

## Elevated oxygen uptake and high rates of nitrogen excretion in early life stages of the cobia *Rachycentron canadum* (L.), a fast-growing subtropical fish

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Physiological energetics of cobia *Rachycentron canadum* were quantified for 18 to 82 days post-hatch (dph) hatchery-reared juveniles to better understand energy transformation and its implications in growth and survival. Mean oxygen consumption rates ( $M_{O_2}$ ;  $\text{mg O}_2 \text{ h}^{-1}$ ) of fish fed *ad libitum* and fish that were starved significantly increased with increasing wet mass ( $M$ ; g),  $M_{O_2} = 1.4291M^{0.8119}$  and  $M_{O_2} = 1.1784M^{0.7833}$ , respectively, with a significant reduction in mean metabolic rates of starved fish (19 to 27% specific dynamic action; SDA). Total ammonia nitrogen excretion rates ( $A_{MM}$ ,  $\mu\text{mol h}^{-1}$ ) also scaled with  $M$  and significantly decreased after starvation. Mean mass-specific  $A_{MM}$  and urea excretion rates are the highest reported in the literature, with urea accounting for approximately half the total nitrogen excretion measured in both fed and starved fish. Relatively high energetic rates may allow cobia to develop rapidly into pre-juveniles and be less susceptible to predation and starvation at a comparatively early age. © 2007 The Authors

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Key words: energetics; juvenile cobia; metabolism; nitrogen excretion; oxygen consumption; specific dynamic action.

### INTRODUCTION

The early developmental stages of fishes are characterized by high mortality rates and rapid morphological and physiological changes over relatively short time scales. It is assumed that the mortality rates of fishes are elevated between hatch and metamorphosis and possibly through the early juvenile stage which make these critical stages of development (Sissenwine, 1984; Houde, 1994). Food availability and predation are key biological variables that influence survival in the early life stages of fishes (Houde, 1997). Hence, growth, which is dependent on food availability and temperature, is considered particularly important since it affects larval duration and may favour decreased mortality due to predation.

The amount of ingested energy available for growth is determined by the energetic losses due to excretion and metabolic maintenance and activity (Brett &

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Groves, 1979). With the exception of scombrids, the bioenergetics or the appropriation and partitioning of energy between metabolism, excretion and growth of rapid growing pelagic fishes is not well understood. In addition, the literature on the energetic physiology of subtropical marine finfish species, particularly in the early stages of development, is relatively limited (Houde & Scheckter, 1983; Houde, 1989; Perez-Pinzon & Lutz, 1991; Pfeiler & Govoni, 1993; Pfeiler, 1996; Torres *et al.*, 1996; Tolley & Torres, 2002; Idrisi *et al.*, 2003).

The cost of growth has become a central issue in studies on young fishes since energy may be a limiting factor during early life history (Pedersen, 1997). In addition, studies on bioenergetics are of fundamental importance to the advancement of aquaculture (Brett & Groves, 1979). While growth increases the size spectrum of potential prey, it also increases consumption requirements. Torres *et al.* (1996) described the 'catch-22' that confronts young fishes that have high energetic requirements and must grow to survive, yet have few lipid reserves to sustain them from one opportunity to the next. Tropical fishes must consume three times as much prey as temperate fishes to meet their metabolic requirements and average growth rates (Houde, 1989). Therefore, the balance between growth energetics and survival may be even more tenuous for tropical fishes since they are theoretically more susceptible to starvation than temperate fishes.

Cobia *Rachycentron canadum* (L.) are a fast growing subtropical fish (Arnold *et al.*, 2002; Denson *et al.*, 2003; Benetti *et al.*, 2006), found worldwide throughout the tropics and warm temperate seas, except for the eastern Pacific Ocean (Shaffer & Nakamura, 1989). Although cobia juveniles bear some gross morphological resemblance to remoras, they are considered to be a closely related sister group of the dolphinfishes based on numerous similar larval characters and are the only species in the Rachycentridae (Johnson, 1984; Ditty & Shaw, 1992; Nelson, 1994). Larvae are found in shelf water, offshore pelagic water, as well as in estuarine environments (Ditty & Shaw, 1992) and develop quickly from larvae into pre-juveniles (12.7–18.0 mm standard length,  $L_S$ ) and 'early juveniles' (27.0–55.0 mm  $L_S$ ) (Dawson, 1971). Cobia continue to grow expeditiously with fish reaching 710 mm fork length ( $L_F$ ) and 3.5 to 6 kg by age 1 year (Franks *et al.*, 1999; Benetti *et al.*, 2006).

This study focused on energetic processes underlying growth and mortality and quantified the physiological energetics of cobia. The routine (resting, post-absorptive) metabolic rates of juvenile cobia and the effect of feeding on metabolism and nitrogen excretion were investigated. Cobia were selected for study because of their economic and ecological significance, extraordinary growth rates and availability. Recent success in the commercial-scale production of cobia has generated worldwide interest in this species from an aquaculture perspective and has also provided unique opportunities for research (Benetti *et al.*, 2003). There is limited general information on cobia from the western Atlantic Ocean (Ditty & Shaw, 1992; Franks *et al.*, 1999) and no studies, as far as is known, on the energetics of cobia in the early stages of development.

## MATERIALS AND METHODS

Juvenile cobia were provided by the Aquaculture Center of the Florida Keys, Inc. (ACFK), a commercial marine fish hatchery located in Marathon, Florida, U.S.A.

Wild-caught broodstock cobia were conditioned to spawn in a temperature-controlled 50 000 l recirculating tank, maintained between 20 and 28° C and 35 salinity. Broodstock cobia were subjected to natural photoperiods (24°46') and spawned during the months of May to August. Fertilized eggs were collected from broodstock tanks and larvae were intensively reared in a flow-through larval rearing system at 25–27° C and 35 salinity (Benetti *et al.*, 2003). Cobia developed into pre-juveniles at *c.* 2 weeks of age.

Juvenile cobia were transported from ACFK to the University of Miami Experimental Hatchery (UMEH). Juvenile cobia were maintained at the UMEH in 700 l fibreglass tanks with 5 µm filtered sea water at 26° C and 34–36 salinity. The holding tanks were in a climate-controlled room set with a 12L:12D photoperiod cycle located adjacent to the laboratory. The cobia were fed *ad libitum* twice daily a diet consisting of mixed commercially available pellets Otohime C1, C2, EP1: 51.0–58.4% protein, 13.7–13.9% lipid (Marubeni Nisshin Feed Co., Tokyo, Japan), Nippai 200–400: 56.0% protein, 8.0% lipid (Nippon Formula Feed Manufacturing Co. Ltd, Yokohama, Japan). Experiments were run on fish that were 18–82 days post-hatch (dph) and all specimens ranged in wet mass (*M*) from 0.0013 to 21.09 g.

## OXYGEN CONSUMPTION

Routine (normal activity, post-absorptive) and feeding (normal activity, fed) metabolic rates were measured on individual pre-juvenile and early juvenile cobia. The feeding respirometry trials were run while guts were observed to be distended with food (0 to 3 h post-feeding). Juveniles were starved for a period of 18 to 24 h in preparation for the routine metabolic trials. At the conclusion of a trial, each fish was mildly sedated with clove oil (10 ppm), blotted dry and weighed on a Mettler AE 163 analytical balance.

