Extended exposure to elevated temperature affects escape response behaviour in coral reef fishes

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The threat of predation, and the prey's response, are important drivers of community dynamics. Yet, environmental temperature can have a significant effect on predation avoidance techniques such as fast-start performance observed in marine fishes. While it is known that temperature increases can influence performance and behaviour in the short-term, little is known about how species respond to extended exposure during development. We produced a startling response in two species of damselfish, the lemon damsel *Pomacentrus moluccensis,* and the Ambon damselfish *Pomacentrus amboinensis,* by the repeated use of a drop stimulus . We show that the length of thermal exposure of juveniles to elevated temperature significantly affects this escape responses. Short-term (4d) exposure to warmer temperature affected directionality and responsiveness for both species. After long-term (90d) exposure, only *P. moluccensis* showed beneficial plasticity, with directionality returning to control levels. Responsiveness also decreased in both species, possibly to compensate for higher temperatures. There was no effect of temperature or length of exposure on latency to react, maximum swimming speed, or escape distance suggesting that the physical ability to escape was maintained. Evidence suggests that elevated temperature may impact some fish species through its effect on the behavioural responses while under threat rather than having a direct influence on their physical ability to perform an effective escape response.

1   **EXTENDED EXPOSURE TO ELEVATED TEMPERATURE AFFECTS**

2   **ESCAPE RESPONSE BEHAVIOUR IN CORAL REEF FISHES**

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14 **Abstract**

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16 Yet, environmental temperature can have a significant effect on predation avoidance techniques

17 such as fast-start performance observed in marine fishes. While it is known that temperature

18 increases can influence performance and behaviour in the short-term, little is known about how

19 species respond to extended exposure during development. We produced a startling response in

20 two species of damselfish, the lemon damsel *Pomacentrus moluccensis,* and the Ambon

21 damselfish *Pomacentrus amboinensis,* by the repeated use of a drop stimulus. We show that the

22 length of thermal exposure of juveniles to elevated temperature significantly affects this escape

23 responses. Short-term (4d) exposure to warmer temperature affected directionality and

24 responsiveness for both species. After long-term (90d) exposure, only *P. moluccensis* showed

25 beneficial plasticity, with directionality returning to control levels. Responsiveness also decreased

26 in both species, possibly to compensate for higher temperatures. There was no effect of

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30 while under threat rather than having a direct influence on their physical ability to perform an

31 effective escape response.

32

33 **INTRODUCTION**

34   Predator-prey interactions are one of the key drivers structuring communities (Lima & Dill, 1990).

35   Predation pressure can alter prey morphology, regulate population size, and control biodiversity

36   (Sih et al., 1985). Similarly, antipredator responses of prey reduce the efficacy of predators and

37   enhance survival. Factors that influence predator attack success or prey escape performance may

38   alter the outcome of these interactions, potentially causing a cascade of trophic effects. Increased

39   temperature has already been shown to affect antipredator responses in terrestrial ectotherms (Cury

40   de Barros et al., 2010), freshwater fishes (Weetman, Atkinson & Chubb, 1998), and marine species

41   (Allan et al., 2015). Understanding how changes in environmental temperature can affect key

42   ecological processes can be useful when predicting the composition of future communities.

43   Rising environmental temperatures can substantially impact the behaviour and locomotor

44   performance components of escape responses in fish (Domenici, 2010). Previous studies have

45   shown that temperature can influence locomotor performance through physiological mechanisms

46   like muscle performance (Johnston, Fleming & Crockford, 1990) and enzyme activity (Johnson &

47   Bennett, 1995). Additionally, temperature can affect behavioural components of escape responses,

48   including responsiveness (Preuss & Faber, 2003) and directionality (Szabo et al., 2008). The effect

49   of environmental temperature will depend on both the level of thermal change compared to the

50   normal thermal conditions, and the length of time thermal change is experienced (Munday,

51   McCormick & Nilsson, 2012). With sufficient exposure time, plasticity can occur resulting in

52   changes to the phenotype, which potentially reduce the negative impacts of rising temperature

53   (Angilletta, 2009). Exposure during the early stages of life is believed to unlock greater plasticity

54   in traits (West-Eberhard, 2003), and this has been found to be true for coral reef fishes (Donelson

55   et al., 2011). While many studies have investigated the effects of short-term exposure to elevated

56   temperatures in adult fishes, to date few studies have examined how juvenile fishes respond to

57   long-term exposure to near-future temperature increases.

