



**Capture-induced physiological stress and post-release mortality for Southern bluefin tuna (*Thunnus maccoyii*) from a recreational fishery**

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1 Capture-induced physiological stress and post-release mortality for  
2 Southern bluefin tuna (*Thunnus maccoyii*) from a recreational fishery

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9 **Abstract:** Southern bluefin tuna (SBT) are a popular component of the recreational large  
10 pelagic game fishery in Australia. The fishery is managed using individual fisher catch limits.  
11 Fifty-nine pop-up archival transmitting (PAT) tags were attached to individual SBT to  
12 estimate post-release survival (PRS) rates. Fish caught on lures configured with J-hooks ( $n =$   
13 44) and those caught on circle hooks ( $n = 8$ ) had similar PRS rates and were combined to  
14 increase sample size, revealing a PRS estimate of 83.0% (95% CI: 75.9 – 90.7%,  $n = 54$ ).  
15 The PRS estimate of fish caught on lures with treble hooks was much lower, 60% (95% CI:  
16 20 – 100%,  $n = 5$ ). By sampling blood from 233 fish, including 56 of the PAT tagged  
17 individuals, we show that angling duration is related to an elevation of lactate, cortisol and  
18 osmolarity in blood plasma, indicative of increased physiological stress. Physical damage  
19 related to hooking location, angling duration, biochemical indicators of physiological stress  
20 and handling duration were not identified as significant factors leading to post-release  
21 mortality. The results quantify a previously un-accounted source of mortality for SBT.

22 *Keywords:* Southern Bluefin Tuna, *Thunnus maccoyi*, recreational fishing, physiological  
23 stress, post-release survival, responsible fishing, animal welfare

## 24 Introduction

25 Southern bluefin tuna (*Thunnus maccoyii*) are a prized target of recreational fishers in  
26 Australia. The species is targeted when the fish seasonally migrate close to the south and  
27 southeast coastline, within range of recreational fishing boats (Green et al. 2012; Tracey et al.  
28 2013). Historical overfishing by the commercial fishery, comprising fleets from several  
29 countries, has reduced the population of southern bluefin tuna (SBT) to 8 – 12% of the pre-  
30 exploitation spawning biomass (CCSBT 2014). While it is unlikely that the Australian  
31 recreational harvest of SBT is significant relative to the global TAC, there is an obligation for  
32 member countries to report all sources of mortality from within their EEZ to the Commission  
33 for the Conservation of Southern Bluefin Tuna (CCSBT) to provide a complete and  
34 transparent estimate of harvest (CCSBT 2014). As such, quantifying fishing mortality arising  
35 from the recreational sector is important (CCSBT 2014; Marcek and Graves 2014).

36 Recreational fishers in Australia are allowed to retain SBT, however this is regulated  
37 by individual possession limits. Some fishers also choose to not retain fish, referred to as  
38 catch and release fishing. Catch limits are traditional tools used globally as a principal means  
39 to manage recreational fisheries (Bartholomew and Bohnsack 2005; Tetzlaff et al. 2013). A  
40 fundamental assumption under-pinning the effectiveness of catch limits, as well as voluntary  
41 catch and release, is that most fish survive (Cooke and Suski 2005; Arlinghaus et al. 2007;  
42 Cooke and Schramm 2007; Tetzlaff et al. 2013) and that fish suffer minimal sub-lethal effects  
43 once released (Arlinghaus et al. 2009). Recent reports indicate that approximately 25% of  
44 SBT caught by recreational fishers in Australia are released (Green et al. 2012; Tracey et al.  
45 2013). Hence, a low post-release survival rate may lead to a significant source of  
46 unaccounted mortality relative to the recreational harvest of SBT.

47 For many species little is known about the fate of fish after release. Over the last  
48 decade, however, there has been a growing body of literature investigating factors that may  
49 cause excessive stress or damage to a fish as well as assessing post-release survival rates.  
50 Significant variability has been reported in post-release survival rates between species  
51 (Muoneke and Childress 1994; Bartholomew and Bohnsack 2005; Arlinghaus et al. 2007;  
52 Cooke and Schramm 2007). This highlights the importance of investigating post-release  
53 survival on a species-by-species basis.

54 Assessing post-release survival of highly migratory fish is logistically challenging  
55 (Moyes et al. 2006; Donaldson et al. 2008). Conventional tagging studies typically yield low  
56 return rates (Kohler et al. 1998). Active tracking of animals for a sufficient period post-  
57 release using acoustic technology is difficult due to the swimming speed and dispersal range  
58 of large pelagic fish (Skomal 2007). While containment experiments are generally not  
59 feasible due to the scale of equipment required to hold the fish (Skomal 2007).

60 The most common approach to assess the fate and behaviour of large pelagic fish after  
61 release is the application of pop-up archival transmitter (PAT) tags. This method has been  
62 applied to assess survival of large sharks (Skomal and Chase 2002), billfish (Graves et al.  
63 2002; Domeier et al. 2003; Kerstetter et al. 2003; Kerstetter and Graves 2006) and tunas  
64 (Stokesbury et al. 2011; Marcek and Graves 2014).

65 The capture of a fish will always have some impact; at the very least the fish is  
66 hooked and fought for a period of time, resulting in some physical exertion (Cooke and Suski  
67 2005; Arlinghaus et al. 2007). Factors such as angling duration, water temperature and air  
68 exposure have all been shown to induce a physiological stress response (Cooke and Suski  
69 2005). The resulting adaptive physiological responses to stressors that occur during capture  
70 can be measured via biochemical reactions (Moyes et al. 2006). Furthermore, stressors can be

71 tested against the fate of the fish to assess whether fishers can alter their practices to  
72 maximise post-release survival and minimise fish welfare impacts (Moyes et al. 2006).

73 Species-specific information on the effects of capture and handling by recreational  
74 fishers can be utilised in the development of scientifically defensible best practices,  
75 minimising the impacts of recreational fishing activities on released fish and fish stocks as a  
76 whole (Cooke et al. 2012). Importantly, this information enhances the sustainable utilisation  
77 of fisheries resources by considering additional sources of mortality in stock assessment and  
78 providing results that facilitate informed decision making by fishers, increasing resource  
79 stewardship.

80 In this study, the post-release survival rate of SBT caught in the Australian recreational  
81 fishery was evaluated. In addition, a range of factors related to the catch, handling and release  
82 of SBT were assessed and tested against physiological stress indicators measured by  
83 biochemical variables. Finally, capture factors and physiological stress indicators were tested  
84 to assess causality of post-release mortality.

## 85 **Methods**

86 Southern bluefin tuna were caught in waters southeast of Tasmania in 2012-14, and in  
87 waters adjacent to southwest Victoria and the South coast of New South Wales (NSW) in  
88 2013 and 2014 (Fig. 1). Sampling occurred between February and July each year. All fish  
89 were caught using standard recreational fishing techniques, specifically rod and reel fitted  
90 with 15, 24 or 37 kg breaking strength monofilament line. Lures were standard, commercially  
91 available products commonly used by the recreational fishery to target SBT, including hard-  
92 body lures with either J-hooks (6/0) or treble hooks (4/0) and skirted lures with single J-  
93 hooks (9/0). All lure configurations were trolled at 6 – 9 knots. Baited circle hooks (7/0) were  
94 used on occasion when fishing adjacent to NSW. These were presented to schools of fish  
95 while the boat was drifting.

