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Influence of fish size and water temperature on the metabolic demand for oxygen by barramundi, *Lates calcarifer* (Bloch), in freshwater

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Abstract

Oxygen demand by all animals is driven primarily by their needs for sustaining metabolism. Typically, larger animals require more oxygen and cellular fuel to carry out respiration than smaller animals. This relationship in most cases is not linear and is usually described by a coefficient and exponent (e.g. ax^b): the exponent b showing the relationship between live-weight and energy/oxygen demand and is often termed the metabolic body weight (MBW) exponent, while the coefficient (a) tends to be temperature specific and describes the relationship between MBW and maintenance metabolic energy and oxygen demand at that specific temperature. Across all temperatures (range 26.0–32.0 °C), the relationship between barramundi (*Lates calcarifer*) live-weight (x; g) and relative oxygen consumption as standard metabolic rate (y; mg O_2 kg^{-1} h^{-1}) at 29.4 ± 1.5 °C (mean ± SD) was described by the exponential curve: $y = 710.19\ x^{-0.3268}$, $R^2 = 0.6875$ ($n = 222$ assessments). Examination of the same data but on a gross oxygen consumption (mg O_2 h^{-1}) basis showed a relationship between live-weight (x; g) and gross oxygen consumption (y; mg O_2 h^{-1}) that was described by the exponential curve: $y = 0.710\ x^{0.6732}$, $R^2 = 0.9033$. Evaluation of the combined relationship between fish live-weight (y; g) and water temperature (x; °C) on gross oxygen consumption rate (z; mg O_2 h^{-1}) was described by the equation: $z = (-20.7818 + 1.4017x - 0.0227x^2) \times y^{0.673}$.

Keywords: oxygen, temperature, Asian seabass, energetics

Introduction

Oxygen demand by all animals is driven primarily by their needs for sustaining metabolism. Typically, larger animals require more oxygen and cellular fuel to carry out respiration than smaller animals (Withers 1992). This relationship, in most cases, is not linear and is usually described by a coefficient and exponent. For example, in barramundi at 28 °C, the relationship of 48.5 (live-weight)$^{0.82}$ has been used to explain this relationship between maintenance metabolic energy demand (kJ) and live-weight (kg) (Lupatsch, Kissil & Sklan, 2002; Lupatsch & Kissil 2003). The exponent of 0.82 shows the relationship between live-weight and energy demand and is often termed the metabolic body weight (MBW) exponent, while the coefficient (in this case 48.5) tends to be temperature specific and describes the relationship between MBW and maintenance energy demand at that specific temperature. In addition to examining the use of cellular fuel (protein, fat and some glycogen) for respiration and maintenance metabolism, it is also possible to examine this relationship based on oxygen consumption. Therefore, it is expected that a similar weight-specific relationship between oxygen consumption and maintenance metabolism energy demand will also be evident in barramundi. This evaluation of oxygen consumption rate by fish requires that the fish have been starved in excess of 24 h, and is referred to as standard metabolic rate (SMR) (Withers 1992).

Fish, being poikilothermic animals, are thermal conformers and as such their metabolic rate is also strictly dependent on the temperature of their envir-

onment (Buentello, Gatlin & Neill 2000; Mallekh & Lagardere 2002). The relationship between water temperature and metabolism in barramundi is assumed to be linear over normal physiological ranges, although this remains to be validated. Similarly, it is not known whether there are any temporal (diurnal) effects on metabolic rate in barramundi. However, results for other finfish species suggest that this is unlikely to be the case (De la Gandara, Garcia-Gomez & Jover 2002). Any increase in oxygen demand has further implications for fish management in that although water oxygenation regimes may be satisfactory for maintenance requirements, feeding the fish may force them into conditions of oxygen debt (Pichavant, Person-Le-Ruyet, Le Bayon, Severe, Le Roux, Quemener, Maxime & Boeuf 2000). The resultant metabolic oxygen limitation may not only mean that the feed is not being utilized efficiently, but mounting evidence suggests that acute, periodic oxygen debt has long-term effects on fish performance (Dalla Via, Villani, Gasteiger & Niederstatter 1998; Buentello *et al.* 2000; Pichavant, Person-Le-Ruyet, Le Bayon, Severe, Le Roux & Boeuf 2001). Therefore, the rationale behind this study was to determine the standard metabolic demand for oxygen by barramundi with varying live-weight, at varying temperatures and under diurnal variation, to help estimate oxygen demands for barramudi in aquaculture systems.

