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ORIGINAL ARTICLE

How differences in the settling behaviour of moths (Lepidoptera) may contribute to sampling bias when using automated light traps

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Abstract. Quantitative community-wide moth surveys frequently employ flight-interception traps equipped with UV-light emitting sources as attractants. It has long been known that moth species differ in their responsiveness to light traps. We studied how the settling behaviour of moths at a light trap may further contribute to sampling bias. We observed the behaviour of 1426 moths at a light tower. Moths were classified as either, settling and remaining still after arrival, or continually moving on the gauze for extended periods of time. Moths that did not move after settling may not end up in the sampling container of the light trap and therefore are under-represented in automated trap samples relative to their true proportions in the community. Our analyses revealed highly significant behavioural differences between moths that differed in body size. Small moths were more likely to remain stationary after settling. As a corollary, representatives of three taxa, which in Europe are predominantly small species (Nolidae, Geometridae: Eupitheciini, Erebidae: Lithosiini), usually settled down immediately, whereas most other moths remained active on or flying around the trap for some time. Moth behaviour was also modulated by ambient temperature. At high temperatures, they were less likely to settle down immediately, but this behavioural difference was most strongly apparent among medium-sized moths. These results indicate the likely extent of the sampling bias when analysing and interpreting automated light-trap samples. Furthermore, to control for temperature modulated sampling bias temperature should always be recorded when sampling moths using flight-interception traps.

INTRODUCTION

Flight-interception traps using UV light as an attractant are the most widely applied method for assessing the diversity of nocturnal moths (Southwood et al., 2003; Lamarre et al., 2012; Jonason et al., 2014; Merckx & Slade, 2014). Like all survey methods light-trap samples do not perfectly mirror the true compositions of animal communities (Southwood et al., 2003; Merckx & Slade, 2014). Light intensity, spectral composition of the emitted light (Cowan & Gries, 2009; van Langevelde et al., 2011; Somers-Yeates et al., 2013), light pollution from nearby alternative illuminations, trap design (Intachat & Woiwod, 1999; Muirhead-Thompson, 2012; Bates et al., 2013) and moonlight all modulate the attraction of moths to light traps (Davies et al., 2012, 2013; Gaston et al., 2013). Other factors that influence the likelihood of moths being captured by light traps include their wing shape or flight times (Beck & Linsenmair, 2006; Beck et al., 2011; Fuentes-Montemayor et al., 2012; Lintott et al., 2014). Nevertheless, using a standardized design of light-trap is a convenient way of characterizing moth communities along ecological gradients.

Comparing catches obtained with automated light traps and hand-sampling revealed that some moth groups, especially small species of Geometridae, tend to be strongly under-sampled (Axmacher & Fiedler, 2004; Merckx & Slade, 2014). However, the reasons for this under-representation of small moths remained obscure. So there are possibly other factors, influencing the effectiveness of automated light traps.

Temperature can modulate activity of nocturnal moths (Hrdy et al., 1996; Pinault et al., 2012) and therefore could also lead to differences in flight behaviour. Another potential, but under-explored source of the variation in capture probability is the difference in behaviour of moths after arrival at a light trap. Bates et al. (2013) report that "observations of moth behaviour at traps have shown that it is not just the proportion of moths captured by a trap but also the proportion of moths retained by a trap, that combine to influence trap capture efficiency". For example, catches of *Noctua pronuba* differed significantly in their numbers depending on the type of trap used by Bates et al. (2013).

Flight-interception light traps usually consist of a sheet or cylinder of a transparent material (such as acrylic glass). Moths that collide with this obstacle may fall through the funnel into the container at the bottom of the trap. The probability of moths falling through the funnel is increased when moths are highly active in flying around the light trap, which results in them colliding many times with the obstacle. Other moths, in contrast, immediately settle on these surfaces or elsewhere on the trap and do not fall into the collecting device.

In this study we recorded moth settling behaviour at a light tower in order to determine whether the species differed in behaviour depending on their body size and taxonomic affiliation.

Our goals were to:

1. Assess whether certain moth groups (defined by body size or phylogenetic relationships) have a higher likelihood of settling down directly upon arrival at the light source, as compared to other groups; and
2. Establish if this behavioural response of moths is contingent on ambient air temperature.

Given that small moths tend to be under-represented in samples obtained using automated light traps, the expectation was that these moths might differ from large moths in their settling behaviour after arrival at a light source.

