

**Coping with climatic extremes: dietary fat content decreased the thermal resilience of barramundi (*Lates calcarifer*)**

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## 27    **Abstract**

28    Aquatic organisms, including important cultured species, are forced to contend with acute changes  
29    in water temperature as the frequency and intensity of extreme weather events worsen. Acute  
30    temperature spikes are likely to threaten aquaculture species, but dietary intervention may play an  
31    important protective role. Increasing the concentration of macronutrients, for example dietary fat  
32    content, may improve the thermal resilience of aquaculture species, however, this remains  
33    unexplored. To evaluate this hypothesis, we used two commercially available diets (20% versus  
34    10% crude fat) to examine if dietary fat content improves the growth performance of juvenile  
35    barramundi (*Lates calcarifer*) while increasing their resilience to acute thermal stress. Fish were fed  
36    their assigned diets for 28-days before assessing the upper thermal tolerance ( $CT_{MAX}$ ) and the  
37    thermal sensitivity of swimming performance ( $U_{CRIT}$ ) and metabolism. We found that feeding fish a  
38    high fat diet resulted in heavier fish, but did not affect the thermal sensitivity of swimming  
39    performance or metabolism over an 18°C temperature range (from 20 – 38°C). Thermal tolerance  
40    was compromised in fish fed the high fat diet by 0.48°C, showing significantly lower  $CT_{MAX}$ .  
41    Together, these results suggest that while a high fat diet increases juvenile *L. calcarifer* growth, it  
42    does not benefit physiological performance across a range of relevant water temperatures and may  
43    even reduce fish tolerance of extreme water temperatures. These data may have implications for  
44    aquaculture production in a warming world, where episodic extremes of temperature are likely to  
45    become more frequent.

46  
47    **Key words:** Temperature stress;  $CT_{max}$ ; swimming performance; oxygen consumption; Asian sea  
48    bass.

49

## 1.0 Introduction

Aquatic organisms are being forced to contend with acute (short-term) changes in water temperature as the frequency and intensity of extreme weather events worsen (IPCC, 2013; Thompson et al., 2013). Habitat temperatures are predicted to suffer daily increases in temperature of up to 10°C (Meehl and Tebaldi, 2004), with temperature spikes of this magnitude already frequently recorded (Ledger and Milner, 2015; Leigh et al., 2015). Ectotherms, including important cultured fish species, are particularly susceptible to acute temperature changes because temperature has an overarching influence on key physiological traits (Brett and Groves, 1979). Temperature increases up to a certain point can be beneficial or benign, however, extreme elevations in temperature beyond optimal limits can push species towards their upper thermal tolerance limit (or critical thermal limit, CT<sub>MAX</sub>) (Pörtner and Peck, 2010). Stressfully high temperatures can have adverse behavioural and physiological effects, marked by pronounced increases in metabolic and oxygen demands (Cross and Rawding, 2008; Steinhausen et al., 2008), haematological alterations (Gollock et al., 2006), and affects whole animal responses such as locomotor performance (Bennett, 1990) and survival in the most extreme cases (Kumar et al., 2011; Pörtner and Knust, 2007).

For fish to survive an acute temperature challenge, they must increase oxygen uptake along the oxygen transport cascade (e.g. increase blood oxygen carrying capacity, cardiac output) and hence, cardiorespiratory oxygen transport capacity is critical in determining resilience to acute temperature changes (Antilla et al., 2014). This inherent relationship between oxygen transport and temperature tolerance has been explored at length (Ern et al., 2015; Norin et al., 2014; Pörtner and Farrell, 2008; Pörtner and Knust, 2007) and suggests that thermal limitation is linked to an organism's capacity to deliver oxygen to tissues at elevated temperatures (i.e. oxygen and capacity-limited thermal tolerance hypothesis; OCLTT). Declines in aerobic capacity are hypothesised to cause consequent declines in fitness-related traits such as locomotion, growth and reproduction (Pörtner and Farrell, 2008; Pörtner and Knust, 2007). However, the generality of this concept is highly debated, especially among tropical species such as barramundi (*Lates calcarifer*) and eurythermal crustaceans (*Penaeus monodon* and *Astacus astacus*) whose upper thermal tolerance appear to be independent of oxygen delivery capacity (Ern et al., 2015; Norin et al., 2014). In fact, at high temperatures, performance is reduced (e.g. growth and locomotor performance; Edmunds et al., 2010; Katersky and Carter, 2007) despite aerobic scope being optimal up to the CT<sub>MAX</sub> suggesting that oxygen limitation may not play a universal role in defining upper thermal limits.

Acute temperature spikes are likely to threaten the productivity of wild fisheries, as well as aquaculture systems globally (Ficke et al., 2007). Given the negative effects that thermal stress can have on aquaculture production, current research aims to develop diets that maintain or enhance fish

84 growth whilst increasing resilience to high temperatures (e.g. Glencross and Rutherford, 2010;  
85 Kumar et al., 2011). The uses of high-energy diets (fats and carbohydrates) in intensive aquaculture  
86 have proven beneficial in increasing fish growth. For instance, increases in dietary fat level have  
87 improved growth related parameters (e.g. final body mass, daily growth rate) in a number of  
88 aquaculture species such as Atlantic salmon (*Salmo salar*) (Grisdale-Helland and Helland, 1997),  
89 European sea bass (*Dicentrarchus labrax*) (Boujard et al., 2004), and barramundi (*Lates calcarifer*)  
90 (Catacutan and Coloso, 1995; Glencross, 2008; Glencross and Bermudes, 2012). Further, high fat  
91 diets have been shown to have either no effect on or improve oxygen transport capacity  
92 (Hammenstig et al., 2014) and may therefore confer greater resilience to high temperatures.

