from 100 seamounts were endemic, but 72% of the data came from just 5 seamounts, so this survey is not definitive. Parin et al. (1997) found that 44% of 171 species of fish caught were endemic on the Nazca and Sala-y-Gómez chain of seamounts in the southeast Pacific. Richer de Forges et al. (2000) surveying the Norfolk Ridge, the Lord Howe seamounts, and the Tasmanian seamounts in the southwest Pacific found that 29 to 34% of all fishes caught were new or potentially endemic. The survey of seamount fishes by Froese & Sampang (2004) estimated that only 12% of fish from 60 seamounts were endemic.

An expectation dependent on endemism is that fish that have evolved on just one seamount or a group of closely placed seamounts would be genetically different from fish that are closely related but on distant seamounts. Evidence reviewed by Stocks & Hart (2007) shows no support for genetic separateness, although the small amount of data available does not make this a strong result.

METHODS AND DATA SOURCES

Testing the GDM with data from the H-E seamount chain

Over the past year and a half we have assembled a database of fish species recorded on seamounts. The data has been extracted from the web-based SeamountsOnline (seamounts.sdsc.edu, last accessed July 5, 2010) (Stocks 2010), from the Ocean Biogeographic Information System website (www.iobis.org/, last accessed July 5, 2010), the Earth Reference Data and Models site (www.earthref.org), from FishBase (www.fishbase.org, last accessed July 5, 2010), and from the Encyclopaedia of Life (www.eol.org, last accessed July 5, 2010). Our database contains 2138 records from all seamounts where fish have been recorded. Apart from species names we have also gathered information on the type of habitat in which the fish lives, the depth of the seamount peak and the total height of the seamount, its latitude and longitude, the distance to the next seamount, and the distance to the nearest continental shelf. Where possible we have also recorded the estimated age of the seamount.

The distribution of seamounts that have been sampled is patchy, and in many cases there is only a very small amount of data recorded from a particular place. To test the GDM, we need to be sure that we are using the most completely sampled set of seamounts; consequently, for the purposes of testing the theory we decided to focus on the H-E seamount chain, as the fish records from this chain make up 34% of all our data. The chain is also well-defined in time, and we know that even if estimated ages are inaccurate (see Baksi 2005), seamounts close to Hawaii will be the youngest (~0.4 Ma) and those furthest from away will be the oldest (~75.8 Ma) (Clouard & Bonneville 2005). The estimated ages of H-E seamounts included in our analysis are shown in Table 1.

Most of the data on fish distributions along the H-E seamount chain comes from Uchida & Uchiyama (1986). The data came from a survey of the chain lasting 5 yr and organised by the Southwest Fisheries Center Honolulu Laboratory, National Marine Fisheries Service (NMFS). Data were gathered on 24 cruises and from 18 other commercial trips operating in the area of the northwest Hawaiian Islands but with no scientific observers on board. The vessels used midwater and bottom trawls, pole and lines, longlines, traps and handlines to catch fish. Bottom fish were mostly sampled with fish traps, lobster pots, shrimp pots, crab nets, dertical longlines, and handlines (Uchida & Uchiyama 1986). No data on the amount of effort used to catch the fish is given in the report.

During the same period that the NMFS survey was carried out (1976–1981) the Soviet fishing fleet was removing large quantities of pelagic armourhead *Pseudo-*

Table 1. The 20 seamounts and banks along the Hawaiian-Emperor (H-E) seamount chain used in the analysis of fish biodiversity. Cross Seamount is not on the main line of seamounts and is an anomaly. For many of the analyses it was removed as an outlier. SSR: Single seamount record: Ma: million yr before present