Materials and methods

Respirometer design and operation

Five flow-through, air-tight containers (respirometers) were supplied with aerated water from a single 500 L supply reservoir tank using a submersible electric pump. Four of the respirometers were 20 L in volume and the other was 40 L. A single 40 L container was used for fish >1000 g. The supply reservoir tank was continuously supplied with additional heated water (normally $\sim 29\,^{\circ}$C) to ensure maintenance of tank water volume at greater than 400 L. The reservoir was continuously aerated. Water was pumped from the reservoir to each individual respirometer and inflow rate to each respirometer was regulated through an in-line tap. Outflow water was collected in a small container (outflow reservoir) before allowing it to discharge to drainage. Inflow rate was maintained at around 200–300 mL min^{-1} to ensure that no appreciable build-up of ammonia occurred.

Fish of a range of sizes were added to each respirometer to contain a biomass of approximately 0.02–0.04 kg L^{-1}. This necessitated fish numbers in any given tank ranging from one to seven. All fish were starved for at least 24 h before being acclimated to respirometers. No fish was confined within a respirometer for a period longer than 36 h. Fish were not reused once they had already been used in any study. Before being placed in the respirometer, each fish was weighed and allowed to acclimate for a 1 h period before any measurements were taken. In addition, each respirometer was covered to ensure that the fish were not disturbed by activity in the vicinity.

Oxygen consumption measurements

During the acclimation period, the oxygen content of the supply and outflow reservoirs was monitored and the flow rate of the inflowing water to each respirometer was regulated to ensure an oxygen differential between the supply and outflow reservoirs of about 2 mg L^{-1}. Once the oxygen consumption rate appeared to have stabilized, measurements were made. Stabilization in oxygen consumption typically occurred after about 30–40 min. To measure the rate of oxygen consumption (VO_2) the oxygen differential between the supply and outflow reservoirs was measured, flow rate was determined by measuring the rate of water discharged over a 30 s period and water temperature was recorded. Oxygen consumption (mg kg^{-1} h^{-1}) was calculated as (Grottum & Sigholt 1998):

$$VO_2 = \frac{(\text{Supply})O_2(\text{mg L}^{-1}) - \text{Outflow}\,O_2(\text{mg L}^{-1})}{\text{fish live} - \text{weight(kg)} \times \text{flow rate(L h}^{-1})}$$

Temporal effects

The temporal variability of oxygen consumption was examined by the 24 h oxygen consumption patterns of groups of 65, 410 and 1699 g fish in separate respirometers ($n = 5$ assessments per size group). The fish were allowed to acclimatize to the respirometer for 1 h before the first measurements were taken. Oxygen consumption and water flow measurements were then taken every 2 h for the ensuing 24 h period. Water temperature ($28.6 \pm 0.2\,^{\circ}$C; mean $\pm$ SD) was also monitored over the experimental period.

Live-weight effects

The influence of fish live-weight on oxygen consumption was examined by placing groups of 59, 144, 410

and 1064 g fish in separate respirometers ($n = 5$ assessments per size group). The fish were allowed to acclimatize to the respirometer for 1 h before the first measurements were taken. Oxygen consumption and water flow measurements were then taken every hour for the ensuing 5 h period to provide mean oxygen consumption measurements for the average temperature of the experimental period. Water temperature was maintained at 28.6 ± 0.1 °C.

A further examination of this effect was undertaken based on the pooled data over all studies undertaken and also at all temperatures (range 26.0–32.0 °C), for all assessments ($n = 222$) undertaken during the study. A comparison of oxygen consumption rates between fish of different sizes and over different water temperatures was also undertaken.

