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The effect of high water temperatures on the allometric scaling effects of energy and protein starvation losses in juvenile barramundi, *Lates calcarifer*

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ABSTRACT

This study was undertaken to examine the effects of high water temperatures on the allometric scaling effects of energy and protein starvation losses by juvenile barramundi, *Lates calcarifer*. The somatic energy and protein loss was examined in fish of varying sizes when starved for 24 or 25 days at temperatures ranging from 23 °C to 38 °C. The amount of energy and protein lost varied according to both size and temperature and was consistent with the function of $a*W^b$, where a is a temperature dependent coefficient, W the animal's weight and b an exponent relating energy loss to live-weight. The coefficient for energy or protein loss varied and was described by a polynomial function, with a general increase from 23 °C to 32 °C, before a dramatic decline after 35 °C. In contrast, the exponent for energy loss was relatively constant between 23 °C and 35 °C, but showed a rapid increase at 38 °C. Both the coefficients and exponents of protein loss mirrored that of energy loss. Analysis of the protein and lipid contributions to energy loss shows that typically lipid loss accounts for the greater part of energy loss (~67%), but also shows that above 35 °C protein energy loss increases (from ~33% to ~39%) while the losses seen from lipid catabolism remain the same. These results show that one of the main nutritional issues associated with heat stress in fish is a dramatic increase in endogenous loss of protein.

1. Introduction

Barramundi (*Lates calcarifer*, Bloch) aquaculture production in Australia occurs over temperatures that range from 18 to 36 °C (Glencross, 2006). Growth has been shown to reach a thermal optima at 30–32 °C and above this temperature range growth plateaus before declining rapidly above 35 °C. Death occurs with further increases in temperature above 38 °C (Bermudes et al., 2010). Above 35 °C thermal stress occurs, with the primary symptoms being a slower growth rate, increased mortality, increased level of cataract formation, reduced rates of protein synthesis and increased levels of endogenous protein turnover (Carter et al., 2007; Glencross and Rutherford, 2010; Bermudes et al., 2010).

Thermal stress is also likely to play an important role in total maintenance energy and protein demand in fish. Traditionally the relationship between fish size and total maintenance energy and protein demand is described by the function $a*W^b$, where a is a temperature dependent coefficient, W the animal's weight and b an exponent relating energy or protein loss to live-weight (Clarke and Johnston, 1999; White, 2010). These functions have also been key elements of bio-energetically based nutritional models for fish (Glencross, 2008).

Bio-energetic models have been around for a long time, but have made a recent resurgence in their application to growth, feed demand and nutrient requirement predictions for aquaculture of a range of fish species (Cuenco et al., 1985; Cacho, 1990; Cho and Bureau, 1998; Lupatsch and Kissil, 1998; Dumas et al., 2009). Factorial models are one version of these bio-energetic models that have been successfully applied to barramundi (Glencross, 2008). These models rely on a suite of empirically determined parameters relating to energy flux; such as growth potential, utilisation coefficients and maintenance demands, with the mathematical linking of these parameters forming the basis of the models. However, like most models they are based on a range of assumptions.

One of the key assumptions of many of the bio-energetic models being used is that the metabolic body weight exponent is constant. However, the value of the determined exponent is often dependent on fish species, experimental design and other exogenous issues that have resulted in metabolic weight exponents ranging between 0.75 and 0.94 (Clarke and Johnston, 1999; White, 2010). Many investigations have determined energy exponent values close to 0.80 and as such many studies use this value to standardise their data (Azevedo et al., 1998; Lupatsch et al., 2001; 2003; Glencross, 2008, 2009). Similarly, an exponent of 0.70 has been used to standardise data relating to protein demands (Lupatsch et al., 2003; Glencross, 2008).

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However, it is probable that under conditions of thermal stress that these weight-related exponents may change, consistent with deterioration in the animals' capacity for protein synthesis and increased rates of protein turnover. Minor alterations to these exponents can have substantial impacts on the outcomes of energetic models to which they applied, particularly so in the determination of maintenance energy demand (Pirozzi, 2009).

This study examined the hypothesis that at elevated water temperatures the allometric scaling exponents for protein and energy loss in barramundi will change. Using a range of fish sizes, groups (tanks) of animals were starved to determine the relative consumption of somatic energy and protein reserves at six different temperatures from 23 °C to 38 °C. The effect of temperature on the maintenance energy demand is discussed.

2. Materials and methods

2.1. Experiment design and fish handling

An experimental system with 24×150 L tanks was used for this study. Because of this limitation the experiment was undertaken as two blocks, each with a range of fish sizes crossed with a range of water temperatures (Table 1). This was done to create the statistical power and resolution needed to effectively examine the weight-specific loss of protein and energy as a function of water temperature. Due to the nature of the planned outputs of the study (regression analysis), it was determined that to increase resolution there was greater merit in more treatments than in replicates. A greater number of replicates were applied to the 38 °C array of treatments due to predicted decline in performance of these treatments relative to the other treatments in the experiment, and also a greater risk of complete tank losses.