96 Hooked fish were retrieved to the boat and carefully lifted on-board and the angling  
97 duration (time from lodging hook until retrieved to the boat) recorded. Fish were placed on a  
98 padded mat and the eyes covered with a wet towel, following the methods considered ‘best  
99 practice’ for responsible fish handling. Fish were not irrigated during the handling process as  
100 this was considered beyond the scope of what is promoted as ‘best practice’ for recreational  
101 fish handling. Immediately after the fish was landed a non-lethal blood sample was taken (0.5  
102 – 3.0 ml) from the lateral artery posterior to the pectoral fin or by cardiac puncture using a  
103 lithium heparin Vacutainer® (Becton-Dickinson) with a 38 mm 21-gauge needle. Blood  
104 samples were placed on ice until further processing. Fork length (*FL*) was measured to the  
105 nearest cm, the condition of the fish was assessed (1 = vigorous, 2 = active, 3 = low active or  
106 4 = dead), the location of the hook and severity of bleeding recorded (1 = none, 2 = minor  
107 external, 3 = minor internal or 4 = major bleeding), and the hook removed. At release, fish  
108 were held alongside the boat, which was slowly moving forward, until it freely kicked from

109 the grip of the handler. This resuscitation technique is recommended to the recreational  
110 fishery as ‘best practice’ for releasing southern bluefin tuna. The release location as reported  
111 by a GPS and the time out of water (handling time) were then recorded.

### 112 **Pop-up archival transmitter tags**

113 Fifty-nine fish were released with pop-up archival transmitter (PAT) tags (MiniPAT;  
114 Wildlife Computers, Redmond, WA, USA) regardless of bleeding or hooking location. To  
115 minimise the potential for mortalities related to carrying a satellite tag only fish greater than  
116 90 cm FL were tagged. All 21 fish caught adjacent to NSW were tagged. Only a proportion  
117 of fish caught from VIC ( $n = 14$ ) and TAS ( $n = 24$ ) were tagged as a greater number were  
118 caught from these locations than tags available, with many fish of a similar size. Fish to be  
119 tagged were selected at random and approximately proportional to the total number of fish  
120 caught within 10 cm length ( $FL$ ) bins, spanning the size distribution of all fish caught. Each  
121 tag was rigged with a Domeier nylon umbrella dart tag anchor via stainless steel wire. The  
122 anchor was inserted into the musculature at the base of the second dorsal fin and through the  
123 pterygiophores. A second Domeier umbrella anchor crimped to a 24 kg monofilament loop,  
124 was attached approximately 5-10 cm behind the primary tagging location to minimise lateral  
125 tag movement.

126 Fifty-four tags were programed to detach after 180 days, the remaining five were  
127 programed for shorter retention durations (Table S1). Each tag was programmed to release  
128 from the anchored tether at the conclusion of the programmed period via a corrodible release  
129 pin. Alternatively, if the tag sank to a depth greater than 1800 m or the depth recorded by the  
130 tag did not change by greater than  $\pm 2.5$  m over a 2-day period the tag was also programmed  
131 to detach from the tether. Detached tags floated to the sea surface and transmitted data to the  
132 Advanced Research and Global Observation Satellite (ARGOS) system. By examination of



133 dive and temperature profiles in the hours and days after release a determination was made as  
134 to the fate of each tagged fish (Fig. 2).

135 Sea-surface temperature (SST) at the time of capture was identified by the first  
136 temperature record on a tag at a depth less than ten meters on the day of tagging. For fish that  
137 were not satellite tagged, location specific SST at time and location of capture were derived  
138 from Advanced Very High Resolution Radiometer (AVHRR) satellite estimates accessed  
139 through the Integrated Marine Observing System (IMOS).

#### 140 **Physiological stress**

141 Whole blood lactate and glucose were measured in the field, the former with a Lactate  
142 Pro LT-1710 (Arkray, Kyoto, Japan), the latter with an Accu-Chek Active (Roche,  
143 Mannheim, Germany). The remaining blood sample was centrifuged for five minutes at  
144 3300rpm using a portable field centrifuge (LW Scientific Portafuge). The resultant blood  
145 plasma was stored in liquid nitrogen (~ -80°C) until subsequent laboratory analysis.

146 For samples where there was sufficient blood plasma, glucose and lactate were  
147 analysed in the laboratory using a GM7 Microstats reader (Analox Instruments, Helena  
148 Laboratories, VIC, Australia). Given the laboratory analysis was a more accurate method the  
149 values from field meters were converted to laboratory values based on the parameters of  
150 significant linear relationships. pH was measured using a Minilab Isfet pH meter, Model  
151 IQ125 (Hach Pacific, Victoria, Australia).

152 Quantitative determination of cortisol was conducted using an ENZO Cortisol  
153 Enzyme Linked Immunosorbant Assay (ELISA) kit (United Bioresearch Products Pty Ltd,  
154 NSW, Australia). Standards and samples diluted 1:5 to 1:100 were prepared and assayed  
155 according to manufacturer instructions. Microtitre plates were read at 405nm using a TECAN

156 Genios plate reader (TECAN Australia Pty Ltd). Parallelism of diluted plasma with the  
157 standard curve was confirmed according to Plikaytis et al. (1994). Slopes for the reference  
158 standards and serially diluted serum were 161.76 and 161.76, respectively. Inter-assay  
159 coefficient of variation (CV) using high and low standard reference points was <10%. Intra-  
160 assay CV using high, moderate and low plasma samples was similarly <10%. Recovery of  
161 cortisol from a spiked plasma sample was 101.2%. Spiked and diluted sample recoveries  
162 percentage limits of within 90–110% were considered acceptable.

### 163 **Statistical analysis**

164 Data analysis was conducted using R 3.0.3 (R Core Team 2014). The relationships  
165 between predictor variables related to the capture process (severity of bleeding, angling  
166 duration, fish length and sea surface temperature at the site of capture) to each of the  
167 biochemical variables were investigated using generalised additive models (GAMs) (Zuur et  
168 al. 2009). Outliers were removed based on visual interpretation of box plots. The error  
169 structure of each GAM was determined by the fit to the data with the aim of satisfying the  
170 assumption of normality. Correlations between variables were explored and the existence of  
171 collinearity between covariates was identified using the variance inflation factor (VIF). The  
172 upper threshold value of the VIF was set at '3' which has been identified as a robust approach  
173 (Zuur et al. 2010). If collinearity was identified the variables with the highest VIF values  
174 were sequentially removed until all VIF values were less than the threshold (Zuur et al. 2009;  
175 Zuur et al. 2010).