93 A handful of studies have examined the role of dietary intervention as a method of  
94 improving thermal tolerance. Dietary manipulation with lecithin (Kumar et al., 2014), pyridoxine  
95 (Kumar et al., 2016; Teixeira et al., 2011), zinc (Kumar et al., 2017), tryptophan (Tejpal et al.,  
96 2014) and microbial levan (Gupta et al., 2010) proved to be potential nutritional components in  
97 enhancing fish tolerance of high temperatures. Contrarily, few studies have examined how  
98 nutritional macronutrients such as dietary fat, protein and carbohydrates influence thermal  
99 tolerance. Hoar et al. (1952; 1949) found that dietary fat type (e.g. pilchard oil, herring oil and lard)  
100 increased survival at high temperatures and was correlated with the degree of unsaturation of fats.  
101 Increasing the concentration of dietary fat may therefore improve thermal tolerance, however, this  
102 remains unexplored.

103 The present study aimed to assess whether dietary fat content influences the thermal  
104 tolerance ( $CT_{MAX}$ ) and thermal sensitivity of swimming performance and metabolism of juvenile  
105 barramundi (*Lates calcarifer*). We used two using two readily available commercial diets differing  
106 primarily in dietary fat content (10% versus 20% crude fat) to test for differences in thermal  
107 tolerance. Exercise (swimming) performance was chosen as an integrative measure of the  
108 physiological status of barramundi in response to acute thermal stress. We also measured  
109 haemoglobin concentration, haematocrit and relative ventricle size, as critical components of the  
110 oxygen transport cascade, along with routine and maximal rates of oxygen uptake ( $\dot{M}O_{2ROUTINE}$  and  
111  $\dot{M}O_{2MAX}$ , respectively) to estimate the metabolic costs of acute thermal stress of fish fed high and  
112 low fat diets. Barramundi were used because of their increasing importance in commercial  
113 aquaculture. Barramundi are a tropical eurythermal fish species, currently cultured over much of  
114 their thermal tolerance range (~22 – 35°C) but can experience large seasonal (18 – 36°C) and daily  
115 ( $\pm 10^\circ\text{C}$ ) thermal fluctuations under both wild (Collins et al., 2013; Newton et al., 2010) and captive  
116 conditions (Pusey et al., 2004; Schipp et al., 2007). Further, barramundi aquaculture has expanded  
117 globally to locations where temperature frequently approaches the species' upper thermal limit

118 (Bermudes et al., 2010; Katersky and Carter, 2005). The feeding of a high fat diet (20%) was  
119 hypothesised to improve growth performance and confer resilience to acute temperature stress by  
120 reducing the thermal sensitivity of swimming performance and metabolism, and improving thermal  
121 tolerance of juvenile barramundi.

## 122 **2.0 Materials and Methods**

### 123 *2.1. Experimental diets*

124 Fish were fed one of two commercial pelleted diets (2 mm pellets) sourced from Ridley  
125 Aqua-feeds (Narangba, Queensland, Australia). The two diets differed in fat content (crude fat %).  
126 A low fat diet (10%, Fry Start, Ridley Aqua-feeds) and a high fat diet (20%, Hatchery Start, Ridley  
127 Aqua-feeds) were used in this experiment. The proximate compositions of the two diets are  
128 displayed in Table 1.

### 129 *2.2. Animal maintenance and experimental design*

130 *Lates calcarifer* were sourced from a commercial hatchery (Kuranda Fish Farm; Kuranda,  
131 Queensland, Australia; hatchery water temperature ~ 28°C) and transported to The University of  
132 Queensland in oxygenated transport bags. Fish (n = 110) were randomly distributed between  
133 twenty-two 40 L glass tanks (60 × 25 × 30 cm; L × W × H) and allowed to habituate to laboratory  
134 conditions for two weeks prior to experimentation. Fish were maintained at 30°C using 600 W  
135 heaters (Schego, Offenbach, Germany) attached to a NEMA 4X digital temperature controller (±  
136 1°C; Aqua Logic, Inc., San Diego, USA). Water parameters (pH, ammonia, nitrite, nitrate) were  
137 monitored on alternate days using an API master test kit (Mars Fishcare North America, Inc.,  
138 Chalfont, USA). Fish were maintained under a constant 12: 12 h light: dark cycle. After the  
139 habituation period, tanks were assigned to one of two diet treatments (high fat or low fat diet, as  
140 above), replicated 11 times at the tank level. Fish were fed once daily (at around 9:00) to apparent  
141 satiety. Food was weighed prior to feeding and any uneaten food was siphoned out of each tank 30  
142 min after feeding and re-weighed to calculate the feed efficiency. Fish were fasted for between 40 –  
143 48 h before all experiments to prevent the metabolic effects of digestion on rates of oxygen  
144 consumption and performance. All experiments were conducted in compliance with The University  
145 of Queensland animal ethics requirements (permit no. SBS/038/15/RSF).

