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Development of a nutritional model to define the energy and protein requirements of cobia, *Rachycentron canadum*

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Abstract

This study assessed the protein and energy requirements of Cobia (*Rachycentron canadum*) using a bio-energetic factorial approach. Using a series of inter-related studies, several parameters were defined to enable the construction of a bio-energetic factorial model for this species. The studies included two controlled laboratory experiments and also extensive field-data collection from commercial and research farms in Vietnam. The devised model includes parameters for both maintenance and protein demands; the effect of fish live-weight on maintenance protein ($LW^{0.697}$), lipid ($LW^{0.972}$), and energy demands ($LW^{0.815}$); the efficiencies of protein, lipid and energy utilisation at various protein, lipid and energy intake levels; and the variability in whole body composition with varying live-weight. The protein utilisation efficiencies (0.456 • [protein intake] – 0.445), lipid utilisation efficiencies (1.292 • [lipid intake] – 1.120) and energy utilisation efficiencies (0.651 • [energy intake] – 48.41) were similar to other carnivorous fish species. However, the maintenance requirements for both energy (74.3 kJ/ $kgBW^{0.8}$/ d^{-} at 28ºC) and protein (0.99 g/ $kgBW^{0.7}$/d at 27.9ºC) were about double to other species. Using this modelling approach it was possible to iteratively derive optimal dietary protein and energy specifications for this species.

1. Introduction

Cobia (*Rachycentron canadum*) is the only species in the family Rachycentridae. The species is distributed worldwide in warm marine waters, accept for the central and eastern Pacific. The species is generally regarded as a fast growing, tropical pelagic animal. In offshore net cage systems, cobia can grow from 0.5 kg fingerling to 6.0 to 8.0 kg marketable size within 6 to 8 months with a feed conversion ratio of 1.5 (Liao *et al.*, 2004) or 6 kg after 1 year at 28ºC (Benetti *et al.*, 2010). Due to their high quality white flesh, cobia is suitable for sashimi or fillet production (Chou *et al.*, 2001). The global aquaculture production of Cobia has increasing rapidly from 2002, reaching to 41,774 MT in 2012 (FAO, 2014). The three main producers of cobia in 2012 were China, Taiwan and Vietnam, where annual production was approximately 38,014 metric tons (MT), 1,384 MT and 2,000 MT, respectively (FAO, 2014). While Cobia cultured in offshore net cage systems is generally reared using formulated feeds (Liao *et al.,* 2004), most cobia production in traditional inshore sea cages is still based on trash fish (Petersen *et al.*, 2015). Currently, the limited supply of trash fish as the main feed source for cobia grow-out has become a major constraint for cobia culture in Viet Nam and other countries.

Cobia culture has been rapidly gaining in popularity since the early 1990s, but formulated feed development for aquaculture of this species is still lagging behind compared with other fish species such as salmon or barramundi (Zhou *et al.,* 2007; Xiao *et al.*, 2009; Liu *et al.*, 2010). Despite, many studies have been undertaken to identify a range of nutritional requirements of this species, the energy and protein requirements are still undefined and pelleted feed are still not well established (Salze *et al.,* 2010). Earlier studies have suggested that the optimum dietary protein and lipid levels in juvenile cobia were 45% and 5–15% dry weight, respectively (Chou *et al.,* 2001; Craig *et al.*, 2006). Maximum growth and the best feed conversion ratios have been recorded at 27–29°C in juvenile cobia with an optimum feed ration level determined at 9% initial body weight per day for fish of 10-200g live-weight (Sun *et al.*, 2006; Webb, 2009; Sun and Chen, 2014).

The requirements for protein and energy for most aquaculture species have traditionally been determined using empirical dose-response studies (Mercer, 1982). More recently, the use of bio-energetic factorial modelling has proven to be a useful alternative method in estimating these requirements (Shearer, 1995; Glencross, 2008; Trung *et al.,* 2011). The benefits of bio-energetic factorial modelling are that it provides a method for estimating nutritional requirements independent of animal size and it results in a series of nutrient specifications that are indexed against energy demand and as such it underpins the potential for a wide range of diet specifications to be developed subject to different formulation strategies (Lupatsch *et al.,* 2003; Booth *et al.,* 2010; Glencross *et al.*, 2011). Additionally, this modelling approach also has an advantage over an empirical approach in that it can also be used to define the optimal feed rations as well as specifications. This has further merits in that total nutrient and energy budgets, including losses through wastage and excretion, and also raw material demands can be determined and strategies examined by which to improve fish production (Glencross, 2010).

This paper describes a series of studies designed to determine the energy and protein requirements of cobia (*R. canadum*). Using farm-collected data, samples and experiments from both Vietnam and Australia, and a series of studies undertaken to determine key parameters of the model. These parameters include; the estimation of growth potential of fish with varying size, changes in body protein and energy composition with fish size; determination of the energy and protein requirements for maintenance, determination of the protein and energy digestibility of a reference diet, and determination of the partial efficiencies of both protein and energy utilisation. From this series of studies the results are then integrated to present an iterative approach to the determination of the protein and energy requirements for this species over a range of fish sizes.

2. Methods

2.1 Study 1 – Endogenous losses of protein, lipid and energy

This experiment was conducted at the Cat Ba National Broodstock Center of Marine Aquaculture of the Research Institute for Aquaculture - 1 (RIA-1), in Vietnam. Twelve 1,000 L tanks were each stocked with ten cobia (*R. canadum*). Fish sizes within each tank were in one of four general size classes (100 g, 200 g, 500 g and 1,000 g fish^{-1}), with three replicates being used for each size class. Additional fish (n=5 for each size class) of similar approximate weights to those four size classes were euthanized at the beginning of the study to determine the dry matter, ash, protein, lipid and energy composition of the fish at the beginning of the study. The experimental tanks were supplied with aeration, flow-through marine water (salinity 32PSU) at 28.4 ± 1.58°C. The transferred fish were kept in the tanks for 21 days, without feeding. After this period the fish were re-weighed and all fish from each tank were used as a replicate to determine weight, energy, lipid and protein loss. Following weighing five of the fish from each size class were euthanized, pooled and assessed for composition change in dry matter, ash, lipid, protein and energy concentrations.

2.2 Study 2 – Energy and protein digestibility

This study was conducted at the Cleveland Laboratory of the CSIRO Aquaculture Program in Australia. A single basal diet was formulated to provide protein and lipid at 489 g/kg and 138 g/kg diet at a gross energy level of 22.2 MJ kg^{-1} (estimated digestible protein and energy of 406 g kg^{-1} and 19.7 MJ kg^{-1}, respectively) (Table 1). The dry ingredients were first blended in a series of batches using a 60 L upright Hobart mixer (HL600, Hobart, Pinkenba, QLD, Australia), to produce a single batch of basal mash which was extruded using a laboratory-scale, twin-screw extruder with intermeshing, co-rotating screws (MPF19:25, Baker Perkins, Peterborough, United Kingdom). The resultant pellets produced through a 3 mm Ø die were cut into 4 to 5 mm lengths using a four-bladed variable speed cutter and collected and dried at 60ºC for 12 h in a fan-forced drying oven. The remaining oil allocation was vacuum infused post-drying according to the methods reported by Diu et al (2015).

TABLE 1 HERE

Three 100 L tanks of flow through seawater (27.9 ± 0.32ºC) were each stocked with 10 juvenile (~200 g) fish. The transferred fish were allowed to acclimate to the tanks and were fed the reference diet for 23 days before faecal collection was initiated. Faeces were collected using stripping techniques similar to that used for barramundi (Blyth *et al.*, 2014).

Diet and faecal samples were analysed for dry matter, yttrium, protein, total lipid, gross energy and ash content (AOAC, 2005). Differences in the concentrations of the protein, lipid, energy and yttrium in the feed and faeces on a dry matter basis in each treatment were calculated to determine the apparent digestibility (AD_{diet}) of each nutritional parameter. Those digestibilities examined were based on the following equation:

$$ADC = \left(1 - \frac{Y_{feed} \times Parameter_{faeces}}{Y_{faeces} \times Parameter_{feed}}\right)$$

Where Y_{diet} and Y_{faeces} represent the yttrium content of the diet and faeces respectively, and $Parameter_{diet}$ and $Parameter_{faeces}$ represent the nutritional parameter of concern (protein, lipid or energy) content of the diet and faeces respectively.