58   The interaction between a predator and its prey is an intricate sequence that, at its end,

59   leads to a predator striking at a prey and the prey being captured or escaping (Lima & Dill, 1990).

60   Many amphibians and fishes undertake a C-start escape response to a startling stimulus (Bullock,

61   1984) involving a sudden acceleration away from the direction of the perceived threat (Domenici

62   & Blake, 1997). C-start responses are controlled by two large neurons called Mauthner cells, one

63   for each lateral half of the body (Eaton, Bombardieri & Meyer, 1977). When a stimulus is detected,

64   the corresponding Mauthner cell triggers the escape response resulting in a rapid contraction of

65   white muscle fibres. At first, the fish bends into a C-shape (stage 1), then contralateral contraction

66   releases this tension which propels the fish to flee the stimulus in the opposite direction (stage 2)

67   (Eaton, Bombardieri & Meyer, 1977). The initiation of C-starts are neurally controlled with

68   maximum performance believed to be limited by muscle power output (Wakeling & Johnston,

69   1998). However, fish rarely perform at their maximal limits (Webb, 1986) and modulation of

70   performance during an escape response has been shown to occur in different behavioural contexts,

71   showing there is potential for optimisation (Korn & Faber, 2005; Domenici, 2010; Ramasamy,

72   Allan & McCormick, 2015). This supports the economic model that individuals balance the

73   energetic cost of escaping with the threat of predation (Ydenberc & Dill, 1986). How long-term

74   exposure to elevated temperature can alter behavioural components of an escape response is still

75   unknown.

76   The present study examines whether short-term or long-term exposure to elevated

77   temperature regimes affect the fast-start escape response in two tropical damselfishes. Using a

78   repeatable drop stimulus (i.e. a stimulus that was consistently replicated), we compared escape

79   responses of juveniles from current-day controls (29 °C) to individuals exposed to 50 and 100 year

80   projections (30 and 31 °C; Collins et al. 2013) for either 4d or 90d. Specifically, we tested: (1) the

81   effects of short-term exposure to elevated temperature as an indication of thermal sensitivity and

82   (2) how short-term exposure differed to long-term exposure at elevated temperature as an

83   indication of plasticity. The inclusion of two species was to explore the species-specific effect that

84   temperature had on escape responses.

85   **METHODS**

86   *Ethics statement and collecting permits*

87   All work reported herein was conducted under permits from James Cook University Animal Ethics

88   Committee (A2079), the Great Barrier Reef Marine Park Authority (G10/33239.1), and

89   Queensland Fisheries (170251).

90   *Study species, collection, and holding facilities*

91   To examine how short-term and long-term exposure to elevated temperatures influences escape

92   performance, two reef fish species were used (the Lemon damselfish, *Pomacentrus moluccensis*,

93   and the Ambon damselfish, *Pomacentrus amboinensis*). These species have been used in past

94   studies on escape performance (Allan et al., 2013) and behaviour (McCormick & Weaver, 2012)

95   making them familiar study species. These species co-occur across the Indo-Pacific from the

96   southern Great Barrier Reef up to Indonesia and Japan (Allen, 1991). Like many reef fish, these

97   species undergo embryonic development during a pelagic larval phase. After 3-4 weeks, larvae

98   make their final metamorphosis and recruit back to the reef to join the adult population. Both

99   species settle to the reef at similar ages, though standard length of *P. amboinensis*, tends to be