Hatchery-reared juvenile barramundi of a range of sizes (Table 2) were grown in indoor 1000 L heated sea-water tanks from several batches of fish obtained from Cell Aquaculture (Fremantle, WA, Australia). Fish were first acclimated to their experimental temperature in two steps over a period of 2 weeks. In the first acclimation step, groups of small and large fish were placed at 23 °C or brought up to 32 °C over a period of 8 days. During this phase fish were held in 1000 L tanks with 100 to 400 fish per tank depending on size (~5 to 650 g). Fish were transferred into the experimental system (150 L tanks) to carry out the second acclimation step and stocked at 28 small or 8 large animals per tank, respectively. The second acclimation step lasted 4 days during which the temperature was raised at a maximum rate of 1.5 °C per day. Acclimation was staggered so that each treatment group reached its experimental temperature simultaneously with the exception of the fish groups already acclimated to 23 and 32 °C. Once each treatment temperature was reached, fish were acclimated for a further 3 days before an initial weighing and sampling, where the numbers of fish in each tank were reduced to a minimum viable number (Table 2).

Table 1
Treatment allocations within the incomplete factorial array from the combined experimental blocks A and B. Each letter indicates which block and how many times it was replicated.

Temperature	Fish weight									Total
	7	20	30	50	95	150	270	500	650	
23 °C	B		A	A	B	A	B	A	B	8
26 °C	B		A	A	B	A	B	A	B	8
29 °C		A		A		A		A		4
32 °C	B	A		A	B	A	B	A	B	8
35 °C	B	A		A	B	A	B	B	A	8
38 °C	B		AB	AB	B	AB	B	AB	B	12
Total	5	3	4	7	5	7	5	7	5	48

Table 2
Experiment variables for each experimental block. Indicated are the mean ± SD fish weights and water temperatures used. Fish numbers used per size class and block indicated: block1/block2.

	Block 1	Block 2	Number of fish/tank
Initial weights	20.6 ± 0.76	7.7 ± 0.25	18/15
	29.1 ± 0.76	58.9 ± 0.26	18/10
	48.9 ± 0.56	92.6 ± 3.24	15/9
	148.9 ± 0.99	267.8 ± 7.58	9/5
	508.4 ± 24.04	468.9 ± 3.13	4/5
		648.2 ± 9.82	4
Temperatures	23.9 ± 0.02	23.5 ± 0.06	
	26.3 ± 0.03	26.2 ± 0.03	
	29.7 ± 0.06	32.5 ± 0.05	
	32.7 ± 0.01	34.9 ± 0.13	
	35.8 ± 0.03	37.4 ± 0.06	
	38.3 ± 0.07		
Duration	25 days	24 days	

Prior to handling the fish were sedated using isoeugenol at 0.002 mL L^{-1} (supplied as AQUI-S™) and individually weighed to 0.1 g accuracy. The fish were allowed to regain equilibrium before being placed within their designated tank.

2.2. Comparative slaughter trial — energy and protein loss trial

Five or six temperatures were allocated to groups of four (or eight in one block) tanks. Groups of fish of a similar size (7, 20, 30, 50, 95, 150, 270, 500 and 650 g) were allocated to replicate tanks within each temperature treatment (23, 26, 29, 32, 35 and 38 °C), but as tank numbers were limited various size classes were used across the experiment in an incomplete factorial array (see Tables 1 and 2 for details). Thus not all treatment combinations were present in each block. In either experimental block each tank was supplied with flow-through (4 L min^{-1}), heated and continuously aerated water. Temperature was controlled to within 0.2 °C by a digital programmable logic controller (PLC) operating a solenoid that missed preheated water of 20 °C and 40 °C in a mixing box prior to distributing water of the correct temperature to each replicate tank. The transferred fish were kept in their respective tanks for the duration of each trial (24 or 25 days), without being fed. After each trial period had elapsed the surviving fish were re-weighed. Following weighing, a minimum of n = 3 fish per replicate tank were euthanized and assessed for composition change in dry matter, protein and energy concentrations. Additional fish of a similar nominal size class as those used to stock each trial were euthanized at the beginning of the study in order to determine dry matter, protein and energy concentrations of the fish prior to starvation. Changes in dry matter, protein and energy concentrations according to treatment allocation were determined by comparative slaughter analysis.

Two tanks of fish (150 g and 500 g initial weigh classes) assigned to the 38 °C temperature regime suffered catastrophic losses. Moribund specimens exhibited an increased incidence of cataract formation.

2.3. Sample processing and chemical analysis

Whole frozen fish were homogenised by two passes through an industrial meat mincer (Reber N22 1.0Hp, Reservoir, VIC, Australia). Samples were then taken and analysed for dry matter and another sample refrozen and freeze-dried for further analysis of dry matter, nitrogen, ash and gross energy content. Dry matter was calculated by gravimetric analysis following oven drying at 105 °C for 24 h. Protein levels were calculated from the determination of total nitrogen by LECO auto-analyser, based on N×6.25. Gross ash content was determined gravimetrically following the loss of mass after combustion of a sample in a muffle furnace at 550 °C for 12 h. Gross energy