2.3 Study 3 – Energy and protein utilisation efficiency

This study was conducted at the Cleveland Laboratory of the CSIRO Aquaculture Program in Australia. Twenty four 100 L tanks were each stocked with 10 cobia juveniles (mean weight 136.2 ± 0.71 g) . A series of six feed ration treatments were assigned in quadruplicate to the array. The same diet as used in the digestibility study was used in this study (Table 1). Each ration level was determined based on satiety, 80%, 60%, 40%, 20% of satiety and starved. The sub-satietal levels were estimated based on feed intake measured in the three days preceding the initiation of the experiment when fish were being acclimated to the tanks. Water temperature was maintained at 27.9 ± 0.32˚C for the duration of the study. The trial was run for 23 days to minimize the time that fish were unfed before a result could be obtained. The apparent satiety ration level was determined based on the loss of feeding activity after the fish being offered food on three or more independent feeding episodes within a one-hour period. Any uneaten food was collected by siphoning and accounted for. After 23 days the weight gain was assessed by weighing all fish within each tank to determine tank mean weight gain. At this point three fish from each tank were also euthanized and whole fish samples were collected for the analysis of dry matter, protein, lipid and energy content.

2.4 Study 4 – Fish composition variation

A range of sizes of cobia from 25 g to 2013 g were collected (n=18) from both laboratory stocks and commercial grow-out producers in northern Vietnam, with further fish also sourced from the Bribie Island Research Centre in Woorim, QLD, Australia. Whole fish were minced and then analysed for dry matter, protein, lipid and energy content. These fish were obviously fed a range of diets, therefore representing the average genetic response of the species to variations in dietary protein and energy balance provision in diets. All analyses were conducted according to the methods specified by the AOAC (2005).

2.5 Study 5 – Assessment of fish growth rates

Growth rates were assessed from a combination of both farm and laboratory data sources. Eight commercial sea cage production facilities in Khanh Hoa, Cat Ba and Nghe An provinces in Vietnam were each assessed at monthly intervals (from April 2010 to April 2011) by weighing around 15 fish from each cage to determine mean weight gain and daily growth rates (g day^{-1}). Growth rates (range 0.23 to 17.77 g day^{-1}) were expressed relative to the geometric mean weight (range 4 to 5,040 g fish^{-1}) of the fish from each measurement. Water temperature (range 18.0 to 29.5 ºC, mean ± SD = 25.6 ± 2.8ºC) was also measured at each sampling time.

2.6 Chemical analyses

Fish and samples of the reference diet were analysed for dry matter, protein, total lipids and energy content. Diet and faecal samples were also analysed for yttrium. Dry matter was calculated by gravimetric analysis following oven drying at 105ºC for 24 h. Gross energy content was determined using ballistic bomb calorimetry or in some cases calculated using protein = 23.6 kJ g^{-1}, lipid = 38.5 kJ g^{-1} and carbohydrate = 17.3 kJ g^{-1}. Protein levels were calculated from the determination of total nitrogen by combustion analysis using a CHNOS autoanalyser in Australia and Kjeldhal in Vietnam and multiplying N by 6.25. Total lipid contents were determined gravimetrically following extraction by chloroform and methanol (2:1 v/v) solution according to the method of Folch (1957). Total yttrium concentrations were determined after digestion with concentrated nitric acid (60%) using inductively coupled plasma atomic emission spectrophotometry (ICP-AES). Carbohydrate was determined as the difference in dry matter content minus protein, ash and total lipids. All of these determinations were conducted according to the methods specified by the AOAC (2005**).**

2.7 Statistical analysis

All values are mean ± SEM unless otherwise specified. Fish weights were converted to the geometric mean (GMW) of initial and final weights prior to plotting on the figures. Graphical presentation was done using Microsoft Excel. Regression analysis on linear, power and polynomial functions (including derivation of error terms) was done using Statistica version 9.0 (StatSoft, Tulsa, OK, USA). Error terms for exponents were determined based on natural logarithmic transformations of the data prior to linear regression analysis.

3. Results

3.1 Study 1 – Starvation energy and protein losses

Protein, lipid and energy losses by cobia over a 21 d period of starvation were shown to vary with the size of the fish (Figures 1, 2 and 3). Overall endogenous protein loss was greater with increasing fish size, and is expressed as an exponential relationship (Equation 1). Concomitant with that protein loss, was also the loss of lipid during this starvation period (Equation 2). The loss of these two nutrients due to starvation is expressed as the energy loss (Equation 3). A greater protein and lipid losses were recorded in larger fish (Fig. 1, 2). Similar to protein losses, the relationship with size and energy losses was also described by an exponential relationship with body weight (BW) (Equation 3). The determined exponents of protein ($BW^{0.697}$), lipid ($BW^{0.989}$), and energy ($BW^{0.822}$), loss were so similar to standard exponents ($BW^{0.70}$, $BW^{1.00}$ and $BW^{0.80}$ respectively) for other fish species that it was decided to standardise their use to these common exponents for further calculations.

(Equation 1) Protein loss (g/fish) = 0.235 • $(GMW)^{0.697}$, (R^2 = 0.844)

(Equation 2) Lipid loss (g/fish) = 0.021 • $(GMW)^{0.989}$, (R^2 = 0.870)

(Equation 3) Energy loss (kJ/fish) = 4.902 • $(GMW)^{0.822}$, (R^2 = 0.874)

FIGURES 1, 2 AND 3 HERE

3.2 Study 2 – Energy, lipid and protein digestibility

Protein, lipid and energy digestibility values of the reference diet were determined. Protein digestibility was measured at 83.0 ± 0.2%. Lipid digestibility was measured at 94.6 ± 0.8% and energy digestibility was measured at 89.0 ± 0.1%. This equated to a digestible protein content of the reference diet of 40.6%, a digestible lipid content of 13.0% and a digestible energy content of 19.7 MJ/kg, each on a dry matter basis.

3.3 Study 3 – Energy and protein utilisation efficiency

The measured utilisation of protein, lipid and energy by cobia, was based on the assessment of net gain in each parameter relative to the varying intake of dietary digestible protein (Figure 4; Equation 4), digestible lipid (Figure 5; Equation 5) and digestible energy (Figure 6; Equation 6). Each relationship followed a linear function.

(Equation 4) Protein gain (g/ $kg^{0.70}$/ d) = 0.456 • (digestible protein intake) – 0.450, (R^2 = 0.938)

(Equation 5) Lipid gain (g/ $kg^{1.00}$/ d) = 1.292 • (digestible energy intake) – 1.120, (R^2 = 0.973).

(Equation 6) Energy gain (kJ/ $kg^{0.80}$/ d) = 0.651 • (digestible energy intake) – 48.411, (R^2 = 0.996)

FIGURES 4, 5 AND 6 HERE

The intercept of each linear function with the X-axis was used to determine the maintenance requirements for each of protein, lipid and energy.

3.4 Study 4 – Fish composition

The composition of the fish varied over the live-weight range of fish examined (Figure 7). Protein content was relatively constant and was described by a linear function (Equation 7). Typically, the live-weight total lipid composition was also observed to increase with increasing live-weight (Figure 7; Equation 8). The increase in total lipid content with increasing fish size was also consistent with an increase in energy density of the fish (Figure 7; Equation 9).

(Equation 7) Live-weight protein (%) = 0.001 • (live-weight) + 0.168, (R^2 = 0.007)

(Equation 8) Live-weight lipid (%) = 0.0054 • $(\text{live-weight})^{0.451}$, ($R^2$ = 0.822)

(Equation 9) Live-weight energy (MJ/ kg) = 2.669 • $(\text{live-weight})^{0.1715}$, ($R^2$ = 0.791)

FIGURE 7 HERE

3.5 Study 5 – Assessment of fish potential growth rate

The growth rates of juvenile cobia showed that larger fish typically had the potential to gain greater total biomass per day than smaller fish (Figure 8). The temperature independent function for this growth can be expressed as Equation 10. This equation derived from growth data of cobia cultured at an average water temperature 25.6 ± 2.8˚C. Where the geometric mean weight (GMW) is in gram per fish.

(Equation 10) Fish growth rate (g/d) = 0.159 • $(\text{GMW})^{0.574}$ (R^2 = 0.847)

FIGURE 8 HERE

3.6 Study 6 – Iterative design of dietary protein and energy specifications

From the starvation data and the calculated maintenance protein, lipid and energy demands a function describing the relationship between fish live-weight and those maintenance demands was derived. Because insufficient data was collected on temperature effects on maintenance demands the determination of a temperature response was not attempted.

(Equation 11) Maintenance Protein Demand (g/ d/ fish) = 0.99 • $(\text{live-weight})^{0.70}$

(Equation 12) Maintenance Lipid Demand (g/ d/ fish) = 0.87 • $(\text{live-weight})^{1.00}$

(Equation 13) Maintenance Energy Demand (kJ/ d/ fish) = 74.3 • $(\text{live-weight})^{0.80}$

TABLE 2 HERE

4. Discussion

Factorial models have proven to be useful in defining both protein and energy demands and total feed ration management for a range of fish species (Lupatsch *et al.*, 2003; Glencross, 2008; Pirozzi *et al.*, 2010). This study reports on the development of a model for a new carnivorous fish species and as such adds to the volume of data on such fish species.

