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Defining the allometric relationship between size and individual fatty acid turnover in barramundi *Lates calcarifer.*

Michael J. Salini[a,b,c*], David Poppi[b,c], Giovanni M. Turchini[a], Brett D. Glencross[d]

[a] Deakin University, Geelong, Australia. School of Life and Environmental Sciences, Warrnambool Campus, Princess Hwy, Warrnambool, VIC., Australia.

[b] CSIRO Agriculture, 144 North Street, Woorim, QLD, Australia.

[c] CSIRO Agriculture, QLD Biosciences Precinct, Services Rd, St Lucia, QLD, Australia.

[d] Institute of Aquaculture, Stirling University, FK9 4LA Stirling, United Kingdom

***Corresponding author at:** Present address: Ridley Aqua Feed, Unit 4, 31 Robart Court, Narangba, QLD, Australia. Tel.: +61 7 3817 9829 Email: Michael.salini@ridley.com.au

Abstract

An experiment was conducted with barramundi (Asian seabass; *Lates calcarifer*) to examine the allometric scaling effect of individual fatty acids. Six treatment size classes of fish were deprived of food for 21 days (Treatment A, 10.5 ± 0.13g; Treatment B, 19.2 ± 0.11g; Treatment C, 28.3 ± 0.05g; Treatment D, 122.4 ± 0.10g; Treatment E, 217.6 ± 0.36g; Treatment F, 443.7 ± 1.48g; mean ± SD) with each treatment comprising of fifteen fish, in triplicate. The assessment of somatic losses of whole-body energy and lipid were consistent with previous studies, validating the methodology to be extended to individual fatty acids. Live-weight (LW) exponent values were determined to be 0.817 ± 0.010 for energy and 0.895 ± 0.007 for lipid. There were significant differences among the fatty acids ranging from 0.687 ± 0.005 for 20:5n-3 (eicosapentaenoic acid) and 0.954 ± 0.008 for 18:1n-9 (oleic acid). The LW exponent values were applied to existing fatty acid intake and deposition data of barramundi fed with either 100% fish oil or 100% poultry oil. From this the maintenance requirement for each fatty acid was determined. The metabolic demands for maintenance and growth were then iteratively determined for fish over a range of size classes. Application of these exponent values to varying levels of fatty acid intake demonstrated that the biggest driver in the utilisation of fatty acids in this species is deposition demand and despite their reputed importance, the long-chain polyunsaturated fatty acids had nominal to no maintenance requirement.

Keywords: Allometric scaling; maintenance; fatty acid; bioenergetics; LC-PUFA; barramundi, Asian seabass.

1. Introduction

A range of different approaches have been used to predict or determine growth as well as feed requirements based on the dynamic flow of nutrients in aquatic systems (Bar et al., 2007; Cho and Bureau, 1998; Glencross, 2008; Lupatsch and Kissil, 1998; Machiels and Henken, 1986). Predictive models started out as relatively simple approaches such as the ubiquitous specific growth rate and thermal growth coefficient calculations; however, progressive extensions of these models now exist that consider the many biological properties of fish (Birkett and Lange, 2007; Dumas et al., 2010). In addition, mass-balance models have also been developed and used in understanding specific nutrient and metabolite flows in a range of model species (Cunnane and Anderson, 1997; Turchini et al., 2006; Turchini et al., 2007) as well as a whole ecosystem approach (Sawyer et al., 2016).

There is a growing body of evidence regarding the essential fatty acid requirements of many species generally determined by various forms *in vivo* feeding assessments (NRC, 2011). The efficient utilisation of these fatty acids within an organism depends on a number of factors and there are many complex interactions potentially affecting their utilisation efficiency (Glencross, 2009; Tocher, 2015). Despite the numerous studies to date, relatively little is known about the maintenance requirements and utilisation efficiencies associated with specific fatty acids and how these may be used in nutrient modelling. An obvious step in the refinement of factorial models is the incorporation of empirically derived utilisation efficiency values. Recently, the marginal efficiency of long-chain polyunsaturated fatty acids (LC-PUFA) was determined for barramundi (Asian seabass; *Lates calcarifer*) and differences were clear among the fatty acids (Salini et al., 2015a). Maintenance requirements of protein, lipid and energy typically described by linear equations of intake to gain ratios can give an insight into the partitioning of production and maintenance costs (Bureau et al., 2006; NRC, 2011; Pirozzi et al., 2010). Similarly, it should be possible to determine estimates of productivity for specific fatty acids using derived body weight scaling exponent values to provide a size independent response (Bar et al., 2007; Glencross and Bermudes, 2011; White, 2011).

Bioenergetic modelling of the nutrient flows in barramundi has been extensively researched and ‘user friendly’ simulation programs are used routinely (Glencross, 2008; Glencross and Bermudes, 2010, 2011, 2012). One of the key assumptions and constraints in the application of these models is that the live-weight (LW) exponent values are constant. Studies have

shown with barramundi, over a range of normal temperatures, that this is generally the case for the energy, protein and lipid exponents (Glencross and Bermudes, 2011). However, it is assumed that when broken down into constituent fatty acids the body weight exponents are also equivalent to that of the lipid as a complete nutrient.

A further refinement of those factorial bioenergetic models could include consideration of the individual fatty acids and potentially amino acids in order to better understand their utilisation in terms of productivity on a size independent basis. Therefore, the aim of the present study was to determine the allometric scaling effect of specific fatty acids in barramundi for use in future bioenergetic studies. In addition, a re-evaluation of previously published data is used to refine the fatty acid demands for maintenance and growth of barramundi using *in silico* predictive modelling.