Temperature effects

To examine the influence of water temperature on oxygen consumption, the temperature of water provided to the supply reservoir tank was varied. Fish of varying sizes were placed in the respirometers, similar to that detailed for sections 2.3 and 2.4. Similarly, the fish were allowed to acclimatize to the respirometers for a minimum of 1 h before any measurements were undertaken. Water temperature was measured at each hour, along with oxygen consumption and flow rates ($n = 222$). Water temperature of the supply tank and respirometers varied from 26.0° to 32.0 °C.

Evaluations of the Q_{10} effects of water temperatures (based on 26–32 °C) on each of the fish live-weight ranges (Fig. 4), on gross oxygen consumption, were calculated using the equation (Das, Pal, Chakraborty, Manush, Chatterjee & Mukherjee 2004):

$$Q_{10} = (\text{rate2}/\text{rate1})^{(10/(\text{temp2}-\text{temp1}))}$$

Statistical analysis

Curve fitting to the data was undertaken using either Microsoft Excel. Multiple regression was performed using STATISTICA v6 (StatSoft Pacific, Melbourne, Vic., Australia).

Results

Temporal effects

Temporal variability of oxygen consumption was related to variability in water temperature. No significant ($P > 0.05$) variation in oxygen consumption, due to any specific time-related effects, was observed. The variability in oxygen consumption was consistent across a range of fish live-weights. The lowest oxygen consumption rates of all fish were observed at 02:00 hours, concomitant with the lowest water temperatures during the experimental period. The highest oxygen consumption rates of all fish were between 12:00 and 16:00 hours, concomitant with the highest water temperatures during the experimental period.

Live-weight effects

The relationship between fish live-weight (x; g) and standardized oxygen consumption as (y; mg O_2 kg^{-1} h^{-1}) for all assessments with an overall average temperature of 29.4 ± 1.5 °C (mean ± SD) (range 26.0–32.0) was described by the exponential curve: $y = 710.19x^{-0.3268}$, $R^2 = 0.6875$ ($n = 222$) (Fig. 1). Examination of the data on a gross oxygen consumption (mg O_2 h^{-1}) basis ($n = 222$ assessments) was described by the exponential curve: $y = 0.7102x^{0.6732}$, $R^2 = 0.9033$ (Fig. 2).

Temperature effects

Across all experiments, water temperature varied from 26.0° to 32.0 °C over the full term of the study period. Natural logarithmic transformation of the data in blocks of 1.0 C over the full temperature range shows that the relationship between temperature and live-weight is relatively consistent across the range (Fig. 3). Most relationships had coefficients of 0.60–0.68, with only one being greater than 0.70 at 0.77. Examination of the gross oxygen consumption (gross metabolic rate; gross MR) data (mg O_2 h^{-1}) as influenced by water temperature showed curvilinear relationships for most fish sizes (Fig. 4). Generally, these were best described by quadratic functions. Only the 409 and 1048 g fish showed oxygen consumption relationships with temperature that were close to linear responses. Evaluations of the combined effects of water temperature and fish live-weight on gross oxygen consumption are presented in Fig. 5 ($n = 222$). The combined relationship between fish live-weight (y; g) and water temperature (x; °C) on gross oxygen consumption rate (mg O_2 h^{-1}) is described by the equation:

$$\text{Gross oxygen conspumption} = (-20.7818 + 1.4017x - 0.0227x^2) \times y^{0.673}$$

Evaluations of the Q_{10} effects of water temperatures on each of the fish live-weight ranges (Fig. 4)

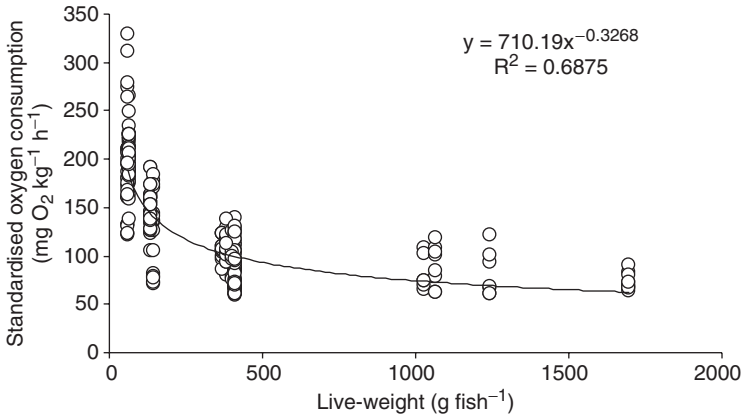

Figure 1 Standard oxygen consumption rates of barramundi of varying live-weight at varying temperatures (26.0–32.0 °C).