4.1 Comparisons to the growth model

There are actually only a few studies examining growth by cobia in the literature. A study by Benetti *et al*., (2010) reported the growth of cobia cultured in open ocean submerged cages in the Caribbean. Growth rates in that study were consistently 94% (at 27.8ºC) and 82% (at 25.5ºC) of those reported in the present model at water temperature of 27.8ºC. A common issue with the comparison of the growth model against much of the published literature is that most of the published literature appears to be with very small fish and often under limiting conditions of feed quality situations and with fish growing much slower than that encountered from our farm-based data collection. Such vagaries in the growth rates throughout the literature highlight the need to develop a benchmark standard against which laboratory studies should be compared.

4.2 Protein requirements

The metabolic weight exponent for protein metabolism in cobia is 0.697. This is similar to the generic protein exponent for most fish species is 0.70. The efficiency of protein use by cobia, based on the regression of the protein gain against the digestible protein intake, was linear over the protein intake range examined and had a coefficient of 0.456. This coefficient value for the partial efficiency of protein gain for this species is also similar to that observed for most other fish species – barramundi: 0.48, gilthead seabream: 0.53, rainbow trout: 0.40 - 0.47, yellowtail kingfish: 0.51, (Lupatsch *et al.,* 2003; Glencross, 2008; 2009; Glencross *et al.,* 2008; Booth *et al.,* 2010). Although in most other studies this relationship between protein gain and protein intake has usually been observed to be curvilinear, in the present study this response was linear over the feed intake ranges studied (Lupatsch *et al.*, 2003; Glencross, 2008; Dumas *et al.*, 2010; Glencross, 2010; Glencross *et al.*, 2011). Such linear responses have been observed before (Lupatsch *et al.,* 2001). Though it has been argued that such linear responses are indicative of underfeeding as even the curvilinear responses reported are close to linear at the lower levels of feed intake (Glencross and Bermudes, 2012).

A notable feature of this study was the higher maintenance protein requirements (DP_{maint}) observed of this species. Based on the point of zero net protein gain a DP_{maint} intake of 0.99 g/ $kg^{0.70}$/d was calculated (Figure 4). This is about 50% higher than the value of 0.66 g /$kg^{0.70}$/d determined for *D. labrax* (Lupatsch *et al.*, 2001), and double the 0.45 g /$kg^{0.70}$/d determined for barramundi

(Glencross, 2008). However, it is only about half that reported for yellowtail kingfish (1.70 g /kg$^{0.70}$/d), another highly active pelagic carnivorous species (Booth *et al.*, 2010).

4.3 Energy requirements

The relationship between this specie's energy metabolism and its body weight also conform to the allometric equation: $a \bullet BW(kg)^b$ as is the case for virtually every other fish species studied (Withers, 1998; Dumas *et al.*, 2010). Similarly, the exponent value of body weight ($BW^{exponent\ value}$) for energy metabolism in cobia was observed to be 0.822 which is similar to the result determined by Watson and Holt (2010) using indirect calorimetry with this species (0.809). It is also similar to other fish species including barramundi (0.80), grouper (0.79), gilthead seabream (0.82), European seabass (0.80), Pangasius catfish (0.84) and tilapia (0.85) (Lupatsch *et al.*, 2003; Glencross, 2008; Glencross and Bermudes, 2011; Glencross *et al.*, 2011; Trung *et al.*, 2011).

The maintenance energy requirements ($DE_{maint\ =}$ 74.3 kJ/kg$^{0.80}$/d), as defined by the point of zero net energy gain, in this study was substantially higher from that seen for other species like rainbow trout (40.1 kJ / kg$^{0.80}$ /d), barramundi (42.6 kJ / kg$^{0.80}$ /d) and mulloway (26.3 kJ / kg$^{0.80}$ /d) (Glencross *et al.*, 2008; Glencross, 2008; Pirozzi *et al.* 2010). However, the DE_{maint} was similar to the 87.4 kJ/kg$^{0.80}$/d reported by Booth *et al.* (2010) for another pelagic carnivorous fish species the yellowtail kingfish (*Seriola lalandi*). This observation poses a question whether it is this active pelagic nature of these animals that results in such a higher or some other feature like the partial endothermy observed in some Scombrid species (Glencross *et al.*, 2001).

The partial efficiency of energy use is determined as the slope of the regression of the energy intake against energy retention, on a metabolic body weight basis (Lupatsch *et al.*, 2001). In the present study for cobia species, the response of full energy intake range was recorded to be linear. This contrasts with the curvilinear response observed with other species (Lupatsch *et al.*, 2003; Bureau *et al.* 2006; Glencross, 2008; Glencross *et al.*, 2008; Trung *et al.*, 2011), but is consistent with the linear response reported in other studies (Cho & Bureau, 1998; Lupatsch *et al.*, 2001).

In the present study, the partial efficiency of energy gain was observed to be 0.651. This value is consistent with other carnivorous fish species e.g. Gilthead Seabream (0.65), white grouper, *Epinephelus aeneus* (0.69), barramundi (0.68), rainbow trout (0.62), yellowtail kingfish (0.65) and mulloway (0.60) (Lupatsch *et al.*, 2003; Glencross, 2008; 2009; Booth *et al.*, 2010; Pirozzi *et al.*, 2010).

4.4 Iterative diet design

Key dietary parameters of energy and protein specifications can be derived iteratively from this model for fish at any phase of its production cycle (Glencross, 2008; Booth *et al.*, 2010; Glencross *et al.*, 2010). This iterative approach was also used to define the energy and protein requirements for cobia from 100g to 2000g at each of three dietary energy densities (Table 2). Based on a combination of the somatic and non-somatic (maintenance) energy demands a simplistic energy budget was created that dictates how much energy the fish needs to consume to achieve a prescribed growth potential. The amount of feed (g/fish) rationed to the animal then being this energy demand divided by the digestible energy density of that feed (Table 2).

Similarly, the needs for protein for both somatic and non-somatic demands can also be defined using this approach which defines the appropriate DP:DE ratio (Table 2). Using the empirically derived equations from studies 1 to 5 the requirements for protein and energy at a range of fish sizes was determined (Table 2). Based on a combination of the predicted growth, the protein and energetic cost of that weight gain, the efficiencies associated with those gains and the maintenance requirements, the total daily requirements for both protein and energy at a range of fish sizes were calculated (Table 2). From this both the daily energy and protein intake requirement were defined. This has subsequently allowed us to iteratively specify a series of hypothetical diets of varying energy density (12 MJ/kg, 16 MJ/kg and or 20 MJ/kg) (Table 2).

In applying this iterative approach, it is assumed that the fish will eat to an energetic demand and as such the energy content of each diet will define total feed consumption. This total feed consumption also influences the amount of dietary protein required to satisfy the daily protein demand (Dumas *et al.*, 2010).

Using this iterative approach the present study shows that there are several strategies that can be employed to define the theoretically optimal diet energy and protein specifications and that these change with fish size, consistent with what has been reported in numerous other similar studies (Lupatsch *et al.*, 2003; Glencross, 2008; Booth *et al* 2010; Trung *et al.*, 2011; Glencross and Bermudes, 2012). When the diet energy density and/or fish size varies the present model demonstrates that there is a need to vary the dietary protein supply for this species. This model also demonstrates how the choice of diet energy density has an effect on the biological feed conversion ratio (FCR). When a lower FCR is achieved with a higher energy density simply due to the energetic demands being satisfied by fewer grams of feed. Importantly though, this lower feed ration combined with the same daily protein requirement also means that the protein concentration required in that diet for it to satisfy the daily protein demands has to increase for it to be effective. Similar to other species, it was noted that the most dramatic changes in the protein demand (based on the required protein : energy ratio) of cobia occur over the first 500 g of its growth, where the optimal DP:DE changes from 36 g/MJ at 50 g to 24 g/MJ at 500 g (Figure 9).

FIGURE 9 HERE

For cobia, the optimal DP:DE ratios at 100 g and 1000 g were 32 and 22 g/MJ, respectively (Table 2) and by comparison barramundi optimal DP:DE ratios at 100 g and 1000 g were 30.2 and 19.9 g/MJ (Glencross, 2008). This contrasts those determined for yellowtail kingfish which had optimal DP:DE ratios at 100 g and 1000 g of 39 and 27 g/MJ, respectively (Booth *et al.,* 2010). It can be seen that for each of the sizes of cobia, examined in the present study that the optimal DP:DE ratios were marginally higher than those of barramundi, but substantially lower than those of yellowtail kingfish (Figure 9).

4.4 Conclusions

This study used a factorial method for determining the protein and energy requirements for cobia. This study adds to the volume of literature using this method to estimate these requirements for a range of fish species. Comparison of the data derived from this study with that obtained for other species indicates a high degree of homology of most energetic parameters. The primary difference, in comparison to the many other models developed for most other carnivorous fish species, is that this species has a marginally higher demand for protein, but most notably its maintenance requirements for protein and energy are substantially higher than other studied species. The only exception to this being the comparison with another pelagic marine fish, the yellowtail kingfish, which also has similarly high maintenance demands.

