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

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Abstract

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

Key words: Temperature stress; CTmax; swimming performance; oxygen consumption; Asian sea bass.

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 10ºC (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_{MAX}) (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_{MAX} 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 – 35ºC) but can experience large seasonal (18 – 36ºC) and daily
115 (± 10ºC) 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

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

2.0 Materials and Methods

2.1. Experimental diets

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

2.2. Animal maintenance and experimental design

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

2.3. Growth experiment

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

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

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

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

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

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

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

2.4. Critical swimming speed

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

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

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

2.5. Oxygen Uptake

The thermal sensitivity of routine and maximal rates of oxygen uptake ($\dot{M}O_{2ROUTINE}$ and $\dot{M}O_{2MAX}$, respectively) were measured using closed system respirometry following published protocols (Cramp et al., 2014) at five test temperatures (20, 25, 30, 35 and 38^{o}C). Briefly, plastic respirometers were fitted with an oxygen-sensitive fluorescent sensor spot (PreSens, Regensburg, Germany) to allow the determination of oxygen partial pressure of the water non-invasively by measuring the fluorescence of the sensor spot through the plastic wall of the respirometer. Fluorescence was captured and recorded using a fibre-optic cable connected to a Fibox 3 reader (Presens). For $\dot{M}O_{2ROUTINE}$, fish were netted from their holding tanks and transferred to respirometers without delay. Fish (n = 6 per diet) were placed into 750 or 1600 ml plastic respirometers (depending on fish size and test temperature) filled with air-saturated water. Respirometers were placed in a water bath (64.5 × 41.3 × 39.7 cm; L × W × H) and temperature was controlled (± 0.5^{o}C) using a Seachill TR-10 aquarium chiller (TECO, USA). Temperature was adjusted at a rate of 4^{o}C h^{-1} to reach the necessary test temperatures. Fish were allowed at least 1 h before $\dot{M}O_{2ROUTINE}$ was measured, after which respirometers were sealed and the decline in oxygen was measured every 10 min for the following ~1-2 h. During the measurement period, oxygen levels did not drop below 70% saturation. The interval which resulted in the lowest $\dot{M}O_2$ reading was taken as $\dot{M}O_{2ROUTINE}$. Although activity was not quantified, fish usually remained still during respirometry trials. Fish movements were limited (e.g. small fin movements) and likely represent 'low routine' $\dot{M}O_2$ (Chabot et al., 2016). $\dot{M}O_{2MAX}$ (n = 6) was assessed following U_{CRIT} measurements by transferring the fatigued fish from the swimming flume into a respirometer filled with air-saturated water. Fish were transferred from the flume to the respirometer within 30 s of fatigue. Due to logistical constraints, the swim tunnel was not used as a respirometer. Air saturation inside the respirometer was then measured every minute for 15 min, and the greatest decline in oxygen saturation was taken as $\dot{M}O_{2MAX}$. Control respirometers (without fish) were used concurrently to determine background $\dot{M}O_2$. The rate of oxygen consumption ($\dot{M}O_2$, mg O_2 h^{-1}) was determined using equation 5 below:

$$(5)\ \dot{M}O_2 = \Delta O_2/\Delta t \times V$$

where ΔO_2 is the rate of change of oxygen saturation of a respirometer containing a fish, Δt is the change in time over which the ΔO_2 was measured, and V is the volume of the respirometer minus the volume of the fish (assuming 1 g displaces 1 ml of water).

2.6. Upper Thermal Tolerance

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

2.7. Haematological analysis and ventricular mass

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

2.8. Statistical analyses

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

analyses, and total length in the U_{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_{CRIT}, $\dot{M}O_{2ROUTINE}$, and $\dot{M}O_{2MAX}$ were calculated as $Q_{10} = [(R_2)\ (R_1)^{-1}]^{[(10)\ (T2 - T1)]}$, 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 ± 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_{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 25ºC in fish fed both diets. Further, a pairwise *post hoc* analysis showed that performance was not significantly different between 30 and 38ºC in fish fed either diet. Fish fed the 20% fat diet treatment showed optimal swimming performance at 35ºC (7.09 ± 0.42 m s^{-1}), while fish fed the 10% fat diet showed optimal swimming performance at 38ºC (7.42 ± 1.12 m s^{-1}). Average thermal sensitivity quotients (Q_{10}) showed that, for U_{CRIT}, thermal sensitivity tended to be greater at lower temperatures (20 – 30ºC), and reached a plateau of thermal independence between 30 and 38ºC (Table 3). Fish size (L_T) was inversely related to U_{CRIT} ($F_{1, 35} = 22.03$, $P < 0.001$), with smaller fish on average having a higher relative swimming speed (BL s^{-1}).

3.3. Oxygen uptake

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

3.4.Upper thermal tolerance

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

3.5. Haematology and ventricular mass

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

4.0 Discussion

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

Growth performance

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

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

Swimming performance

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

Oxygen Uptake

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

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

Upper thermal tolerance

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

In order to cope with increases in temperature up to the CT_{MAX}, fish must increase oxygen carrying capacity (e.g. increase blood variables, ventilation). In the present study, diet treatment did not induce changes to oxygen carrying capacity, as measured by H_{CT} and [Hb], and indirectly by relative ventricular mass, and so it is possible that fish fed the low fat diet were capable of making other physiological adjustments (e.g. increasing cardiac/ventilatory output) to explain the observed 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_{MAX} 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 immediatly below the CT_{MAX}, 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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Declarations of interest: none

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Figure captions:

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

Figure 2. Thermal sensitivity of routine (A) and maximal (B) rates of oxygen uptake ($\dot{M}O_2$) of juvenile barramundi (*Lates calcarifer*) fed either a low fat (10%) or a high fat (20%) diet. Fish were fed their assigned diets for four week at 30ºC and tested acutely at five test temperatures (20, 25, 30, 35 and 38ºC). Routine and maximal $\dot{M}O_2$ were thermally sensitive but were not affected by dietary fat treatment. Data are presented as individual data points (n = 6 per treatment).

Figure 3. Critical thermal maximum (CT_{MAX}, A) and haematological parameters (B, haemoglobin concentration mg dL^{-1}; C, haematocrit [%]; and D, relative ventricular mass (% body mass) of juvenile *Lates calcarifer* fed either a low fat (10%) or a high fat (20%) diet for 28-days. An asterisk represents statistical significance between dietary treatments. Data (n = 10) are presented as means ± S.E.

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		**Fry Start-Low fat (10 %)**	**Hatchery Start-High fat (20 %)**
598	*Ingredients (% inclusion)*		
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			
	Chemical composition		
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

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

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

634 **Table 3.** Thermal sensitivity quotients (Q_{10}) for the critical swimming speed (U_{CRIT}), routine
635 ($\dot{M}O_{2ROUTINE}$) and maximal ($\dot{M}O_{2MAX}$) 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 38ºC), as well as the upper
638 (30 and 38ºC) and lower (20 and 30ºC) test temperatures.

Temperature Range	Fry start Low fat (10%)			Hatchery start High fat (20%)		
	U_{CRIT}	$\dot{M}O_{2ROUTINE}$	$\dot{M}O_{2MAX}$	U_{CRIT}	$\dot{M}O_{2ROUTINE}$	$\dot{M}O_{2MAX}$
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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