Seamount	Estmated age (Ma)	No. of benthic and reef fish	Depth of peak (m)	Area (n miles^2)	SSRs
Academician Berg	34	7	500	102.12	0
Bank 8	28	22	64	308.53	3
Bank 9	20	5	115	147.8	2
Brooks Banks	12	21	51	143	8
Colahan	39	6	274	68.8	0
Cross Seamount	0.5	4	595	81.6	0
Equator (Pacific)	30	6	20	27.23	0
Hancock (NW and SE)	34	42	265	30.63	8
Kimmei and Koko	44	5	500	142.9	0
Ladd	30	26	64	340.4	4
Lira	50	1	500	190.6	0
Middle Bank	7	30	35	572.55	16
Milwaukee Group (Yuryaku and Kammu)	45	16	20	424.4	0
Nero	28	26	62	217.85	2
Raita Bank	27	14	16.4	2389	2
Saint Rogatien Bank	12	4	22	1838.16	0
Salmon Bank	27	17	55	925.09	2
Turnif	28	4	20	122.544	0
Twin Banks	2	12	20	1531.8	0
Zapadnaya	28	10	500	183.816	3

pentaceros wheeleri and alfonsino *Beryx splendens* from the chain (Clark et al. 2007). This fishery would not only have removed these fish but would also have damaged habitats for reef-dwelling and benthic fish. There is no way that these removals can be factored into our data set, but should be borne in mind when evaluating the outcome of our analysis.

Endemism on the H-E seamount chain

Species recorded from only one seamount along the H-E seamount chain are the largest group in our dataset (Fig. 2). Each one of the 49 species is a putative endemic, but www.fishbase.org and the Encyclopaedia of Life (www.eol.org) were used to check synonyms and distributions. Using these sources, we found that of the 49 fish species found on only one seamount in our database, 41 are also found elsewhere in the Pacific. Of the remaining 8 species, only one is recorded from just one seamount whilst the others are endemic to the H-E seamount chain. The only putative single seamount endemic is *Tosanoides filamentosus* (Serranidae) found on the Hancock seamounts. As a result of this analysis we could not test whether the number of endemics has a humped relationship with seamount age as predicted by the GDM.

In the quest for factors that might predict the number of single seamount records (SSR) we plotted their number against the combined number of reef-associated and benthic species found on the associated seamount and the relationship is Number SSRs = –1.25 + 0.27 (Benthic + Reef fish). The model accounted for a 53% of the variation in SSR number, with $F = 18.63$, $p < 0.0005$, df = 1, 17. A further factor in determining the number of SSRs could be the depth of the peak with deeper peaks being harder to sample adequately. The number of SSRs has a weak negative correlation with depth. Including this variable with the number of benthic and reef fish in a multiple regression with the number of benthic and reef-based fish resulted in the model accounting for 53% of the variation, with $F = 9.73$, $p = 0.0015$, df = 2, 17. Adding the additional variable does not increase significantly the amount of variance explained.

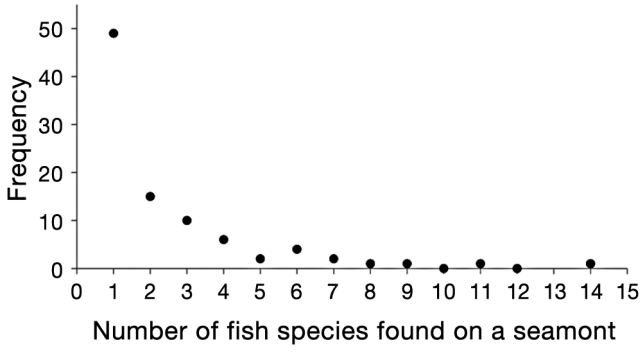

Fig. 2. Frequency of the number of fish species recorded from a seamount. The 49 species each recorded on only one seamount are potential endemics

Number of species on H-E seamount chain

GDM predicts that the number of species on seamounts should be a humped function of age. To test this we plotted the number of species of reef and benthic fish against seamount age, and the relationship is shown in Fig. 3. There is considerable scatter in the data, but a fitted polynomial, constrained to pass through zero, shows that Diversity = 1.34 Age – 0.03 Age^2. An analysis of variance shows that this fitted curve accounts for a significant proportion of the variance in diversity with $F = 15.12$, df = 2, 18, and $p = 0.0001$.