The specific dynamic action (SDA, *y*) cost of digestion and absorption of food (Jobling, 1981) was calculated as the difference between feeding metabolic rate ( $R_f$ ) and routine metabolic rate ( $R_r$ ),  $y = R_f - R_r$ . Standard metabolic rate ( $R_s$ ) is not part of SDA since it is a subcomponent of the  $R_f$  and  $R_r$  measurements and was negated in the above equation. In Table I,  $R_f$  and  $R_r$  were determined by linear regression with each respective metabolic variable dependent on mass. The difference in metabolic rates was converted from mg O<sub>2</sub> h<sup>-1</sup> to J h<sup>-1</sup>.

The respirometer consisted of six 1302 microcathode oxygen electrodes inserted into six independent respiration chambers and connected to a Strathkelvin Instruments (Motherwell, Scotland, U.K.) 928 interface (Idrisi *et al.*, 2003, 2006). Strathkelvin Instruments 928 system software version 2.2 was used to analyse oxygen consumption rates ( $M_{O_2}$ ). Electrodes were stabilized for a minimum of 1 h and calibrated at 26° C prior to each set of experiments. A two-point calibration procedure was performed with air-saturated sea water (high end: 6.63 mg O<sub>2</sub> l<sup>-1</sup> at 26° C, salinity 35; Green & Carritt, 1967) and a zero-oxygen seawater solution (low end: sea water and sodium sulphite)

TABLE I. Specific dynamic action (SDA) for fed and starved (routine) juvenile cobia at 26° C for a given wet mass (*M*)

<i>M</i> (g)	Fed (J h <sup>-1</sup> )	Unfed (J h <sup>-1</sup> )	Heat increment (%, SDA)
0.005	4.62	3.39	26.6
0.05	5.13	3.80	25.8
0.5	10.22	7.93	22.4
1.0	15.89	12.52	21.2
5.0	61.19	49.21	19.6
10.0	117.82	95.08	19.3
15.0	174.45	140.94	19.2
20.0	231.08	186.81	19.2

according to the manufacturer's directions. Oxygen levels within the chambers were recorded every 1 s in  $\text{mg O}_2 \text{ ml}^{-1}$ .

Respiration chambers were acrylic and either 700 ml (manufactured by Strathkelvin Instruments) or 2 l in volume (depending on the size of the individual fish). The chambers were filled with 1  $\mu\text{m}$  filtered sea water (26° C, salinity 35) and mixed by a magnetic air-driven stir bar located beneath a perforated screen to minimize disturbance to the fish. The respiration chambers were partially submerged in a water-bath which maintained temperature at 26.0° C, range  $\pm 0.3^\circ \text{C}$  by a recirculating heating and chilling unit (Forma Scientific, model 2095; Marietta, OH, U.S.A.). All fluorescent lighting was turned off and only a minimum of ambient light was permitted through the shaded windows of the laboratory.

The day prior to an experiment, fish were placed in a 40 l aquarium to make capture and placement into the chambers relatively easy and stress free. Young specimens were gently scooped into a glass beaker to minimize handling trauma, whereas larger fish were gently netted and transferred to the respiration chambers. The first 30 min of the trial were omitted to allow time for the specimens to acclimate to the containers. The smoothest portion of the respiration slopes were subjectively chosen for analysis. The time increments used to determine  $M_{\text{O}_2}$  varied between 17 and 120 min per trial. Dissolved oxygen levels remained  $>4.6 \text{ mg O}_2 \text{ ml}^{-1}$  (70% saturation) during respiration measurements in the majority of the trials (62 of 77) with oxygen levels depleted to an average of 77% saturation.

A seawater control (no fish) was simultaneously run with each trial to make corrections for microbial respiration and electrode  $\text{O}_2$  consumption. The control rate of oxygen consumption was subtracted from the raw respiration rate of each chamber to determine the corrected rate of oxygen consumption ( $\text{mg O}_2 \text{ individual}^{-1} \text{ h}^{-1}$ ). The mean  $\pm$  s.d. background levels for routine and feeding control trials were  $0.104 \pm 0.065$  and  $0.094 \pm 0.077 \text{ mg O}_2 \text{ h}^{-1}$ , respectively.

## NITROGEN EXCRETION

Ammonia ( $\text{NH}_3$  and  $\text{NH}_4^+$ ) ( $A_{\text{MM}}$ ) and urea ( $U_{\text{E}}$ ) excretion were measured concurrently with the oxygen respiration trials. After an individual fish was transferred to a respiration chamber, a c. 5 ml sample of chamber water was removed and stored in a 25 ml scintillation vial. The volume of water removed was replaced with filtered sea water and the chamber was sealed for the respiration trial. At the end of a trial, the time was recorded and a second water sample was taken. Samples were stored in a  $-80^\circ \text{C}$  freezer for a period of c. 6 months.

Triplicate 200  $\mu\text{l}$  sub-samples were added to individual wells on a 96 well microtiter plate for the total ammonia assay (Ivancic & Degobbi, 1984). The sample plate was then read in a Molecular Devices SpectraMax (Sunnyvale, CA, U.S.A.) microplate reader at 635 nm and the results were standardized to a 100  $\mu\text{M}$   $\text{NH}_4\text{Cl}$  serial dilution. The mean net total ammonia flux rate was calculated in  $\mu\text{M h}^{-1}$  and  $\mu\text{M g}^{-1} \text{ h}^{-1}$ . The urea assay (Price & Harrison, 1987) was also micro-modified for use on a 96 well microtiter plate. Three 200  $\mu\text{l}$  sub-samples of water from each respiration chamber were added to individual wells. The plates were read on the microplate reader at 540 nm and standardized to a 1 mM urea serial dilution and the mean value was reported.

The O:N ratios were calculated on the mass-specific oxygen consumption rates and mass-specific ammonia excretion rates ( $\mu\text{mol } \mu\text{mol}^{-1}$ ). The values were determined only on individual fish for which both respiration and excretion rates were measured. The O:N ratios reflect total mass-specific nitrogen excretion ( $A_{\text{MM}} + U_{\text{E}}$ ), with the  $U_{\text{E}}$  concentration defined as  $[\text{urea}] \times 2$  since urea has two nitrogen atoms.

## STATISTICAL ANALYSES

Diagnostic tests to determine if parametric tests were appropriate were conducted prior to statistical analyses. Error residual analysis was used to test for normality

and homogeneity of variance and partial  $F$  tests ( $\alpha = 0.05$ ) were used to test for significant differences in hypotheses. Non-linear regression analysis (proc NLIN) in SAS (SAS Institute Inc., version 9.1) was used to determine parameter estimates for non-linear responses. The regression (proc REG) and the general linear models (proc GLM) calculated the parameter estimates for ANCOVA using estimation of least squares.  $\log_{10}$  transformation of the data was applied where appropriate if the response was non-linear or for comparison to the literature.

The  $M_{O_2}$  dependent on  $M$  is expressed by the allometric relationship:  $M_{O_2} = \alpha M^\beta$ , where  $\alpha$  is the intercept and  $\beta$  is the slope. The non-transformed linear model was chosen, however, for statistical comparison of oxygen consumption between fed and unfed fish because the coefficients of determinations were high ( $r^2 > 0.96$ ). The  $\log_{10}$  transformed non-linear model was used to compare nitrogen excretion rates and O:N ratios of fed and unfed fish.

ANCOVA was used to make statistical comparisons between fed and unfed fish. The full model was  $Y = \beta_0 + \beta_1 X + \beta_2 Z + \beta_3 XZ + \varepsilon$ , where  $Y$  was the response variable,  $X$  was the covariate,  $Z$  was the dummy variable index which identified if fish were fed or starved,  $\beta_0$  was the  $Y$  intercept,  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  are the respective regression slope coefficients and  $\varepsilon$  was the random error term. The two individual lines were defined as  $Y = \beta_0 + \beta_1 X + \varepsilon$  and  $Y = (\beta_0 + \beta_2) + (\beta_1 + \beta_3) X + \varepsilon$ .