2. Materials and Methods

2.1 Fish husbandry and management

Juvenile barramundi (*Lates calcarifer*) were sourced from the Betta barra fish hatchery (Atherton, QLD, Australia), originally from two shipments and on-grown to various sizes using commercial feeds (Ridley Marine Float; Ridley Aquafeeds). The fish were graded multiple times during on-growing phase in order to generate an appropriate range of size classes. Before commencement of the experiment the fish were individually weighed on an electronic balance and sorted into a series of experimental tanks (600 L). Each tank was alimented with heated flow-through seawater (3L/min) and maintained at a temperature of 30.0 ± 0.2 °C and dissolved oxygen of 6.6 ± 0.3 mg/L, under fluorescent lighting 12L:12D. At the beginning of the experiment each of the tanks held fifteen fish. The six treatment size classes were randomly distributed among the tanks with each treatment having three replicate tanks (Treatment A, 10.5 ± 0.13g; Treatment B, 19.2 ± 0.11g; Treatment C, 28.3 ± 0.05g; Treatment D, 122.4 ± 0.10g; Treatment E, 217.6 ± 0.36g; Treatment F, 443.7 ± 1.48g). The fish were then fasted for 21 days. Whole fish samples were collected prior to and after fasting from each of the treatment size classes and frozen at -20 °C before laboratory analysis. Ethical clearance was approved for the experimental procedures by the CSIRO animal ethics committee (Approval A3/2015).

2.2 Laboratory analysis

The initial and final fish were processed using the following methods. The frozen whole fish were passed through a commercial meat mincer (MGT – 012, Taiwan) twice to obtain a homogeneous mixture. A sample was taken for dry matter analysis and another sample was freeze-dried until no further loss of moisture was observed (Alpha 1-4, Martin Christ, Germany). Dry matter was calculated by gravimetric analysis following oven drying at 105ºC for 24 h. Crude protein was calculated after the determination of total nitrogen by organic elemental analysis (CHNS-O Flash 2000, Thermo Scientific, USA), based on N x 6.25. Total lipid content was determined gravimetrically following extraction of the lipids using chloroform:methanol (2:1) following Folch et al. (1957). Gross ash content was determined gravimetrically following loss of mass after combustion of a sample in a muffle furnace at 550 ˚C for 24 h. Gross energy was determined by adiabatic bomb calorimetry (Parr 6200 Calorimeter, USA). All methods were consistent with (AOAC, 2005).

Fatty acid composition was determined following the methods of Christie (2003). Lipids were esterified by an acid-catalysed methylation and 0.3 mg of an internal standard was added to each sample (21:0 Supelco, PA, USA). The fatty acids were identified relative to the internal standard following separation by gas chromatography (GC). An Agilent Technologies 6890N GC system (Agilent Technologies, California, USA) fitted with a DB-23 (60m x 0.25mm x 0.15 μm, cat 122-2361 Agilent Technologies, California) capillary column and flame ionisation detection was used. The temperature program was 50–175 ºC at 25 ºC /min then 175–230 ºC at 2.5 ºC /min. The injector and detector temperatures were set at 250 ºC and 320 ºC, respectively. The column head pressure was set to constant pressure mode at 170 kPa using hydrogen as the carrier gas. The peaks were identified by comparing retention times to the internal standard and further referenced against known standards (37 Comp. FAME mix, Supelco, PA, USA). The resulting peaks were then corrected by the theoretical relative FID response factors (Ackman, 2002) and quantified relative to the internal standard.

2.3 Assessment of energy, lipid and fatty acid loss

The assessment of somatic losses was based on the formula previously reported by Glencross and Bermudes (2011):

$$Energy\ loss\ (kJ/day) = \frac{Wi * Ei - Wf * Ef}{t}$$

Where the *Wi* and *Wf* are the initial and final weights of the fish respectively. *Ei* and *Ef* are the initial and final energy content of the whole fish on a live-weight basis respectively. The duration of the assessment is denoted as *t*. The determination of lipid and fatty acid loss was calculated in the same way by substituting the appropriate *Ei* and *Ef* values with the corresponding values for either lipid or fatty acids.

2.4 Iteratively determined demands for fatty acids

Maintenance demands for each fatty acid were calculated based on the multiplication of the maintenance requirement by the regression of the transformed intake and deposition and then the proportion of each fatty acid present in the whole body lipids following methodology presented in Glencross (2008). Calculation and determination of fatty acid maintenance requirements were previously reported in barramundi (Salini et al., 2015a). The fatty acid gained was calculated as the mass of each fatty acid present in the lipid multiplied by the predicted daily growth following the growth equation developed for barramundi (Glencross, 2008):

$$Gain\ (g/fish/d) = \ (K + xT + yT^2 + zT^3) * (weight)^{ax+b}$$

where K and *b* are constants and *x, y, z* and *a* are determined coefficients of the functional growth response model. *T* is the temperature within an operating range of 16 to 39°C and *weight* is the geometric mean weight of the fish in grams ($GMW=(W_{initial} \times W_{final})^{0.5}$). The fatty acid requirement for growth was calculated as the fatty acid gained as a function of its utilisation efficiency (Salini et al., 2015a). The total fatty acid demand is the sum of the requirement for maintenance and growth.

2.5 Statistical analysis

All values are presented as mean ± standard error of the mean (SEM) unless otherwise stated. Energy, lipid and fatty acid losses were examined relative to the geometric mean weight in grams of the initial and final fish from each treatment size class. All relationships were examined using a power function ($y=aX^b$) or a logarithmic function ($y=b\ ln(x)+a$). Microsoft Excel (Microsoft Office 2007) was used to generate the equations and figures. A bootstrapping approach was used to generate replications of exponent values of energy, lipid and fatty acid loss in order to analyse the data statistically. Fatty acid exponents were then analysed by one-way ANOVA using the RStudio package v.0.98.501. Levels of significance were compared using Tukey's HSD test with significance defined as $P<0.05$.

3. Results

3.1 Fish compositional changes

The initial and final weights of the fish are presented in Table 1. In all size groups of fish weight loss was between 12.9 % for the smallest fish to 5.3 % for the largest fish. The condition factor was also lower in the fish after fasting. No fish died during the experiment. The initial and final chemical composition of the fish were analysed and reported in Table 1. The energy density of the barramundi of varying size before and after fasting was best fitted to a power function with high R^2 values of 0.845 and 0.844 respectively (Fig. 1). There was a decrease in the energy density of the fasted fish (Fig. 1). The lipid density of the barramundi was best fitted to a logarithmic function with initial and final R^2 values of 0.711 and 0.744 respectively (Fig. 2). The logarithmic response after fasting appears to be driven by Treatment F.