176 The initial, full factorial model was:

$$V_{blood} = \alpha + s_{FL}(FL) + s_{AD}(AD) + s_{SST}(SST) + BL + \epsilon$$

177 where  $\alpha$  is the GAM intercept,  $FL$  is fork length,  $AD$  is angling duration,  $SST$  is sea surface  
178 temperature,  $BL$  is the ordinal severity of bleeding index,  $\epsilon$  is an error term and  $s$  are thin-

179 plate spline smoothers. The amount of smoothing ( $k$ ) applied to the splines was restricted to  
180 avoid over-fitting due to sample size, but adequate to describe the non-linearity between the  
181 response and explanatory variable.

182 For each GAM, a stepwise backward selection method was applied beginning with all  
183 predictor variables. Non-significant variables with the lowest significance levels were  
184 excluded at each step and the model run again until only significant predictors remained. The  
185 goodness of fit for each of the reduced models was considered using the unbiased risk  
186 estimator (UBRE), the level of deviance explained, and the lowest Aikake's Information  
187 Criterion (AIC) as per (Zuur et al. 2009). The GAMs were fitted using the *mgcv* package in  
188 R. The significance level was set to  $\alpha = 0.05$  for all tests.

189 Post-release survival was categorised as a binary fate ('survived' or 'died'). A  
190 decision rule was implemented to assign a mortality as either related to the capture event or  
191 as a natural mortality. A Kaplan-Meier survival analysis was used to visualise tag retention  
192 and mortality events through time. The maximum duration considered was 180 days  
193 governed by the detachment date and time programmed on-board the tag. Mortality related to  
194 the fishing event was considered to have occurred if the tag indicated the fish had died within  
195 10-days post-release. This assumption was based on the behaviour of the fish prior to the  
196 mortality event determined from the recorded dive profile, a natural break in the cumulative  
197 number of fish identified as dying after this time and existing literature. Mortalities beyond  
198 this point were considered natural mortalities. The 95% confidence interval associated with  
199 post-release survival estimates were calculated using the release mortality software (version  
200 1.1.0) (Goodyear 2002). Confidence intervals were based on 10,000 simulations. One  
201 premature release that occurred within the 10-day period was included in the model as a  
202 'survivor' based on the interpretation of depth data from this tag. Although the fishing and  
203 handling techniques replicated 'best practice' recreational fishing methods, the additional

204 processing, including drawing blood samples and application of tags may bias the post-  
205 release survival estimate downward.

206 Factors related to the capture of the fish, physiological stress imparted during capture  
207 and handling duration (time out of water) were modelled against post-release fate using a  
208 GAM with a binomial error term. The initial, full factorial model was:

$$\begin{aligned} Fate = & \alpha + s_{FL}(FL) + s_{AD}(AD) + s_{SST}(SST) + BL + s_{Glu}(Glu) + s_{pH}(pH) + s_{Lac}(Lac) + s_{Cor}(Cor) + s_{Osm}(Osm) \\ & + s_{HT}(HT) + RL + \epsilon \end{aligned}$$

209 where  $\alpha$  is the GAM intercept,  $FL$  is fork length,  $AD$  is angling duration,  $SST$  is sea surface  
210 temperature,  $BL$  is the ordinal severity of bleeding index,  $Glu$  is plasma glucose,  $pH$  is plasma  
211 pH,  $Lac$  is plasma lactate,  $Cor$  is plasma cortisol,  $Osm$  is plasma osmolarity,  $HT$  is handling  
212 time,  $RL$  is the ordinal release condition index,  $\epsilon$  is a binomial error term and  $s$  is a thin-plate  
213 spline smoother. The same process of identifying collinearity and stepwise backward  
214 selection described previously was applied to identify the best candidate model.

215 Two fish were observed to be predated on immediately post-release. These fish were  
216 removed from this predictive analysis, although included in the post-release survival  
217 estimates. It was assumed that these fish did not die because of factors tested in this model.

218

## 219 **Results**

220 A total of 233 fish were landed during the study ranging in length from 78 – 188 cm  
221 FL, with a median size of 98 cm FL, excluding five large fish. The five large fish were caught  
222 adjacent to Victoria (187 cm FL) and Tasmania (162, 172, 184 and 188 cm FL). Six fish  
223 (3%) were landed either dead or in a non-responsive state with this fate attributed directly to  
224 the capture event. Deep hooking, leading to gill damage accounted for five of these cases.  
225 The other fish became tail wrapped towards the end of an extended angling duration, leading  
226 to the fish being retrieved tail-first to the boat.

227 In three cases, blood samples could not successfully be drawn from satellite tagged  
228 fish due to adverse weather conditions and/or the behaviour of the fish. While no blood was  
229 taken from these fish an attempt was made, including the insertion of a needle. The  
230 assumption was made that this constitutes a standardisation of method, but due to limited  
231 sample size we were unable to test whether the process of sampling blood affected survival.  
232 In other cases, there was an insufficient amount of blood plasma available to analyse several  
233 of the biochemical variables. Cortisol levels were not obtained for 12 tagged fish. Osmolarity  
234 and pH were not obtained for eight of these fish.

## 235 **Post-release survival**

236 Sixteen of the 59 satellite tagged fish were determined to have died during the period  
237 they had tags attached, 11 of which were attributed to the catch and release event. Four  
238 mortalities were considered natural, occurring greater than 19-days after release (Fig. 3). One  
239 individual was recaptured by a commercial longline vessel less than 24-hrs after release. It  
240 was categorised as not dying due to the recreational catch and release event, as taking a  
241 baited hook was evidence of feeding behaviour. It was assumed that this reflects that the fish  
242 was not significantly stressed or injured at the time of recapture.

243 Seven fish (63% of mortalities associated with the recreational fishing event) died  
244 within 24 hours of release (Table 1). Four of these fish were classified as direct catch-induced  
245 mortalities, one was inferred as a post-release predation based on dive and temperature data  
246 recorded by the tag and two were observed post-release predation by seals (Tables 1 & 2).

247 The post-release survival (PRS) rate of fish caught on lures rigged with J-hook,  
248 excluding the two fish that were observed to be predated upon immediately once they were  
249 returned to the water, was 86.6% (95% CI: 77.3 – 95.5%,  $n = 44$ ). For fish caught with circle  
250 hooks the PRS rate was 87.4% (95% CI: 70 – 100%,  $n = 8$ ), and for fish caught on lures with  
251 treble hooks the PRS rate was 60% (95% CI: 20 – 100%,  $n = 5$ ). As the PRS rates of fish  
252 caught with J-hooks and circle hooks were similar, these categories were combined to  
253 increase sample size. The resulting PRS rate, with the inclusion of the two fish that were  
254 observed to be predated on, was 83.0% (95% CI: 75.9 – 90.7%,  $n = 54$ ). Given the PRS rate  
255 of fish caught using lures with treble hooks was much lower, these individuals were not  
256 pooled with the other two hook categories. Noting however, that the sample size of treble  
257 hooked fish is low and the result is therefore indicative rather than statistically robust.