### 146 *2.3. Growth experiment*

147 The growth experiment lasted for a period of 28 days. A four week feeding trial was chosen  
148 as it has been shown to be sufficient time to change the body composition of barramundi fed high  
149 fat diets (Glencross and Rutherford, 2010). Initial individual body mass ( $B_M$ , g) and total length  
150 ( $L_T$ ; cm) of each fish were measured and a tank averages calculated. All fish were re-weighed and  
151 measured at the end of the 28-day feeding trial. Fish were checked daily and any dead fish were

152 removed and accounted for when calculating feed efficiency. All data from the growth experiment  
153 is presented in Table 2. Growth variables were calculated using equations (1) – (3):

154 
$$(1) \text{ BMG (\%)} = \frac{M_F - M_I}{M_I} \times 100$$

155 
$$(2) \text{ FER} = \frac{\text{BMG}}{\text{PA}}$$

163

156 where *BMG* is the body mass gain (%) and  $M_F$  and  $M_I$  are the final and initial masses (g) of the fish,  
157 respectively. *FER* is the feed efficiency ratio,  $P$  is the mass of the pellets recovered from each tank  
158 and  $A$  is a water absorption factor accounting for water absorption by the pellets. Absorption ( $A$ )  
159 was determined by placing 2 g of pellets in an empty tank (without fish) filled with aquarium water  
160 and measuring the mass of the pellets recovered after ten min ( $n = 10$  per diet; Goosen et al., 2011).  
161 The water absorption factor was calculated as  $A = (F_D)/(F_W)$ , where  $F_D$  is the dry mass of the feed  
162 and  $F_W$  is the wet mass of the feed.

164 
$$(3) K = 100 \times \left( \frac{B_M}{L_T^3} \right)$$

165 where  $K$  is Fulton's condition factor, and  $B_M$  and  $L_T$  are body mass and total length of the fish,  
166 respectively.

#### 167 2.4. Critical swimming speed

168 Critical swimming speed ( $U_{\text{CRIT}}$ ) was examined at five test temperatures (20, 25, 30, 35, and  
169 38°C) to generate a thermal performance curve. Swimming performance was tested in a 10 L, flow-  
170 controlled hydraulic flume (Loligo, Tjele, Denmark; swimming-chamber dimensions = 40 × 10 ×  
171 10 cm;  $L \times W \times H$ ). A flow meter (Hontzsch, Bonby, Denmark) was used to calibrate water  
172 velocity produced by the flume. Fish ( $n = 6$  per diet at each temperature) were individually placed  
173 in the flume filled with filtered water at 30°C. Fish were allowed a minimum of one hour to  
174 habituate to flume conditions. Water temperature was adjusted to test temperature using a TU4-  
175 Unistat heat circulator (Thermoline Scientific, NSW, Australia; temperature stability  $\pm 0.1^\circ\text{C}$ ) to  
176 heat and a Seachill TR10 chiller (Teco, Ravenna, Italy) to cool the water at a rate of  $4^\circ\text{C h}^{-1}$  as  
177 required. Swimming performance tests began at a water velocity of  $0.2 \text{ m s}^{-1}$  ( $1.5 - 2$  mean  $L_T$  of the  
178 fish) and progressively increased every five minutes at a rate of  $0.05 \text{ m s}^{-1}$  until the fish fatigued.  
179 Fatigue was defined as the fish resting against the back wall of the flume for  $\geq 3 \text{ s}$  (Brett, 1967).  
180 Once fatigued, fish were weighed and measured. Total swimming time and water velocity at fatigue  
181 were recorded to calculate  $U_{\text{CRIT}}$  using Brett's (1964) equation (4):

182 
$$(4) U_{\text{CRIT}} = U_F + \left( U_I \left( \frac{T_F}{T_I} \right) \right)$$

183 where  $U_F$  is the highest water velocity maintained for the entire five minute interval ( $\text{m s}^{-1}$ ),  $U_I$  is  
184 the water velocity increment ( $0.05 \text{ m s}^{-1}$ ),  $T_F$  is the time swum during the final increment (s) and  $T_I$   
185 is an entire velocity interval (300 s). Swimming performance data were expressed in terms of body  
186 lengths per second ( $\text{BL s}^{-1}$ ).

