

1 **Growth efficiency of juvenile barramundi, Lates calcarifer, at high temperatures**

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24 **Abstract**

25 Temperature is recognized to be the most important environmental factor
26 affecting growth in fish. Barramundi are cultured over a wide range of temperatures and
27 extreme high temperatures which they can experience under culture approach the upper
28 thermal tolerance limit for this species. A growth trial was conducted on juvenile
29 barramundi to examine the effects of high temperatures ranging from the minimum
30 optimal temperature (27 °C) for growth efficiency to the extreme upper thermal limits (39
31 °C) for feed intake, growth and growth efficiency. Juveniles (4.87 ± 0.32 g) were held at
32 four different temperatures 27, 33, 36 and 39 °C and fed twice daily to satiation (503.5 g
33 kg^{-1} crude protein, 182.5 g kg^{-1} lipid, 150.1 g kg^{-1} ash, 20.52 GE MJ kg^{-1}). Feed intake
34 ($\text{g}\cdot\text{d}^{-1}$) and SGR ($\%\cdot\text{d}^{-1}$) increased with increasing temperature up to 36° C. At 39 °C
35 feed intake, growth, feed efficiency ratio, protein efficiency ratio and productive energy
36 value were significantly lower than at the other temperatures. This demonstrates that
37 growth was optimized at temperatures from 27 to 36 °C and that barramundi have a much
38 wider range for maximum growth efficiency than preciously thought.

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40 *Keywords:* barramundi; Asian sea bass; Lates calcarifer; temperature; growth efficiency;
41 feed intake

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43 **1. Introduction**

44 Temperature has a marked effect on many key physiological processes in fish
45 (Brett and Groves, 1979) and there have been numerous studies (Jobling, 1981;
46 McCarthy, et al., 1998; Jonassen, et al., 1999) and reviews (Elliott, 1982) which have

47 examined the effects of temperature on growth. Every species has a range of
48 temperatures over which it survives and where growth occurs. However, growth is
49 maximized at an optimal temperature within this thermal tolerance range (Jobling, 1997).
50 As temperature increases, feed intake will increase to a maximum and then decrease
51 rapidly prior to the upper limit for thermal tolerance (Jobling, 1994). Maximum feed
52 intake will occur at a temperature which is generally a few degrees above the optimal
53 temperature for growth. Metabolic rate increases exponentially as the temperature
54 increases and, at any given temperature, the difference between feed intake and metabolic
55 rate will determine the energy available for growth (Brett and Groves, 1979; Jobling,
56 1994). For cultured species, these parameters are extremely important in order to
57 understand and maximize the efficiency with which the consumed food is converted into
58 growth (Jobling, 1994; Carter, et al., 2001).

59 Barramundi or Asian sea bass is a commercially important aquaculture species in
60 Australia and southeast Asia. Production of these fish in Australia has steadily increased
61 for the past 15 years and this trend is expected to continue (Boonyaratpalin and Williams,
62 2002). Barramundi have an extremely wide thermal tolerance range (15-40°C) and they
63 are cultured in temperatures from 22-35°C (Tucker et al., 2002). However, the more
64 extreme temperatures under which they are cultured approach the thermal tolerance for
65 this species. Recent research has focused on determining optimal feeding practices
66 (Williams and Barlow, 1999) and nutritional requirements for juvenile barramundi
67 (Catacutan and Coloso, 1997; Boonyaratpalin, et al., 1998; Coloso et al., 1999; Williams
68 and Barlow, 1999; Murillo-Gurrea et al., 2001). However, studies have not examined
69 temperatures above 30°C. Previous research has shown that growth efficiency had an

70 optimal range of 27 to 33°C (Katersky and Carter, unpublished data). However, the
71 highest temperature examined was 33°C and the full temperature range for maximum
72 growth efficiency was not determined. This study examines feed intake and growth
73 efficiency at temperatures which range from the minimum optimal temperature (27°C)
74 for growth efficiency to the extreme upper thermal limits (39°C) for juvenile barramundi
75 in order to determine the entire range of temperatures where growth is optimized.