This study represents a series of estimations based on a series of inter-related studies and their derived parameters. As such the estimations deduced from this modelling exercise are only as robust as the weakest data estimates. It would be prudent to take the outputs from this model and independently validate them and also test some of the assumptions used to increase the robustness of this model.

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References

AOAC (Association of Official Analytical Chemists). 2005. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th edition. Association of Official Analytical Chemists. Washington, DC, USA.

Benetti, D.D., B. O'Hanlon, J.A. Rivera, A.W. Welch, C. Maxey, Orhun M.R., 2010. Growth rates of cobia (*R. canadum*) cultured in open ocean submerged cages in the Caribbean. Aquaculture 302, 195-201.

Bermudes, M., Glencross, B.D., Austen, K., Hawkins, W., 2010. Effect of high water temperatures on nutrient and energy retention in barramundi (*Lates calcarifer*). Aquaculture 306, 160-166.

Blyth, D., Tabrett, S.J., Glencross, B.D., 2014. A study of the effects of faecal collection method and acclimation time on the digestibility of diets and ingredients when fed to juvenile barramundi (*Lates calcarifer*). Aquaculture Nutrition 21, 248–255. doi: 10.1111/anu.12159.

Booth, M.A., Pirozzi, I., Allan, G.A., 2010. Estimation of digestible protein and energy requirements of yellowtail kingfish *Seriola lalandi* using a factorial approach. Aquaculture 307, 247-259.

Bureau, D.P., Hua, K., Cho, C.Y., 2006. Effect of feeding level on growth and nutrient deposition in rainbow trout (*Oncorhynchus mykiss* Walbaum) growing from 150 to 600 g. Aquacult. Res., 37, 1090–1098.

Cho, C.Y., Bureau, D.P., 1998. Development of bioenergetic models and the Fish-PrFEQ software to estimate production, feeding ration and waste output in aquaculture. Aquatic Living Resources 11, 199-210.

Chou R.L, Su M.S., Chen H.Y., 2001. Optimal dietary protein and lipid levels for juvenile cobia (*Rachycentron canadum*). Aquaculture 193 (1-2), 81-89.

Chou, R.-L., M.-S. Su, Chen H.Y., 2001. Optimal dietary protein and lipid levels for juvenile cobia (*Rachycentron canadum*). Aquaculture 193, 81-89.

Craig, S.R., Schwarz, M.H., McLean, E., 2006. Juvenile cobia (*Rachycentron canadum*) can utilize a wide range of protein and lipid levels without impacts on production characteristics. Aquaculture 261, 384–391.

Diu, N.T., Pirozzi, I., Glencross, B.D., 2015. Digestibility of canola meals in barramundi (Asian seabass; *Lates calcarifer*). Aquaculture 435, 442–449.

Dumas, A., France, J., Bureau, D.P., 2010. Modelling growth and body composition in fish nutrition: Where have we been and where are we going? Aquaculture Research 41, 161-181.

FAO 2014. Fisheries and aquaculture software. FishStatJ - software for fishery statistical time series. In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 22 July 2014.

Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226, 497–509.

Fraser, T.W.K., Davies S.J., 2009. Nutritional requirements of cobia, *Rachycentron canadum* (Linnaeus): a review. Aquaculture Research 40, 1219-1234.

Glencross, B.D., Carter, C.G., Gunn, J, van Barneveld, R.J., Rough, K.M., Clarke, S.M., 2001. Southern bluefin tuna. In: Nutrient requirements and Feeding of Finfish for Aquaculture (Webster and Lim, Eds). CABI Publishing, Wallingford, UK. pp 112-124.

Glencross B.D., Michael R., Austen K., Hauler R., 2008. Productivity, carcass composition, waste output and sensory characteristics of large barramundi *Lates calcarifer* fed high-nutrient density diets. Aquaculture 284 (1-4),167-173.

Glencross, B.D., 2008. A factorial growth and feed utilisation model for barramundi, *Lates calcarifer* based on Australian production conditions. Aquaculture Nutrition 14, 360-373.

Glencross, B.D., 2009. Reduced water oxygen levels affect maximal feed intake, but not protein or energy utilisation by rainbow trout (*Oncorhynchus mykiss).* Aquaculture Nutrition 15, 1-8.

Glencross, B.D., 2010. Using modelling approaches to understand the implications of physiological challenges and raw material demands on aquaculture feed designs. In: Cruz-Suarez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A.,Gamboa-Delgado, J. (Eds), Avances en Nutrición Acuícola X - Memorias del Décimo Simposio Internacional de Nutrición Acuícola, 8-10 de Noviembre, San Nicolás de los Garza, N. L., México. ISBN en trámite. Universidad Autónoma de Nuevo León,Monterrey, México, pp. 341-365.

Glencross B.D. and Bermudes M., 2011. The effect of high water temperatures on the allometric scaling effects of energy and protein starvation losses in juvenile barramundi, *Lates calcarifer*. Comp Biochem Physiol. A. Mol. Integr. Physiol., 159(2),167-74. doi: 10.1016/j.cbpa.2011.02.013.

Glencross, B.D., Phuong, N.T., Hien, T.T.T., Cam Tu, T.T., Phu, T.M., 2011. A factorial growth and feed utilisation model for Tra catfish, *Pangasianodon hypopthalamus*. Aquaculture Nutrition 17, e396-e405.

Glencross B.D. and Bermudes M., 2012. Using a bioenergetic modelling approach to understand the implications of heat stress on barramundi (*Lates calcarifer*) growth, feed utilisation and optimal protein and energy requirements - Options for adapting to climate change? Aquaculture Nutrition 18(4), 411-422.

Glencross, B.D., Bermudes, M., 2012. Using a bioenergetic modelling approach to understand the implications of heat stress on barramundi (*Lates calcarifer*) growth, feed utilisation and optimal protein and energy requirements – Options for adapting to climate change? Aquaculture Nutrition 18, 411-422.

Liao, I.C., Huang T.S., Tsai W.S., Hsueh C.M. , Chang S.L. and Eduardo M.L., 2004. Cobia culture in Taiwan: current status and problems. Aquaculture 237, 155-165.

Liu, K., X.J. Wang, Q. Ai, K. Mai, and Zhang W., 2010. Dietary selenium requirement for juvenile cobia, *Rachycentron canadum* L. Aquaculture Research 41, e594-e601.

Liu, K., Q.H. Ai, K.S. Mai, W.B. Zhang, L. Zhang, Zheng S.X., 2012. Dietary manganese requirement for juvenile cobia, *Rachycentron canadum* L. Aquaculture Nutrition 19:461-467.

Lupatsch, I., Kissil, G.W., Sklan, D., 2001. Optimization of feeding regimes for European sea bass *Dicentrarchus labrax*: a factorial approach. Aquaculture 202, 289-302.

Lupatsch, I., Kissil, G.W., Sklan, D., 2003. Comparison of energy and protein efficiency among three fish species *Sparus aurata, Dicentrarchus labrax* and *Epinephelus aeneus*: energy expenditure for protein and lipid deposition. Aquaculture 225, 175-189.

Mercer, L.P., 1982. The quantitative nutrient-response relationship. J. Nutr. 112, 560-566.

Niu, J., Y.J. Liu, L.X. Tian, K.S. Mai, H.J. Yang, C.X. Ye, Zhu Y., 2008. The effect of different levels of dietary phospholipid on growth, survival and nutrient composition of early juvenile cobia (*Rachycentron canadum*). Aquaculture Nutrition 14, 249-256.

Petersen E.H., Glencross B.D., Tien N.V., Tuan L.A., Tuan V.A., Phuong T.H., 2015. Recent Changes in the Bioeconomic of Finfish Mariculture in Vietnam. J. Aquac. Res. Development 6 (3), 311. doi:10.4172/2155-9546.1000311

Pirozzi, I., Booth, M.A., Allan, G.L., 2010. A factorial approach to deriving dietary specifications and daily feed intake for mulloway, *Argyrosomus japonicus*, based on the requirements for digestible protein and energy. Aquaculture 302, 235-242.

Salze, G., McLean,E., Battle, P.R., Schwarz, M.H., Craig, S.R., (2010) Use of soy protein concentrate and novel ingredients in the total elimination of fish meal and fish oil in diets for juvenile cobia, *Rachycentron canadum*. Aquaculture 298, 294–299.

Shearer, K.D., 1995. The use of factorial modeling to determine the dietary requirements for essential elements in fishes. Aquaculture 133, 57–72.

Sun, L., Chen, H., Huang, L., 2006. Effect of temperature on growth and energy budget of juvenile cobia (*Rachycentron canadum*). Aquaculture 261, 872–878.

Sun, L., Chen, H. 2014. Effects of water temperature and fish size on growth and bioenergetics of cobia (*Rachycentron canadum*). Aquaculture 426–427, 172–180.

Trung D.V., Diu, N.T., Hao, N.T., Glencross, B.D., 2011. Development of a nutritional model to define the energy and protein requirements of Tilapia, *Oreochromis niloticus*. Aquaculture 320, 69-75.