MATERIAL AND METHODS

Observations were recorded at seven locations on 13 nights in Pineta san Vitale, Parco regionale del Delta del Po (Ravenna, Italy). We sampled hygrophil forest, pine forest, downy oak forest, wetland with reed vegetation and dry and open grassland habitats. Observations were recorded from twilight (depending on the season, which on average was 9:00 PM) till 12:00 PM. Observations were terminated when hardly any moths arrived at the light trap due to low temperatures (i.e. when the temperature dropped to about 15°C in June to October 2013). As the light source, an Osram 500 W HWL lamp powered by a Honda EM 500 gasoline generator was used. The light tower consisted of a gauze cuboid, 1.80 m high with a top edge length of 0.40 m (Fig. 1). Temperature was measured directly at the light tower using a digital thermometer (Febi Bilstein 37476 Sensor). Mean night temperatures during observations ranged from 14.5°C to 28.5°C. Depending on their behaviour immediately upon arrival, moths were classified either as “settled” (if they remained at their initial landing place for longer than 30 s) or “restless” (if moths behaved otherwise). Temperature was recorded at the time of a moth’s arrival. Since this was not always possible, for a couple of records there are no temperatures. To avoid pseudoreplication all moths were then caught and kept for later determination. For practical reasons, we only considered so-called “large” moths belonging to the families Cossidae and Limacodidae and to the “Macroheterocera” sensu Regier et al. (2013).

Moths were divided into three size classes based on their wing span (big: > 40 mm, mean = 51 mm, standard error = 8.54, n = 131; medium: 30–39 mm, mean = 34 mm, standard error = 2.08, n = 765; small: < 30 mm, mean = 23 mm, standard error = 2.41, n = 317). Inspection of the frequency distribution of our data revealed that this classification yielded a rather even partitioning. Data on wing span were obtained from http://ukmoths.org.uk/ (last visited 11.10.2015) and by direct measurements of specimens when data was not available. Moths were also classified according to their systematic affiliation. Six families were explicitly considered (Geometridae, Nolidae, Noctuidae, Erebidae, Notodontidae, Lasiocampidae) plus two tribes (Eupitheciini within the Geometridae; Lithosiini within the Erebidae). Three other moth families (Cossidae, Limacodidae, Drepanidae) were too poorly represented in our data to warrant representation as distinct taxa in our statistical model, but were included in the analyses of size classes. Sphingidae did not appear at our light traps and hence were not included in our analysis.

Fig. 1. The light trap used in this study.

We analyzed our data using the logistic regressions in the package Statistica 7.1 (StatSoft Inc.). In this analysis only those species for which more than eight individuals were observed were included. Furthermore, Generalized Linear Models (GLM) with binomial error structure and logit link function were used.

RESULTS

The regression analysis revealed that the settling behaviour of moths of the three body size classes differed. Small moths were far more likely to settle down immediately upon arrival than medium or large moths (Wald’s χ^2_{2df} = 62.20, p < 0.0001; Fig. 2).

The probability of settling down immediately decreased with increase in temperature (regression coefficient in logistic model: $b = -0.0882$, $t_{1399df} = 5.612$, $p < 0.0001$), but this effect was largely restricted to medium-sized moths (Fig. 3). For this size category, the temperature effect was much stronger (regression coefficient in logistic model: $b = -0.1608$; $t_{821df} = 7.525$; $p < 0.0001$).

Settling behaviour was also strongly contingent on ambient temperature, but in a more complex way. GLM revealed that settling behaviour was significantly correlated with body size (df = 2, Wald’s $\chi^2 = 17.591$, p = 0.0002) and

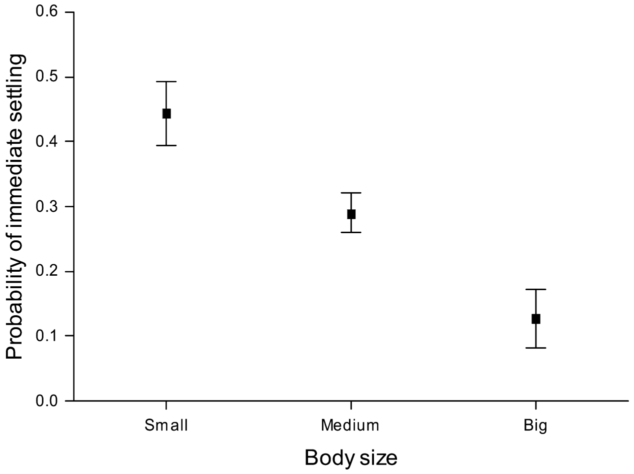

Fig. 2. Probability of small, medium and big moths settling down immediately after arrival at a light source. Given are the means ± 95% confidence intervals. The small moths were more likely than the medium and big moths to settle down immediately after arrival at the light source.

temperature (df = 1, Wald's χ^2 = 5.284, p = 0.0215). This effect was even more obvious when body size and temperature were combined (df = 2, p < 0.0001).