100   slightly larger (McCormick & Weaver, 2012). Here, thermal plasticity is ecologically important

101   as individuals often settle to reefs with thermal regimes different to what they experienced as

102   larvae. Recently settled recruits (aged 1-2 months) were collected from the Cairns region (-

103   16°78´S, 146°26´E) of the Great Barrier Reef, Australia in January 2014. At this latitude, these

104   species do not mature for at least one year, making our individuals immature juveniles. Therefore,

105   gender was not observed as it is not relevant at this age. Once mature, individuals will sex-change

106   depending on social structure (Fishelson, 1998).

107   Fish were transported back to a closed system holding facilities at the Marine and

108   Aquaculture Research Facilities Unit (MARFU), James Cook University, Townsville. Fish were

109   housed in densities of three of varying sizes (e.g. one big, medium, and small) in 40 litre replicate

110   tanks. This sizing order helped formed a natural hierarchy within tanks and reduced within tank

111   fighting. Tanks were randomly allocated into one of three temperatures: 29.0 °C (current-day

112   summer average for the collection region; control; AIMS 2014), 30.0 °C, or 31.0 °C (projected 50

113   and 100 year future temperatures; Collins et al. 2013). Holding tank temperatures were computer

114   monitored via Innotech Genesis II controller V5 temperature system capable of controlling random

115   fluctuation less than 0.1 °C for the system. The system was continuously supervised by the in-

116   house staff at the facility and automatically notified of any irregularities. To avoid thermal stress,

117   the temperature was raised 1 °C per day (Gardiner, Munday & Nilsson, 2010; Nilsson, Östlund-

118   Nilsson & Munday, 2010). Elevated temperatures were split into two exposure lengths: 4d or 90d.

119   A 90d period was chosen for our long-term exposure treatment based on previous developmental

120   studies on this species (Grenchik, Donelson & Munday, 2013) and a closely related species of

121   damselfish (Donelson et al., 2011). A 4d period was chosen for our short-term exposure treatment

122   to observe the effects of increased temperature without causing a thermal stress response

123   (Gardiner, Munday & Nilsson, 2010). No thermal benefit in plasticity has been shown to occur in

124   *P. moluccensis* for up to 22 days of exposure to similar elevated temperatures supporting our 4d

125   treatment would not be confounded by reversible plasticity (Nilsson, Östlund-Nilsson & Munday,

126   2010). Temperature variation and photoperiod followed natural diurnal cycle for the collection

127   region, ± 0.6 °C around the mean and 12:12h respectively (AIMS, 2014). Food quantity was not

128   controlled for and all fish were given a high performance commercial fish pellet (NRD 0.5-0.8

129   mm; Primo aquaculture, QLD, Australia) once or twice daily to satiation. Excess food was

130   removed and tanks were cleaned via a vacuum siphon weekly or as needed.

131   *Experimental design*

132   The escape performance of fishes was quantified using a repeatable drop stimulus (i.e. a stimulus

133   with mechanical release that could be replicated consistently). Thermal sensitivity to elevated

134   temperature (aim 1) was measured by exposing individuals to either 30 or 31 °C for a 4d period.

135   To explore the extent of thermal plasticity (aim 2), separate treatments were held at either 30 or 31

136   °C for 90d. The control treatment remained at 29 °C for the entirety of the experiment. In total,

137   there were 10 treatments: 2 short-term exposures, 2 long-term, 1 control, and repeated for each

138   species. Holding tank temperatures were raised 1 °C/day to reduce the effect of heat shock

139   (Gardiner, Munday & Nilsson, 2010). To control for any effect of time spent in captivity, the 4d

140   treatments were first held at control temperature, then exposed to their randomly assigned

141   temperature 4 days before the end of the long-term duration. This allowed all fish to finish

142   temperature treatments at the same time.