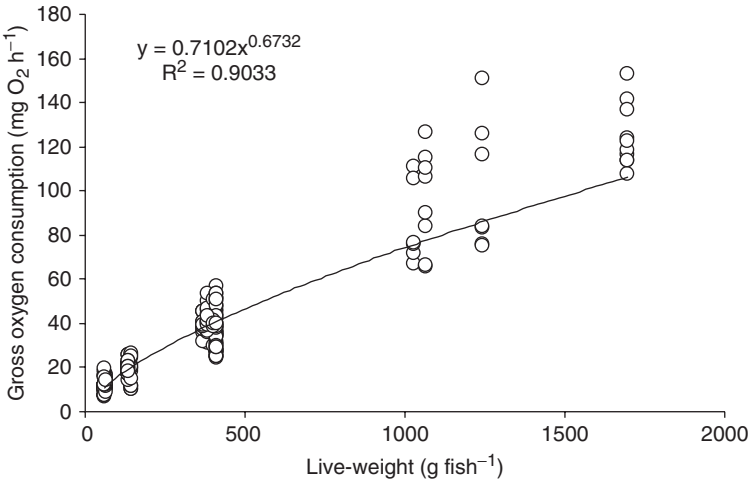

Figure 2 Relationship between fish live-weight and oxygen consumption rate at a range of temperatures (26.0–32.0 °C).

ranged from 2.4–5.0. The higher end of this range differed substantially from the remainder of the Q_{10} values, with an average value of 3.1 ± 1.02 (mean ± SD) being determined across all fish sizes.

Discussion

This study has shown that there are numerous influential factors on oxygen consumption demands of juvenile barramundi. The findings are generally consistent with what has been observed for many other fish species, although the subtle variability between the species is still important to note as it has important implications for fish management regimes, particularly in oxygen-sensitive scenarios.

Temporal effects

The key outcome from this experiment was that based on the observations of the barramundi, it was assumed that oxygen consumption measurements taken from any time period (assuming independence from feed intake and disturbance/activity) would be relevant to SMR estimations.

The temporal variability of oxygen consumption, when examined as SMR, of fish unfed for over 24 h and undisturbed, was relatively constant. The marginal variability observed in the SMR was largely consistent with influences of water temperature. These findings are consistent with those observed by other researchers on fish species such as Atlantic cod (*Gadus morhua*) and European sea bass (*Dicentrarchus labrax*) (Dalla Via *et al.* 1998; Hunt von Herbing & White 2002).

However, the limited diurnal variability observed in barramundi contrasts with the observations of Maxime (2002), who noted a distinct diurnal variation in routine metabolism in smoulting Atlantic salmon (*Salmo salar*). It is of note, though, that the work of Maxime (2002) was based primarily around the transfer of the fish to salt water from freshwater and the examination of smoulting stage on the metabolic