## 187 2.5. Oxygen Uptake

188 The thermal sensitivity of routine and maximal rates of oxygen uptake ( $\dot{\text{M}}\text{O}_{2\text{ROUTINE}}$  and  
189  $\dot{\text{M}}\text{O}_{2\text{MAX}}$ , respectively) were measured using closed system respirometry following published  
190 protocols (Cramp et al., 2014) at five test temperatures (20, 25, 30, 35 and  $38^\circ\text{C}$ ). Briefly, plastic  
191 respirometers were fitted with an oxygen-sensitive fluorescent sensor spot (PreSens, Regensburg,  
192 Germany) to allow the determination of oxygen partial pressure of the water non-invasively by  
193 measuring the fluorescence of the sensor spot through the plastic wall of the respirometer.  
194 Fluorescence was captured and recorded using a fibre-optic cable connected to a Fibox 3 reader  
195 (Presens). For  $\dot{\text{M}}\text{O}_{2\text{ROUTINE}}$ , fish were netted from their holding tanks and transferred to  
196 respirometers without delay. Fish ( $n = 6$  per diet) were placed into 750 or 1600 ml plastic  
197 respirometers (depending on fish size and test temperature) filled with air-saturated water.  
198 Respirometers were placed in a water bath ( $64.5 \times 41.3 \times 39.7 \text{ cm}$ ;  $L \times W \times H$ ) and temperature  
199 was controlled ( $\pm 0.5^\circ\text{C}$ ) using a Seachill TR-10 aquarium chiller (TECO, USA). Temperature was  
200 adjusted at a rate of  $4^\circ\text{C h}^{-1}$  to reach the necessary test temperatures. Fish were allowed at least 1 h  
201 before  $\dot{\text{M}}\text{O}_{2\text{ROUTINE}}$  was measured, after which respirometers were sealed and the decline in oxygen  
202 was measured every 10 min for the following ~1-2 h. During the measurement period, oxygen  
203 levels did not drop below 70% saturation. The interval which resulted in the lowest  $\dot{\text{M}}\text{O}_2$  reading  
204 was taken as  $\dot{\text{M}}\text{O}_{2\text{ROUTINE}}$ . Although activity was not quantified, fish usually remained still during  
205 respirometry trials. Fish movements were limited (e.g. small fin movements) and likely represent  
206 'low routine'  $\dot{\text{M}}\text{O}_2$  (Chabot et al., 2016).  $\dot{\text{M}}\text{O}_{2\text{MAX}}$  ( $n = 6$ ) was assessed following  $U_{\text{CRIT}}$   
207 measurements by transferring the fatigued fish from the swimming flume into a respirometer filled  
208 with air-saturated water. Fish were transferred from the flume to the respirometer within 30 s of  
209 fatigue. Due to logistical constraints, the swim tunnel was not used as a respirometer. Air saturation  
210 inside the respirometer was then measured every minute for 15 min, and the greatest decline in  
211 oxygen saturation was taken as  $\dot{\text{M}}\text{O}_{2\text{MAX}}$ . Control respirometers (without fish) were used  
212 concurrently to determine background  $\dot{\text{M}}\text{O}_2$ . The rate of oxygen consumption ( $\dot{\text{M}}\text{O}_2$ ,  $\text{mg O}_2 \text{ h}^{-1}$ ) was  
213 determined using equation 5 below:

214 
$$(5) \dot{\text{M}}\text{O}_2 = \Delta\text{O}_2 / \Delta t \times V$$

215 where  $\Delta O_2$  is the rate of change of oxygen saturation of a respirometer containing a fish,  $\Delta t$  is the  
216 change in time over which the  $\Delta O_2$  was measured, and  $V$  is the volume of the respirometer minus  
217 the volume of the fish (assuming 1 g displaces 1 ml of water).

## 218 2.6. Upper Thermal Tolerance

219 Upper thermal tolerance was assessed at the end of the 28-day feeding trial using critical  
220 thermal methodology (Becker and Genoway, 1979). Critical thermal maximum ( $CT_{MAX}$ ) were  
221 conducted in a WiseCircu WCR-P22 refrigerated bath circulator (Witeg, Germany; bath capacity=  
222 22 L; effective space= 350 × 250 × 150 mm; L × W × H) filled with filtered water at 30°C, and  
223 continuous aeration was provided during  $CT_{MAX}$  determinations. Fish ( $n = 10$  per diet) were  
224 randomly selected and individually placed into the water bath. Water temperature was increased at a  
225 rate of 0.3° C min<sup>-1</sup> until loss of equilibrium (LOE) was reached, defined as the failure to maintain  
226 dorsal-ventral orientation for greater than 10 s (Becker and Genoway, 1979). Once LOE was  
227 reached, fish were transferred to their holding tanks and monitored for the next 24 h. No mortality  
228 was recorded following  $CT_{MAX}$  trials.

## 229 2.7. Haematological analysis and ventricular mass

230 Fish ( $n = 10$  per diet) were euthanised with an overdose of an aquatic anaesthetic (250 mg L<sup>-1</sup>  
231 <sup>1</sup>; Aqui-S TM, Aqui-S Pty LTD, Lower Hutt, New Zealand) for 5 – 10 minutes. Once opercular  
232 ventilations ceased, a scalpel was used to sever the caudal peduncle. Blood was collected directly  
233 into two heparinised haematocrit tubes. After blood had been collected, the ventricle was dissected  
234 from fish and individually weighed to obtain wet ventricular mass (g) and expressed as a relative  
235 measure in terms of per cent body mass. Haematocrit ( $H_{CT}$ ) was measured by centrifuging (micro-  
236 haematocrit centrifuge; Hawksley, Sussex, UK) the blood in one of the haematocrit tubes for 2 min  
237 at 5000 g.  $H_{CT}$  was calculated as the proportion of red blood cells in whole blood. Blood from the  
238 remaining haematocrit tube was transferred to a 1.5 mL Eppendorf tube and placed on ice for  
239 haemoglobin concentration ( $[H_B]$ ) analysis.  $[H_B]$  was determined spectrophotometrically at 405 nm  
240 and quantified against a standard curve of known  $[H_B]$  using a Sigma-Aldrich haemoglobin assay  
241 kit (MAK115, St Louis, MO, USA).