## RESULTS

### OXYGEN CONSUMPTION

$M_{O_2}$  (mg  $O_2$   $h^{-1}$ ) of fed juveniles increased significantly ( $P < 0.001$ ) with  $M$  (g) (Fig. 1). The data were best described by a linear regression (Fig. 1) with  $r^2 = 0.98$ . The allometric equation for fed juveniles was defined by the equation,  $M_{O_2} = 1.4291M^{0.8119}$ ;  $P < 0.001$ . Individual  $M_{O_2}$  values ranged from 0.050–17.903 mg  $O_2$   $h^{-1}$  ( $n = 35$ ). The  $R_R$  also increased linearly with  $M$  (Fig. 1) with  $r^2 = 0.96$  ( $P < 0.001$ ). The routine allometric metabolic relationship was expressed as:  $M_{O_2} = 1.1784M^{0.7833}$ ;  $P < 0.001$ . Individual routine  $M_{O_2}$  rates ranged from 0.084 to 9.375 mg  $O_2$   $h^{-1}$  ( $n = 42$ ).

This metabolic cost of feeding ( $R_f$ ), also referred to as SDA, reflects the energetics of mechanical and biochemical processes associated with digestion and assimilation of ingested food. It is quantified as the measurable increase in  $M_{O_2}$  following the consumption of a meal (Brett & Groves, 1979; Jobling, 1981). An ANCOVA on the linear models indicated the two slopes ( $b$ ) ( $F_{1,75}$ ,  $P < 0.05$ ) and the two lines ( $F_{2,75}$ ,  $P < 0.05$ ) were significantly different. The SDA of feeding results in an increase of 19 to 27% above the metabolic rates of starved fish (Table I).

Mass-specific oxygen consumption rates (mg  $O_2$   $g^{-1}$   $h^{-1}$ ) ( $Q_{O_2}$ ) decreased significantly with increasing size. The data were  $\log_{10}$  transformed to linearize the relationship between  $Q_{O_2}$  and  $M$ . The linear regressions for  $R_f$  ( $r^2 = 0.71$ ,  $P < 0.001$ ) and  $R_r$  ( $r^2 = 0.54$ ,  $P < 0.001$ ) are given in Fig. 2(a). The mean  $\pm$  s.d. mass-specific feeding metabolic rate ( $Q_{O_2}$ ) was  $8.481 \pm 26.952$  and the routine mass-specific metabolic rate ( $Q_{O_2}$ ) was  $2.176 \pm 4.378$ .

### NITROGEN EXCRETION

The  $A_{MM}$  ( $\mu\text{mol } h^{-1}$ ) of fed cohiba increased with increasing  $M$  according to the equation,  $A_{MM} = 4.8680M^{0.9201}$  ( $n = 18$ ,  $P < 0.001$ ). Ammonia excretion

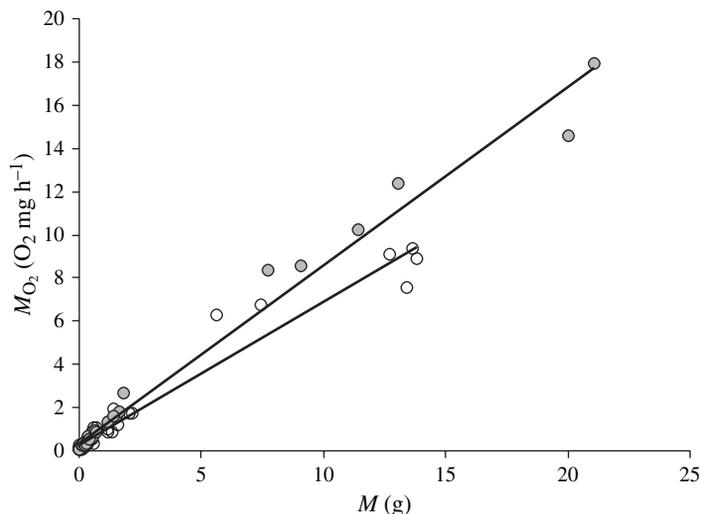


FIG. 1. Mean oxygen consumption rate ( $M_{O_2}$ ) and wet body mass ( $M$ ) for fed (● —),  $y = 0.8255x + 0.3324$  and starved (routine) (○ —),  $y = 0.6680x + 0.2438$ , juvenile cobia at 26°C.

rates of starved cobia significantly decreased after a period of starvation (ANCOVA,  $F_{2,29}$ ,  $P < 0.05$ ), with  $A_{MM}$  increasing with  $M$  according to the equation,  $A_{MM} = 3.1808M^{0.7615}$  ( $n = 15$ ,  $P < 0.001$ , although  $A_{MM}$  rates scaled the same with respect to  $M$  (*i.e.*  $b$  were not different) (ANCOVA,  $F_{1,29}$ ,  $P > 0.05$ ). Wet mass-specific ammonia excretion rates ( $\mu\text{mol g}^{-1} \text{h}^{-1}$ ) declined with an increase  $M$  for both feeding and starved fish (Fig. 3). Mass-specific ammonia excretion rates for starved larvae varied between 2.425 and 13.873  $\mu\text{mol g}^{-1} \text{h}^{-1}$  with a mean  $\pm$  s.d. value of  $4.333 \pm 2.809 \mu\text{mol g}^{-1} \text{h}^{-1}$ . Fed larval  $A_{MM}$  ranged between 3.093 and 16.355  $\mu\text{mol g}^{-1} \text{h}^{-1}$  with a mean  $\pm$  s.d. value of  $6.793 \pm 3.983 \mu\text{mol g}^{-1} \text{h}^{-1}$ .

Absolute urea excretion rates [ $(U_E) \mu\text{mol h}^{-1}$ ] of fed and starved cobia were more variable compared to  $A_{MM}$  and did not scale significantly with  $M$ . Mass-specific urea excretion rates ( $\mu\text{mol g}^{-1} \text{h}^{-1}$ ), however, declined dramatically with increasing  $M$  in both fed ( $n = 10$ ,  $P = 0.01$ ) and starved ( $n = 12$ ,  $P < 0.001$ ) cobia (Fig. 4). Mass-specific urea excretion rates varied between 0.532 and 59.064  $\mu\text{mol g}^{-1} \text{h}^{-1}$ . The mean  $\pm$  s.d. urea flux rates for fed and starved larvae were  $8.719 \pm 8.339$  and  $9.906 \pm 16.266 \mu\text{mol g}^{-1} \text{h}^{-1}$ , respectively.

## O:N RATIOS

The O:N values increased significantly with  $M$  for both fed ( $n = 8$ ) and starved ( $n = 12$ ) fish ( $P < 0.001$ ). An ANCOVA indicated the slopes for fed and starved fish were parallel ( $F_{1,16}$ ,  $P > 0.05$ ) and that the lines were not significantly different ( $F_{2,16}$ ,  $P > 0.05$ ). Consequently, the O:N ratios ( $y$ ) for both fed and starved fish were pooled and expressed by the equation:  $y = 5.7631M^{0.3027}$  ( $n = 20$ ,  $P < 0.001$ ) (Fig. 5).