Ursin, E., 1967. A mathematical model of some aspects of fish growth, respiration and mortality. Journal of the Fisheries Research Board of Canada 24, 2355-2453.

Watson, A.M., Holt, G.J., 2010. Energy Budget of Early Juvenile Cobia, *Rachycentron canadum*. Journal of the World Aquaculture Society 41, 224-234.

Webb, K.A., L.T. Rawlinson, and Holt G.J., 2009. Effects of dietary starches and the protein to energy ratio on growth and feed efficiency of juvenile cobia, *Rachycentron canadum*. Aquaculture Nutrition 16, 447-456.

Withers, P.C., 1992. Comparative Animal Physiology. Brooks/Cole, Singapore.

Xiao, L.D., K.S. Mai, Q.H. Ai, W. Xu, X.J. Wang, W.B. Zhang, Z.G. Liufu. 2009. Dietary ascorbic acid requirement of cobia, *Rachycentron canadum* Linneaus. Aquaculture Nutrition 16, 582-589.

Zhou, Q.C., Z.H. Wu, B.P. Tan, S.Y. Chi, Yang Q.H., 2006. Optimal dietary methionine requirement for Juvenile Cobia (*Rachycentron canadum*). Aquaculture 258, 551-557.

Zhou, Q.C., Z.-H. Wu, S.Y. Chi, Yang Q.H., 2007. Dietary lysine requirement of juvenile cobia (*Rachycentron canadum*). Aquaculture 273, 634-640.

Table 1. Reference diet formulation (% as used) and composition (% dry basis unless otherwise indicated)

Ingredients	**(%)**
Brown fish meal	65.5
Wheat flour	14.5
Wheat gluten	10.0
Fish oil	9.4
Mineral and vitamin premix*	0.5
Marker (yttrium oxide)	0.1
Composition	
Dry matter (% as fed)	95.8
Crude protein	48.9
Digestible protein	40.6
Total lipid	13.8
Digestible lipid	13.0
Crude ash	11.0
Carbohydrate**	21.1
Gross energy (kJ/g)	22.2
Digestible Energy (kJ/g)	19.7

* Vitamin and mineral premix includes (IU kg^{-1} or g kg^{-1} of premix): Vitamin A, 1.3MIU; Vitamin D3, 0.5 MIU; Vitamin E, 0.17 MIU; Vitamin K,3, 3.4 g; Vitamin B1, 6.7 g; Vitamin B2, 5.8 g; Vitamin B6, 6.7 g; Vitamin B12, 0.003 g; Folic acid, 0.8 g; D-Calpan, 20 g; Niacin, 11.7 g; Biotin, 0.17 g; Vitamin C, 33 g; Inositol, 45 g; Iron, 8.3 g; Zinc, 16.7 g; Copper, 8.3 g; Manganese, 3.0 g; Cobalt, 0.67 g; Iodine, 0.17 g; Selenium, 0.07 g.

** Calculated only

Table 2 Calculations of dietary energy and protein requirements for growing cobia at 26˚C including feed specifications based on a series (12, 16 and 20 MJ/kg) suggested dietary DE densities

Fish live-weight (g/fish)	100	500	1000	2000	100	500	1000	2000	100	500	1000	2000
Growth (g/fish/day) @ 25.6 ºC [a]	2.23	5.62	8.37	12.47	2.23	5.62	8.37	12.47	2.23	5.62	8.37	12.47
Energy												
Metabolic BW ($kg^{0.80}$)	0.158	0.574	1.000	1.741	0.158	0.574	1.000	1.741	0.158	0.574	1.000	1.741
DE_{maint} (kJ/fish/day) [b]	11.78	42.67	74.30	129.36	11.78	42.67	74.30	129.36	11.78	42.67	74.30	129.36
Energy gain (kJ/fish/day) [c]	13.12	43.58	73.07	122.53	13.12	43.58	73.07	122.53	13.12	43.58	73.07	122.53
DE_{growth} (kJ/fish/day) [d]	20.16	66.94	112.25	188.21	20.16	66.94	112.25	188.21	20.16	66.94	112.25	188.21
DE_{total} (kJ/fish/day) [e]	31.93	109.62	186.55	317.58	31.93	109.62	186.55	317.58	31.93	109.62	186.55	317.58
Protein												
Metabolic Protein BW ($kg^{0.80}$)	0.200	0.616	1.000	1.625	0.200	0.616	1.000	1.625	0.200	0.616	1.000	1.625
$DPro_{maint}$ (g/fish/day) [f]	0.20	0.61	0.99	1.61	0.20	0.61	0.99	1.61	0.20	0.61	0.99	1.61
Protein gain (g/fish/day) [g]	0.37	0.94	1.41	2.09	0.37	0.94	1.41	2.09	0.37	0.94	1.41	2.09
$DPro_{growth}$ (g/fish/day) [h]	0.82	2.07	3.08	4.59	0.82	2.07	3.08	4.59	0.82	2.07	3.08	4.59
$DPro_{total}$ (g/fish/day) [i]	1.02	2.68	4.07	6.20	1.02	2.68	4.07	6.20	1.02	2.68	4.07	6.20
Feed specifications												
DE content of feed (MJ/kg)	12	12	12	12	16	16	16	16	20	20	20	20
Feed intake (g/fish/day)	2.66	9.13	15.55	26.46	2.00	6.85	11.66	19.85	1.60	5.48	9.33	15.88
Feed intake (%BW)	2.7	1.8	1.6	1.3	2.0	1.4	1.2	1.0	1.6	1.1	0.9	0.8
DPro content of feed (g/kg)	383	294	262	234	511	391	350	312	639	489	437	391
Expected FCR	1.19	1.62	1.86	2.12	0.89	1.22	1.39	1.59	0.72	0.97	1.11	1.27
DPro : DE ratio (g/MJ)	32	24	22	20	32	24	22	20	32	24	22	20

[a]Based on Equation 10 and a temperature of 25.6°C. [b]Digestible energy required for maintenance = 74.3 kJ / $kg^{0.80}$ /day. [c]Energy content of body based on Equation 9. [d]Amount of digestible energy required for growth based on a partial energy utilization efficiency of 0.651. [e]Total digestible energy required per day = DE_{maint} + $DE_{growth.}$ [f]Digestible protein required for maintenance = 0.99 g /$kg^{0.70}$ /day. [g] Protein content of body based on 16.89% of live-weight. [h]Amount of digestible protein required for growth based on a partial protein utilization efficiency of 0.456. [i]Total digestible protein required per day = DP_{maint} + DP_{growth}

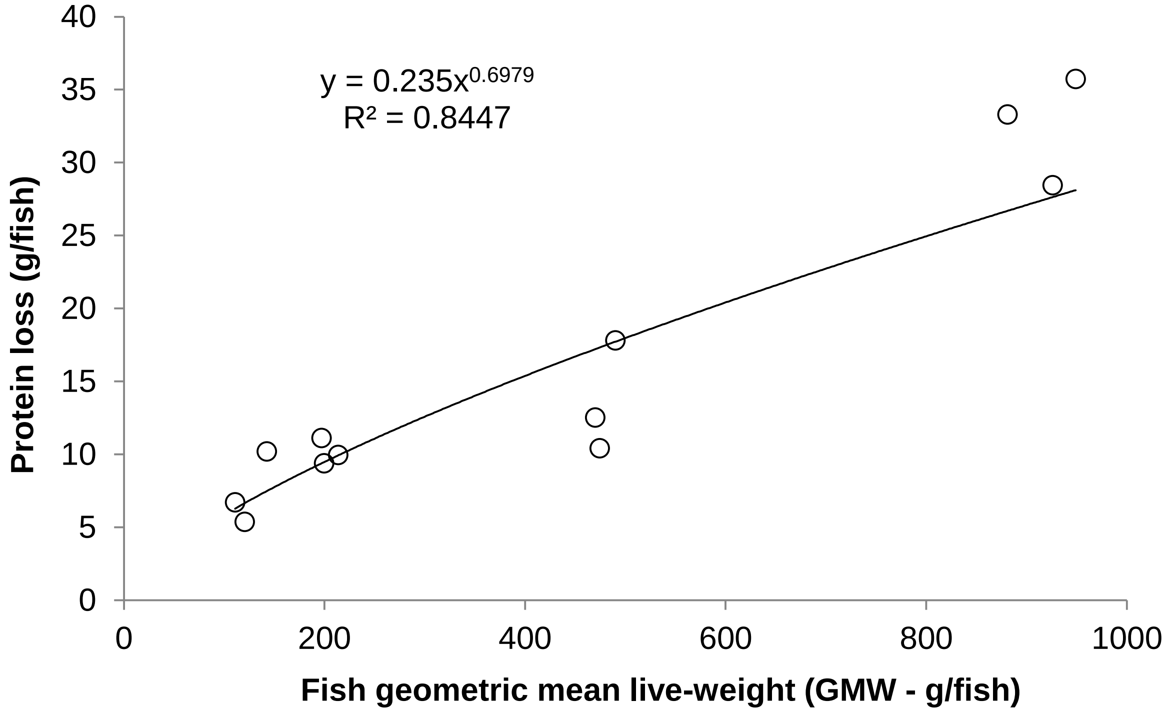

Figure 1. Protein loss (g/fish) by cobia starved for 21 days at 28°C. Regression equation is: Protein loss = 0.235*(fish geometric mean live-weight)$^{0.697}$, (R^2 = 0.844)