258 No explanatory variables were identified as significantly related to post-release  
259 survival by GAMs. A visual inspection of the relationships between angling duration, fish  
260 length, SST and each biochemical variable confirmed no obvious differences between fish  
261 that were classified as ‘survived’ or ‘died’ (Fig. 4).

## 262 **Physiological stress**

263 Plasma cortisol, lactate, and osmolarity levels were all identified to be significantly  
264 affected by angling duration (Table 3). The response of each of the significant biochemical  
265 variables increased with angling duration, the rate of increase however reduced for angling  
266 durations greater than approximately 15 minutes (Fig. 4). Sea surface temperature at the

267 location of capture was significantly related to the response of glucose and lactate in blood  
268 plasma and fish size was significantly related to lactate response (Table 3). pH was not  
269 related to any factors tested.

270 Several biochemical variables were significantly correlated. Lactate was significantly  
271 correlated with all other biochemical variables with the strongest correlation identified with  
272 cortisol ( $r = 0.49$ ). Cortisol was also significantly correlated with glucose ( $r = 0.27$ ) and  
273 osmolarity ( $r = 0.46$ ).

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274 **Discussion**275 **Post-release survival**

276 This is the first study to assess the survival rate of recreationally caught SBT after  
277 release. The reported post-release survival estimate should be considered conservative as the  
278 effects of processing, in particular drawing blood samples and attaching satellite tags are  
279 unknown and may have biased the results towards a higher mortality rate (Cooke and  
280 Schramm 2007). With the exception of hook type, no other factors tested were found to  
281 significantly influence the post-release survival rate. Therefore, the survival estimate is  
282 representative of a broad range of recreational fishing activities which differ by factors such  
283 as size distribution of fish caught, angling duration or sea surface temperature. Fish caught  
284 on lures configured with treble hooks had a lower post-release survival rate than fish caught  
285 on either baited circle hooks or lures configured with J-hooks. Given the low sample size of  
286 fish caught on treble hooks, however, it was not possible to determine if this result was  
287 significant. Different hook types have been shown to significantly influence post-release  
288 survival for other species (Skomal et al. 2002; Horodysky and Graves 2005), and as such  
289 further research into the effects of treble hooks on the post-release survival of SBT is  
290 warranted.

291 There have been no comprehensive studies to date on the national recreational  
292 harvest, or release rates, of SBT within Australia. However, two studies have been conducted  
293 that provide estimates of harvest and release rates from the recreational SBT fishery at a state  
294 level. The first was conducted in 2011 in Victoria (Green et al. 2012). Using a comprehensive  
295 onsite creel method, the recreational harvest of SBT was estimated at 240 t and the release  
296 rate reported as 25%, which equates to approximately 42 t assuming released fish had the  
297 same size composition as retained fish. By applying a post-release mortality rate (19%, all  
298 hook methods combined in lieu of no information on the proportion of fish caught by each



299 hook type) an estimated 7.8 t were lost as post-release mortality from the Victorian  
300 recreational fishery in 2011. The second survey was conducted in 2012 using an offsite  
301 longitudinal phone-diary survey in Tasmania (Tracey et al. 2013). A total harvest of 79 t was  
302 estimated and a release rate of 24%. Applying the same principals as for the Victorian survey  
303 this equates to 14 t of fish released with 2.6 t lost to post-release mortality. These estimates  
304 indicate that post-release mortality of SBT adds approximately 3% to the total recreational  
305 harvest as unaccounted mortality and that this additional tonnage is insignificant relative to  
306 the Australian allocation of the TAC (5,193 t in 2014).

307 Previous studies on recreationally caught Atlantic bluefin tuna, *Thunnus thynnus*  
308 (ABT) have reported post-release survival rates of 100% for juveniles (Marcek and Graves  
309 2014) and 94-97% for adults (Stokesbury et al. 2011). While the estimates presented for SBT  
310 are lower than the estimates presented for ABT, they are similar to those presented for other  
311 large pelagic fishes caught by recreational fishing methods, including white marlin  
312 *Tetrapturus albidus* (82.5%) (Horodysky and Graves 2005), black marlin *Istiompax indica*  
313 (89%) (Musyl et al. 2015), Sailfish *Istiophorus platypterus* (91.8%) (Musyl et al. 2015), and  
314 striped marlin *Kajikia audax* (74%) (Domeier et al. 2003).

315 The majority of SBT that were attributed to have died due to the capture process  
316 occurred within 24-hours after release (64%). This is also consistent with other studies on  
317 large pelagics showing mortality occurring shortly after release (Domeier et al. 2003;  
318 Horodysky and Graves 2005; Kerstetter and Graves 2006; Kerstetter and Graves 2008).

319 Predation of released fish by Australian fur seals *Arctocephalus pusillus* was observed  
320 on two occasions. On both occasions the seals interacted with the fish prior to landing (seals  
321 chased the fish while they were being retrieved to the boat – inflicting minor superficial  
322 grazing), and even though efforts were made to move on from the area before releasing the  
323 fish, the seals chased the boat and re-engaged with the fish. Direct observation of predation

324 on fish after release has been reported in other studies (Danylchuk et al. 2007). Although, due  
325 to the logistics of observing fish for an extended period post-release, predation events are  
326 more commonly identified from data recorded on the PAT tags. The depth, temperature and  
327 light level data often reveal clear evidence of a predatory event, and in some cases, the data  
328 can provide insight into the predator's taxa or even species (Kerstetter et al. 2004; Beguer-  
329 Pon 2012; Marcek and Graves 2014).

330 In this study the satellite tags indicated predatory events occurred on six individuals.  
331 Two tags recorded temperatures of approximately 38°C, typical of the body temperature of a  
332 mammal, in this case most probably a seal (Austin et al. 2006). The temperature increases  
333 were concurrent with reduced light levels.

334 Four tags, two within 10-days post-release and two well after release, indicated an  
335 abrupt increase in temperature to approximately 26°C, 6 – 8°C above the ambient water  
336 temperature recorded prior to the predation event (Fig 2C). These events were also concurrent  
337 with a sustained drop in light level. The most likely candidate predator was Lamnid sharks,  
338 specifically Shortfin Mako (*Isurus oxyrinchus*), which are commonly found in the offshore  
339 waters adjacent to NSW and the east coast of Tasmania where these predatory events  
340 occurred. These shark species tend to maintain body temperatures 7–10°C above ambient  
341 (Carey and Teal 1969). The recorded depth profiles during the period when the tags were  
342 ingested were also consistent with the behaviour of Lamnid shark (Sepulveda et al. 2004).

343 In addition to the fish determined to have died as a response to being captured, four  
344 PAT tagged fish were assessed to have died due to natural causes. Natural mortality estimates  
345 for SBT are non-linear and age dependent, with higher mortality rates for young fish, ranging  
346 from 0.20 – 0.42 yr<sup>-1</sup> (CCSBT 2009). Given these relatively high rates some degree of natural

347 mortality during this study was not unexpected with tags programmed to detach six months  
348 after deployment.