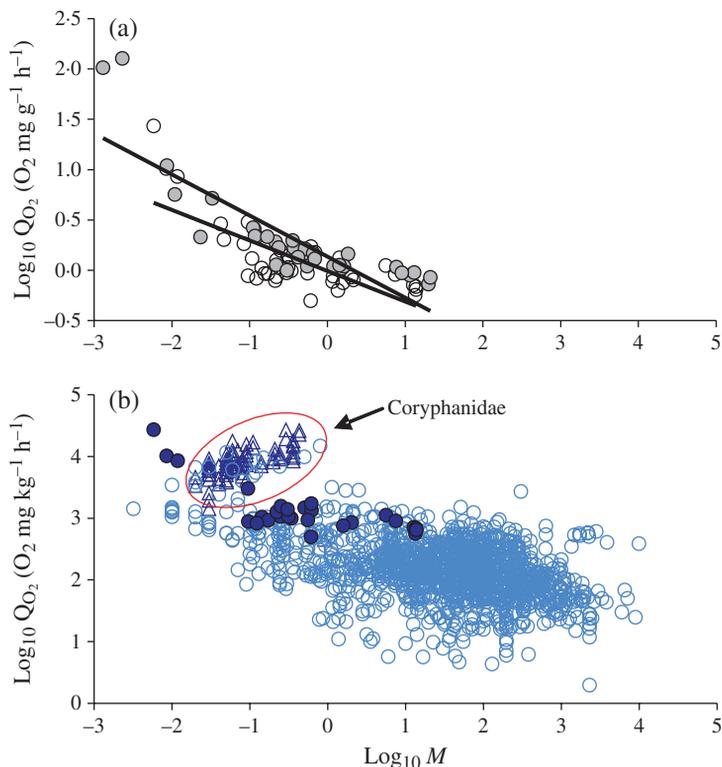


FIG. 2. (a)  $\text{Log}_{10}$  mass-specific oxygen consumption rates ( $Q_{O_2}$ ) and wet body mass ( $M$ ) for fed (●—),  $y = -0.4082x + 0.1366$  and starved (routine) (○—),  $y = -0.3031x - 0.0083$ , juvenile cobia at 26° C and (b) routine  $\log Q_{O_2}$  for cobia (●) juveniles adjusted to  $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$  for comparison to juvenile dolphinfish ( $\Delta$ ) ( $n = 72$ ) and other juvenile fishes (○) ( $n = 1357$ ) (Froese & Pauly, 2003).

## DISCUSSION

### OXYGEN CONSUMPTION

Cobia have relatively high  $R_r$  compared to other species of similarly sized juvenile fishes. At similar temperatures, the routine  $M_{O_2}$  of juvenile cobia are very similar to the  $M_{O_2}$  of common dolphinfish *Coryphaena hippurus* L. (26° C) (Benetti, 1992) and are greater than the  $M_{O_2}$  of juvenile carp *Cyprinus carpio* L. (25° C) (Oikawa & Itazawa, 1995). The routine  $M_{O_2}$  of cobia also appear greater than the oxygen consumption rates of juvenile Japanese flounder *Paralichthys olivaceus* (Temminck & Schlegel) (Liu *et al.*, 1997) and juvenile spotted dogfish *Scyliorhinus canicula* L. (Sims, 1996), although these rates were measured at cooler temperatures (20° and 15° C, respectively) (Fig. 6). These results are not unexpected since tropical fish larvae have elevated metabolic rates compared to larvae from cold waters since oxygen uptake increases significantly with temperature (Houde, 1989). The routine mass-specific oxygen consumption rates ( $Q_{O_2}$ ) of juvenile cobia are also compared to two species of Coryphaenidae and other juvenile fishes in Fig. 2(b).

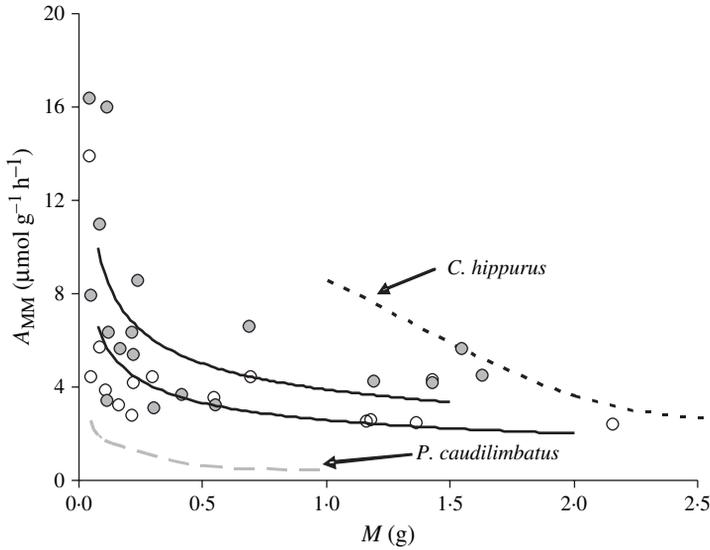


FIG. 3. Ammonia excretion rates ( $A_{MM}$ ) for fed (● —),  $y = 3.8906x^{-0.3632}$  and starved (○ —),  $y = 2.5818x^{-0.3640}$ , juvenile cobia at 26° C in relation to wet body mass. Mass-specific trend lines for juvenile *Paraconger caudilimbatus* (- - -) from Bishop & Torres (1999) and *Coryphaena hippurus* (- - -) extrapolated from Benetti (1992) are included for comparison.

High metabolic rates and fast growth (protein synthesis) may seem counter-intuitive, since metabolic needs and growth requirements ‘compete’ for the same finite pool of energy. Nevertheless, Boggs & Kitchell (1991) suggest that it is because of high metabolic rates that fishes such as tunas are able to grow

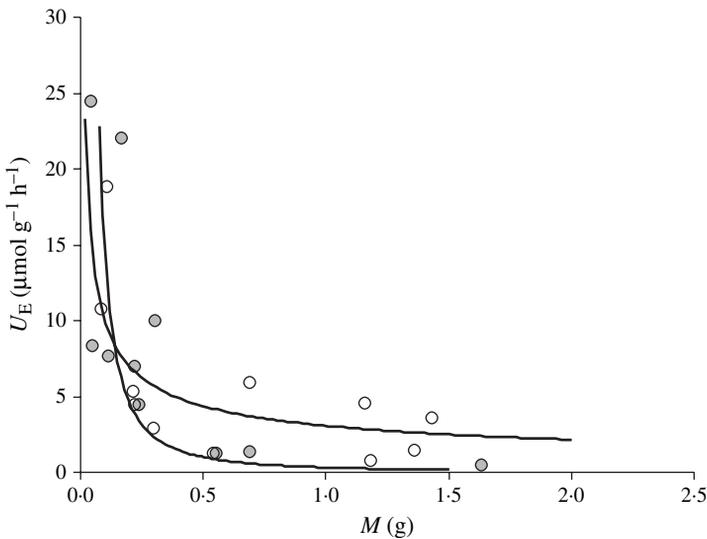


FIG. 4. Urea excretion rates ( $U_E$ ) for fed (● —),  $y = 3.6071x^{-0.5096}$  and starved (○ —),  $y = 0.3150x^{-1.6541}$ , juvenile cobia at 26° C in relation to wet body mass ( $M$ ).