76 **2. Materials and Methods**

77 *2.1 Experimental diet*

78 A standard diet (2-mm pellets) was formulated according to known dietary
79 requirements for barramundi (Boonyaratpalin and Williams, 2002). The diet was
80 formulated to contain 50% crude protein and 19.7 MJ kg⁻¹ gross energy (Table 1). Fish
81 meal and fish oil were supplied by Skretting (Cambridge, TAS, Australia). Vitamins and
82 minerals were supplied by Sigma-Aldrich Pty. Ltd (Sydney, NSW, Australia), Vitamin C
83 was supplied as Stay-C (Roche Vitamins Australia Ltd., Sydney, NSW, Australia).

84 *2.2 Growth experiment*

85 Juvenile barramundi, *Lates calcarifer*, (1 g) were obtained from WBA Hatcheries
86 (West Beach, SA, Australia). Fish were maintained at the University of Tasmania under
87 constant environmental conditions (salinity: 10 ‰; photoperiod: 24h light (Barlow et al.,
88 1995); temperature: 27.0°C). Fish were initially stocked into 4 150-l aquaria and
89 maintained at 27.0°C. Temperature was adjusted 1°C d⁻¹ towards the experimental
90 temperatures of 33.0, 36.0 and 39.0°C, with the exception of the 27.0°C aquarium which
91 was maintained at a constant temperature. After 12 days all fish were at their
92 experimental temperature. The experiment was conducted in four identical recirculating

93 systems each consisting of 3 19-l carboys with a trickle biofilters on each system. Each
94 system was held at a constant temperature (either 27, 33, 36 or 39°C) with submersible
95 heaters each controlled with an individual thermostat. The system was modified from that
96 used by (Engin and Carter, 2001). The fish were fed to satiation twice daily during this
97 acclimation period. The standard diet was fed to all fish at all times (Table 1).

98 At the start of the experiment, 60 fish from each treatment were anesthetized (100
99 mg l⁻¹, benzocaine) and individual weight (g) was measured. These fish were then
100 randomly separated into 3 19-l recirculating tanks to give 20 fish per experimental tank.
101 Ten fish were killed with an overdose of benzocaine (400 mg l⁻¹) and frozen in liquid
102 nitrogen for whole-body chemical analysis. Water quality was monitored 3 times week⁻¹
103 and water changes (~50%, with preheated water) were performed as necessary to keep
104 water quality within the limits for barramundi (Tucker et al., 2002). Dissolved oxygen
105 was maintained at >80 % saturation with constant aeration in all temperature treatments.
106 Temperature was recorded twice daily with a mercury thermometer.

107 Fish were fed to satiation twice daily at 0900 and 1800 for 20 d. A pre-weighed
108 ration was provided to each tank, and if completely consumed additional pellets were
109 counted out and provided until feeding ceased. Any uneaten pellets were siphoned out
110 after 10 min and counted in order to determine total daily food consumption. On day 20,
111 fish from 1 replicate from each of the four temperature treatments were starved for 24 h.
112 Following this starvation period, individual weight (g) and total length (mm) were
113 measured for all fish. Five fish from each tank were killed with an overdose of
114 benzocaine (400 mg l⁻¹), autoclaved and freeze-dried to a constant weight in order to
115 determine whole-body chemical composition. On days 21 and 22 fish from the

116 remaining 2 replicates of each treatment were starved for 24 h and sampled as described
 117 above.

118 *2.3 Calculations*

119 The following equations were used to calculate specific growth rate (SGR), feed
 120 efficiency ratio (FER), protein efficiency ratio (PER), productive protein value (PPV) and
 121 productive energy value (PEV):

$$122 \quad \text{SGR (\%}\cdot\text{d}^{-1}\text{)} = [(\ln\text{BW}_F - \ln\text{BW}_I)/d]*100 \quad (1)$$

$$123 \quad \text{FER (\%)} = \text{BW gain, g (wet)} / \text{Mass of food consumed, g (dry)} \quad (2)$$

$$124 \quad \text{PER (\%)} = \text{BW gain (wet)} / \text{Mass of protein fed (dry)} \quad (3)$$

$$125 \quad \text{PPV} = (\text{fish protein gain (g CP)}/\text{total protein consumed (g CP)})*100 \quad (4)$$

$$126 \quad \text{PEV} = (\text{fish energy gain (g MJ)}/\text{total energy consumed (g MJ)})*100 \quad (5)$$

127 *2.4 Chemical analysis*

128 Standard methods were used to determine dry weight (freeze drying to a constant
 129 weight); crude protein (Kjeldahl); total lipid (Bligh and Dyer, 1959); energy (bomb
 130 calorimeter); ash by combustion at 550°C for 16 h (AOAC, 1995).