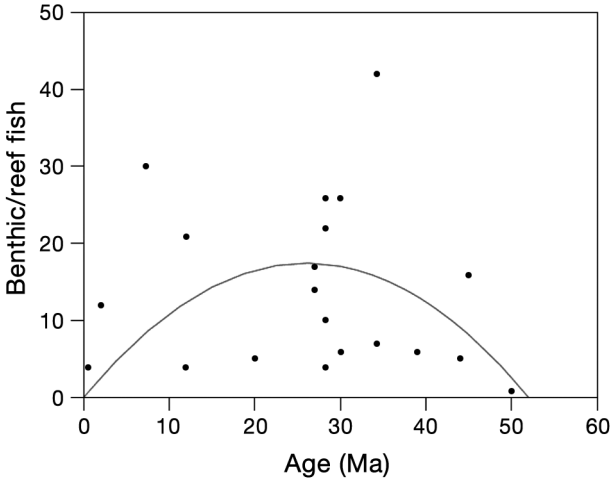

Fig. 3. Number of benthic and reef-dwelling fish found on seamounts and banks of the Hawaiian-Emperor (H-E) seamount chain plotted against the estimated age of the seamount. The line is a fitted polynomial with the origin constrained to pass through zero

Number of species on H-E seamounts is predicted by seamount area and age

The GDM expands on the model from the previous section and includes seamount area as an additional explanatory variable so that $N = a + b(\text{Age}) + c(\text{Age}^2) + d(\log \text{Area})$. Applied to the data on the number of reef and benthic fish recorded from the H-E seamount chain, the equation only accounts for 22% of the variance. An ANOVA shows that the model is not accounting for a significant portion of the variance, with $F = 1.38$, df = 3, 15, and p = 0.29.

Looking for alternative predictors of species abundance on H-E seamounts

The depth of the seamount peak is correlated with age, and there is a positive relationship between these 2 variables, so that Depth of peak (m) = −71.54 + 8.50 Age (Ma), which explains 31% of the variance. An ANOVA shows that the model accounts for a significant amount of the variance, with $F = 1.77$, df = 1,17, and p = 0.01. As older seamounts might be expected to have accumulated more species over time, it is possible that deeper and older seamounts have more species. A plot of the number of reef and benthic fish combined against seamount depth shows that the relationship is in fact negative and accounts for only 19% of the variance. An ANOVA shows that the model does not account for a significant amount of the variance, with $F = 4.10$, df = 1,17, and p = 0.06.

DISCUSSION

Although 49 species have only been recorded from one seamount on the H-E chain, further analysis showed that most of these have also been found elsewhere in the Pacific. The total number of species found on a seamount is positively correlated with the number of species found on only one seamount. This echoes the thesis of Emerson & Kolm (2005a,b), who argue that high species numbers on an island accompany a high proportion of single island endemics. In this case the result obtained in the present study probably implies that the number of single seamount records is an artefact of the poor sampling rather than a reflection of the true rarity of species. Establishing endemism is always difficult but made harder on seamounts by the difficulties of sampling.

Data from other seamount chains reviewed by Stocks & Hart (2007) implies that endemism could be significant, but even in the cases cited the paucity of sampling is still an important consideration.

Our analysis of the relationship between the number of benthic and reef fish and seamount age follows the prediction from the GDM. Seamounts of medium age will be expected to have the greatest number of niches and the highest carrying capacity. These factors lead to the expectation that speciation will lead to a greater number of species on seamounts of medium age. This confirmation of the GDMs prediction has to be interpreted with caution, given the nature of the data. With so few species in the data confined to just one or a few seamounts, the role of speciation in generating diversity is not well supported.

The prediction of the GDM that species abundance will be determined by seamount age and area, factors identified in the original MacArthur & Wilson (1967) theory, is not supported by our analysis. Seamount age alone does bear a good relation with species abundance, but the addition of area renders the combined predictive effect non-significant. One factor to consider is that seamount area is hard to determine accurately from maps. We have measured area above the 500 m depth isocline. As with a mountain on an oceanic island, conditions change significantly as the seamount approaches the sea surface. This will mean that a seamount with a large area, most of which is between 500 and 300 m depth, will offer very different conditions to fish from seamounts with most of its area between 300 and 100 m. The depth values we have are for the shallowest point, but this might just be a peak, with most of the habitable area on the seamount being at a much deeper depth. A more refined analysis of area is required before this aspect of the GDM can be tested properly.