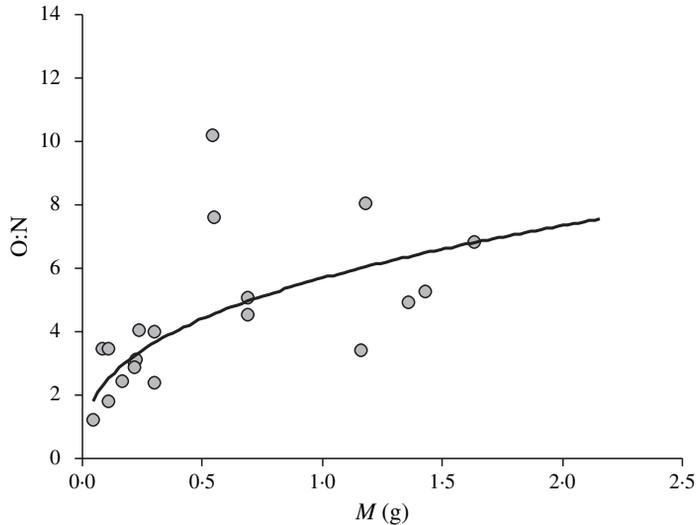


FIG. 5. Oxygen nitrogen (O:N) ratios for fed and starved (pooled data) juvenile cobia  $y = 5.7631x^{0.3027}$  at  $26^{\circ}\text{C}$  in relation to wet body mass ( $M$ ).

as rapidly as they do. It is hypothesized that pelagic species, such as tunas, billfish and dolphins, are able to grow, digest and recover from exhaustive exercise so rapidly because of their ability to deliver oxygen and metabolic substrates to tissues (Brill, 1996). In addition, the rate of oxygen uptake, rather than food supply, may be the key to anabolism and ultimately the limiting factor of

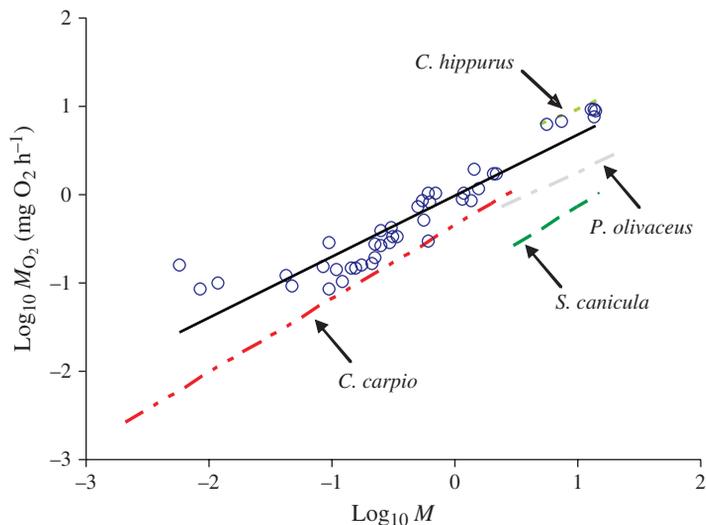


FIG. 6. Routine  $\log_{10}$  oxygen consumption rate ( $M_{\text{O}_2}$ ) of starved juvenile cobia ( $\circ$ ) at  $26^{\circ}\text{C}$  compared to juvenile *Coryphaena hippurus* (---) (Benetti, 1992), *Cyprinus carpio* (---) (Oikawa & Itazawa, 1995), *Paralichthys olivaceus* (---) (Liu *et al.*, 1997) and *Scyliorhinus canicula* (—) (Sims, 1996) in relation to  $\log_{10}$  wet body mass ( $M$ ). Exponent values from other studies were adjusted to  $\text{mg O}_2 \text{ h}^{-1}$  and  $\text{g}$  wet body mass.

growth in fishes (Pauly, 1981). Hence, cobia, although they are not obligate pelagics and may frequent coastal environments, possess similar 'high performance' physiological capabilities.

Routine metabolic rates of young juvenile cobia increase exponentially with mass at approximately the same scale as dolphinfish (cobia  $b = 0.67-0.78$ ; dolphinfish  $b = 0.71$ ). Unfortunately, the overlap in size of the juvenile dolphinfish data set was not that substantial, but routine respiration rates appear parallel for these two closely related species. The slope of increase for  $M_{O_2}$  at this stage of development was lower than the isometric value of one considered typical for larval fishes (Giguere *et al.*, 1988). This probably reflects the increase in mass of the juveniles and consequential reduction of cutaneous respiratory capabilities present in earlier stages of development. Initially, when surface to volume ratios are high, most small larval fishes rely on the skin and fins as important sites for respiration prior to the development of gill filaments and lamellae (de Sylva, 1974; Hughes & Al-Kadhomi, 1988; Rombough, 1988, 1992; Rombough & Ure, 1991). The exponent value was within the slope value of 0.70–0.86 that is typically cited for juvenile and adult fishes (Brett & Groves, 1979).

#### SPECIFIC DYNAMIC ACTION

The elevation of the metabolic rates of feeding fish relative to fish that are starved is known as specific dynamic action (SDA) or the heat increment and is the metabolic expenditure associated with the biochemical transformation of ingested food (Brett & Groves, 1979; Jobling, 1981). Jobling (1981) also suggests that the energetic costs associated with SDA are also indicative of growth rates, citing evidence that growth rates are greatest when oxygen consumption above maintenance metabolism is greatest. Oxygen serves as the final receptor in the electron transport system, meeting the basal metabolic requirements for animal maintenance in addition to activities such as growth, excretion and swimming (Beamish, 1990). Fishes that are in their asymptotic phase of growth demonstrate little increase in metabolic rates compared to routine metabolic rates (Jobling, 1985). The 19–27% increase in feeding metabolic rates indicated that the cost associated with feeding was a considerable percentage of the total energetic budget for juvenile cobia. In addition, the SDA data indicate that cobia specific-growth rates may be decreasing with increasing size. Metabolic rates for starved small pre-juveniles dropped 27% compared to *c.* 19% for larger early juveniles which suggests that relative growth is decreasing. This reduction was lower than the 40–60% reduction of SDA observed in larval redbfish *Sciaenops ocellatus* L. 3–14 dph (Torres *et al.*, 1996) and the 50% reduction in routine metabolic costs observed in juvenile dolphinfish (Benetti, 1992).

#### NITROGEN EXCRETION

The nitrogen excretion rates for cobia reported here make an important contribution to the understanding of the energetics of young subtropical teleosts. There is limited information in the literature on nitrogen excretion rates of young juvenile fishes and in particular, information pertaining to subtropical species. Reported flux rates may be slightly elevated since the protein content

of the feed used in this study was a little higher than what is considered optimal for this species (44.5%) (Chou *et al.*, 2001). Early stage juvenile cobia mean specific ammonia excretion rates are the highest reported in the literature; however, this mainly reflects the lack of data currently available for similar sized tropical species. Values for cobia are greater than those reported for juvenile dolphinfish ( $0.29 \mu\text{mol g}^{-1} \text{h}^{-1}$ ), although the dolphinfish were considerably larger (10–40 g) (Benetti, 1992). If ammonia excretion rates for larval redfish ( $30.6 \mu\text{mol g}^{-1}$  dry mass  $\text{h}^{-1}$ ; Torres *et al.*, 1996) are approximated to g wet mass (by 0.20 conversion factor), larval redfish nitrogen excretion rates are comparable ( $6.1 \mu\text{mol g}^{-1}$  wet mass  $\text{h}^{-1}$ ) to those of juvenile cobia. Mass-specific endogenous ammonia excretion rates of cobia are an order of magnitude greater compared to larger temperate juveniles (Iwata, 1970; Kikuchi *et al.*, 1992; Fraser *et al.*, 1998; Walsh *et al.*, 2001; Terjesen *et al.*, 2002) (Table II).

Endogenous ammonia excretion rates ( $\mu\text{mol h}^{-1}$ ) of starved cobia were significantly lower than fed cobia, which reflects the lower metabolic rates of fasting cobia. The coefficient of determination for starved fish indicated less variability in this data set compared to feeding fish, which probably reflects the randomness of when the rates of fed fish were measured relative to feeding (0–3 h). The temporal response of ammonia excretion to feeding varies with size and species (Brett & Groves, 1979). For example, a strong pulse of ammonia occurred *c.* 4 h after the start of feeding of fingerling sockeye salmon *Oncorhynchus nerka* (Walbaum) (Brett & Zala, 1975). Ammonia excretion rates of juvenile *P. olivaceus*, however, remained elevated compared to starved fish for >12 h after feeding (Kikuchi *et al.*, 1992).