349

### 350 **Physiological stress**

351 Cortisol is considered to be the best quantitative indicator of physiological stress  
352 (Ellis et al. 2007) and responds to a variety of both acute and chronic stressors (Pickering  
353 1992; Barton 2000). Chronically elevated levels are often associated with adverse  
354 consequences such as reduced growth rate (Jentoft et al. 2005) and immune-suppression  
355 (Watanuki et al. 2002) as it shifts energy investment from anabolic to catabolic activities,  
356 such as energy mobilisation and maintenance of homeostasis (Bonga 1997).

357 In this study, a typical stress response was observed in relation to angling duration.  
358 Plasma cortisol concentrations from SBT were elevated and sharply increased in association  
359 with angling duration with peak levels observed within 10-30 mins, although cortisol  
360 concentrations will likely increase post-release as values do not typically peak until 1-2 hours  
361 following exercise (Barton et al. 2002). Plasma glucose and lactate concentrations followed a  
362 similar pattern and were significantly associated with cortisol and angling duration,  
363 concurring with other studies (Gustaveson et al. 1991).

364 Longer angling durations have been shown in many studies to increase physiological  
365 disturbance and the time required for recovery (Cooke and Suski 2005; Cooke et al. 2008),  
366 however, several studies have found no relationship between angling duration and post-  
367 release mortality (Diodati and Richards 1996), including species such as, rainbow trout,  
368 *Salmo trutta* (Schisler and Bergersen 1996) and striped marlin (Domeier et al. 2003). The  
369 results here do not indicate that angling duration effects survival of SBT. Extended angling  
370 durations do, however, increase the physiological effect on the fish. Hence consideration  
371 should be given to using appropriate fishing tackle relative to the size of the fish to minimise

372 the angling duration, subsequently improving the welfare of the animal (Cooke and Suski  
373 2005; Iwama 2007).

374 Water temperature at the location of capture was not related to the fate of SBT post-  
375 release. This is not surprising given the broad thermal niche of the species. Satellite tags  
376 indicated that the fish spent time in water ranging from 8 - 22°C normally distributed around  
377 a mean of 16°C. The ability of Bluefin tuna to tolerate such a wide range of temperatures is  
378 due to their endothermic physiology, whereby they can retain metabolic heat.

379 Hooking location has been reported as the single most important factor related to a  
380 fish's fate as a result of recreational capture (Bartholomew and Bohnsack 2005). When fish  
381 are deep hooked they tend to experience increased bleeding and damage to vital organs (Lyle  
382 et al. 2007). This often equates to high rates of immediate and short-term mortality  
383 (Bartholomew and Bohnsack 2005; Cooke and Suski 2005; Arlinghaus et al. 2007; Lyle et al.  
384 2007). Seven of the 59 fish satellite tagged in this study were caught using baited circle hooks  
385 while drifting over schools of SBT. Six of these fish were hooked in the corner of the mouth  
386 and one was deep hooked, in the latter case the fishing line was cut and the hook left in the  
387 fish, this fish survived post-release. This practice has been shown to reduce mortality rates  
388 relative to removing the hook, with the fish eventually shedding the hook (Schill 1996;  
389 Tsuboi et al. 2006; Lyle et al. 2007).

390 In this study 59% of the SBT that had PAT tags attached were identified as having  
391 little to no bleeding, 37% had minor bleeding associated with the hooking location in the  
392 mouth and 2% had major bleeding, one around the mouth and the other due to major external  
393 damage ventral to the operculum from a treble hook. Blood loss due to hooking damage,  
394 however, was not significantly related to the fate of the fish post-release. One fish that died  
395 within 10-days post-release had minor internal bleeding from the gill region inflicted by a

396 treble hook and was predated upon within hours after release. The two fish that were recorded  
397 as having major bleeds both survived. There are many instances where injuries include minor  
398 or moderate bleeding that is unlikely to result in mortality (Arlinghaus et al. 2007).

399         These results indicate that voluntary catch-and-release fishing as well as release due  
400 to catch limit regulation, are not a significant source of mortality based on current estimates  
401 of recreational release rates of SBT. It should be noted however that if either the harvest rate  
402 or the release rate were to increase the absolute tonnage lost through post-release mortality  
403 will also increase. Recent management regulations in New South Wales have reduced the  
404 bag limit from two to one fish per person, and a boat limit of four fish has recently been  
405 implemented in Tasmania, both of which may lead to increased release rates. As the  
406 population of SBT rebuilds it is also expected that effort and subsequent harvest from the  
407 recreational fishery will increase.

408         While the post-release mortality rate was relatively low, there was evidence of  
409 adverse physiological response to capture, and although this was not found to relate to  
410 survival in this study, maintaining or improving fish handling practices is fundamental to  
411 minimising the unintended impacts of recreational fishing on SBT. This, in turn, will improve  
412 animal welfare and stewardship by the recreational fishing sector. The results presented here,  
413 in concert with estimates of recreational harvest for which methods are currently being  
414 developed, will provide greater transparency of an unaccounted source of mortality.  
415 Subsequently these results will improve the completeness of data available for stock  
416 assessment to facilitate effective international management strategies aimed at continuing to  
417 rebuild the southern bluefin tuna population.

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- 570

571 TABLES

572 **Table 1.** The number of fish identified as not surviving after release (up to 10-days). Fish caught on J-  
 573 hooks (J) and circle hooks (C) are combined as the PRS rates were similar. Fish caught on treble  
 574 hooks (T) are reported separately as the PRS was much lower than for the other two hook categories.  
 575 Noting however, that the sample size of fish caught on treble hooks ( $n = 5$ ) was small, therefore this  
 576 result is indicative.

Day of mortality post-release	Moribund		Predation		Total died		Cumulative number died		PRS (%)	
	J & C	T	J & C	T	J & C	T	J & C	T	J & C	T
1	4	0	2	1	6	1	6	1	88.9	80.0
3	0	0	1	0	1	0	7	1	87.0	80.0
5	1	0	0	0	1	0	8	1	85.2	80.0
8	0	1	0	0	0	1	8	2	85.2	60.0
10	0	0	1	0	1	0	9	2	83.3	60.0

577

578 **Table 2.** Fate of the 59 fish fitted with PAT tags. Mortalities occurring within 10-days post-release  
 579 were attributed to the capture event ( $PRM_{CI}$ ), with the exception of a recapture. Mortalities occurring  
 580 10-days or more post-release were considered natural ( $PRM_N$ ).