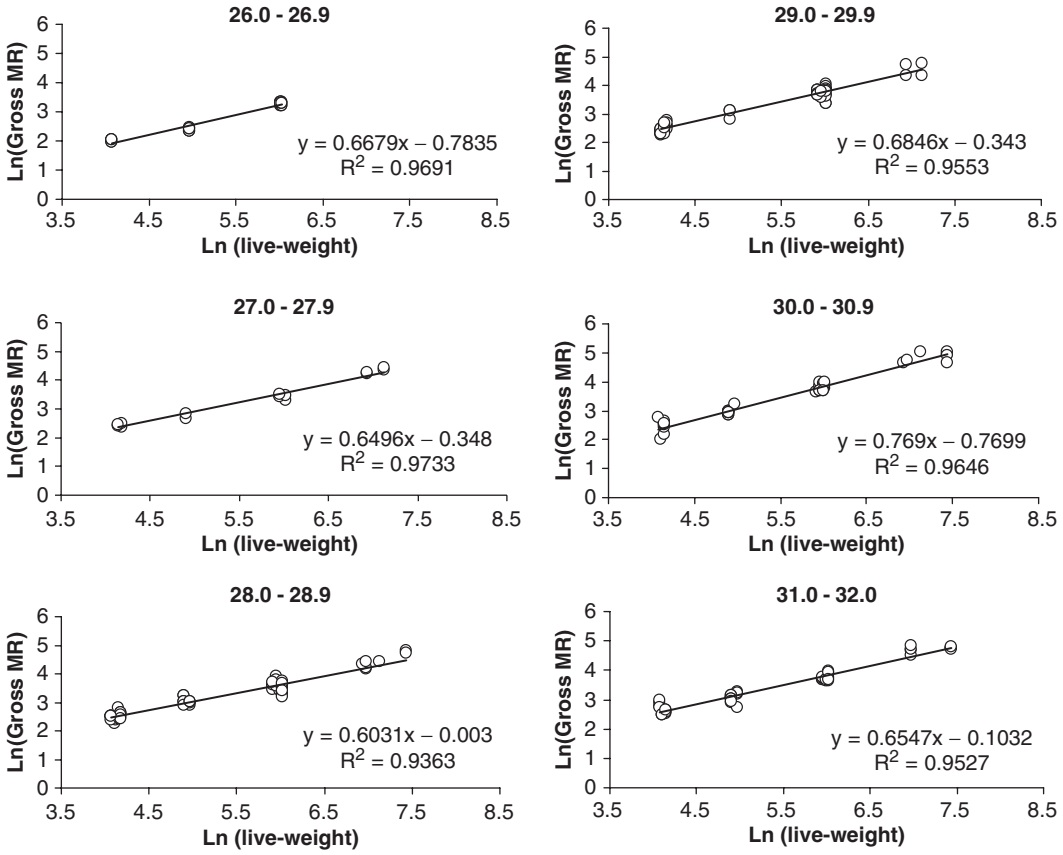

Figure 3 Relationship between Ln(fish live-weight) and Ln(gross oxygen consumption rate) at a range of temperatures (26.0–32.0 °C), grouped in 1 °C increments.

rates of the fish as a consequence. Interestingly, Altinok and Grizzle (2003) also noted differences in oxygen consumption rates among fish when exposed to various salinity regimes. The response in oxygen consumption rate of a euryhaline species such as Rainbow trout (*Oncorhynchus mykiss*) to alterations in water salinity was far greater than that observed for a stenohaline species such as Channel catfish (*Ictalurus punctatus*). Based on this, it would be expected that barramundi, also a euryhaline species, would also show substantial variation in oxygen consumption depending on water salinity and this should be investigated further.

Live-weight effects

The relationship between fish live-weight and SMR shows that smaller fish have a distinctly higher SMR than larger fish (Fig. 1). The nature of this relationship makes it difficult to compare SMR among individuals or even other species unless the fish are of the same weight or a weight exponent transformation of the SMR is undertaken. In this case, all fish live-weights would need to be transformed by an exponent of −0.3268. Extrapolation of the SMR vs. live-weight curve indicates that fish even smaller than those used in this study are likely to have significantly higher oxygen demands. This has important implications for management of larval and juvenile fish rearing, particularly under conditions of static culture systems, where oxygenation regimes will be critical.

The relationship between fish live-weight and gross oxygen consumption, often considered an indirect measurement of the animal's metabolic rate, shows a non-linear relationship consistent with the power curve: $y = 0.7102x^{0.6732}$, $R^2 = 0.9033$ (Fig. 2). The exponent of the curve is often referred to as the metabolic weight exponent and essentially it describes the relationship between the animals' live-