## 242 2.8. Statistical analyses

243 Statistical analyses were carried out using RStudio (v0.99.491) statistical software. Linear  
244 mixed effects models were used to determine the effect of dietary fat level (two levels; 10% and  
245 20% fat) on the growth, FER, K,  $CT_{MAX}$ , as well as the thermal sensitivity of  $U_{CRIT}$  and  $\dot{M}O_2$ .  
246 Measurements of oxygen uptake were log transformed to meet the assumptions of normality and  
247 homoscedasticity. Body mass was included as a covariate in the oxygen uptake and  $CT_{MAX}$



analyses, and total length in the  $U_{\text{CRIT}}$  analysis. Test temperature (where appropriate) was included as a fixed effect and tank (22 levels) as a random effect. Minimal adequate model were determined using maximum likelihood (ML) simplification. The *lme* function in the *nlme* package (Pinheiro et al., 2015) were used for all analyses. *Post hoc* pairwise comparisons between test temperatures were performed using the *lsmeans* function of the *lsmeans* package (Russel, 2015). Thermal sensitivity coefficients ( $Q_{10}$ ) for  $U_{\text{CRIT}}$ ,  $\dot{\text{M}}\text{O}_{2\text{ROUTINE}}$ , and  $\dot{\text{M}}\text{O}_{2\text{MAX}}$  were calculated as  $Q_{10} = [(R_2) (R_1)^{-1}]^{(10) (T_2 - T_1)}$ , where  $R$  represents the rate at temperature ( $T$ ) 1 and 2. Statistical significance was accepted at  $P < 0.05$ , and data are presented as mean  $\pm$  standard error unless otherwise stated.

## 3.0 Results

### 3.1. Growth performance

Growth performance measures are presented in Table 2. A significant effect of diet was observed on the final body mass of the fish after the 28-day feeding trial. Fish fed the high fat diet (20%) had significantly higher final body mass ( $M_F$ ) and body mass gain (BMG) compared to fish fed the low fat (10%) diet ( $M_F$ ,  $F_{1, 19} = 8.80$ ,  $P = 0.007$ ; BMG,  $F_{1, 19} = 19.33$ ,  $P < 0.001$ ). Neither fish condition ( $K$ ,  $F_{1, 19} = 2.66$ ,  $P = 0.12$ ) nor feed efficiency (FER;  $F_{1, 19} = 0.41$ ,  $P = 0.32$ ) was affected by dietary fat level.

### 3.2. Critical swimming speed

The critical swimming performance ( $U_{\text{CRIT}}$ ) of juvenile *L. calcarifer* was unaffected by dietary treatment ( $F_{1, 19} = 0.35$ ,  $P = 0.56$ ). Swimming performance was affected by test temperature ( $F_{4, 35} = 22.03$ ,  $P < 0.001$ , Fig. 1), and was reduced significantly at 20 and 25°C in fish fed both diets. Further, a pairwise *post hoc* analysis showed that performance was not significantly different between 30 and 38°C in fish fed either diet. Fish fed the 20% fat diet treatment showed optimal swimming performance at 35°C ( $7.09 \pm 0.42 \text{ m s}^{-1}$ ), while fish fed the 10% fat diet showed optimal swimming performance at 38°C ( $7.42 \pm 1.12 \text{ m s}^{-1}$ ). Average thermal sensitivity quotients ( $Q_{10}$ ) showed that, for  $U_{\text{CRIT}}$ , thermal sensitivity tended to be greater at lower temperatures (20 – 30°C), and reached a plateau of thermal independence between 30 and 38°C (Table 3). Fish size ( $L_T$ ) was inversely related to  $U_{\text{CRIT}}$  ( $F_{1, 35} = 22.03$ ,  $P < 0.001$ ), with smaller fish on average having a higher relative swimming speed ( $\text{BL s}^{-1}$ ).

### 276 3.3. Oxygen uptake

277 Dietary fat level did not influence routine ( $\dot{M}O_{2ROUTINE}$ ;  $F_{1, 19} = 1.46$ ,  $P = 0.24$ ) or maximal  
278 ( $\dot{M}O_{2MAX}$ ;  $F_{1, 32} = 0.21$ ,  $P = 0.65$ ) rates of oxygen uptake. Both  $\dot{M}O_{2ROUTINE}$  ( $F_{4, 34} = 95.54$ ,  $P <$   
279  $0.0001$ ) and  $\dot{M}O_{2MAX}$  ( $F_{4, 32} = 72.63$ ,  $P < 0.0001$ ) were affected by test temperature, increasing  
280 exponentially with each temperature increment from 20 to 38°C (Fig. 2AB). Further,  $\dot{M}O_{2ROUTINE}$   
281 tended to be more thermally sensitive than  $\dot{M}O_{2MAX}$ , irrespective of dietary fat treatment (Table 3).

### 282 3.4. Upper thermal tolerance

283 The mean critical thermal maximum ( $CT_{MAX}$ ; Fig. 3A) for fish fed the 10% fat diet ( $CT_{MAX}$   
284  $= 42.24 \pm 0.06^{\circ}C$ ) was significantly higher ( $F_{1, 17} = 9.57$ ,  $P = 0.006$ ) than the mean  $CT_{MAX}$  of fish  
285 fed the 20% fat diet ( $41.76 \pm 0.08^{\circ}C$ ). There was no significant effect of body mass on  $CT_{MAX}$  and  
286 was therefore excluded from the analysis.