Fate description	No. of fish ( $\leq 10$ days)	No. of fish ( $> 10$ days)
$PRM_{CI}$ - Early onset catch induced	4	-
$PRM_{CI}$ - Delayed onset catch induced	2	-
$PRM_{CI}$ - Catch induced post-release predation	3	-
$PRM_{CI}$ - Observed post-release predation	2	-
$PRM_N$ - Natural mortality	-	2
$PRM_N$ - Natural predation	-	2
$PRM_R$ - Recapture	1	-
PRS - Premature tag shedding	-	31
PRS - Full term	-	12

581

582

583 **Table 3.** The results of generalised additive modeling between the suite of biochemical indicators and  
 584 angling duration (*AD*), fork length (*FL*), sea surface temperature (*SST*) and an ordinal blood loss index  
 585 (*BL*). The full initial model was  $y \sim s_{AD}(AD) + s_{FL}(FL) + s_{SST}(SST) + BL$ . The model was reduced for each  
 586 response variable using a backwards stepwise process. The significant explanatory variables for each  
 587 reduced model are shown for each response variable.

588

Response variable ( <i>y</i> )	Explanatory variables	n	<i>GCV</i>	<i>F</i>	<i>P</i>	<i>R</i> <sup>2</sup> <i>adj</i>	<i>Deviance explained</i>	<i>Error family(link)</i>
Glucose (mmol/L)		262	1.09			0.05	6.5%	Gaussian (identity)
	<i>s(FL)</i>			4.68	<b>0.009</b>			
	<i>s(SST)</i>			2.69	0.048			
Lactate (mmol/L)		262	15.15			0.36	37.4%	Gaussian (identity)
	<i>s(AD)</i>			42.97	<b>&lt;0.001</b>			
	<i>s(FL)</i>			7.90	<b>&lt;0.001</b>			
	<i>s(SST)</i>			6.11	<b>0.014</b>			
Cortisol (ng/ml)		177	2237.70			0.58	58.9%	Gaussian (identity)
	<i>s(AD)</i>			50.0	<b>&lt;0.001</b>			
Osmolarity (Osm/L)		180	0.004			0.27	29.9%	Gamma(log)
	<i>s(AD)</i>			22.51	<b>&lt;0.001</b>			

589

590

591

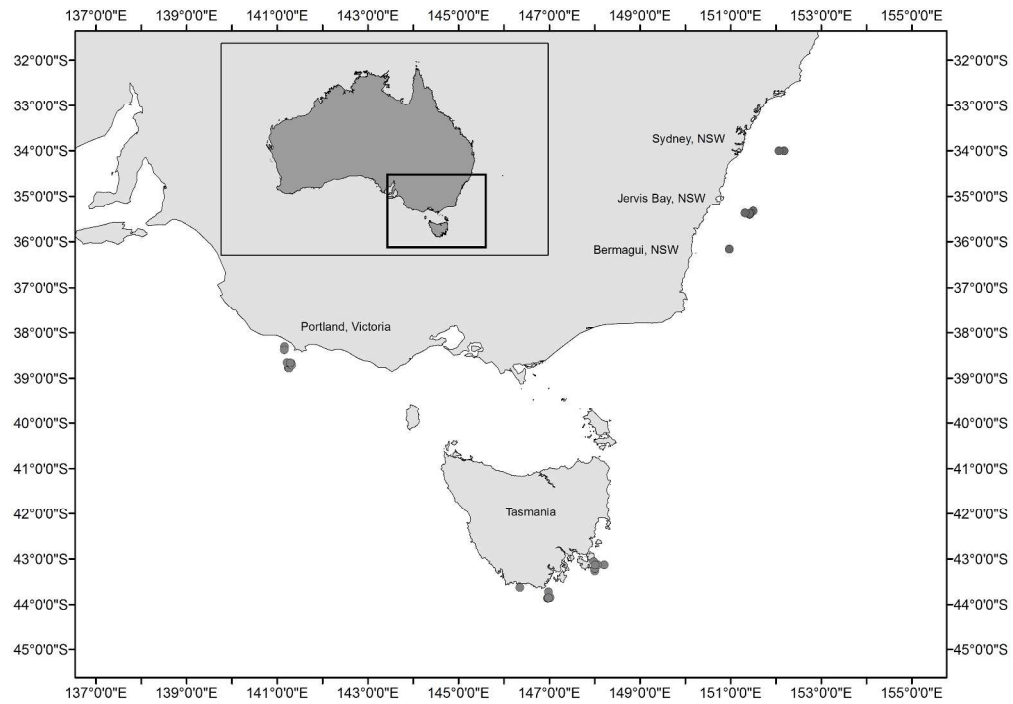


Fig. 1. Capture and release locations (grey circles) of satellite tagged southern bluefin tuna caught using recreational fishing methods around southeast Australia, including Tasmania.

297x209mm (300 x 300 DPI)

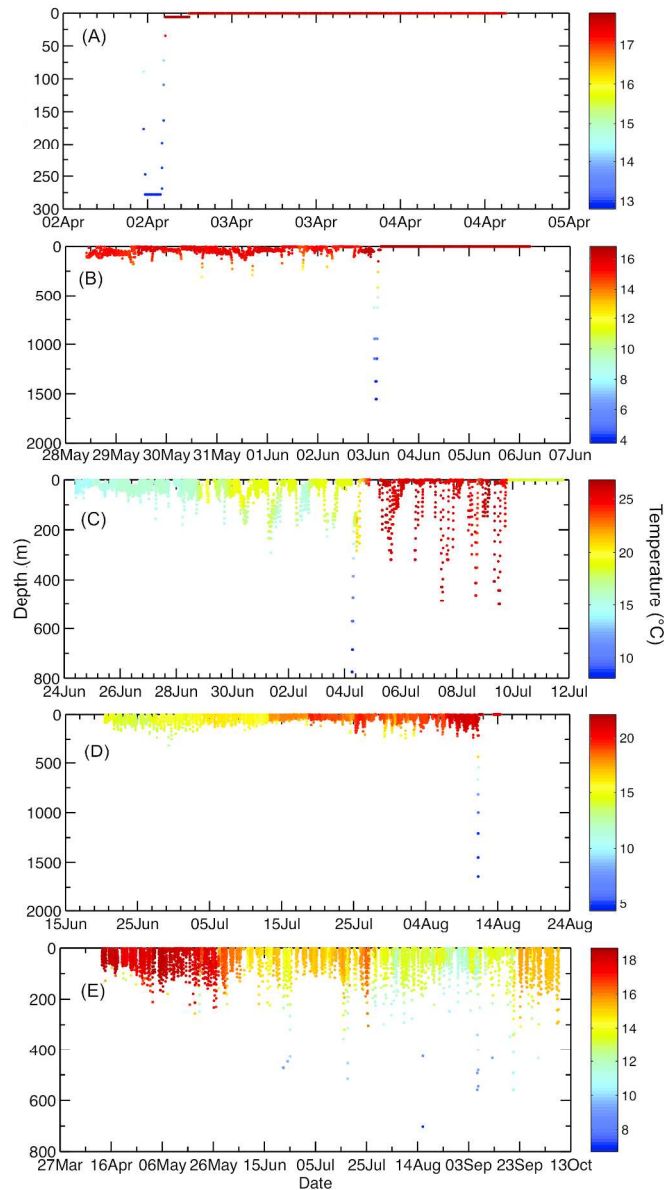


Fig. 2. PAT tag data illustrating different fates of fish post release. (A) Early onset catch induced post-release mortality, occurring within 24-hours after release. (B) Delayed onset catch induced post-release mortality, occurring within 10-days after release. (C) Catch induced post-release predation, indicated by rapid increase in temperature. (D) Natural mortality, occurring later than 10-days post-release. (E) Survival full term deployment (180 days).