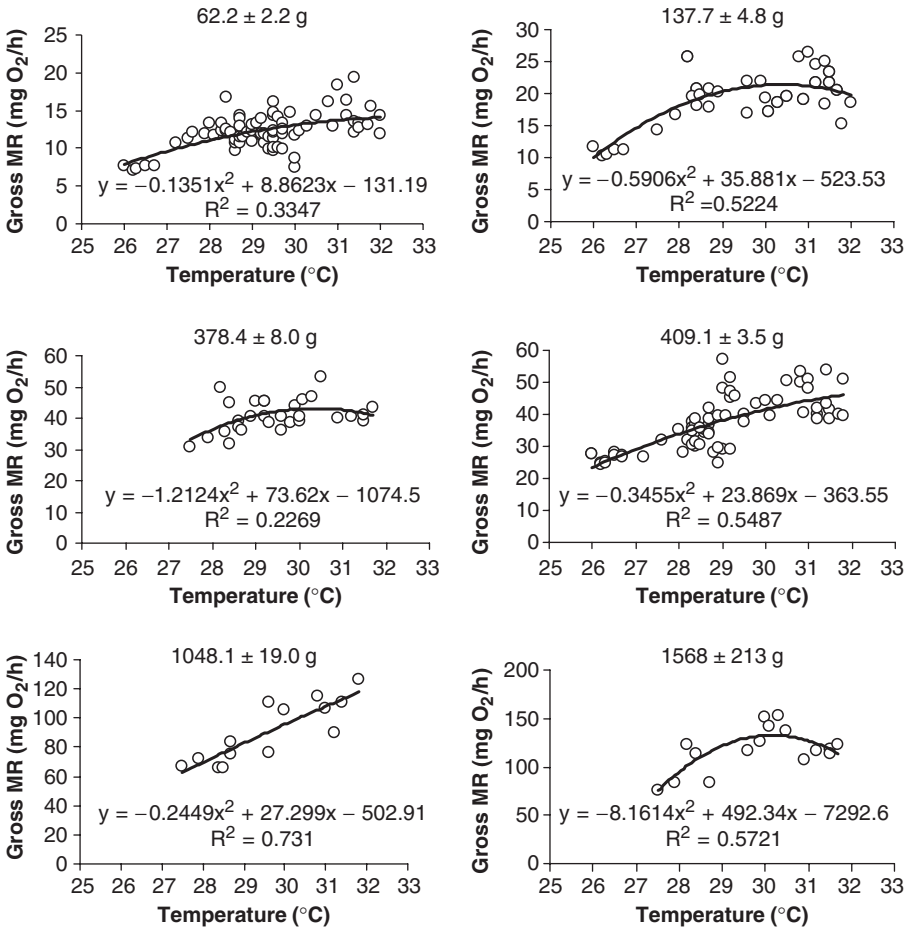

Figure 4 Influence of temperature (°C) on gross metabolic rate (mg O_2 h^{-1}) of discrete groups of fish arranged by live-weight.

weight and metabolic rate. Lupatsch and Kissil (2003), using direct assessment of energy loss through starvation, estimated metabolic energy demand at 27.0 °C of $y = 31.0x^{0.82}$. Notably, the weight exponent was substantially greater in this direct estimate than that observed in the present study (0.82 cf. 0.67). It is noted that the exponent determined in this study is at the lower range of those identified for most fish species, although the greater number of larger fish used in the assessment may be pertinent (Withers 1992; Lupatsch *et al.*, 2002).

The direct implications of the metabolic weight exponent being lower than that observed in the study of Lupatsch and Kissil (2003) are that with a larger exponent, there is a more direct relationship between live-weight and metabolic rate. Clearly, the upper temperature ranges of the assessments by Lupatsch and Kissil (2003) were below the minimal ranges of the present study, which make a direct comparison difficult. Notably, the study of Lupatsch and Kissil (2003) was conducted with all temperatures below those recognized as optimal (27–30 °C) for growth of barramundi (Williams & Barlow 1998). It would be of value to extend the lower temperature range of the present study to explore more fully the relationship between live-weight and metabolic rate.

It is important to note that the relationship between fish live-weight and gross oxygen consumption was among the strongest of those identified in this study. The response of barramundi oxygen consumption to increasing fish live-weight is considerably greater than that seen due to the increase in water temperature over the range used in this study (Fig. 5). However, this does by no means diminish the importance of the influence of water temperature on oxygen demand to barramundi.