143   *Experimental procedure*

144   After the exposure period, individuals were measured (standard length, $\bar{x} \pm$ SD; *P. amboinensis* n

145     $= 58, 30.57 \pm 5.79$ mm, *P. moluccensis* n $= 83, 28.08 \pm 3.59$ mm) and tested for escape performance

146 the following day. Treatments of *P. amboinensis* were significantly larger than *P. moluccensis* ($F_{1,131}$ = 9.52, p = 0.002), however there were no differences across treatments for each species.

148 Escape performance was tested in a circular arena (30 cm diameter) filled to a depth of 10 cm to

149 reduce movement in the vertical plane and illuminated with fluorescent lighting strips. Water

150 temperature of the arena reflected the treatment temperature that fish were held in. Water

151 temperature was monitored with a C20 Comark thermometer (Comark corp., Norfolk, UK).

152 Temperature was maintained with a glass bar heater (Aqua One, 300W) and air stone for aeration.

153 Complete water exchanges were conducted every 10 minutes or after 3 fish. The walls and top had

154 opaque covers to prevent outside disturbance. To elicit an escape response, a startling stimulus

155 consisting of a weight with a tapered end was released through a tube suspended above the centre

156 of the arena. The suspended tube stopped ~1 cm above the water's surface concealing the stimulus

157 weight as it fell thus creating a more sudden disturbance. A monofilament line was used to raise

158 the weight and was long enough that the end touched just the water's surface when released,

159 preventing collision with the individual. To start a trial, fish were placed into a central habituation

160 ring (10 cm diameter) via a water filled sample jar and given a one-minute habituation period.

161 Following the habituation period, the central ring was gently raised and the stimulus weight was

162 released, striking the water in an attempt to elicit an escape response. If a C-start was performed,

163 no further attempts were conducted and the fish was returned to its holding tank. If no reaction

164 was observed, fish were replaced into the habituation ring for another minute and the second

165 attempt was conducted. Fish were given three attempts to perform a C-start before being recorded

166 as "no reaction". The escape response was recorded using a high-speed camera (Casio Exilim Ex-

167 F1; 600fps) directed at a 45° angled mirror placed beneath the testing tank.

168 *Response variables*

169   Response variables were measured using the fish's centre of mass (~35% SL from the snout; Webb

170   1976) and tracked using ImageJ software (v1.48) with the manual tracking plugin. We chose our

171   variables as they have been used in previous study as a good indicator of escape ability (Walker et

172   al., 2005). Only stages 1 and 2 of the C-start, described as directional changes in the anterior part

173   of the body (Domenici & Blake, 1997) were quantified.

174   *Non-locomotor*

175   a) Responsiveness: proportions of fish that responded with a "C-start" or "no C-start". No C-

176   start was subdivided into "avoid" to the stimulus by swimming away but not performing a

177   desired C-start and "no reaction" to the stimulus.

178   b) Directionality: left and right side were split anteroposteriorly from directly above the fish.

179   Directionality was determined by whether the head turned "away" or "toward" the side the

180   stimulus occurred during stage 1 of the response.

181   c) Response latency (ms): time between the stimulus onset and first movement of the individual.

182   *Locomotor*

183   d) Escape distance (mm): distance travelled during the response to the end of stage 2.

184   e) Maximum swim speed (body lengths s^{-1}): maximum velocity achieved at any time during the

185   escape response.

186   *Data analysis*

187   Statistical analyses were performed using IBM SPSS statistics (v23.0.0.2). Proximity of fish to the

188   stimulus at the onset and body size can affect escape responses (Webb, 1976). The central

189   habituation ring helped reduce differences in starting proximity to stimulus and the remaining

190   distances of fish to the stimulus did not differ among treatments (one-way ANOVA $F_{4,110} = 0.959$,

191 $P = 0.433$). Maximum swim speed (m s^{-1}) was converted to body length s^{-1} to control for any effect

192 of size. Control temperature did not consist of two exposure combinations like the elevated

193 temperatures, therefore a full-factorial 3x2 design was not possible. Instead, the five treatments

194 (control, 2 short-term, and 2 long-term) were categorised into one "temperature treatment" factor.