The individual fatty acid density of barramundi for each of the treatment size classes is presented in Table 2 and the LC-PUFA density is plotted in Fig. 3. There was a general increase in the fatty acid density with increasing size, concomitant with the lipid composition of the fish (Table 1). The response after fasting was best fitted to a logarithmic function that appears to be driven by the lipid content of Treatment F.

3.2 Determination of metabolic live-weight exponents

Somatic losses of energy, lipid and individual fatty acids were well described by the function $a*X^b$ (Table 3). A bootstrapping approach was used to generate replications of coefficient (slope) and exponent values of energy, lipid and fatty acid loss. An energy live-weight (LW) exponent of 0.817 was derived based on energy losses after fasting and the equation is presented in Eqn. 1. Similarly, a lipid loss LW exponent of 0.895 was derived based on lipid loss after fasting and the equation is presented in Eqn. 2. The relationships between fatty acid losses and the geometric mean weight over the range of sizes in the present study are presented in Fig. 4 (A and B).

$$\text{Energy loss (kJ/fish/day)} = 0.104(\pm 0.003)*(\text{Live-weight})^{0.817(\pm 0.010)},\ R^2 = 0.949 \quad (1)$$

$$\text{Lipid loss (g/fish/day)} = 0.002(\pm 0.000)*(\text{Live-weight})^{0.895(\pm 0.007)},\ R^2 = 0.985 \quad (2)$$

There was a significant difference in the derived LW exponent values for specific fatty acids confirming that they are for most, different from that of the total lipid ($LW^{0.895}$). However, there was no difference in the exponent values of 16:0 and 18:3n-3 (LNA) and 18:0 and 20:4n-6 (ARA) (Table 3). The LW exponent values for 22:6n-3 (DHA), 22:5n-3 (DPA) and 20:5n-3 (EPA), 0.792, 0.748 and 0.687 respectively, were all significantly lower than that of lipid while 18:1n-9 was higher at 0.954 (Table 3). The weighted exponent values were calculated and the sum of all fatty acids presented was equal to 0.854 ± 0.033 (Table 3; sum not presented).

3.3 Metabolic demands for fatty acids

A re-evaluation of the marginal utilisation efficiencies of individual fatty acids using the fatty acid LW exponents derived from the present experiment is presented in Table 4. This re-evaluation was performed on data from three prior experiments (Glencross and Rutherford, 2011; Salini et al., 2015a; Salini et al., 2016). The linear equations of the marginal intake to marginal gain ratio were extrapolated to zero ($0 = b(x) + a$) in order to obtain estimated maintenance requirement values. In the first experiment, the LC-PUFA all produced negative requirement values whereas all other shorter-chain length and more saturated fatty acids have a determined requirement. In the subsequent studies, the marginal utilisation efficiencies for ARA, EPA and DHA were higher, contrasting those of the first study. There was no requirement value established for EPA and DHA, however there was a maintenance requirement of 0.012 $g/kg^{0.880}/d$ determined for ARA.

Iteratively determined fatty acid maintenance, fatty acid gain, fatty acid for growth and total requirements are presented in Table 5. For each of the size classes the values are presented for barramundi fed either 100% fish oil or 100% poultry oil diets adapted from Salini et al. (2015a).

4. Discussion

One of the key assumptions of nutritional modelling is that the allometric scaling exponent values for biological variables ascribed to transform live-weight (LW) are constant (Glencross and Bermudes, 2011; Lupatsch et al., 2003). In reality, exponent values for the

metabolic LW for energy in aquatic species usually fit around an average value of 0.80, which has been adopted and used routinely (Bureau et al., 2002; Cho and Kaushik, 1990; Cui and Liu, 1990; Lupatsch et al., 2003; NRC, 2011). Similarly for protein, a range of LW exponents have been used to describe the allometric relationship and the value of 0.70 is routinely used under normal physiological conditions (Lupatsch et al., 1998; Pirozzi et al., 2010). Arguably the average of the weighted LW exponents for lipid and protein energy should be equal to that of gross energy, therefore 0.90 can be ascribed to LW exponent of lipid (Glencross and Bermudes, 2011). The development of predictive models of energy transactions that also consider the individual compounds of nutrients rather than aggregates of energy would help in understanding the discrete biochemical relationships that exist and some attempts at compartmentalising these have been made in monogastric animals (Birkett and Lange, 2007). However, these are not common in the literature or in practice for aquatic species. The present study therefore investigated the allometric scaling effect of specific fatty acids in barramundi held at a constant temperature.

In the present study, the assessment of somatic energy before and after fasting was highly consistent with the study of Glencross and Bermudes (2011). This suggests that over the variable size range of fish used in the present study and held at an optimal temperature, fasting losses are quite predictable, further validating the methodology to be extended to specific fatty acids. One caveat of the present study was that the size class selection of the fish was limited to two initial shipments of fish that were held in stock aquaria and subsequently graded. Therefore we cannot conclude on what may happen outside this range or within the range if additional treatments were available. The increasing live-weight as a function of energy and lipid density of the fish was best fitted to power and natural logarithmic equations respectively. The lower than expected analytical values obtained for lipid in Treatment F are likely related to the nutritional status of the fish prior to the commencement of the study however there is no consistent explanation for this. Consistent with other studies, the loss of lipid was concomitant to the loss of energy, confirming that lipid is preferentially metabolised under fasting conditions in order to retain protein (Glencross and Bermudes, 2011; Lupatsch et al., 1998).

The somatic losses determined in the present study were best described by power functions, following the equation $y=a*X^b$, where a represents a temperature dependent coefficient, X is the live-weight (LW) and b the scaling exponent. An important finding of the present study was that the energy LW exponent value (0.817 ± 0.010) is consistent with the commonly

reported value of 0.80. This is an important finding as previous studies have found that even slight variations in the reported exponent values can lead to substantially different outcomes when applied to the determination of maintenance energy demands (Pirozzi, 2009). The lipid exponent value of 0.895 ± 0.007 was also highly consistent with the values previously described for barramundi (Glencross and Bermudes, 2011). One caveat of the present study was that only a single temperature range was examined; however, it is reported that provided barramundi are held within their normal temperature range then the values should be mostly consistent (Glencross and Bermudes, 2011).