131 *2.5 Statistical analysis*

132 Data are presented as mean \pm standard error. The normality and homogeneity of
 133 data were explored by examining the residual plots. Results were analyzed using a one-
 134 way ANOVA (SPSS, version 11.5) and significant results were compared using Tukey's
 135 HSD. Growth data were analyzed using ANCOVA (SPSS, version 11.5) between the
 136 initial and final weight measurements as significant differences in mean weights of fish
 137 were found between treatments at the start of the experiment.

138 **3. Results**

139 Feed intake ($\text{g}\cdot\text{d}^{-1}$) was significantly higher at 33 and 36°C than at 27 and 39°C
140 ($F=29.40$; $df=3,8$; $P<0.001$), which were not significantly different from one another
141 (Table 2). At 33 and 36°C feed intake was 10.48 ± 0.50 and $9.74 \pm 0.99 \text{ g}\cdot\text{d}^{-1}$,
142 respectively. This was approximately double the intake at 27 and 39°C. Significant
143 differences found among initial weights ($F=21.64$; $df= 3,236$; $P<0.001$) did not have a
144 significant effect on final weights ($F=1.31$; $df= 3,225$; $P=0.253$). Body weight gain (g)
145 ($F=63.40$; $df=3,8$; $P<0.001$) and specific growth rate (SGR)($F=72.38$; $df=3,8$; $P<0.001$)
146 were both significantly higher at 33 and 36°C than at 27 and 39°C. Fish reared at 39 °C
147 had significantly lower growth than at the other temperatures. Overall, as temperature
148 increased there was an increase in both feed intake and SGR up to 36°C. At 39°C a sharp
149 decrease in feed intake and growth was observed (Table 2).

150 Whole-body crude protein was significantly ($F=9.35$; $df=3,20$; $P<0.001$) lower in
151 the 39°C treatment than the other temperatures which were not significantly different
152 from one another. Crude lipid was also significantly ($F=4.70$; $df= 3,22$; $P=0.011$) lower
153 at 39°C than at 33 and 27°C but not different from the 36°C treatment. Ash content at
154 39°C was significantly ($F=8.03$; $df=3,20$; $P=0.001$) higher than at the 33 and 27°C groups
155 but not different from the 36°C. Energy did not differ among the temperature treatments
156 ($F=2.89$; $df=3,20$; $P=0.061$), (Table 3).

157 Growth efficiency expressed as feed efficiency ratio (FER), protein efficiency
158 ratio (PER) and productive energy value (PEV) were significantly lower for fish held at
159 39°C, than the remaining treatments (FER, $F=68.72$; $df=3,8$; $P<0.001$; PER, $F=68.25$;
160 $df=3,8$; $P<0.001$, Table 2; PEV, $F=84.23$; $df=3,8$; $P<0.001$, Fig. 1). Productive protein
161 value (PPV) was also significantly lower for fish held at 39°C than the remaining

162 treatments, however the 27°C was significantly lower than the 33°C treatment ($F=148.36$;
163 $df=3,8$; $P<0.001$, Fig. 1).

164 **5. Discussion**

165 The present study was the first to examine feed intake, growth and growth
166 efficiency in juvenile barramundi over a wide range of high temperatures. The results
167 show that feed intake and SGR were highest at 33 and 36°C. However, when growth
168 efficiency (PEV, PER and FER) was examined there was no significant difference from
169 27 to 36°C. It was only when temperatures exceeded 36°C that a decline in efficiency
170 was observed.