### 287 3.5. Haematology and ventricular mass

288 Dietary fat level (10 versus 20%) did not influence any of the blood variable measures,  
289 including haemoglobin concentration (Fig. 3B;  $F_{1, 17} = 0.16$ ,  $P = 0.69$ ), haematocrit (Fig. 3C;  $F_{1, 17} =$   
290  $0.26$ ,  $P = 0.61$ ), or the relative ventricular mass (Fig. 3D;  $F_{1, 17} = 1.44$ ,  $P = 0.24$ ) of fish.

## 291 4.0 Discussion

292 Acute temperature spikes are set to imperil aquaculture species if the intensity of extreme  
293 weather events worsen, but nutritional supplementation may play an important buffering role. Here  
294 we examined the potential for dietary fat to improve the fish tolerance to high temperatures. The  
295 feeding of a high fat diet (20%) improved fish growth performance, but did not influence the  
296 thermal sensitivity of swimming performance or metabolism. Moreover, contrary to our hypothesis,  
297 fish upper thermal tolerance ( $CT_{MAX}$ ) was reduced in fish fed the high fat diet indicating a potential  
298 trade-off between growth performance and thermal tolerance.

### 299 Growth performance

300 The present study shows that the growth-related parameters were improved in fish fed a  
301 high fat (20% crude fat) compared to a low fat (10% crude fat) diet. This result is consistent with  
302 several previous reports (Boujard et al., 2004; Glencross et al., 2014; Keramat et al., 2012; Koskela  
303 et al., 1998; Williams et al., 2003) and indicates that the feeding of high fat diets facilitates a higher  
304 growth rate in various fish species. The growth rate and feed efficiency ratios presented here agree  
305 with previous growth trials involving *L. calcarifer* (e.g. BMG: 300 – 500%; Catacutan and Coloso,  
306 1995; Katersky and Carter, 2007; Williams et al., 2003) (FER: 1.1 – 1.5; Katersky and Carter, 2005;  
307 Katersky and Carter, 2007; Williams et al., 2003) and suggest good growth and feed conversion.  
308 However neither FER nor condition factor (K) differed between dietary treatments. Together, the

309 data from the growth experiment suggests that the use of a high fat diet supports a higher growth  
310 rates and hence may be beneficial for aquacultural production.

### 311 *Swimming performance*

312 Contrary to our hypothesis, swimming performance was independent of dietary fat content  
313 in juvenile barramundi. Our results are consistent with a previous study (Hammenstig et al., 2014),  
314 which found no effect of dietary fat level (10 vs. 20%) on the swimming performance of Atlantic  
315 salmon (*Salmo salar*). It is likely that lipid composition, rather than cumulative dietary fat and lipid  
316 content, may play a role in fish swimming performance (McKenzie et al., 1998). For example, some  
317 lipids have been shown to improve (e.g. anchovy oil) while others reduce performance (e.g. poultry  
318 fat; Wagner et al., 2004). Dietary fat level also did not influence the thermal sensitivity of  
319 swimming performance. Optimal swimming performance was maintained across a wide range of  
320 test temperatures (30 – 38°C) in fish fed both diet treatments and indicates that juvenile barramundi  
321 are unlikely to be negatively impacted by acute thermal increases. A seemingly innate thermal  
322 insensitivity of particular traits may be characteristic of species exposed to high thermal fluctuations  
323 (Healy and Schulte, 2012; Huey and Hertz, 1984). For example, in the eurythermal killifish  
324 (*Fundulus heteroclitus*) who experience substantial season and daily thermal variations, swimming  
325 performance remained unchanged over a 25°C temperature range (Fangue et al., 2008). In both  
326 natural and farmed environments, barramundi may experience acute changes temperatures with  
327 significant daily and seasonal thermal fluctuations (Pusey et al., 2004; Schipp et al., 2007). The  
328 capacity to minimise the effect of temperature on key traits may make this species particularly  
329 valuable in light of forecast climate warming and weather extremes. However, it is important to  
330 consider a suite of physiological performance matrices (e.g. growth, reproduction etc.) to  
331 adequately gauge a species susceptibility to high temperature.

### 332 *Oxygen Uptake*

333 Dietary fat content did not influence the thermal sensitivity of routine ( $\dot{M}O_{2MOUTINE}$ ) or  
334 maximal ( $\dot{M}O_{2MAX}$ ) rates of oxygen uptake. In general, the effects of temperature on metabolism  
335 were as expected for ectotherms, increasing exponentially (from 20 to 38°C) with temperature and  
336 reflects this species' tolerance of high temperatures (Ern et al., 2015; Healy and Schulte, 2012;  
337 Norin et al., 2014). The temperature sensitivity quotients ( $Q_{10}$ ) presented here are within the  
338 predicted values for teleost fishes, including previous work on *L. calcarifer* (Norin et al., 2014),  
339 showing an approximate doubling or tripling ( $Q_{10} \approx 2 - 3$ ) with every 10°C increase in temperature.  
340  $\dot{M}O_{2ROUTINE}$  appears to be more thermally sensitive than  $\dot{M}O_{2MAX}$ , represented by higher  $Q_{10}$  values  
341 over the entire temperature range tested (20 – 38°C). This may be indicative of a metabolic trade-off  
342 whereby the  $\dot{M}O_{2MAX}$  of barramundi is less thermally sensitive, but comes at the cost of increased