195 A binomial logistic regression was used to ascertain the effects of temperature treatment on the

196 likelihood of an individual to elicit a C-start after the stimulus and the likelihood of that individual

197 to turn away from the stimulus. Response latency, maximum swim speed, and escape distance

198 were used as dependent variables analysed separately with one-way ANOVAs ($\alpha = 0.05$) and

199 temperature treatment as the fixed factor. These analyses were repeated for both species (replicates

200 ranged from 7-12 for *P. amboinensis* and 9-19 for *P. moluccensis*). Preliminary analysis of

201 response latency, maximum swim speed, and response distance used a linear mixed effects model

202 with holding tank as a random factor to test for a tank effect. However, AIC values were lower for

203 models not including random tank factor with ΔAIC < 2 to models that did include random tank

204 factor. Consequently, this term was dropped from the final model. We predicted that short-term

205 exposure to elevated temperature would have a negative impact on escape responses. If beneficial

206 plasticity was possible with long-term exposure, individuals exposed for 90d would exhibit

207 improvement in performance that either approached or returned to present-day control levels.

208 **RESULTS**

209 There was no significance in the logistic regression model for responsiveness in *P. amboinensis*

210 ($\chi^2 (4) = 4.18$, $p = 0.38$; Fig 1*a*). At control, 84.62% of individuals responded with a C-start, 7.69%

211 showed no reaction, and 7.69% displayed avoidance behaviour. Exposure of 4 days to either 30 or

212 31 °C had very little effect on responsiveness. However, for individuals that did not display a C-

213 start, all showed no reaction to the stimulus and none displayed avoidance behaviour. After 90

214   days, fish were 2.19 times less likely (71.43%) to perform a C-start at 30 °C and 3.14 times less

215   likely (63.64%) at 31 °C. Additionally, avoidance behaviour was returned (14.29%) and divided

216   non C-start individuals in half with those showing no reaction (14.29%). There was significance

217   in the regression model for directionality in *P. amboinensis* (χ^2 (4) = 11.23, *p* = 0.024; Fig 1*b*).

218   The model explained 28.70% (Nagelkerke R^2) of the variance in directionality of C-starts and

219   correctly classified 67.60% of cases. Compared to controls, fish exposed to 30 °C for 4 days were

220   5 times more likely (33.33%) to turn towards the stimulus and 6.67 times more likely (40%) after

221   90 days. At 31 °C, fish were 25 times more likely (71.43%) to turn towards the stimulus, which

222   was the same for both exposure durations. Temperature nor exposure duration had any significant

223   effect on response latency ($F_{4,42}$ = 1.32, *p* = 0.27, partial η^2 = 0.053; Fig 2*a*), maximum swim speed

224   ($F_{4,41}$ = 0.6, *p* = 0.66 partial η^2 = 0.056; Fig 2*b*), or response distance ($F_{4,42}$ = 0.65, *p* = 0.62, partial

225   η^2 = 0.058; Fig 2*c*) for *P. amboinensis*.

226   For *P. moluccensis*, logistic regression model for responsiveness was statistically significant (χ^2

227   (4) = 16.58, *p* = 0.002; Fig 1*a*). The model explained 28% (Nagelkerke R^2) of the variance in

228   proportion of individuals that elicited a C-start and correctly classified 80% of cases. At control,

229   82.61% of fish displayed a C-start. Of the fish that did not C-start, most showed avoidance

230   behaviour (13.04%) and no reaction (4.35%) least of all. Responsiveness rose to absolute (i.e.