The fatty acid allometric scaling exponent values derived in the present study were significantly different and ranged from 0.687 ± 0.005 for EPA to 0.954 ± 0.008 for 18:1n-9. With the exception of ARA, all the LC-PUFA exponent values were significantly lower than the more dominant shorter-chain length and more saturated fatty acids. The individual fatty acids presented as weighted exponent values are also consistent with that of lipid as a complete nutrient (0.854 ± 0.033 vs. 0.895 ± 0.007 respectively). The lower exponent values recorded for the LC-PUFA suggest that there is likely to be a greater turnover of these fatty acids in the juvenile fish indicating more specific biological demands. While the higher exponents (eg. 18:1n-9) suggest there is less effect of size and lower biological demands for those fatty acids. Additionally, the LC-PUFA with lower exponent values also have marginal utilisation efficiencies that are considerably lower than other more dominant fatty acids (Salini et al., 2015a). This lends further support to the theory that they are more biologically important and that they are selectively retained in the tissues, corroborating evidence from past studies in barramundi (Glencross and Rutherford, 2011; Salini et al., 2015c). Moreover, the significance of LC-PUFA is also be supported by their anti-inflammatory role in the production of eicosanoids and specialised proresolving mediators (Bannenberg and Serhan, 2010; Rowley et al., 1995; Serhan, 2010).

The energy from metabolizable food in juvenile animals can only really be partitioned into maintenance and growth as reproductive effort is essentially zero (Lucas, 1996; NRC, 2011). Moreover, the concept of maintenance and growth demands are additive in terms of productivity (Bureau et al., 2002; Clarke and Fraser, 2004). A range of data pools were used in the analysis of the present study in order to iteratively determine the metabolic demands for specific fatty acids in growing barramundi. The partial (marginal) efficiency values from Salini et al. (2015a) were re-calculated with the newer exponent values derived in the present study. The LW exponent of lipid (0.90) was applied to specific fatty acids and acknowledged

as an assumption in that earlier study. This re-calculation allowed a more accurate determination of maintenance fatty acid demands given the acknowledged impact that this transformation can have on the determination of that parameter (Pirozzi, 2009). We also assessed the suitability of marginal efficiency values for ARA, EPA and DHA determined from subsequent studies (Glencross and Rutherford, 2011; Salini et al., 2016). However these values were inconsistent with those of Salini et al. (2015a) and not included in the final analysis (Table 5). Reasons for these inconsistencies are likely to be due to the large differences in the initial size of the fish and the feeding regime utilised.

The results of the present study demonstrate that the different fatty acids are utilised with different efficiencies. However, contrary to what might be expected, the levels of LC-PUFA required in barramundi fed a fish oil based diet are numerically higher than those fed a poultry oil based diet. This apparent difference is driven largely by the deposition demands of individual fatty acids rather than catabolism or other processes. Based on the demands (requirements) for maintenance presented in Table 5, we could conclude that the LC-PUFA requirements are negligible (Birkett and Lange, 2007). This conclusion may be more generally applied to larger growing barramundi however, evidence suggests that essential fatty acid requirements are more pronounced during the rapid growth phase of juveniles, and virtually negligible at larger fish sizes (Salini et al., 2015c).

The relative contribution of the more dominant shorter-chain length and more saturated fatty acids for the provision of energy is clear. This corroborates with data recently obtained in barramundi where the monounsaturated and to a lesser extent saturated fatty acids ‘spared’ LC-PUFA for deposition and were preferentially utilised as energy sources (Salini et al., 2015b). This supports that the available lipids are partitioned into either those fatty acids directed towards oxidative fates for generating energy or those directed towards other downstream biological purpose such as eicosanoid production.

There are many potential assumptions in the application of energetic models (Glencross, 2008). The current allometric assessment only considers a single phenotypic parameter (live-weight). Not surprisingly, past reports have concluded that temperature plays a key role in the metabolism of ectotherms, including fish (Clarke and Fraser, 2004; Clarke and Johnston, 1999; Glencross and Bermudes, 2011; Pirozzi et al., 2010). With barramundi, Glencross and Bermudes (2011) demonstrated that the allometric scaling over a range of temperatures did change; however, the response was not dramatic under normal thermal conditions for the

species. Therefore, we assume that the effect of temperature would be minimised by using a constant ‘optimal’ temperature of 30°C.

Additionally, there are many studies investigating the metabolic rate in animals and these relationships with size can usually be described similarly using non-linear power equations or variants of these (Clarke and Johnston, 1999; White, 2011). The assumption in the present study is that the standard metabolic rate does not change under fasting conditions as this could further impact the somatic losses incurred. There is evidence to suggest that in fish and crustaceans, the standard metabolic rate is reduced by up to 50% during fasting and this is due partly to decreased protein synthesis (O'Connor et al., 2000; Simon et al., 2015). Without an estimation of oxygen consumption or another measure of standard metabolic rate, we cannot conclude on what might happen on a temporal basis under fasting conditions.

The present study demonstrated that allometric scaling exponents of specific fatty acids varied after food deprivation for 21 days in barramundi. The underlying assumption so far has been that the scaling exponent of lipid (0.90) could be applied at a nutrient level to any situation involving fatty acids, including the calculation of maintenance demands. The results of the present study indicate that there are differences allometric scaling values of the individual fatty acids and in the utilisation efficiencies of individual fatty acids, corroborating evidence from past studies. After re-evaluating data from three separate experiments we have concluded that the biggest driver in our understanding of LC-PUFA metabolism in barramundi is that of deposition demand. Empirically based models should now attempt to consider the energetic costs associated with the lipid metabolic pathway, as this would be the logical progression of the current work.