154x276mm (300 x 300 DPI)



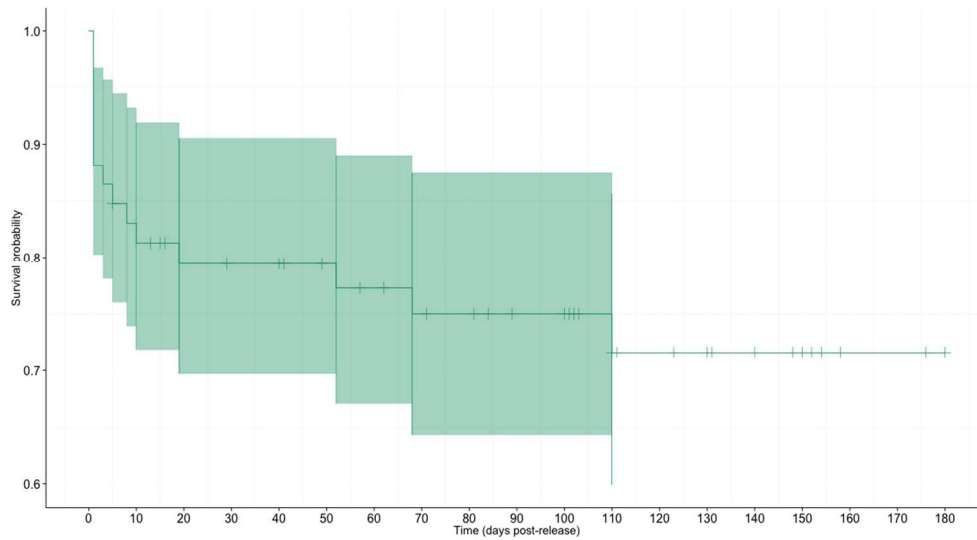


Fig. 3. The proportion of southern bluefin tuna surviving on each day post release as estimated using a Kaplan-Meier survival function ( $n = 59$ ). The shaded area indicates the 95% confidence intervals. The small vertical lines on the plot indicate premature tag shedding.

463x263mm (72 x 72 DPI)

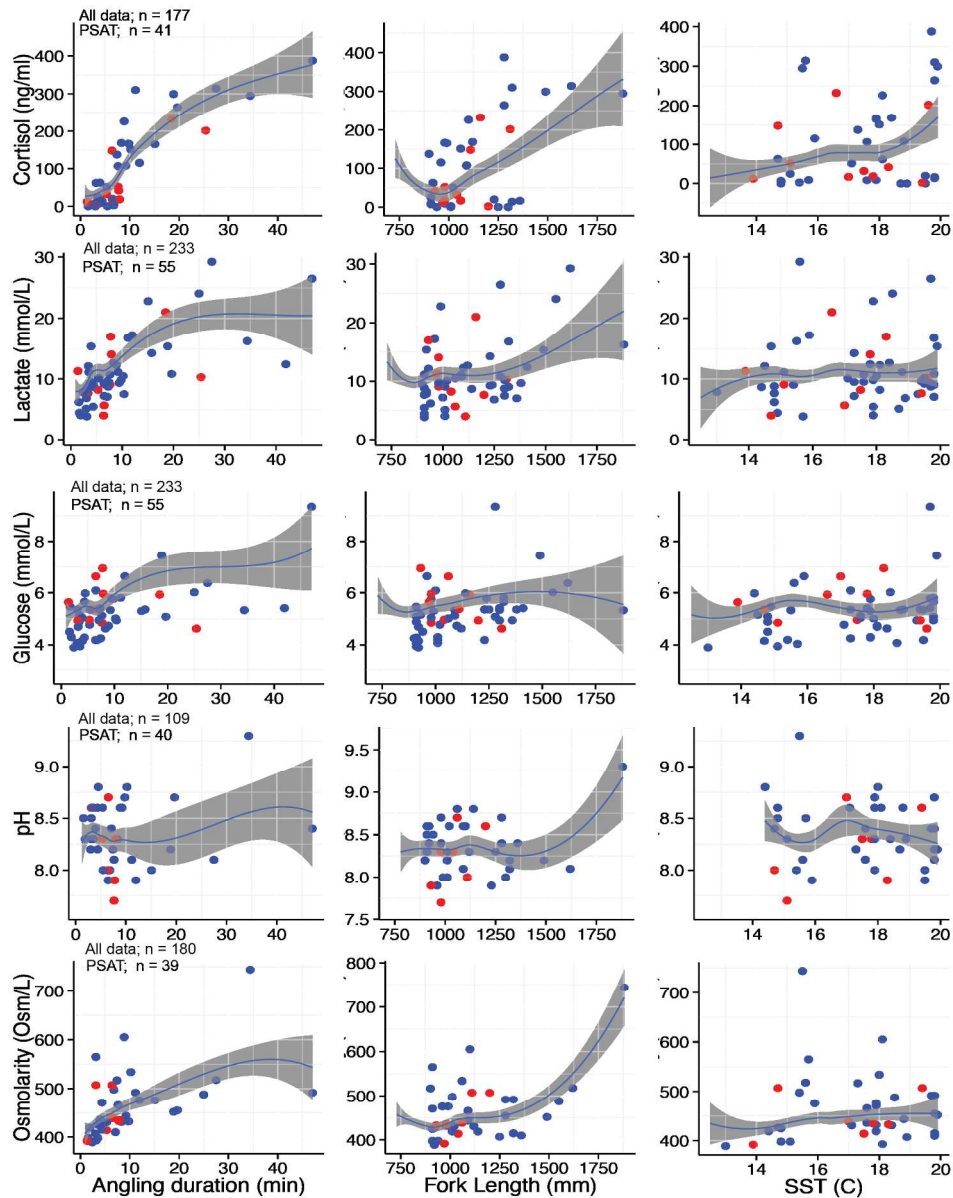


Fig. 4. Response of biochemical blood plasma variables to angling duration, fish length (FL) and SST. The fitted line is a LOESS smoother applied to all available data and the grey shading illustrates the 95% confidence intervals of the smoother fit. Blue points indicate southern bluefin tuna (SBT) that were PAT tagged and survived greater than 10-days after release, red points indicate SBT that were PAT tagged and did not survive to 10-days after release. Data points from SBT that were not tagged have been removed to aid the visualisation of biochemical values of the tagged fish against the expected fits to all data.

1 Table S1. Details of satellite tagged southern bluefin tuna. Deployment details include release date  
 2 and location, fish length and programmed detachment day. Pop-up transmission data includes the  
 3 date and location that the tag released from the fish as well as the number of days each tag was  
 4 attached.