The connectivity of the H-E seamounts is an important issue for the analysis of endemics. As so many marine fish have planktonic larvae, the potential is high for distribution of species across several seamounts in an area. The main flow of water across the Hawaiian portion of the chain is from southwest to northeast (Uchida & Uchiyama 1986), and this could mean that connectivity is not as good as might be expected. However, eddies are caused by the seamounts as the current flows through them, and this may retain larvae in the area and facilitate transfer from one seamount to the next (Lavelle & Mohn 2010, Shank 2010). The evidence we reviewed on the wider distribution of the 49 species recorded from only one seamount showed that if they are to be found elsewhere in the Pacific it is usually to the west towards the Indonesian archipelago and the northeast coast of Australia (as shown on the Encyclopaedia of Life; www.eol.org). The Hawaiian seamounts are often the eastern-most location for many of these species. This ties in with the hypothesis proposed by Mora et al. (2003) that the Indonesian archipelago acts as a speciation hotspot, with fish dispersing from there to islands and reefs far and near, depending on the length of their larval phase.

The argument presented depends on the contention that within the sea there are boundaries at different depths that define the patterns of ecological and evolutionary processes. The chief boundary is between the euphotic and abyssal zones, essentially a boundary defining habitats with and without light. The lack of primary production in the abyssal zone defines a trophic regime, which is fundamentally different from the trophic opportunities that exist in the euphotic zone. As a result, one might expect different selection pressures to hold, and this is borne out by the very marked adaptations that deep sea fish have because they live in a food poor habitat (Marshall 1971). These differences mean that in any analysis of diversity on seamounts, depth must be a prime variable, but within a given depth zone, we are claiming that the same theory can be used to predict patterns of diversity. Within this depth zone, seamounts are equivalent to oceanic islands at the surface.

A further point to make is that studies of diversity need to take trophic level into account. The main theories of diversity on oceanic islands, particularly Hubbell's (2001) neutral theory, apply only to organisms at the same trophic level. The theory of Whittaker et al. (2008) is not specific about its trophic status, but as it proposes that competition on an increasingly populated island will lead to speciation, the theory implies that it only treats species living at the same trophic level.

The chief problem with any study of diversity and endemism on seamounts is the shortage of data and the difficulty inherent in obtaining it. Seamounts are often remote, and it is expensive to carry out surveys at the level of detail required. In addition, the sampling methods most often employed are non-selective and lacking in discrimination with respect to depth and precise habitat. Dredges and trawls just catch a collection of species from between 2 depths, but there is little chance of obtaining more spatially defined information on the species caught. Remote vehicles with cameras allow a more detailed survey of the spatial relations between organisms on a seamount, but such equipment is expensive and can only sample small areas at a time. We need good data from many seamounts within an oceanic region before it will be possible to determine whether or not seamounts are equivalent to oceanic islands in terms of their species diversity.

This paper has proposed a conceptual and theoretical framework within which the diversity of fish on seamounts can be analysed and discussed. Using data on benthic and reef fish found on seamounts belonging to the H-E seamount chain we have illustrated how the GDM of species diversity developed by Whittaker et al. (2008) could be used to analyse patterns and predict aspects of fish diversity. The thoughts expressed in this paper are just a start to the problem of understanding what the level of species diversity is on seamounts and whether seamounts are centres of speciation. The analysis presented is unsatisfactory because there is so little good data, making it impossible to test adequately the ideas proposed against the state of nature. From the point of view of conservation and management of seamount fish, we should be thinking in terms of closing all seamounts to exploitation until we understand their status more fully. At present we have only a weak scientific basis on which to plan conservation and management (Pitcher et al. 2007).

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