TABLE II. Comparative flux rates for ammonia ( $A_{\text{MM}}$ ) and urea ( $U_{\text{E}}$ ) excretion of juvenile and larval fishes

Species	Temperature (° C)	Wet mass (g)	$A_{\text{MM}}$	$U_{\text{E}}$	Source
Juvenile					
<i>Paralichthys olivaceus</i>	20	1.5–6.5	0.42	0.02	Kikuchi <i>et al.</i> (1992)*
<i>Coryphaena hippurus</i>	26	9.8–43.8	0.29		Benetti (1992)†
<i>Carassius auratus</i> L.	20	1	0.24		Iwata (1970)‡
<i>BathYGONUS nigripinnis</i>	11–13	4–10	0.59	0.10	Walsh <i>et al.</i> (2001)
<i>Hippoglossus</i> <i>hippoglossus</i> L.	6	0.01	0.44	0.06	Terjesen <i>et al.</i> (2002)
<i>H. hippoglossus</i>	11–12	62–100	0.21	0.01	Fraser <i>et al.</i> (1998)§
<i>Opsanus beta</i>	23–25	0.04	0.24	1.02	Barimo <i>et al.</i> (2004)
<i>Rachycentron canadum</i>	26	0.04–2.16	4.33	9.91	This study
Larval					
<i>Sciaenops ocellatus</i>	25		30.6		Torres <i>et al.</i> (1996)¶
<i>C. hippurus</i>	26		2.1		Benetti (1992)¶

\*Converted from  $\text{mg } 100 \text{ g}^{-1} \text{h}^{-1}$ .

†Converted from  $\text{mg } \text{g}^{-1} \text{h}^{-1}$ .

‡Converted from  $\text{mg } \text{kg}^{-1} \text{h}^{-1}$ .

§Converted from  $\mu\text{g } \text{g}^{-1} \text{h}^{-1}$ .

||Size, pers. comm.

¶Values are  $\mu\text{mol } \text{g}^{-1}$  dry mass  $\text{h}^{-1}$ .

Given the relatively high rates of ammonia excretion, the finding that juvenile cobia (<2 g wet mass) excrete more than half of their total nitrogenous wastes as urea was somewhat unexpected. This finding is intriguing from an energetic perspective, since it is metabolically more costly to produce equivalent amounts of urea than ammonia (Wood, 1993). Bony fishes are considered primarily to be ammoniotelic, although urea can contribute a substantial portion of the waste budget in several fish species (Sayer & Davenport, 1987; Wood, 1993; Walsh, 1998; Walsh *et al.*, 2001). In general, marine fishes (40% of  $A_{MM}$ ) tend to produce more urea compared to their freshwater counterparts (5–35%) (Wood, 2001). The gulf toadfish *Opsanus beta* (Goode & Bean) may release >90% of its nitrogenous waste from its gills as urea in a single pulse (Wood *et al.* 1995, 1997, 1998; Gilmour *et al.*, 1998). Juvenile gulf toadfish are also pulsatile ureotelic, with their mass-specific urea flux rate an order of magnitude higher than in adults (Barimo *et al.*, 2004). It has also been shown that several species of fishes are ureotelic as embryos but become ammoniotelic as adults (Wright *et al.*, 1995; Chadwick & Wright, 1999), suggesting that urea may play a more important role in the nitrogen budget of larval and juvenile teleosts than previously thought (Walsh *et al.*, 2001).

The mean specific urea excretion rates for cobia are also the highest reported in the literature for teleosts. This may be partially due to the lack of studies done on similar sized tropical juveniles and possibly the flexible nature of urea excretion. Under intensive rearing conditions, several hatchery studies on salmonids have shown the role of urea in the nitrogen budget may be considerable, although somewhat fluctuating (Burrows, 1964; McLean & Fraser, 1974; Wood, 2001). Overall, daily urea production varied substantially (0–78% of total nitrogen), but tended to prevail on higher light intensity days and appeared less important with increasing biomass and temperatures (Wood, 2001). A more precise estimate of urea flux in cobia could be determined by undertaking a study of excretion patterns over a longer period of time.

Mass-specific urea excretion rates scaled strongly with increasing mass, which is not unexpected since metabolic rates in organisms scale exponentially with mass (Withers, 1992). There was a strong demarcation of mass-specific urea flux at a body size of *c.* 0.05 g, with excretion rates higher in fishes that are smaller. Fed juvenile cobia >0.05 g have excretion rates that are similar to those reported for small (0.043 g; J. F. Barimo, pers. comm.) juvenile gulf toadfish ( $1.02 \mu\text{mol g}^{-1} \text{h}^{-1}$ ) (Barimo *et al.*, 2004). The present results suggest that both urea and ammonia are important for the excretion of nitrogenous wastes with a possible decrease in the relative importance of urea production as juvenile cobia grow larger. Although recent studies recognize that the summation of  $A_{MM}$  and urea is the typical method of measuring nitrogen excretion, it may significantly underestimate the proportion of total nitrogen wastes by 10–60% (Walsh *et al.*, 2001; Kajimura *et al.*, 2004).

## O:N RATIO

The O:N ratios significantly increased as juvenile cobia grew larger, from *c.* 3.8 at 0.25 g to 6.5 at 1.5 g wet mass. The O:N ratio indicated that initially the energetic substrate for juvenile cobia was primarily protein (97%) and the

utilization of protein decreased (57%) as the juveniles grew larger [interpreted from the inverse of the O:N ratio (Idrisi *et al.*, 2006) based on the nitrogen quotient ( $Q_N$ ) defined by van den Thillart & Kesbeke (1978) and discussed by Lauff & Wood (1996):  $P = 3.70Q_N$ , where  $P$  is the proportion of the utilized diet that is protein and  $Q_N$  is N:O. There was no significant difference in the ratio for fed and starved fish, which may indicate the lack of lipid reserves present in juvenile cobia. When fishes are fed, however, protein utilization is typically expected to subsequently peak compared to unfed fishes (Wood, 2001). This point may be clarified by increasing the period of starvation (18–24 h) in future studies on this species.

Juvenile cobia utilize an energetic strategy for survival that emphasizes high metabolic rates, which allow for high rates of ingestion (based on high nitrogenous flux rates) which support rapid growth. Juvenile cobia (120 dph) grew 2.46% body mass day<sup>-1</sup> under sub-optimal conditions (23.9° C) and may grow as rapidly as dolphinfish (4.33% body mass day<sup>-1</sup>; Benetti *et al.*, 1995) in warmer water (Denson *et al.*, 2003). Growth provides an ecological refuge of increased size by decreasing the number of potential predators and increasing the number of potential prey (Torres *et al.*, 1996). A lower than isometric metabolic scaling coefficient means pre-juvenile and early juvenile stage cobia have reached a stage of development that is less susceptible to starvation than their larval counterparts. The absolute need for energy does not increase as rapidly and use of energy on a mass-specific basis was lower when compared to larval fish. This may give cobia a competitive advantage over tropical reef species since they can metamorphose to juvenile in as little as 2 weeks post-hatch. This type of precocious larval development appears typical for other pelagic predatory species of fishes such as Atlantic sailfish *Istiophorus platypterus* (Shaw), Atlantic blue marlin *Makaira nigricans* Lacepède, dolphinfish and swordfish *Xiphias gladius* L. (Prince *et al.*, 1991; Benetti, 1992; Govoni *et al.*, 2003; Luthy *et al.*, 2005).