231   100% of individuals performed a C-start) after 4 days of exposure to either 30 or 31 °C. When 90

232   day exposure was compared to controls, individuals were 1.45 times less likely (76.47%) to

233   perform a C-start at 30 °C and 3.55 times less likely (57.14%) at 31 °C. For individuals that did

234   not display a C-start, there was an increase in no reaction up to 38% at 31 °C (from 4.35% at

235   control). There was no significance in the regression model for directionality (χ^2 (4) = 5.98, *p* =

236   0.20; Fig 1*b*). Fish with 4 days of exposure to 30 °C were 2.8 times more likely (50%) to turn

237 towards the stimulus compared to controls and 4.2 time more likely (60%) at 31 °C. However, this

238 was reduced after prolonged exposure where directionality was very similar to control (23.08%

239 and 25% for 30 and 31 °C, respectively). There was no significant effect of temperature and

240 exposure duration on response latency ($F_{4,60} = 1.23$, $p = 0.30$, partial $\eta^2 = 0.052$; Fig 2*a*), maximum

241 swim speed ($F_{4,60} = 0.19$, $p = 0.94$, partial $\eta^2 = 0.013$; Fig 2*b*), nor response distance ($F_{4,60} = 0.85$,

242 $p = 0.49$, partial $\eta^2 = 0.054$; Fig 2*c*) for *P. moluccensis*.

243 **DISCUSSION**

244 Studying how escape responses are affected by elevated temperature can be important when

245 making predictions on the ability of prey to escape predators under climate change conditions

246 (Gilman et al., 2010). Supporting our predictions, we found short-term exposure to elevated

247 temperature negatively impacted directionality for both species. However, there was little effect

248 on responsiveness, response latency, maximum, swim speed, and escape distance. After long-term

249 exposure, directionality in *P. moluccensis* followed our predictions by reducing the number of

250 turns towards the stimulus and returning to control levels. For *P. moluccensis*, long-term exposure

251 also altered responsiveness with fish responding less often with a C-start response at elevated

252 temperatures. Such alterations in the C-start responsiveness to predators is likely to change prey

253 survival, while the differential effects of temperature on the two closely related species suggests

254 that future temperature elevation may affect community composition through differential survival.

255   The ability for prey to perceive and avoid a predatory threat is essential to survival.

256 Directionality in escape responses is important as turning towards a stimulus would increase

257 exposure time to a predator (Domenici, 2010). Short-term exposure to elevated water temperature

258 negatively impacted directionality by increasing number of turns towards the stimulus. Previous

259 study on goldfish also found directionality was impaired at increased temperatures compared to

260   controls (Szabo et al., 2008). Altered directionality may have been due to an impairment of

261   sensorimotor control (Foreman & Eaton, 1993). While not significant, short-term exposure to

262   elevated temperatures increased C-start responsiveness in *P. moluccensis* from 80% to 100%, and

263   reduced avoidance reactions to 0% for both species. Generally, proportion of C-starts has been

264   shown to increase with exposure to temperature both above (Szabo et al., 2008) and below control

265   treatments (Preuss & Faber, 2003), suggesting responsiveness may increase with thermal stress, at

266   either extreme. Hyperactivity under stress can be beneficial to survival, as a fish that does not react

267   during a predator attack will most likely get eaten. However, this strategy can risk expending

268   additional energy on a false or non-threatening stimulus.

269   Extended exposure to new environmental conditions can alter the physiological and

270   behavioural response of individuals (Angilletta, 2009). We found directionality in *P. moluccensis*

271   showed signs of beneficial plasticity after long-term exposure by returning to control levels. Long-

272   term exposure may have reduced the negative impact of sensorimotor control brought on by

273   thermal stress. Physiological plasticity to similar temperatures (+2 °C) in this species has also been

274   shown to occur for aerobic metabolism (Grenchik, Donelson & Munday, 2013) potentially

275   demonstrating that *P. moluccensis* could exhibit beneficial plasticity to future projected

276   temperatures in a range of traits. The proportion of C-starts reduced while avoidance behaviour

277   increased with long-term exposure in both species. Decreasing the proportion of C-starts deviates

278   further from control levels, therefore cannot be considered beneficial. However, future sea

279   temperature increases are expected to exceed the thermal optimum for many reef fish (Tewksbury,

280   Huey & Deutsch, 2008). forcing species to adopt energy saving strategies (Ydenberc & Dill, 1986).

281   Reducing the proportion of C-starts and replacing with avoidance behaviour may help compensate

282   for the costs of increasing thermal conditions.