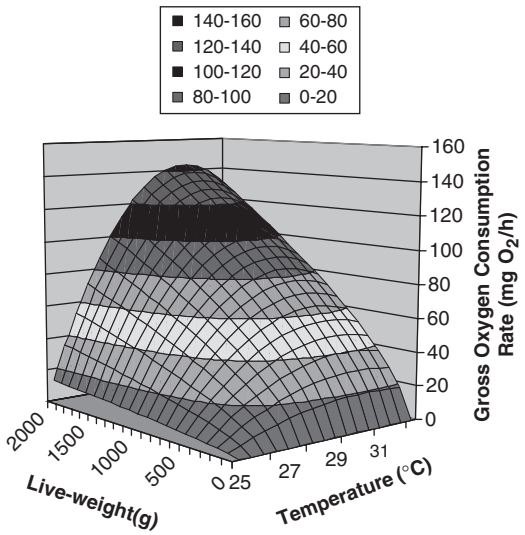

Figure 5 Smoothed surface relationship between live-weight (g) and temperature (°C) effects on gross metabolic rate ($mg\,O_2\,h^{-1}$). Functional equation of the relationship is: Gross oxygen consumption = $(-20.7818+1.4017x-0.0227x^2)\times y^{0.673}$.

Temperature effects

Temperature is well recognized, in addition to live-weight, as one of the key influencing factors of a fish's metabolic rate and oxygen demand. The findings from this study are certainly no different in this regard, with multiple regression identifying that these two factors alone explain in excess of 92% of the variation observed in oxygen consumption by the barramundi.

One of the key functions in examining the relationship between an animal's oxygen consumption and temperature is the Q_{10} effect, which is the increase in rate for an increase in 10 °C (Brett & Groves 1979). Typically, this has been observed to be around 2.3 for goldfish and 2.2 for some Asian carp species (Brett & Groves 1979; Das *et al.* 2004). In the present study on barramundi, the Q_{10} was observed to range from 2.1 to 5.0, but averaged closer to 3.1., this higher value potentially being skewed by the higher than normal Q_{10} value of 5.0, which was observed for large 1048 g fish, whose oxygen consumption did not decline at the same rate as the other fish sizes, at the upper thermal limits of the study. Most of the other fish sizes had Q_{10} effects close to 2.4, which is in the expected range for fish (Brett & Groves 1979).

The relationship between water temperature and gross oxygen consumption showed highly significant relationships in this study (Fig. 4). In addition, these relationships may have even been stronger if the temperature range used had been further below the known temperature optima for this species (Williams & Barlow 1998). It is important to note that the temperature range used in this study was only from 26.0° to 32.0 °C. While there is considerable confidence in the data over this temperature range, estimates for water temperatures above and below this range could only be approximate. It would be of some value to consolidate these data at the temperatures outside this range, particularly those below 26 °C and above 32 °C.

The relative influence of temperature on the smaller fish was greater than that on larger fish, as evidenced by the broader spread of the data for the smaller fish compared with the larger fish (Fig. 1). However, examination of the gross variability in oxygen consumption (Figs 2 and 5) suggests that there is greater absolute variation in oxygen consumption by larger fish. This suggests that larger fish may be at greater risk of oxygen debt-associated constraints due to high water temperatures, but that the metabolic demand for oxygen of small fish will vary more as a consequence of temperature variation.

Conclusions

The relationships between the key factors of water temperature and fish live-weight on oxygen demand are relatively clear. A multi-factorial functional equation was derived based on the data and can be used to estimate gross oxygen consumption (GMR) for any fish live-weight and at any temperature, within reasonable practical constraints.

This work could be further enhanced by an improved understanding of the implications of dietary energy density intake on oxygen consumption (Jobling & Davis 1980). However, the specific value of this may be dependent on dietary energy type also provided (i.e. protein, lipid and carbohydrate components) and there are also implications for feeding activity to be considered. In addition, an evaluation of the influences of sub-optimal water oxygenation on not only GMR but also fish growth and feed intake would also be valuable in estimating the production cost of hypoxia on barramundi performance.

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