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Legends

Figure 1. Energy density of barramundi of varying live-weight before (●=4.221(±0.010)$x^{0.088(\pm0.001)}$, R^2 = 0.845) and after (○=3.359(±0.011)$x^{0.118(\pm0.001)}$, R^2 = 0.844) fasting for 21 days.

Figure 2. Lipid density of the barramundi of varying live-weight before (●=0.807(±0.019)ln(x) + 2.312(±0.002), R^2 = 0.711) and after (○=0.981(±0.014)ln(x) – 0.083(±0.003), R^2 = 0.744) fasting for 21 days.

Figure 3. Fatty acid density (mg/g lipid) in barramundi of varying live-weight after fasting for 21 days. Values were fitted to a logarithmic curve and equations are presented in Table 2.

Figure 4. Fatty acid (A and B) loss in fasted barramundi of varying live-weight. Data are (n=3) mean ± SEM. Values were fitted to a power function and equations are presented in Table 3.

Table 1. Performance parameters and chemical composition for initial and final barramundi of varying sizes. Data are presented as mean ± SEM and composition data are presented on a wet-weight basis.

	Treatment A	Treatment B	Treatment C	Treatment D	Treatment E	Treatment F
Biometric parameters						
Initial weight (g/fish)	10.5 ± 0.1	19.2 ± 0.1	28.3 ± 0.1	122.4 ± 0.1	217.6 ± 0.4	443.7 ± 1.5
Final weight (g/fish)	9.2 ± 0.1	17.3 ± 0.1	25.6 ± 0.1	114.3 ± 0.1	206.5 ± 0.3	420.0 ± 1.0
Weight loss (g/fish)	1.4 ± 0.1	1.9 ± 0.0	2.7 ± 0.2	8.1 ± 0.2	11.1 ± 0.2	23.7 ± 1.6
Weight loss (%)	12.9 ± 0.7	9.9 ± 0.1	9.6 ± 0.5	6.6 ± 0.2	5.1 ± 0.1	5.3 ± 0.3
Condition initial*	1.2 ± 0.0	1.2 ± 0.0	1.3 ± 0.1	1.2 ± 0.0	1.2 ± 0.1	1.2 ± 0.0
Condition final*	1.1 ± 0.0	1.0 ± 0.0	1.1 ± 0.1	1.1 ± 0.0	1.1 ± 0.1	1.1 ± 0.0
Initial composition						
Dry matter (%)	24.4	24.4	27.2	27.2	29.7	30.9
Protein (%)	16.0	15.2	17.1	17.2	17.9	19.2
Ash (%)	3.8	3.8	3.7	3.1	4.1	4.8
Lipid (%)	3.8	4.0	6.0	6.3	7.2	6.4
Gross energy (MJ/kg)	5.1	5.2	6.2	6.5	7.0	6.9
Final composition						
Dry matter (%)	21.7 ± 0.7	22.0 ± 0.1	25.6 ± 0.3	27.4 ± 0.3	28.1 ± 0.3	29.6 ± 0.2
Protein (%)	15.1 ± 0.6	15.4 ± 0.1	16.7 ± 0.4	17.6 ± 0.5	18.2 ± 0.3	18.5 ± 0.1
Ash (%)	4.5 ± 0.1	4.4 ± 0.3	4.2 ± 0.1	3.7 ± 0.1	4.2 ± 0.1	5.4 ± 0.1
Lipid (%)	1.6 ± 0.2	1.9 ± 0.1	4.3 ± 0.1	5.3 ± 0.2	5.2 ± 0.2	5.1 ± 0.1
Gross energy (MJ/kg)	4.2 ± 0.1	4.4 ± 0.1	5.4 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	6.4 ± 0.1

*Condition factor=weight/length^3

Table 2. Fatty acid density in the fish (mg/g/fish) after 21 days of fasting. Data were fitted to logarithmic functions and presented as mean ± SEM.

	Treatment A	Treatment B	Treatment C	Treatment D	Treatment E	Treatment F	Equation	R^2
16:0	30.0 ± 1.0	35.1 ± 0.7	66.0 ± 2.9	97.9 ± 0.5	94.8 ± 0.9	99.1 ± 0.3	y=19.728(±0.101)ln(x) – 9.833(±0.398)	0.883
18:0	10.8 ± 0.4	11.6 ± 0.2	18.6 ± 0.8	28.1 ± 0.3	27.0 ± 0.4	27.2 ± 0.1	y=4.935(±0.028)ln(x) + 0.466(±0.104)	0.873
18:1n-9	39.3 ± 0.5	47.8 ± 0.2	90.0 ± 4.0	164.3 ± 0.3	161.0 ± 0.4	147.8 ± 0.1	y=34.580(±0.245)ln(x) – 32.392(±0.824)	0.849
18:2n-6	14.0 ± 0.0	17.3 ± 0.1	28.2 ± 1.1	57.9 ± 0.1	58.3 ± 0.5	51.9 ± 0.1	y=12.511(±0.083)ln(x) – 13.006(±0.281)	0.858
18:3n-3	1.3 ± 0.3	1.8 ± 0.1	3.5 ± 0.0	5.8 ± 0.2	5.9 ± 0.2	5.0 ± 0.2	y=1.195(±0.008)ln(x) – 0.985(±0.027)	0.799
20:4n-6	2.6 ± 1.8	2.5 ± 0.9	2.6 ± 0.1	3.6 ± 1.3	4.2 ± 2.9	3.1 ± 2.4	y=0.325(±0.004)ln(x) + 1.771(±0.018)	0.531
20:5n-3	4.1 ± 6.2	5.0 ± 1.6	8.6 ± 0.3	9.0 ± 5.5	8.8 ± 7.2	10.1 ± 5.0	y=1.408(±0.009)ln(x) + 1.884(±0.045)	0.763
22:5n-3	3.3 ± 1.1	3.7 ± 0.4	5.5 ± 0.1	6.5 ± 0.9	6.3 ± 1.4	7.1 ± 0.6	y=0.954(±0.005)ln(x) + 1.510(±0.023)	0.867
22:6n-3	13.9 ± 4.4	15.2 ± 1.0	20.2 ± 0.5	22.2 ± 2.7	21.7 ± 4.7	21.8 ± 1.8	y=2.224(± 0.017)ln(x) + 10.649(± 0.073)	0.756

Table 3. Coefficient and exponent values derived from the power function ($y=aX^b$) of fatty acid loss over a wide range of fish sizes from ~10 g to ~440 g. Replication was derived by manually bootstrapping each individual value and presented as mean ± SEM (n=18).