283   While other studies have shown that elevated temperature affects the locomotor

284   performance in fast-starts (Webb & Zhang, 1994; Beddow, Leeuwen & Johnston, 1995), we found

285   projected increased sea temperatures had no effect on maximum swim speed or escape distances

286   in our species, regardless of exposure duration. Similarly, we found no effect on response latency

287   with temperature and exposure duration. The lack of change in performance at these temperatures

288   indicates once a C-start was initiated, the physical ability to escape is maintained. This suggests

289   future warming may have little effect on mortality rates. Although promising, this may be

290   problematic because metabolic demands for fish increase with rising ambient temperature

291   (Nilsson, Östlund-Nilsson & Munday, 2010; Grenchik, Donelson & Munday, 2013) and C-starts

292   are high energy manoeuvres (Jayne & Lauder, 1993). If an individual's energy budget is limited,

293   then more resources must be allocated to the rising cost of metabolic demands and potentially

294   leave less available for other activities such as escape behaviour (Careau et al., 2008). Thus, the

295   behavioural modulation of reducing C-start responsiveness after long-term exposure observed,

296   may act as a means of balancing the increasing cost of C-starts at higher temperature.

297   To conclude, both species showed negative effects of increased temperature after short-

298   term, though the impact was greater for *P. amboinensis*. After long-term exposure, *P. moluccensis*

299   adjusted to temperature with beneficial plasticity and energy saving strategies. These results show

300   the C-start escape response possesses thermal plasticity and extended exposure can induce

301   beneficial changes. The difference in response by species matches a previous study showing *P.*

302   *moluccensis* possessed a higher capacity for thermal plasticity in aggressive interactions (Warren

303   et al., 2016). Changes in escape behaviour will ultimately affect mortality rates in prey and the

304   species-specific response suggests some will better cope with future temperatures. This study is

305   important as it illustrates the specific contribution that elevated temperature will have on individual

306 performance. However, comprehensive climate change scenarios will also include responses to

307 changes in ocean chemistry (CO_2 levels and pH). Furthermore, precise measurement of escape

308 success will require future study with only the prey, only the predator, and both exposed to elevated

309 temperature as predators will also likely have specific responses to thermal conditions. Future

310 studies may also consider a longitudinal approach with several populations from differing sites to

311 consider adaptation to local thermal environments. Studies investigating the factors that influence

312 predator-prey interactions, like this one, are crucial because these interactions determine

313 population sizes and replenishment, leading to changes in community structure.

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Figure 1

Responsiveness and directionality to the startling stimulus.

C-start responsiveness (*a*) of fish to a drop stimulus for juvenile *Pomacentrus amboinensis* (left) and *Pomacentrus moluccensis* (right) at control, 4d or 90d exposure durations to elevated temperatures. Types of reaction are: no reaction (grey), avoidance (open), and C-start (solid). Directionality (*b*) categorises only C-start individuals by whether first movement of head was towards (solid) or away (open) from the stimulus.