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## References

- Arnold, C. R., Kaiser, J. B. & Holt, G. J. (2002). Spawning of cobia *Rachycentron canadum* in captivity. *Journal of the World Aquaculture Society* **33**, 205.
- Barimo, J. F., Steele, S. L., Wright, P. A. & Walsh, P. J. (2004). Dogmas and controversies in the handling of nitrogenous wastes: ureotely and ammonia tolerance in early life stages of the gulf toadfish, *Opsanus beta*. *Journal of Experimental Biology* **207**, 2011–2020.

- Beamish, F. W. H. (1990). Swimming metabolism and temperature in juvenile walleye, *Stizostedion vitreum vitreum*. *Environmental Biology of Fishes* **27**, 309–314.
- Benetti, D. D. (1992). Bioenergetics and growth of dolphin, *Coryphaena hippurus*. PhD Thesis, University of Miami, Coral Gables, FL, USA.
- Benetti, D. D., Iversen, E. S. & Ostrowski, A. C. (1995). Growth rates of captive dolphin, *Coryphaena hippurus*, in Hawaii. *Fishery Bulletin* **93**, 152–157.
- Benetti, D. D., Alarcon, J. F., Stevens, O. M., O'Hanlon, B., Rivera, J. A., Banner-Stevens, G. & Rotman, F. J. (2003). Advances in hatchery and growout technology of marine finfish candidate species for offshore aquaculture in the Caribbean. *Proceedings of the Gulf and Caribbean Fisheries Institute* **54**, 473–487.
- Benetti, D. D., Brand, L., Collins, J., Orhun, R., Benetti, A., O'Hanlon, B., Danylchuk, A., Alston, D., Rivera, J. & Cabarcas, A. (2006). Can offshore aquaculture of carnivorous fish be sustainable? *World Aquaculture* **37**, 44–47.
- Bishop, R. E. & Torres, J. J. (1999). Leptocephalus energetics, metabolism and excretion. *Journal of Experimental Biology* **202**, 2485–2493.
- Boggs, C. H. & Kitchell, J. F. (1991). Tuna metabolic rates estimated from energy losses during starvation. *Physiological Zoology* **64**, 502–524.
- Brett, J. R. & Groves, T. D. D. (1979). Physiological energetics. In *Fish Physiology*, Vol. VIII (Hoar, W. S., Randall, D. J. & Brett, J. R., eds), pp. 279–352. New York: Academic Press, Inc.
- Brett, J. R. & Zala, C. A. (1975). Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon (*Oncorhynchus nerka*) under controlled conditions. *Journal of the Fisheries Research Board of Canada* **32**, 2479–2486.
- Brill, R. W. (1996). Selective advantages conferred by the high performance physiology of tunas, billfishes, and dolphin fish. *Comparative Biochemistry and Physiology* **113A**, 3–15.
- Burrows, R. E. (1964). Effects of accumulated excretory products on hatchery-reared salmonids. *Bureau of Sport Fisheries Wildlife Research Report 66*. Washington, DC: US Fish and Wildlife Service.
- Chadwick, T. D. & Wright, P. A. (1999). Nitrogen excretion and expression of urea cycle enzymes in the Atlantic cod (*Gadus morhua* L.): a comparison of early life stages with adults. *Journal of Experimental Biology* **202**, 2653–2662.
- Chou, R.-L., Su, M.-S. & Chen, H.-Y. (2001). Optimal dietary protein and lipid levels for juvenile cobia (*Rachycentron canadum*). *Aquaculture* **193**, 81–89.
- Dawson, C. E. (1971). Occurrence and description of prejuvenile and early juvenile Gulf of Mexico cobia, *Rachycentron canadum*. *Copeia* **1971**, 65–71.
- Denson, M. R., Stuart, K. R., Smith, T. I. J., Weirich, C. R. & Segars, A. (2003). Effects of salinity on growth, survival, and selected hematological parameters of juvenile cobia *Rachycentron canadum*. *Journal of the World Aquaculture Society* **34**, 496–504.
- Ditty, J. G. & Shaw, R. F. (1992). Larval development, distribution, and ecology of cobia *Rachycentron canadum* (family, Rachycentridae) in the northern Gulf of Mexico. *Fishery Bulletin* **90**, 668–677.
- Franks, J. S., Warren, J. R. & Buchanan, M. V. (1999). Age and growth of cobia, *Rachycentron canadum*, from the northeastern Gulf of Mexico. *Fishery Bulletin* **97**, 459–471.
- Fraser, K. P. P., Lyndon, A. R. & Houlihan, D. F. (1998). Protein synthesis and growth in juvenile Atlantic halibut, *Hippoglossus hippoglossus* (L.), application of <sup>15</sup>N stable isotope tracer. *Aquaculture Research* **29**, 289–298. doi: 10.1046/j.1365-2109.1998.00210.x
- Giguere, L. A., Cote, B. & St-Pierre, J. F. (1988). Metabolic rates scale isometrically in larval fishes. *Marine Ecology Progress Series* **50**, 13–19.
- Gilmour, K. M., Perry, S. F., Wood, C. M., Henry, R. P., Laurent, P., Pärt, P. & Walsh, P. J. (1998). Nitrogen excretion and the cardiorespiratory physiology of the gulf toadfish, *Opsanus beta*. *Physiological Zoology* **71**, 492–505.
- Govoni, J. J., Laban, E. H. & Hare, J. A. (2003). The early life history of swordfish (*Xiphias gladius*) in the western North Atlantic. *Fishery Bulletin* **101**, 778–789.
- Green, E. J. & Carritt, D. E. (1967). New tables for oxygen saturation of seawater. *Journal of Marine Research* **25**, 140–147.