171 Previous research on the feed intake and growth of juvenile barramundi at
172 different temperatures (Williams and Barlow, 1999) examined temperatures ranging from
173 20 to 29°C and observed a plateau in food conversion ratio (FCR) and feed intake from
174 26°C to 29°C. This is consistent with previous results (Katersky and Carter, unpublished
175 data), where feed intake ($\%BW \cdot d^{-1}$) remained constant between 27 and 30°C but there
176 was a significant increase between 30 and 33°C. In the present study, feed intake was
177 significantly higher at 33°C than at 27°C.

178 There is an inverse relationship between SGR and fish weight, SGR decreases as
179 fish weight increases (Jobling, 1994). The majority of research on barramundi has been
180 on larger slower growing juvenile fish, this partly explains the higher SGR's found in the
181 present study. Williams and Barlow (1999) determined SGR of different size barramundi
182 at 29°C and found SGR decreased from 3.6 $\% \cdot d^{-1}$ for 40 g fish, through 1.7 for 100g fish
183 and 1.5 for 170g fish to 1.1 for 270g fish. A few studies have looked at growth rates of
184 small juvenile barramundi, Eusebio and Coloso (2000) determined SGR to be 4.1 $\% \cdot d^{-1}$

185 for 40g fish raised at 27-28°C and Catacutan and Coloso (1997) found SGR of 5.0 %·d⁻¹
186 when water temperatures ranged from 26.5 to 29°C for 57 g fish. In the present study,
187 using 5 g fish, SGR was 5.6 %·d⁻¹ at 27°C and increased to 7.1 %·d⁻¹ at 33 and 36°C.

188 Temperature has been shown to have an effect on the biochemical composition of
189 fish (Cui and Wootton, 1988; Koskela et al., 1997; Bendiksen et al., 2003; Tidwell et al.,
190 2003). In the present study, as temperatures exceeded the optimal range for growth
191 efficiency there was a significant decrease in whole-body protein. This differs from
192 Baltic salmon reared at high temperatures where protein did not change with increasing
193 temperature (Koskela et al., 1997). However, whole-body lipid levels peaked at optimal
194 growth temperatures for Baltic salmon (Koskela et al., 1997) and then significantly
195 decreased as the temperature continued to increase. In the present study, lipid levels
196 remained elevated up to 36°C and then significantly declined. The decrease in both
197 protein and lipid at the high temperatures can be attributed to the increased metabolism
198 which is encountered at temperatures nearing the upper limits of thermal tolerance
199 (Jobling, 1997).

200 Growth efficiency has been shown to remain constant over a wide range of
201 temperatures (Forseth et al., 2001). The present study showed this to be the case for
202 juvenile barramundi, with maximum growth efficiency occurring between 27 and 36°C.
203 As temperatures approached the upper limit for thermal tolerance (39°C), growth
204 efficiency declined due to an increase in metabolism and a decrease in feed intake
205 (Jobling, 1997). Previous research on barramundi has been focused on temperatures
206 between 27 and 30°C (Williams and Barlow, 1999; Murillo-Gurrea et al., 2001; Tian and
207 Qin, 2003; Williams et al., 2003) as this was believed to be the extent of the optimal

208 range for growth efficiency, however, this research shows that juvenile barramundi have
209 a much wider optimal range for culture than previously believed. This is possibly a
210 biological adaptation that barramundi have developed based on their wide geographical
211 distribution around northern Australia and southeast Asia. Barramundi are an incredibly
212 robust fish thus allowing them to grow and thrive in a wide range of environmental
213 conditions.

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Table 1. Ingredient and chemical composition of experimental diet

<i>Ingredient composition (g kg⁻¹)</i>	
Fish meal	730
Fish oil	70
Starch	119
CMC	10
Choline chloride	10
Phosphorus (NaPO ₄)	10
Vitamin C (Stay-C)	20
Ytterbium-oxide	1
Vitamin premix ^a	15
Mineral premix ^b	15
<i>Chemical composition (g kg⁻¹ DM)</i>	
Dry matter (g kg ⁻¹)	946.9
Crude protein	503.5
Crude lipid	182.5
Ash	150.1
Energy (MJ kg ⁻¹)	20.52

^aVitamin premix (mg kg⁻¹): Vitamin A (7.50), Vitamin D (9.00), Rovimix E50 (150.00), Menadone sodium bisulphate (3.00), Riboflavin (6.00), Calcium D-pantothenate (32.68), Nicotinic Acid (15.00), Vitamin B-12 (0.015), d-biotin (0.23), Folic acid (1.50), Thiamin HCL (1.68), Pyridoxine HCl (5.49), myo-Inositol (450.00), α -cellulose (817.91).