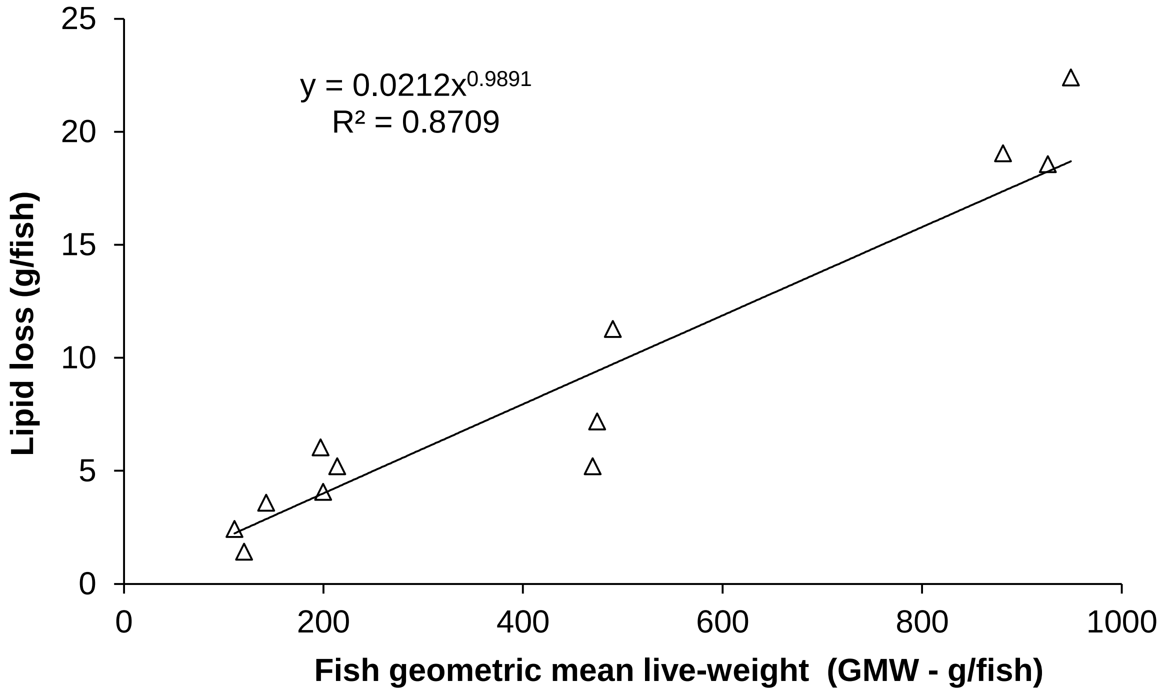

Figure 2. Lipid loss (g/fish) by cobia starved for 21 days at 28ºC. Regression equation is: Lipid loss (g/fish) = 0.021*(fish geometric mean live-weight)$^{0.989}$, (R^2 = 0.870).

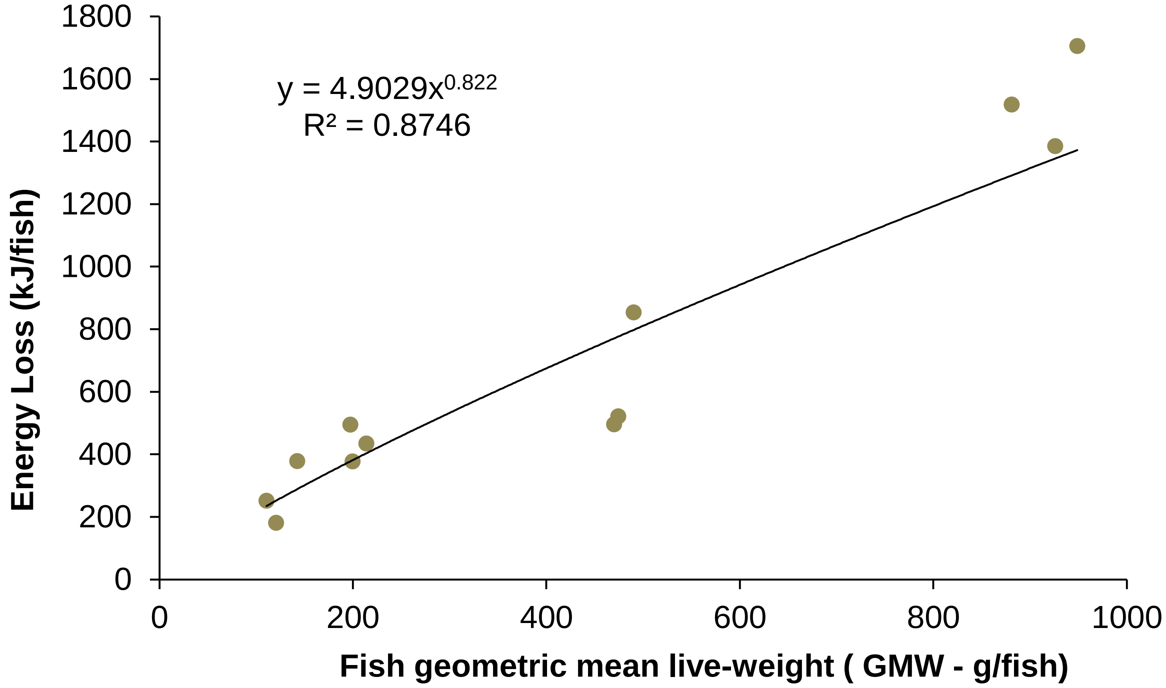

Figure 3. Energy loss (kJ/fish) by cobia starved for 21 days at 28°C. Regression equation is: Energy loss (kJ/fish) = 4.902*(fish geometric mean live weight)$^{0.822}$, (R^2 = 0.874).

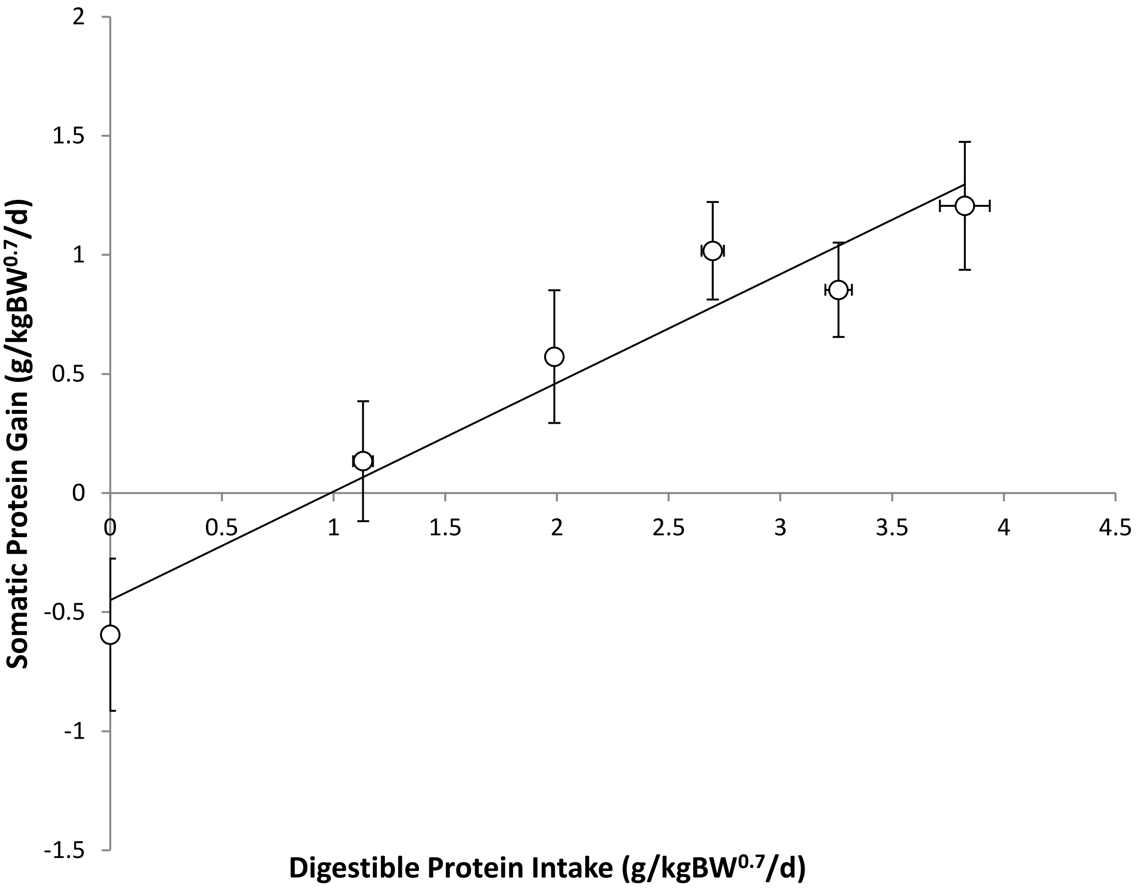

Figure 4 Protein gain (g/kgBW$^{0.7}$/d) by cobia fed increasing amounts of an experimental feed at 27.9 ± 0.3°C. Overall regression equation is: Protein gain = 0.456•(digestible protein intake) – 0.450, (R^2 = 0.938). Maintenance digestible protein intake level is estimated at 0.99 g/kgBW$^{0.7}$/d.