	Coefficient (*a*)	Exponent (*b*)	R^2	Weighted Exponent*
Energy	0.104 ± 0.003	0.817 ± 0.010	0.949	NA
Lipid	0.002 ± 0.000	0.895 ± 0.007[a]	0.985	NA
16:0	0.346 ± 0.010	0.890 ± 0.024[a]	0.992	0.208 ± 0.012
18:0	0.107 ± 0.003	0.876 ± 0.010[b]	0.991	0.061 ± 0.004
18:1n-9	0.399 ± 0.011	0.954 ± 0.008[c]	0.992	0.350 ± 0.007
18:2n-6	0.182 ± 0.005	0.915 ± 0.009[d]	0.992	0.120 ± 0.003
18:3n-3	0.026 ± 0.001	0.899 ± 0.007[a]	0.991	0.013 ± 0.000
ARA	0.012 ± 0.000	0.796 ± 0.007[b]	0.942	0.009 ± 0.001
EPA	0.114 ± 0.002	0.687 ± 0.005[e]	0.990	0.021 ± 0.001
DPA	0.038 ± 0.001	0.748 ± 0.008[f]	0.985	0.015 ± 0.001
DHA	0.138 ± 0.003	0.792 ± 0.006[g]	0.990	0.058 ± 0.004
P value	NA	$<2.2^{-16}$	NA	NA

NA, not analysed.

* Calculated as geometric mean weight of each fatty acid x exponent (*b*).

Table 4. Re-evaluation the marginal efficiency of fatty acid utilisation in barramundi. Data were transformed to LW exponent values determined from the present study. Maintenance requirements and intake to gain ratio for each fatty acid are presented.

Fatty acid	Slope	Intercept	R^2	Req[#]	1/k[*]
Salini et al., (2015)					
16:0	2.258	-0.536	0.712	0.237	0.443
18:0	1.539	-0.068	0.837	0.044	0.650
18:1	1.111	-0.178	0.951	0.161	0.900
LOA	0.821	-0.025	0.751	0.031	1.218
LNA	1.040	-0.010	0.881	0.010	0.962
ARA	0.192	0.005	0.427	-0.025	5.222
EPA	0.305	0.005	0.953	-0.017	3.279
DPA	0.340	0.008	0.783	-0.023	2.943
DHA	0.271	0.014	0.952	-0.050	3.693
Salini et al., (2016)					
ARA	0.919	-0.011	0.965	0.012	1.088
EPA	0.621	0.000	0.975	-0.000	1.610
Glencross and Rutherford, (2011)					
DHA	1.065	0.046	0.961	-0.043	0.939

Maintenance requirement ($g/kg^x/d$) determined by extrapolation to $0 = b\ (x) + a$.

* Intake to gain ratio.

Table 5. Fatty acid demands in growing barramundi fed either 100% fish oil (FO) or 100% poultry oil (PO) diets maintained at 30°C. Calculations are based on the predictive growth models and utilisation efficiencies from published studies for this species.

Fish live weight (g/fish)	50	100	500	1000	2000	50	100	500	1000	2000
Expected growth (g/day)[1]	2.13	2.88	5.81	7.85	10.61	2.13	2.88	5.81	7.85	10.61
Diet[2]	FO	FO	FO	FO	FO	PO	PO	PO	PO	PO
16:0 demands										
16:0-maint (mg/fish/d)[3]	0.004	0.007	0.030	0.055	0.103	0.004	0.007	0.027	0.051	0.094
16:0 gain (mg/fish/d)[4]	0.028	0.042	0.108	0.163	0.245	0.025	0.038	0.099	0.150	0.225
16:0-growth (mg/fish/d)[5]	0.012	0.018	0.048	0.072	0.109	0.011	0.017	0.044	0.066	0.100
16:0-total (mg/fish/d)[6]	0.016	0.026	0.078	0.127	0.211	0.015	0.023	0.071	0.117	0.194
18:0 demands[7]										
18:0-maint (mg/fish/d)	0.000	0.000	0.002	0.003	0.006	0.000	0.000	0.002	0.003	0.005
18:0 gain (mg/fish/d)	0.008	0.012	0.032	0.048	0.072	0.008	0.012	0.031	0.047	0.070
18:0-growth (mg/fish/d)	0.005	0.008	0.021	0.031	0.047	0.005	0.008	0.020	0.030	0.046
18:0-total (mg/fish/d)	0.005	0.008	0.022	0.034	0.052	0.005	0.008	0.022	0.033	0.051
18:1 demands[7]										
18:1-maint (mg/fish/d)	0.003	0.006	0.027	0.051	0.099	0.004	0.007	0.032	0.062	0.120
18:1 gain (mg/fish/d)	0.038	0.057	0.148	0.222	0.335	0.046	0.069	0.179	0.269	0.406
18:1-growth (mg/fish/d)	0.034	0.051	0.133	0.200	0.302	0.041	0.062	0.161	0.242	0.365
18:1- total (mg/fish/d)	0.037	0.057	0.159	0.251	0.401	0.045	0.069	0.193	0.304	0.485
18:2 demands[7]										
18:2-maint (mg/fish/d)	0.000	0.000	0.001	0.002	0.005	0.000	0.000	0.002	0.004	0.007
18:2 gain (mg/fish/d)	0.010	0.014	0.037	0.056	0.085	0.014	0.021	0.055	0.084	0.126
18:2-growth (mg/fish/d)	0.012	0.017	0.045	0.068	0.103	0.017	0.026	0.067	0.102	0.153
18:2-total (mg/fish/d)	0.012	0.018	0.047	0.071	0.108	0.018	0.026	0.069	0.105	0.160
18:3 demands[7]										
18:3-maint (mg/fish/d)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
18:3 gain (mg/fish/d)	0.001	0.001	0.004	0.005	0.008	0.002	0.002	0.006	0.009	0.013
18:3-growth (mg/fish/d)	0.001	0.001	0.003	0.005	0.008	0.001	0.002	0.006	0.009	0.013
18:3-total (mg/fish/d)	0.001	0.001	0.004	0.005	0.008	0.001	0.002	0.006	0.009	0.013