Tag	Location	Releases					Pop-up transmission			
		Date	Latitude	Longitude	LCF (cm)	Programmed duration (d)	Date	Latitude	Longitude	Actual duration (d)
<b>2012</b>										
115746	TAS	16/05/2012	-43.22	148.01	91	40	3/06/2012	-44.38	149.27	18
115748	TAS	16/05/2012	-43.22	148.01	91	40	26/06/2012	-37.60	139.20	40
115743	TAS	11/06/2012	-43.63	146.36	93	40	23/07/2012	-38.43	139.62	40
115749	NSW	23/06/2012	-36.17	150.97	123	100	3/10/2012	-36.18	158.75	100
115750	NSW	23/06/2012	-36.17	150.97	100	100	2/10/2012	-41.81	149.10	100
<b>2013</b>										
121772	TAS	4/03/2013	-43.85	147.00	109	180	31/08/2013	-43.86	150.77	180
121774	TAS	4/03/2013	-43.86	147.01	106	180	8/04/2013	-44.44	148.91	35
121776	TAS	4/03/2013	-43.86	146.97	116	180	9/03/2013	-43.84	146.91	5
121777	TAS	4/03/2013	-43.85	147.01	123	180	31/08/2013	-36.07	151.96	180
121780	TAS	4/03/2013	-43.85	147.00	112	180	27/08/2013	-39.17	152.72	176
115742	VIC	10/04/2013	-38.78	141.25	109	180	9/05/2013	-39.27	142.42	29
115745	VIC	10/04/2013	-38.78	141.25	101	180	7/10/2013	-38.77	135.60	180
115751	VIC	10/04/2013	-38.78	141.25	110	180	16/09/2013	-41.49	149.45	159
121778	VIC	10/04/2013	-38.78	141.26	106	180	9/10/2013	-35.78	122.66	180
128677	VIC	11/04/2013	-38.66	141.22	91	180	4/07/2013	-39.85	143.10	84
121775	TAS	1/05/2013	-43.13	148.05	188	180	27/06/2013	-39.04	149.17	57
128666	TAS	1/05/2013	-43.13	148.05	102	180	29/10/2013	-42.43	150.47	180
121842	VIC	10/05/2013	-38.31	141.16	90	180	5/10/2013	-36.06	137.64	148
128691	VIC	10/05/2013	-38.42	141.31	93	180	15/05/2013	-38.68	142.96	5
128697	VIC	10/05/2013	-38.38	141.15	92	180	19/06/2013	-38.11	140.77	40
128689	TAS	18/05/2013	-43.12	148.07	184	180	21/05/2013	-43.19	148.11	3
128694	TAS	18/05/2013	-43.13	148.07	162	180	5/10/2013	-39.13	152.19	140
121779	TAS	19/05/2013	-43.26	148.01	111	180	1/06/2013	-43.87	148.04	13
115744	TAS	28/05/2013	-43.86	146.96	101	180	18/08/2013	-37.15	153.21	82
121773	TAS	28/05/2013	-43.86	146.98	98	180	6/06/2013	-40.52	150.76	9
121781	TAS	29/05/2013	-43.13	148.22	91	180	26/10/2013	-38.20	151.31	150
115747	TAS	20/06/2013	-43.83	147.01	91	180	15/08/2013	-31.23	154.76	56
128671	TAS	20/06/2013	-43.73	146.98	92	180	1/11/2013	-40.67	144.53	134
128674	TAS	20/06/2013	-43.86	146.98	94	180	21/08/2013	-41.15	150.41	62
128665	TAS	21/06/2013	-43.85	147.02	91	180	6/07/2013	-43.14	145.69	15
128664	NSW	28/06/2013	-35.34	151.50	149	180	24/10/2013	-38.23	154.23	118
128670	NSW	28/06/2013	-35.38	151.45	135	180	4/10/2013	-41.92	151.10	98

128667	NSW	29/06/2013	-35.39	151.42	131	180	29/07/2013	-35.02	151.13	30
128679	NSW	29/06/2013	-35.39	151.42	130	180	4/12/2013	-41.65	156.70	158
128680	NSW	29/06/2013	-35.39	151.42	136	180	20/10/2013	-43.78	153.52	113
128682	NSW	29/06/2013	-35.41	151.42	132	180	4/07/2013	-32.17	152.50	5
128692	NSW	29/06/2013	-35.39	151.42	128	180	13/11/2013	-43.01	149.10	137
128695	NSW	29/06/2013	-35.41	151.42	132	180	23/10/2013	-38.86	160.32	116
128698	NSW	29/06/2013	-35.39	151.42	128	180	17/10/2013	-43.99	145.84	110
128672	NSW	12/07/2013	-34.01	152.18	138	180	21/12/2013	-44.15	146.71	162
128675	NSW	12/07/2013	-34.01	152.18	130	180	9/12/2013	-46.66	166.64	150
128678	NSW	12/07/2013	-34.01	152.18	130	180	1/10/2013	-35.79	166.46	81
128681	NSW	12/07/2013	-34.01	152.18	124	180	13/11/2013	-42.10	148.34	124
128688	NSW	12/07/2013	-34.01	152.18	120	180	23/07/2013	-34.94	151.09	11
128699	NSW	12/07/2013	-34.01	152.18	125	180	8/11/2013	-39.37	166.63	119
<b>2014</b>										
128669	VIC	2/04/2014	-38.68	141.31	104	180	4/04/2014	-38.67	141.32	2
128676	VIC	2/04/2014	-38.68	141.31	98	180	14/05/2014	-40.05	143.32	42
128685	VIC	2/04/2014	-38.67	141.28	99	180	10/05/2014	-38.64	140.55	38
128686	VIC	2/04/2014	-38.67	141.28	98	180	4/04/2014	-38.62	141.48	2
128696	VIC	2/04/2014	-38.67	141.28	101	180	10/06/2014	-38.37	140.82	69
128684	VIC	3/04/2014	-38.72	141.32	98	180	30/09/2014	-36.98	124.13	180
128683	TAS	27/04/2014	-43.07	147.98	96	180	10/05/2014	-43.0	147.4	13
128668	TAS	8/05/2014	-43.86	146.97	114	180	12/08/2014	-39.4	143.2	96
12868301	TAS	24/06/2014	-43.13	148.01	97	70	4/07/2014	-40.3	150.0	10
128690	TAS	14/07/2014	-43.22	148.01	92	180	11/01/2015	-43.22	148.01	180
133520	NSW	23/07/2014	-34.00	152.07	155	180	27/11/2015	-42.80	152.16	130
128693	NSW	25/07/2014	-35.38	151.32	141	180	12/09/2015	-35.64	160.67	49
133519	NSW	25/07/2014	-35.38	151.32	120	180	05/11/2105	-42.99	154.46	103
133521	NSW	25/07/2014	-35.38	151.32	131	180	19/01/2015	-41.51	168.90	180

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