P. amboinensis

P. moluccensis

Control 4d 90d Control 4d 90d

A | No reaction | Avoid | C-start

Percent of reactions

C

B | Away | Towards

Percent of turns

D

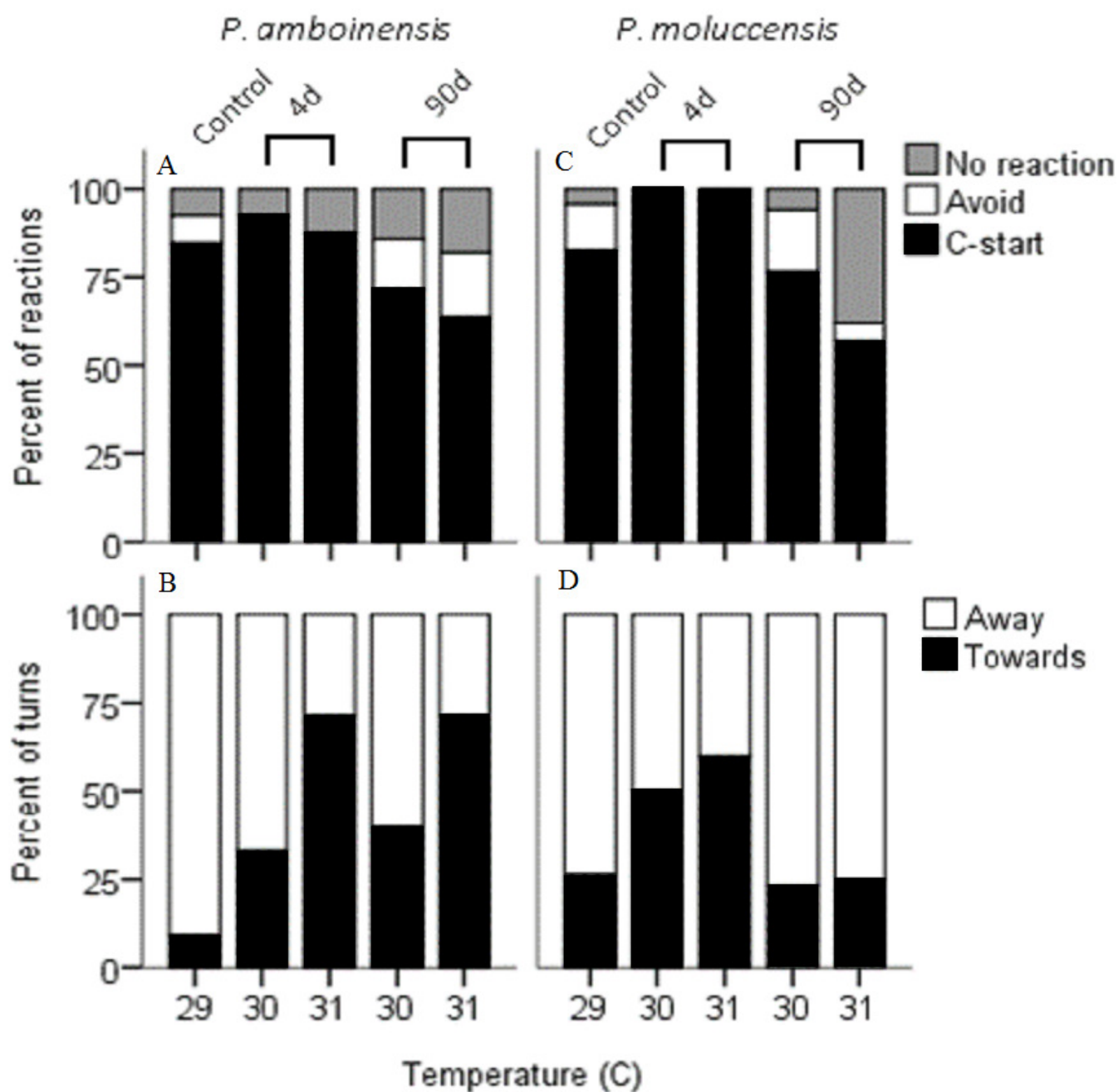
29 30 31 30 31 29 30 31 30 31

Temperature (C)

Figure 2

Measures of kinematic variables to the drop stimulus.

Kinematic performance of fast starts by juvenile *P. amboinensis* (left) and *P. moluccensis* (right) at control (solid), 4d (open) or 90d (grey) exposure durations to elevated temperatures. Variables measured were response latency (*a*), max speed (body lengths s^{-1}; *b*), and escape distance (*c*).