ARA demands[7]										
ARA-maint (mg/fish/d)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ARA gain (mg/fish/d)	0.001	0.001	0.003	0.005	0.007	0.001	0.001	0.002	0.003	0.005
ARA-growth (mg/fish/day)	0.004	0.006	0.016	0.023	0.035	0.003	0.004	0.011	0.016	0.024
ARA-total (mg/fish/day)	0.004	0.006	0.016	0.024	0.036	0.003	0.004	0.011	0.016	0.024
EPA demands[7]										
EPA-maint (mg/fish/d)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
EPA gain (mg/fish/d)	0.005	0.007	0.018	0.027	0.040	0.001	0.001	0.003	0.005	0.008
EPA-growth (mg/fish/day)	0.015	0.022	0.058	0.087	0.131	0.003	0.004	0.011	0.017	0.025
EPA-total (mg/fish/day)	0.015	0.022	0.058	0.087	0.131	0.003	0.004	0.011	0.017	0.025
DPA demands[7]										
DPA-maint (mg/fish/d)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DPA gain (mg/fish/d)	0.001	0.002	0.006	0.009	0.013	0.001	0.001	0.003	0.004	0.006
DPA-growth (mg/fish/day)	0.004	0.007	0.017	0.026	0.039	0.002	0.003	0.008	0.012	0.017
DPA-total (mg/fish/day)	0.004	0.007	0.017	0.026	0.039	0.002	0.003	0.008	0.012	0.017
DHA demands[7]										
DHA-maint (mg/fish/d)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DHA gain (mg/fish/d)	0.005	0.007	0.019	0.029	0.043	0.002	0.003	0.008	0.012	0.018
DHA-growth (mg/fish/day)	0.018	0.027	0.070	0.106	0.159	0.008	0.011	0.029	0.044	0.067
DHA-total (mg/fish/day)	0.018	0.027	0.070	0.106	0.159	0.008	0.011	0.029	0.044	0.067

1 Modelled daily growth based on 30°C water temperature (Glencross, 2008; Glencross and Bermudes, 2012).

2 Data for the calculation of fatty acid demands were taken from previously published studies (Salini et al., 2015).

3 Maintenance digestible fatty acid requirements based on extrapolated values (Table 4), per exponent transformed fatty acid body weight (Table 3) and multiplied by the whole body fatty acids (g/kg/fish).

4 Fatty acid content of the modelled live-weight gain.

5 Digestible fatty acid demand based on the gain through modelled growth divided by the utilisation efficiency of that fatty acid.

6 Combined digestible demand for both maintenance and growth.

7 Refer to *16:0 demands*.

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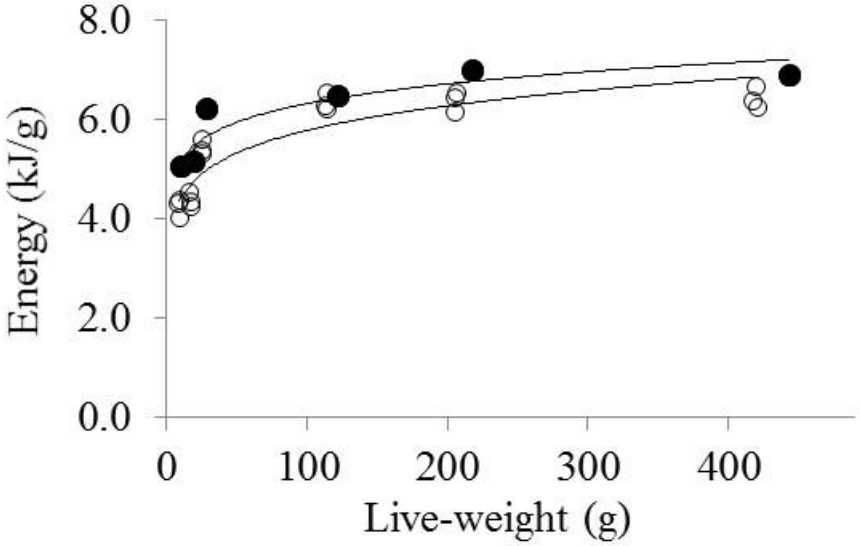
499 Figure 1



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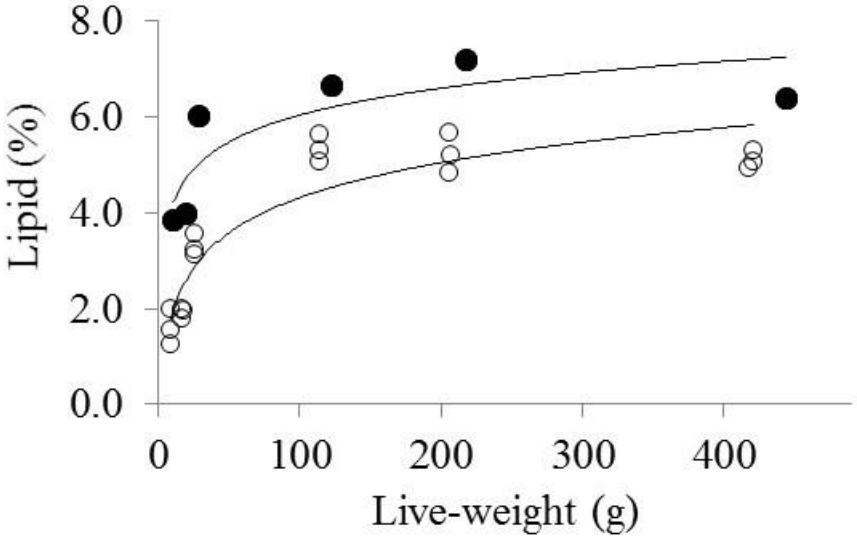
502 Figure 2