^bMineral premix (mg kg⁻¹): CuSO₄ 5H₂O (35.37), FeSO₄ 7H₂O (544.65), MnSO₄ H₂O (92.28), Na₂SeO₃ (0.99), ZnSO₄ 7H₂O (197.91), KI (2.16), CoSO₄ 7H₂O (14.31), α -cellulose (612.33).

Table 2. Survival, feed intake, growth and growth efficiency (mean \pm standard error) of juvenile barramundi at four different temperatures.

Temperature (°C)	27	33	36	39
Measured temperature (°C)	26.98 \pm 0.04	33.16 \pm 0.13	35.36 \pm 0.32	38.86 \pm 0.05
Mean body weight _{initial} (g)	4.23 \pm 0.13	5.06 \pm 0.19	4.49 \pm 0.12	5.69 \pm 0.17
Mean body weight _{final} (g)	13.11 ^b \pm 0.45	22.47 ^a \pm 1.71	20.09 ^a \pm 1.76	10.33 ^c \pm 0.14
Survival (%)	98.33 \pm 1.67	96.67 \pm 1.67	98.33 \pm 1.67	95.00 \pm 2.89
Feed intake(g·d ⁻¹)	5.58 ^b \pm 0.14	10.48 ^a \pm 0.50	9.74 ^a \pm 0.99	4.32 ^b \pm 0.08
SGR (%·d ⁻¹)	5.38 ^b \pm 0.19	7.10 ^a \pm 0.07	7.11 ^a \pm 0.42	2.84 ^c \pm 0.09
FER (g·g ⁻¹)	1.41 ^a \pm 0.05	1.46 ^a \pm 0.01	1.46 ^a \pm 0.02	0.86 ^b \pm 0.04
PER (g·g ⁻¹)	2.80 ^a \pm 0.09	2.91 ^a \pm 0.02	2.82 ^a \pm 0.05	1.72 ^b \pm 0.08

Means with similar superscripts were not significantly different (P<0.05, n=3).

Table 3. Body composition (mean \pm standard error) of juvenile barramundi at four temperatures.

Temperature ($^{\circ}$ C)	27	33	36	39
Dry Matter ($\text{g}\cdot\text{kg}^{-1}$)	262.5 ^b \pm 0.17	275.8 ^a \pm 0.14	275.6 ^a \pm 0.08	263.0 ^b \pm 0.23
Crude Protein ($\text{g}\cdot\text{kg}^{-1}\text{WW}$)	154.9 ^b \pm 1.01	164.2 ^a \pm 1.25	163.8 ^a \pm 1.54	147.4 ^c \pm 1.29
Crude Lipid ($\text{g}\cdot\text{kg}^{-1}\text{WW}$)	63.5 ^a \pm 1.27	63.1 ^{a,b} \pm 2.42	64.4 ^a \pm 2.96	54.0 ^b \pm 2.39
Ash ($\text{g}\cdot\text{kg}^{-1}\text{WW}$)	37.8 ^b \pm 0.37	39.6 ^b \pm 0.78	42.1 ^{a,b} \pm 1.31	44.2 ^a \pm 1.53
Energy ($\text{MJ}\cdot\text{kg}^{-1}\text{WW}$)	5.76 ^{a,b} \pm 0.07	5.96 ^a \pm 0.09	6.09 ^a \pm 0.08	5.48 ^b \pm 0.09

Initial group (mean \pm SD: n=10): Dry matter, 245.3 $\text{g}\cdot\text{kg}^{-1}$, Crude protein, 153.3 \pm 0.54 $\text{g}\cdot\text{kg}^{-1}\text{WW}$, Total lipid, 56.0 \pm 3.19 $\text{g}\cdot\text{kg}^{-1}\text{WW}$, Ash, 34.1 \pm 1.20 $\text{g}\cdot\text{kg}^{-1}\text{WW}$, Energy, 5.14 \pm 0.01 $\text{MJ}\cdot\text{kg}^{-1}\text{WW}$. Means with similar superscripts ($P < 0.05$, $n=3$) were not significantly different between temperatures.