343  $\dot{M}O_{2MOUTINE}$ . It is likely that aspects of a species' biology may dictate how energy budget is  
344 allocated to cope with temperature changes (Huey and Hertz, 1984). Eurythermal species may have  
345 a decreased sensitivity of maximal performance, as reported for barramundi (Norin et al., 2014),  
346 killifish (Healy and Schulte, 2012) and eurythermal crustaceans (*Penaeus monodon* and *Astacus*  
347 *astacus*; Ern et al., 2015) while the opposite pattern has been observed in stenothermal fish like the  
348 rainbow trout (*Oncorhynchus mykiss*) (Chen et al., 2015). Further examination of these trends  
349 however, is required in order to reach concrete conclusions. In the present study, measurements of  
350 oxygen uptake were made on fasted fish and may explain the lack of an observed effect between  
351 diet treatments. However, acute elevations in temperature may impact fish during or after feeding,  
352 as post-prandial metabolism almost doubles that of standard values (Katersky et al., 2006) and may  
353 have more pronounced thermal sensitivity quotient. Measurements of oxygen uptake on fish in a  
354 continuous feeding regime where fish are fed *ad libitum*, such as those experienced in aquaculture  
355 facilities, may elucidate if dietary fat content has attributable metabolic costs throughout the day.

#### 356 *Upper thermal tolerance*

357 Critical thermal maximum represents the breakdown of whole animal functioning at the  
358 upper end of the thermal tolerance range. In terms of aquaculture species, diets that enhance  $CT_{MAX}$   
359 provide an obvious benefit as it means that the collapse of performance is extended up to a higher  
360 temperature. In the present study, fish fed the low fat (10%) diet had a higher  $CT_{MAX}$  than fish fed  
361 the high fat diet. The effect was small, with a 0.48°C difference between the two diet treatments.  
362 The values presented here are similar to other published results on barramundi (41 – 44.5°C) (Norin  
363 et al., 2014; Rajaguru, 2002) and indicate extreme tolerance of high temperatures in this species.  
364 Although fat content is the main difference between our two experimental diets, other  
365 macronutrients also differed, for example oil and vegetable protein, and may explain the observed  
366 differences in  $CT_{MAX}$ . Perhaps, differences in oil content can explain the observed effect on  $CT_{MAX}$ ,  
367 as described by Hoar et al. (1952; 1949) where dietary fat type (e.g. pilchard oil, herring oil and  
368 lard) increased survival at high temperatures and was correlated with the degree of unsaturation of  
369 fats. Further research is needed to fully understand whether thermal limits are affected by fat  
370 content, oils, or other macronutrients.

371 In order to cope with increases in temperature up to the  $CT_{MAX}$ , fish must increase oxygen  
372 carrying capacity (e.g. increase blood variables, ventilation). In the present study, diet treatment did  
373 not induce changes to oxygen carrying capacity, as measured by  $H_{CT}$  and [Hb], and indirectly by  
374 relative ventricular mass, and so it is possible that fish fed the low fat diet were capable of making  
375 other physiological adjustments (e.g. increasing cardiac/ventilatory output) to explain the observed  
376 differences in  $CT_{MAX}$  (Wang et al., 2014). Although the effect was small, small changes in  $CT_{MAX}$

may indicate significantly different performance at thermal extremes. For example, at a cellular level, a small increase in the CT<sub>MAX</sub> of milkfish (*Chanos chanos*) fed 50 mg of pyridoxine was accompanied by a higher expression of liver heat shock protein (HSP 70) relative to fish fed a control diet (Kumar et al., 2016). The elevated expression of protective mechanisms may mean that fish are more thermally tolerant of temperatures immediately below the CT<sub>MAX</sub>, indicating that a low fat diet may provide a slight advantage if extreme thermal exposures become more frequent.

## 5. Conclusion

The results of the present study show that juvenile barramundi fed a high fat diet (20%) have higher growth performance than fish fed a low fat diet (10%), but provides no benefit towards the thermal sensitivity of metabolism or swimming performance. However, thermal tolerance was reduced in fish fed the high fat diet, indicating a potential trade-off. Long-term or chronic thermal stress may alter thermal tolerances and sensitivities of measured traits in fish fed high fat diets and provide a logical link for future direction. Nonetheless, the results presented here suggest that the feeding of high fat diets improves growth performance in juvenile *L. calcarifer* while maintaining performance across a range of temperatures hence it may be beneficial for aquacultural production in the face of greater thermal variability as long as variability does not result in frequent exposures to temperatures near the critical thermal limit of this species.

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562 **Figure captions:**

563 **Figure 1.** Thermal dependence of critical swimming speed ( $U_{\text{CRIT}}$ ) of juvenile barramundi (*Lates*  
564 *calcarifer*; n = 6 fish per temperature) fed either a low fat (10%) or a high fat diet (20%).  
565 Swimming performance was adjusted for body length and expressed in terms of body lengths s<sup>-1</sup>  
566 (BL s<sup>-1</sup>).  $U_{\text{CRIT}}$  was unaffected by dietary treatment but was reduced at the low (20 and 25°C) test  
567 temperatures. Data are presented as individual data points (n = 6 per treatment).

568  
569 **Figure 2.** Thermal sensitivity of routine (A) and maximal (B) rates of oxygen uptake ( $\dot{M}\text{O}_2$ ) of  
570 juvenile barramundi (*Lates calcarifer*) fed either a low fat (10%) or a high fat (20%) diet. Fish were  
571 fed their assigned diets for four week at 30°C and tested acutely at five test temperatures (20, 25,  
572 30, 35 and 38°C). Routine and maximal  $\dot{M}\text{O}_2$  were thermally sensitive but were not affected by  
573 dietary fat treatment. Data are presented as individual data points (n = 6 per treatment).