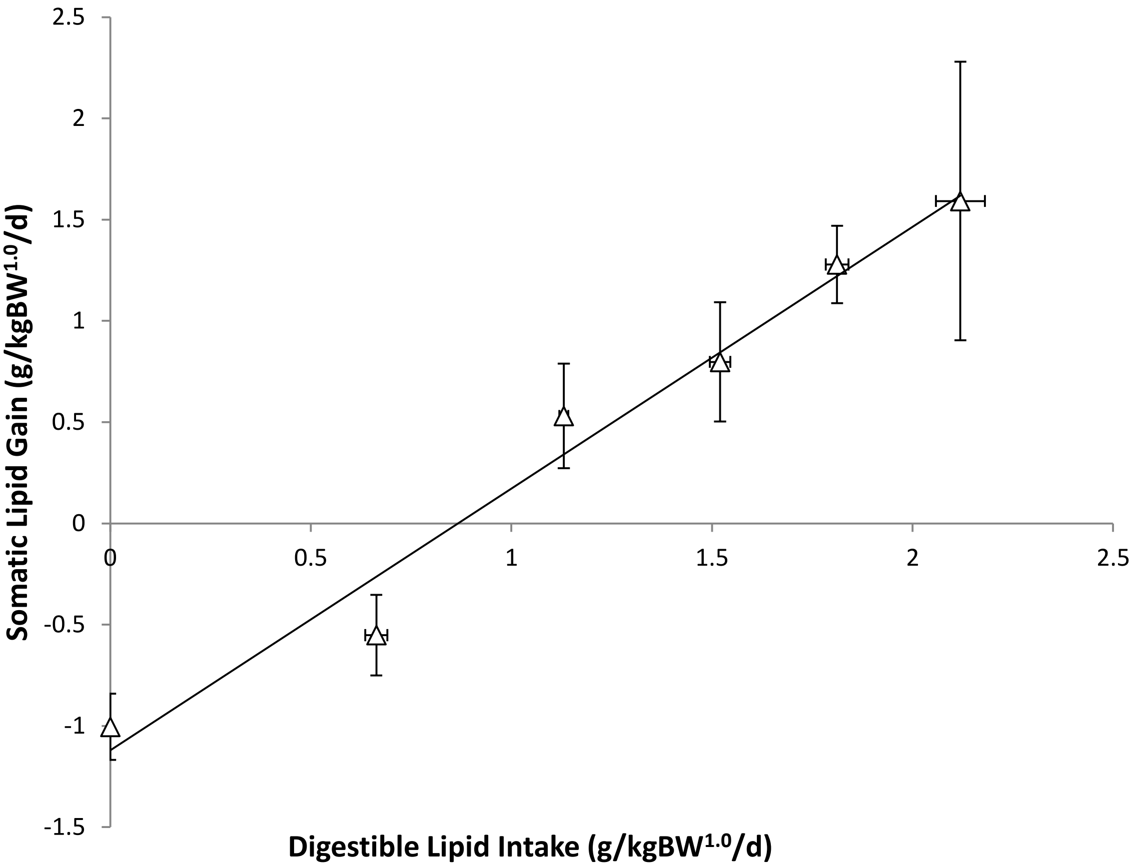

Figure 5 Lipid gain (g/kg$BW^{1.0}$/d) by cobia fed increasing amounts of an experimental feed at 27.9 ± 0.3°C. Overall regression equation is: Lipid gain (g/kg$BW^{1.0}$/d) = 1.292•(digestible lipid intake) – 1.120, (R^2 = 0.973). Maintenance digestible lipid intake level is estimated at 0.87 g/kg$BW^{1.0}$/d

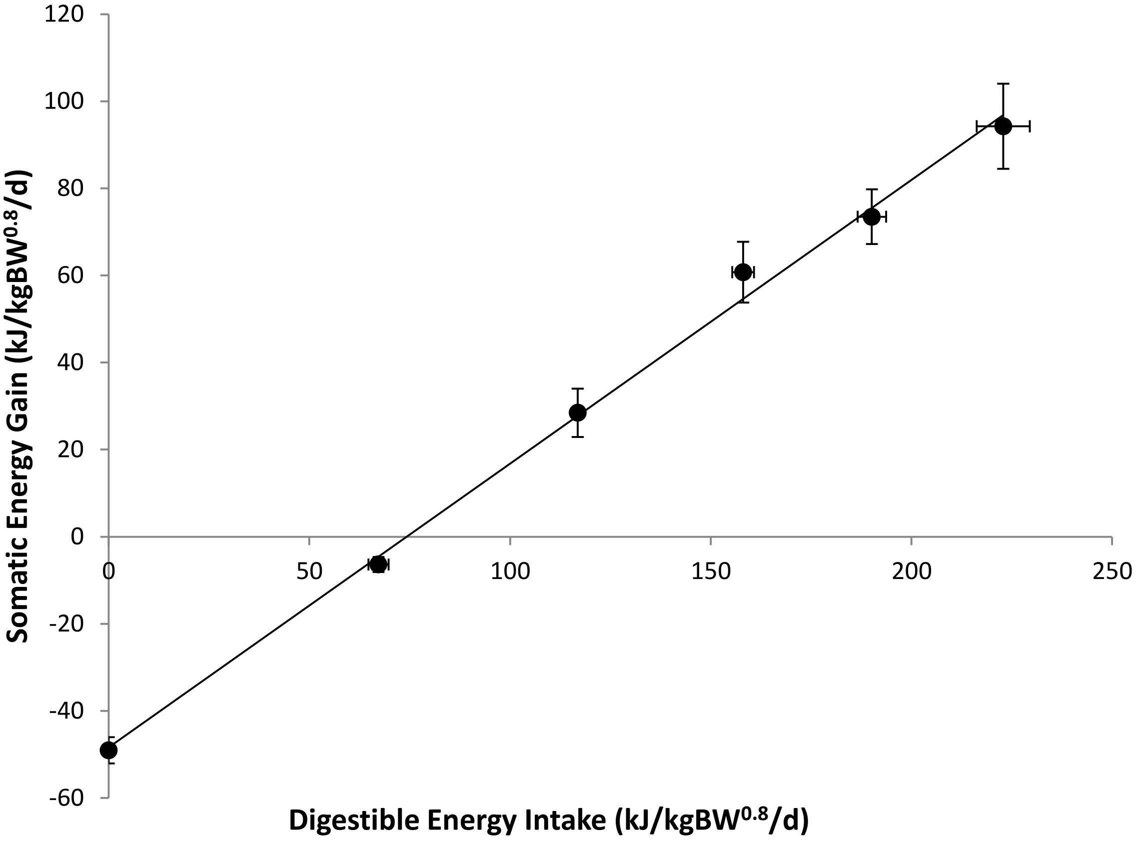

Figure 6 Energy gain (kJ/kgBW$^{0.8}$/d) by cobia fed increasing amounts of an experimental feed at 27.9 ± 0.3°C. Overall regression equation is: Energy gain = 0.651•(Digestible energy intake) – 48.411, (R^2 = 0.996). Maintenance digestible energy intake level is estimated at 74.3 kJ/kgBW$^{0.8}$/d.