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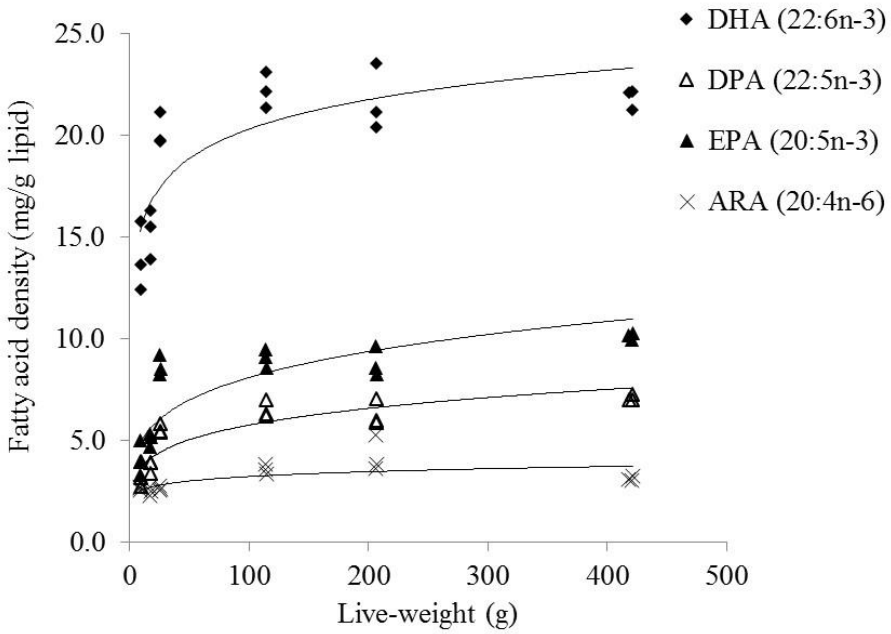
506 Figure 3



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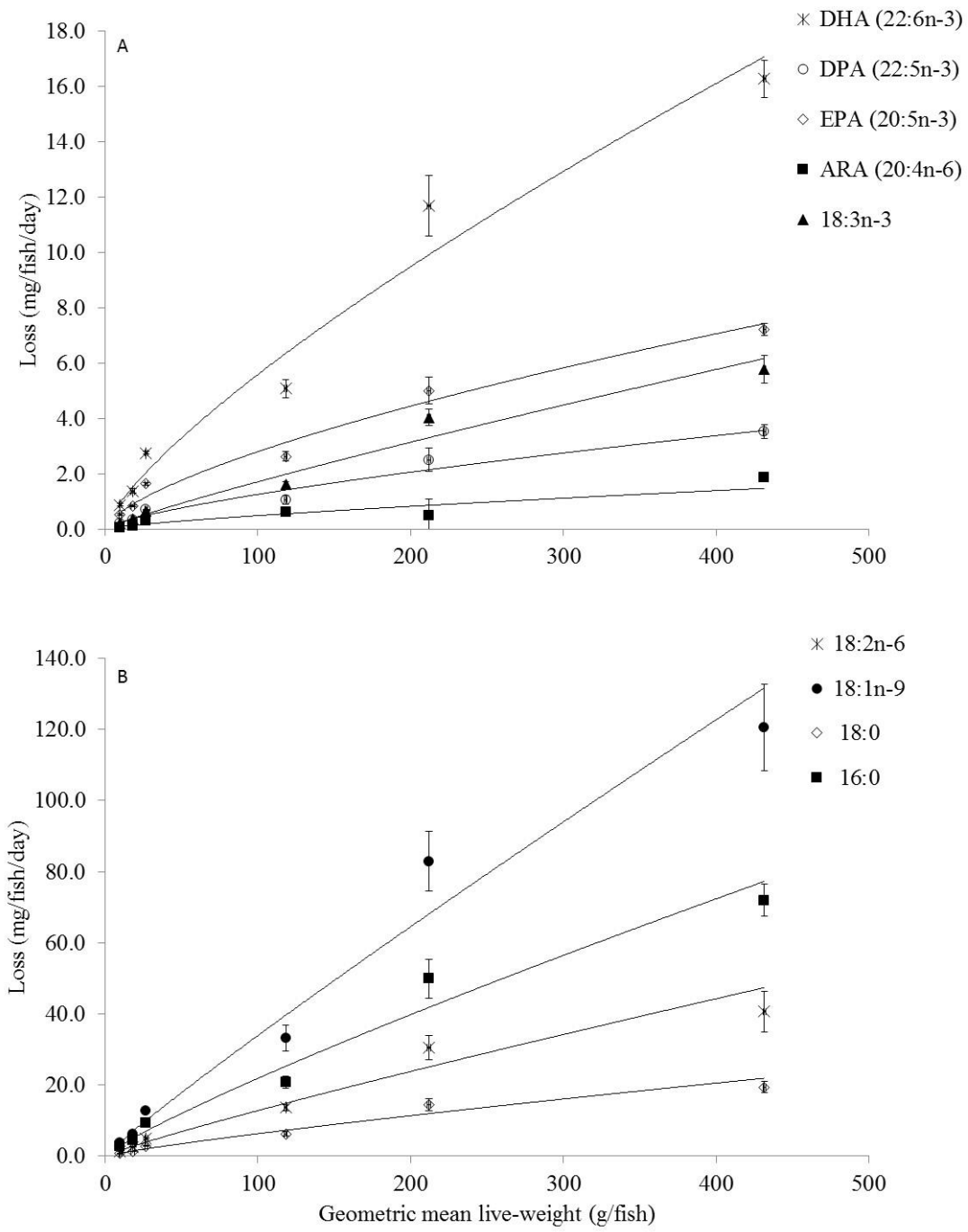
510 Figure 4



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