Figure 1. Growth efficiency (mean \pm standard error) of juvenile barramundi expressed as productive protein value (PPV %) and productive energy value (PEV %). Means with similar letters were not significantly different ($P < 0.05$, $n=3$).

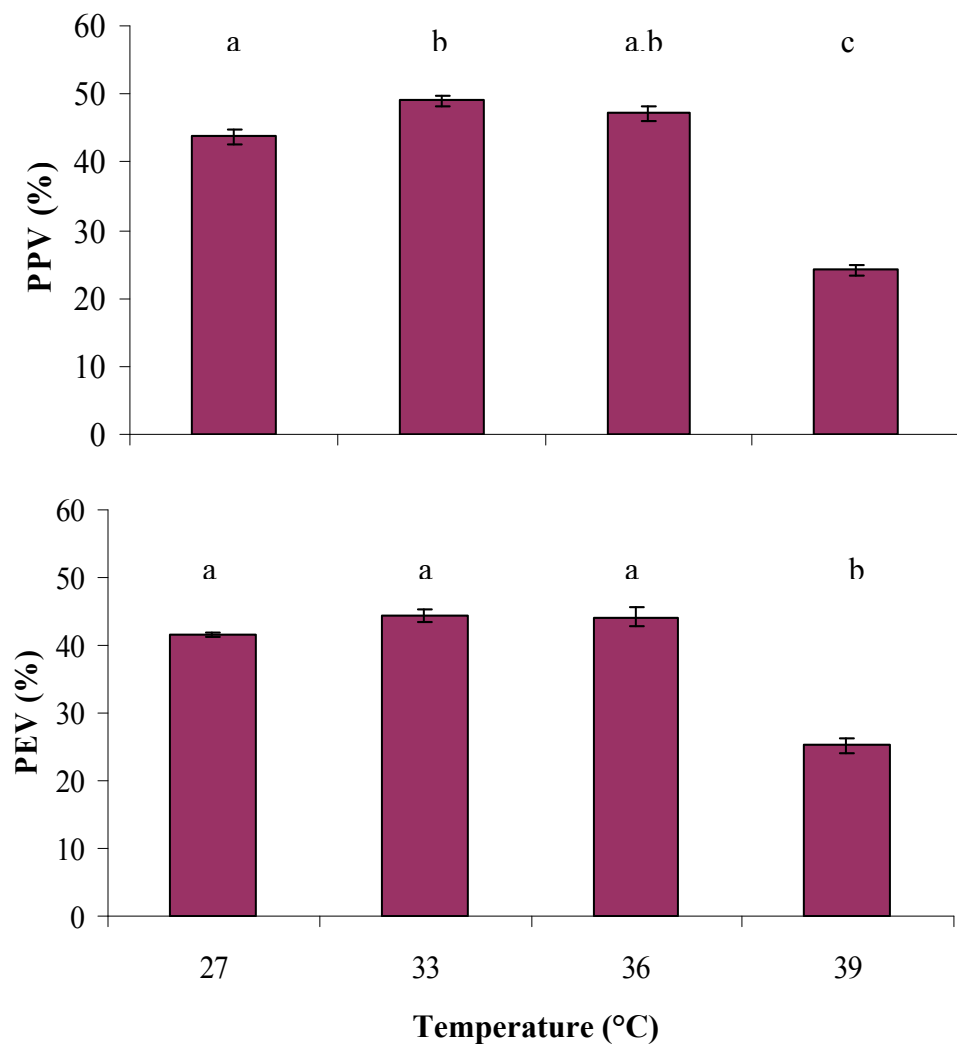


Figure 1. Katersky and Carter