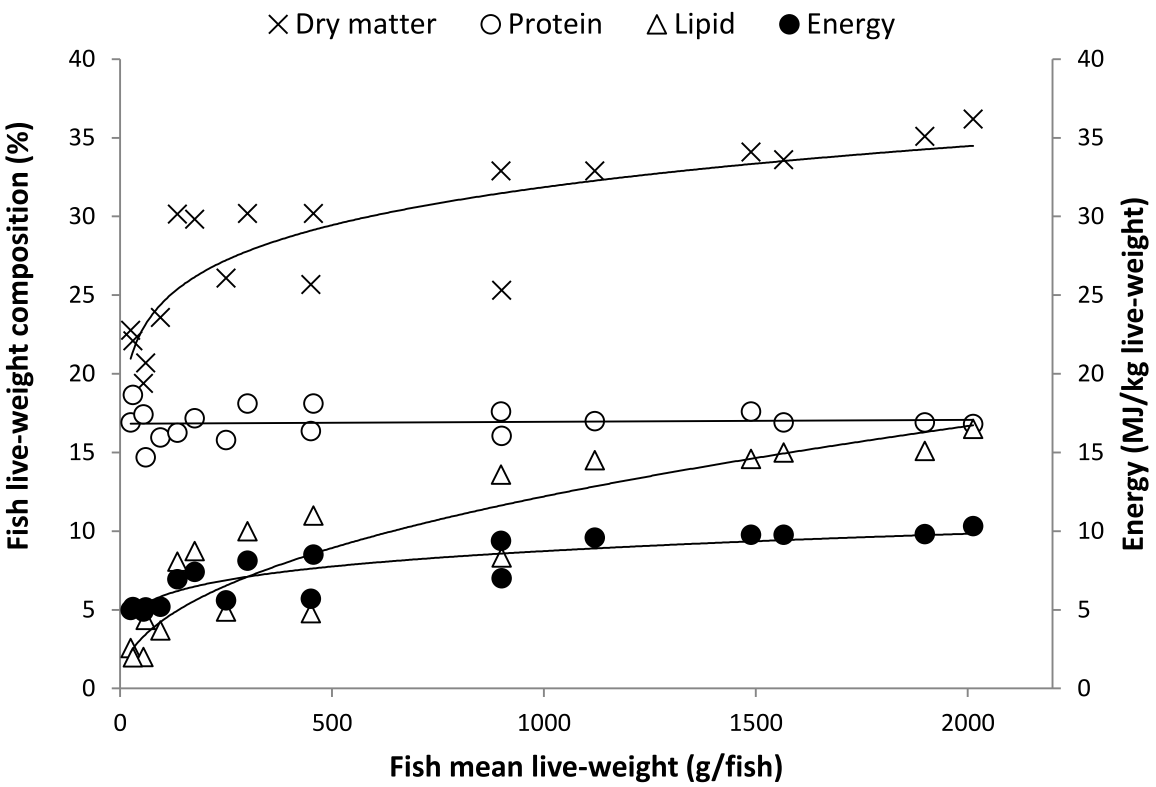

Figure 7. Live-weight body compositions of cobia (n = 18) from 25g to 2013g. Fish were a combination of laboratory and commercial farmed stocks fed either laboratory or commercial diets.

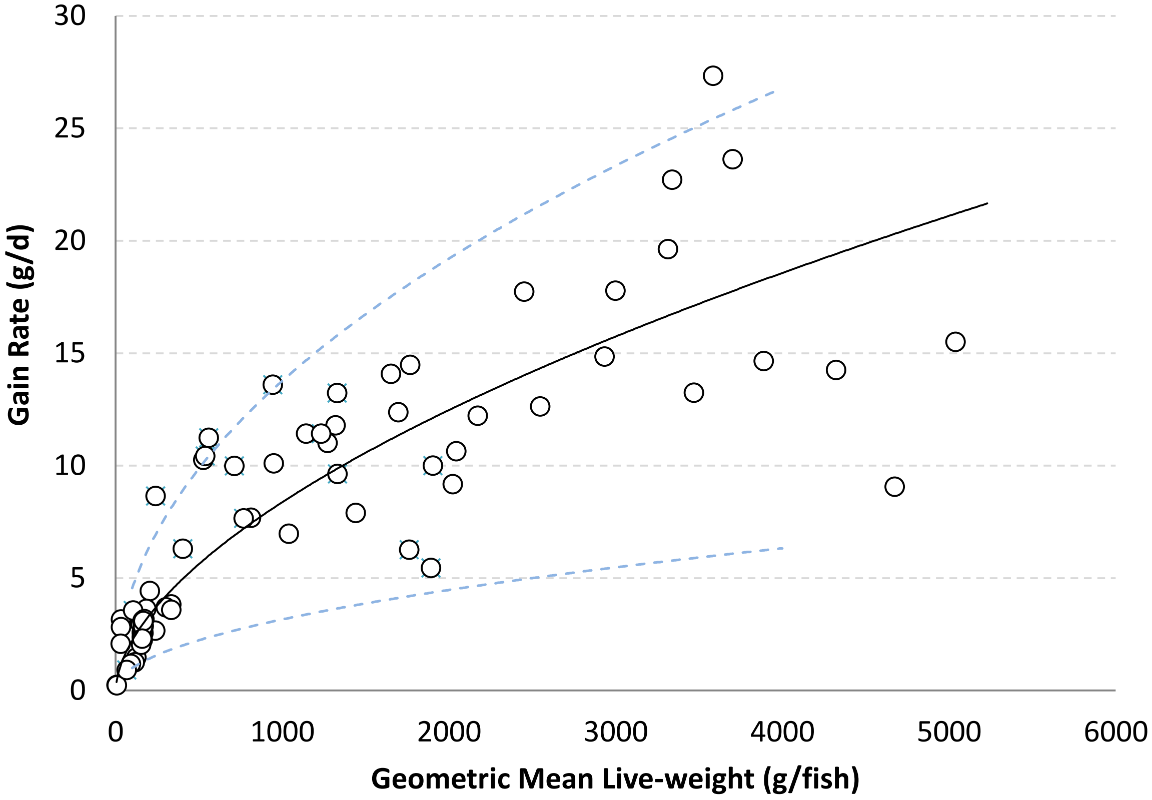

Figure 8 Growth rates of cobia with varying live-weight size range (expressed as geometric mean live-weight). Growth rate (g/d) was defined by the equation $y = 0.1586x^{0.574}$ (R^2 0.847). Average temperature across all data is 25.6 ± 2.8ºC (mean ± SD).

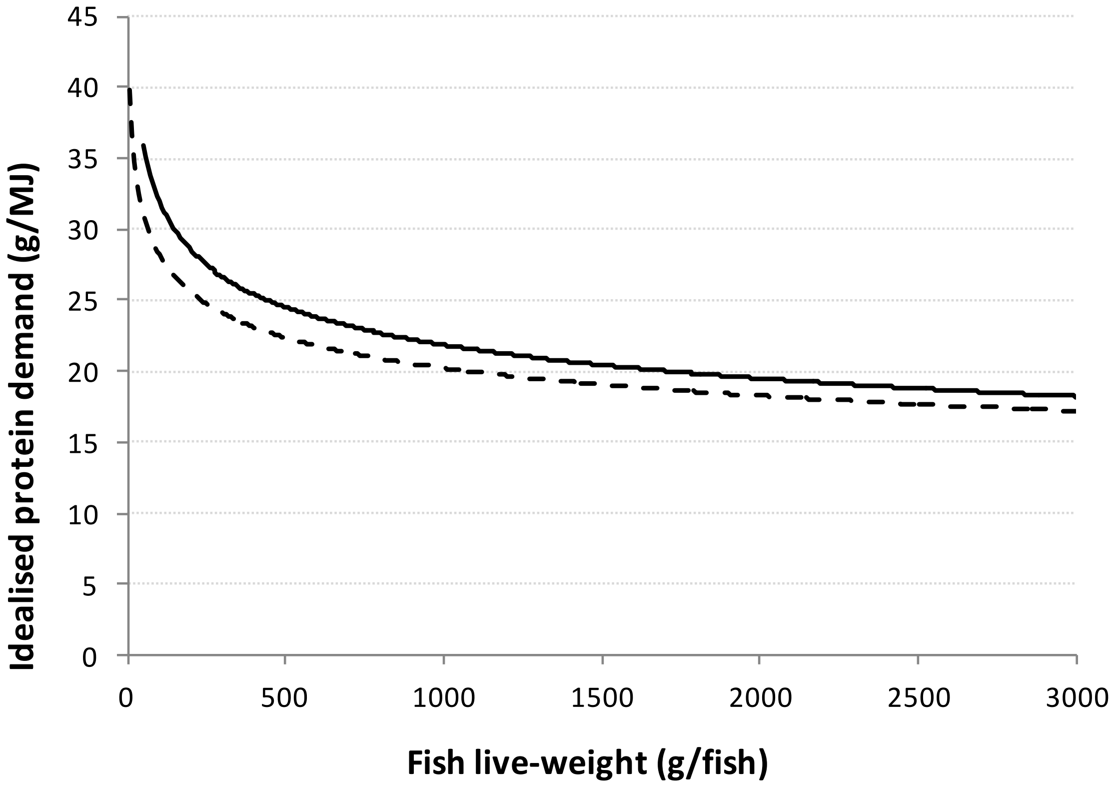

Figure 9 A comparison of the idealised protein demand (g/MJ) of cobia (solid line) with Asian seabass (dashed line) with varying live-weight size range (Asian seabass data derived from Glencross and Bermudes, 2012). Shown is the marginally higher demand for protein by cobia relative to this other tropical carnivorous species.