574  
575 **Figure 3.** Critical thermal maximum ( $\text{CT}_{\text{MAX}}$ , A) and haematological parameters (B, haemoglobin  
576 concentration mg dL<sup>-1</sup>; C, haematocrit [%]; and D, relative ventricular mass (% body mass) of  
577 juvenile *Lates calcarifer* fed either a low fat (10%) or a high fat (20%) diet for 28-days. An asterisk  
578 represents statistical significance between dietary treatments. Data (n = 10) are presented as means  
579  $\pm$  S.E.

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593 **Tables**

594 **Table 1.** Proximate composition of the two experimental diets used in the present study. Protein, fat  
595 and fibre values are for dry matter (%).  
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| 597 |                                  | <b>Fry Start-</b>     | <b>Hatchery Start-</b> |
|-----|----------------------------------|-----------------------|------------------------|
| 598 |                                  | <b>Low fat (10 %)</b> | <b>High fat (20 %)</b> |
|     | <i>Ingredients (% inclusion)</i> |                       |                        |
| 599 | Starch                           | 19                    | 15                     |
|     | Vegetable Protein                | 17.4                  | 9.5                    |
| 600 | LAP                              | 13                    | 15.1                   |
| 601 | Oil (marine and terrestrial)     | 3.9                   | 13.2                   |
|     | Marine protein                   | 44.6                  | 44.8                   |
| 602 | Vitamins and Minerals            | 2.1                   | 2.4                    |
| 603 | Total                            | 100                   | 100                    |
| 604 | <i>Chemical composition</i>      |                       |                        |
| 605 | Crude protein (%)                | 54                    | 50                     |
|     | Crude fat (%)                    | 10                    | 20                     |
| 606 | Crude fibre (%)                  | 4                     | 4                      |
| 607 | Gross energy (MJ/Kg)             | 20.4                  | 22.4                   |
|     | Digestible energy (MJ/Kg)        | 16.5                  | 18.7                   |
| 608 | Phosphorus (%)                   | 1.4                   | 1.8                    |
| 609 |                                  |                       |                        |

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617 **Table 2.** Growth performance and feed utilization of juvenile *Lates calcarifer* fed two experimental  
618 diets differing in crude fat content (%). Values expressed as means  $\pm$  se. Abbreviations = Feed  
619 Efficiency Ratio (FER); Body Mass Gain (BMG). Significant differences between diets are denoted  
620 by an asterisk (\* $P < 0.01$ ; \*\*  $P < 0.001$ ).

|                      | Fry start          | Hatchery start       |
|----------------------|--------------------|----------------------|
|                      | Low fat (10%)      | High fat (20%)       |
| Initial mass (g)     | 3.13 $\pm$ 0.21    | 3.29 $\pm$ 0.15      |
| Initial length (cm)  | 6.23 $\pm$ 0.08    | 6.35 $\pm$ 0.06      |
| Final mass (g)       | 18.79 $\pm$ 1.62   | 24.75 $\pm$ 1.3*     |
| Final length (cm)    | 11.54 $\pm$ 0.17   | 12.51 $\pm$ 0.13*    |
| Condition Factor (k) | 1.22 $\pm$ 0.01    | 1.24 $\pm$ 0.01      |
| Survival (%)         | 92.73 $\pm$ 5.57   | 100 $\pm$ 0.0        |
| BMG (%)              | 495.83 $\pm$ 29.96 | 656.09 $\pm$ 31.58** |
| FER                  | 1.46 $\pm$ 0.17    | 1.34 $\pm$ 0.07      |

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634 **Table 3.** Thermal sensitivity quotients ( $Q_{10}$ ) for the critical swimming speed ( $U_{\text{CRIT}}$ ), routine  
 635 ( $\dot{M}O_{2\text{ROUTINE}}$ ) and maximal ( $\dot{M}O_{2\text{MAX}}$ ) rates of oxygen uptake of juvenile barramundi (*Lates*  
 636 *calcairfer*) fed either a low fat (10%) or a high fat (20%) diet for 28-days. Thermal sensitivity  
 637 quotients were calculated over the entire test temperature range (20 and 38°C), as well as the upper  
 638 (30 and 38°C) and lower (20 and 30°C) test temperatures.

| Temperature Range | Fry start Low fat (10%) |                              |                          | Hatchery start High fat (20%) |                              |                          |
|-------------------|-------------------------|------------------------------|--------------------------|-------------------------------|------------------------------|--------------------------|
|                   | $U_{\text{CRIT}}$       | $\dot{M}O_{2\text{ROUTINE}}$ | $\dot{M}O_{2\text{MAX}}$ | $U_{\text{CRIT}}$             | $\dot{M}O_{2\text{ROUTINE}}$ | $\dot{M}O_{2\text{MAX}}$ |
| 20-38             | 1.34                    | 2.22                         | 1.71                     | 1.25                          | 2.25                         | 1.84                     |
| 20-30             | 1.61                    | 2.24                         | 2.08                     | 1.61                          | 2.68                         | 2.35                     |
| 30-38             | 1.06                    | 2.19                         | 1.35                     | 0.91                          | 1.81                         | 1.36                     |

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