In addition, we observed highly significant differences in settling behaviour of the different moth taxa (Fig. 4; p < 0.001). Species of Eupitheciini, Lithosiini and Nolidae were more likely (60–80%) to settle down immediately after arrival at the light source. In all the other groups caught in sufficient numbers, 80% or more of the moths continued flying or crawling around after arriving at the trap. Of the Cossidae 6 of 19 moths (31.6%) settled upon arrival, of the Drepanidae 0 of 7 and of the Limacodidae 0 of 5 moths.

DISCUSSION

The settling behaviour of moths at light traps was strongly associated with their wing span, with small moths more

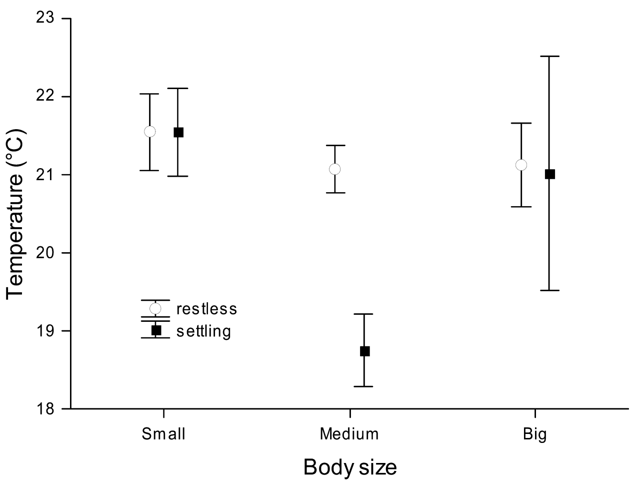

Fig. 3. Mean temperatures ± 95% confidence intervals at which moths in the three body size classes settled down immediately after arrival at the light source (solid squares) or remained active (empty circles). Medium-sized moths mostly tended to settle at low and remained active at high temperatures, which differs from the behaviour recorded for the small and large moths.

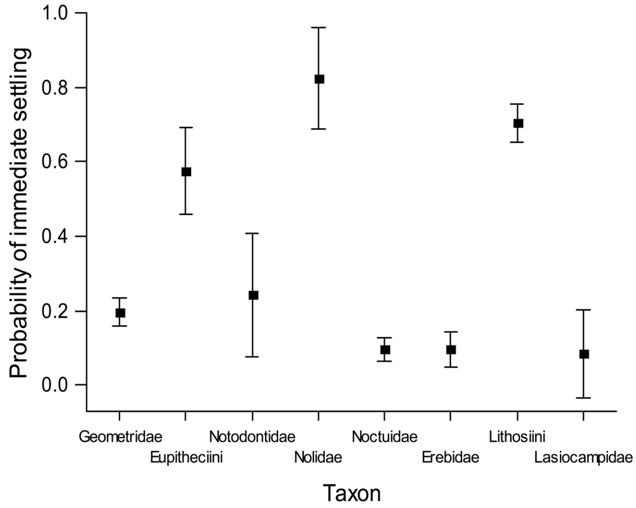

Fig. 4. Probability of moths belonging to eight taxa settling down immediately on arrival at a light source. Given are the means ± 95% confidence intervals. Species of Eupitheciini, Lithosiini and Nolidae were highly significantly more likely to settle down than those of the other groups (Wald's χ^2_{7df} = 433.57, p < 0.0001).

likely to remain stationary after settling. This behaviour reflects the sampling bias towards large moths that is reported in two earlier studies (Intachat & Woiwod, 1999; Bates, 2013). We suggest that this big difference in behaviour is one of the main causes of the under-representation of small moths in the catches of automated light-traps. Our findings also indicate that moth behaviour after arrival at a trap may be modulated by ambient temperature. While in general ectothermic moths are obviously more active at high temperatures (Van Dyck, 2012), especially in medium-sized moths the probability of remaining active for some time was much higher at high air temperatures. This also makes it more likely that medium-sized moths will be caught by automated flight-interception traps at high temperatures, whereas at low temperatures the same moths are more likely to immediately settle down when coming into contact with a light trap and thus escape being collected. If this is a general phenomenon it indicates that the efficiency of automated light traps will be constrained at low temperatures (Summerville, 2013; Jonason et al., 2014) by some kind of size-temperature interaction effect.

Generally, large moths flew around the light source for longer and were less likely to settle down immediately than small moths. This may be explained by their greater ability to store heat even at low temperatures, since moths with a large thorax have a physiological advantage in being able to retain for longer more of the heat they produce through muscle activity, whereas convective cooling acts more rapidly in small insects (Heinrich, 2013).

Since we collected all the moths that settled and remained stationary on the gauze it is unknown whether these moths would have remained inactive throughout the night or would have become active again. If moths resume activity sometime after arrival at the light trap, this could again increase their likelihood of their being caught. Furthermore, the material the light trap is made of might influence the moths settling behaviour. It might be easier, especially for large heavy moths, to settle down on gauze than on acrylic glass. To test this further studies are needed.

In this study, small moths did not differ in their behaviour at different temperatures. Both, settling and restless individuals were recorded at 21 to 22°C. However, most of these moths were caught in early summer, when night temperatures often reach 21 or 22°C. So most of these moths are likely to have been sampled at these temperatures and this result is an effect of sampling mainly on warm nights. To better understand the settling behaviour of small moths, further data needs to be collected for cold nights.

Light source and intensity can affect the species of moths sampled (Cowan & Gries, 2009; van Langevelde et al., 2011; Somers-Yeates et al., 2013). In addition, the comparison of the results of studies using manual sampling and automated light traps also reveal irregularities in the number of species and specimens sampled. For example, in our study, Eupitheciini made up 13.6% of all Geometridae species and 14.4% of all Geometridae specimens caught. Unpublished data of manually light-trapped moths sampled in the botanical garden of Bayreuth also indicate that *Eupithecia* made up 8.0% of all Geometridae species caught (and 4.1% of the Geometridae individuals).

However, in a study in the Swiss Alps using automated traps (Beck et al., 2010), the genus *Eupithecia* accounted for 2.3% of the species of Geometridae, but these made up only 0.4% of the geometrids caught. In samples collected by Truxa & Fiedler (2012), Eupitheciini made up 8.6% of the 140 automatically light trapped species of Geometridae, but accounted for only 2.1% of all the specimens of that family. Comparing the percentage of Eupitheciini in all four studies, manual light trapping recorded more specimens of Eupitheciini (although the percentage of *Eupithecia* species recorded was more or less the same). So, based on our behavioural data reported above, the contribution to the community in terms of Eupitheciini seems under-recorded by automated light traps. Based on our results and in line with earlier direct comparisons of automated versus hand sampling at light traps we conclude that automated traps may inaccurately quantitatively characterize assemblages of small lepidopterans (Bates et al., 2013).

In automatically sampled data we therefore expect an under-representation of families with small species like Tortricidae, Gelechiidae or Pyralidae, which are abundant and species-rich in many habitats. For example, in a comparison of moth assemblages in different types of flood-plain forests in Central Europe based on catches by automated light-traps (Truxa & Fiedler, 2012), the predominantly small Pyraloidea accounted for 17.2% of the species recorded, but made up only 8.1% of the total catch of >32,000 individuals. As a consequence, functionally important moth guilds predominantly composed of small species might be under-represented, such as species with endophagous larvae (like many Eupitheciini, but also micro-moths like Tortricidae or Pyraloidea) or detritivorous species (e.g. the genus *Idaea*).

Our observations of moth behaviour at a light trap also indicate that lichen moths (Erebidae: Lithosiini) might sometimes be under-represented in automatic samples. This could be important when numbers of lichenophagous species caught are used as indicators of ecosystem status (Thorn et al., 2015). However, in the study of Truxa & Fiedler (2012) no such under-representation was obvious, as lichen moths made up 20.5% of the Erebidae species caught, but accounted for 84.0% of the individuals, mainly due to the massive representation of one species (*Pelosia muscerda*) in the trap samples.

These examples illustrate that differences in moth settling behaviour at light traps associated with their size and/or phylogenetic position, may account for the deviations between capture rates in surveys and their abundance in their respective habitats, but that these relationships do not allow for simple generalizations. Moreover, it should be stressed that this does not devalue light trap samples as sources of information on moth biodiversity or community ecology (Merckx et al., 2012a, b; Truxa & Fiedler, 2016). As long as the same light sources and types of traps are used in studies carried out along ecological gradients, there is little reason to assume that sampling bias will result in seriously distorted ecological patterns. Nevertheless, in future studies the fact that small moths and other taxa might be undersampled, especially when sampling different habitats, should be considered.

However, in view of our observations it would be desirable to further elucidate the relationships between sampling results and behaviour. More studies on the individual flight behaviour of a wide range of different groups of moths that simultaneously address the effect of temperature, light conditions (moonlight, spectral characteristics of light sources) and body size of nocturnal moths are needed.

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