- Houde, E. D. (1989). Comparative growth, mortality, and energetics of marine fish larvae: temperature and implied latitudinal effects. *Fishery Bulletin* **87**, 471–495.
- Houde, E. D. (1994). Differences between marine and freshwater fish larvae: implications for recruitment. *ICES Journal of Marine Science* **51**, 91–97.
- Houde, E. D. (1997). Patterns and consequences of selective processes in teleost early life histories. In *Early Life History and Recruitment in Fish Populations* (Chambers, R. C. & Trippel, E. A., eds), pp. 173–196. London: Chapman & Hall.
- Houde, E. D. & Scheckter, R. C. (1983). Oxygen uptake and comparative energetics among eggs and larvae of three subtropical marine fishes. *Marine Biology* **72**, 283–293.
- Hughes, G. M. & Al-Kadhomy, N. K. (1988). Changes in scaling of respiratory systems during the development of fishes. *Journal of the Marine Biological Association of the United Kingdom* **68**, 489–498.
- Idrisi, N., Capo, T., Luthy, S. & Serafy, J. E. (2003). Behavior, oxygen consumption and survival of stressed juvenile sailfish (*Istiophorus platypterus*) in captivity. *Marine and Freshwater Behavior and Physiology* **36**, 87–95.
- Idrisi, N., Barimo, J. F., Hudder, A., Capo, T. R. & Walsh, P. J. (2006). Rates of nitrogen excretion and oxygen consumption in the California Sea hare, *Aplysia californica*. *Bulletin of Marine Science* **79**, 231–237.
- Ivancic, I. & Degobbi, D. (1984). An optimal manual procedure for ammonia analysis in natural waters by the indophenol blue method. *Water Research* **18**, 1143–1147.
- Iwata, K. (1970). Relationship between food and growth in young crucian carps, *Carassius auratus cuvieri*, as determined by the nitrogen balance. *Japanese Journal of Limnology* **31**, 129–151.
- Jobling, M. (1981). The influences of feeding on the metabolic rate of fishes: a short review. *Journal of Fish Biology* **18**, 385–400. doi: 10.1111/j.1095-8649.1981.tb03780.x
- Jobling, M. (1985). Growth. In *Fish Energetics: New Perspectives* (Tytler, P. & Calow, P., eds), pp. 213–230. Baltimore, MD: John Hopkins University Press.
- Johnson, G. D. (1984). Percoidae: development and relationships. In *Ontogeny and Systematics of Fishes* (Moser, H. G., Richards, W. J., Cohen, D. M., Fahay, M. P., Kendall, A. W. & Richardson, S. L., eds), pp. 464–498. Lawrence, KS: Allen Press.
- Kajimura, M., Croke, S. J., Glover, C. N. & Wood, C. M. (2004). Dogmas and controversies in the handling of nitrogenous wastes: the effect of feeding and fasting on the excretion of ammonia, urea and other nitrogenous waste products in rainbow trout. *Journal of Experimental Biology* **207**, 1993–2002.
- Kikuchi, K., Takeda, S., Honda, H. & Kiyono, M. (1992). Nitrogenous excretion of juvenile and young Japanese flounder. *Nippon Suisan Gakkaishi* **58**, 2329–2333.
- Lauff, R. F. & Wood, C. M. (1996). Respiratory gas exchange, nitrogenous waste excretion, and fuel usage during starvation in juvenile rainbow trout, *Oncorhynchus mykiss*. *Journal of Comparative Physiology* **165A**, 542–551.
- Liu, H., Sakurai, Y., Munehara, H. & Shimazaki, K. (1997). Diel rhythms of oxygen consumption and activity level of juvenile flounder *Paralichthys olivaceus*. *Fisheries Science* **63**, 655–658.
- Luthy, S. A., Cowen, R. K., Serafy, J. E. & McDowell, J. R. (2005). Toward identification of larval sailfish (*Istiophorus platypterus*), white marlin (*Tetrapturus albidus*), and blue marlin (*Makaira nigricans*) in the western North Atlantic Ocean. *Fishery Bulletin* **103**, 588–600.
- McLean, W. E. & Fraser, F. J. (1974). Ammonia and urea production of coho salmon under hatchery conditions. *Surveillance Report EPS, 5-PR-74-5*. Vancouver: Environmental Service Pacific Region.
- Nelson, J. S. (1994). *Fishes of the World*. New York: John Wiley & Sons, Inc.
- Oikawa, S. & Itazawa, Y. (1995). Comparison of oxygen consumption of larval and juvenile carp determined by three different methods. *Fisheries Science* **61**, 487–490.
- Pauly, D. (1981). The relationships between gill surface area and growth performance in fish: a generalization of von Bertalanffy's theory of growth. *Meeresforschung* **28**, 251–282.

- Pedersen, B. H. (1997). The cost of growth in young fish larvae, a review of new hypotheses. *Aquaculture* **155**, 259–269.
- Perez-Pinzon, M. A. & Lutz, P. (1991). Activity related cost of osmoregulation in the juvenile snook (*Centropomus undecimalis*). *Bulletin of Marine Science* **48**, 58–66.
- Pfeiler, E. (1996). Energetics of metamorphosis in bonefish (*Albula* sp.) leptocephali: role of keratan sulfate glycosaminoglycan. *Fish Physiology and Biochemistry* **15**, 359–362.
- Pfeiler, E. & Govoni, J. J. (1993). Metabolic rates in early life history stages of elopomorph fishes. *Biological Bulletin* **185**, 277–283.
- Price, N. M. & Harrison, P. J. (1987). Comparison of methods for the analysis of urea in seawater. *Marine Biology* **94**, 307–313.
- Prince, E. D., Lee, D. W., Zweifel, J. R. & Brothers, E. B. (1991). Estimating age and growth of young Atlantic blue marlin *Makaira nigricans* from otolith microstructure. *Fishery Bulletin* **89**, 441–459.
- Rombough, P. J. (1988). Respiratory gas exchange, aerobic metabolism and effects of hypoxia during early life. In *Fish Physiology*, Vol. XIA (Hoar, W. S. & Randall, D. J., eds), pp. 59–161. San Diego, CA: Academic Press, Inc.
- Rombough, P. J. (1992). Intravascular oxygen tensions in cutaneously respiring rainbow trout (*Oncorhynchus mykiss*) larvae. *Comparative Biochemistry and Physiology* **101A**, 23–27.
- Rombough, P. J. & Ure, D. (1991). Partitioning of oxygen uptake between cutaneous and branchial surfaces in larval and young juvenile Chinook salmon *Oncorhynchus tshawytscha*. *Physiological Zoology* **64**, 717–727.
- Sayer, M. D. J. & Davenport, J. (1987). The relative importance of the gills to ammonia and urea excretion in five seawater and one freshwater teleost species. *Journal of Fish Biology* **31**, 561–570. doi: 10.1111/j.1095-8649.1987.tb05258.x
- Shaffer, R. V. & Nakamura, E. L. (1989). *Synopsis of biological data on the cobia *Rachycentron canadum* (Pisces, Rachycentridae)*. *FAO Fisheries Synopsis* **153**, NOAA Technical Report NMFS **82**, 1–21.
- Sims, D. W. (1996). The effect of body size on the standard metabolic rate of the lesser spotted dogfish. *Journal of Fish Biology* **48**, 542–544. doi: 10.1111/j.1095-8649.1996.tb01447.x
- Sissenwine, M. P. (1984). Why do fish populations vary? In *Exploitation of Marine Communities* (May, R. M., ed.), pp. 59–94. Berlin: Springer-Verlag.
- de Sylva, D. P. (1974). Development of the respiratory system in herring and plaice larvae. In *The Early Life History of Fish* (Blaxter, J. H. S., ed.), pp. 465–485. Berlin: Springer.
- Terjesen, B. F., Finn, R. N., Norberg, B. & Ronnestad, I. (2002). Kinetics and fates of ammonia, urea, and uric acid during oocyte maturation and ontogeny of the Atlantic halibut (*Hippoglossus hippoglossus* L.). *Comparative Biochemistry and Physiology* **131A**, 443–455.
- Tolley, S. G. & Torres, J. J. (2002). Energetics of swimming in juvenile common snook, *Centropomus undecimalis*. *Environmental Biology of Fishes* **63**, 427–433.
- Torres, J. J., Brightman, R. I., Donnelly, J. & Harvey, J. (1996). Energetics of larval red drum, *Sciaenops ocellatus*. Part I: oxygen consumption, specific dynamic action, and nitrogen excretion. *Fishery Bulletin* **94**, 756–765.
- van den Thillart, G. & Kesbeke, F. (1978). Anaerobic production of carbon dioxide and ammonia by goldfish *Carassius auratus* (L.). *Comparative Biochemistry and Physiology* **59A**, 393–400.
- Walsh, P. J. (1998). Nitrogen excretion and metabolism. In *The Physiology of Fishes* (Evans, D. E., ed.), pp. 199–214. Boca Raton, FL: CRC Press.
- Walsh, P. J., Wang, Y., Campbell, C. E., DeBoeck, G. & Wood, C. M. (2001). Patterns of nitrogenous waste excretion and gill urea transporter mRNA expression in several species of marine fish. *Marine Biology* **139**, 839–844.
- Withers, P. C. (1992). *Comparative Animal Physiology*. Fort Worth, TX: Saunders College Publishing.
- Wood, C. M. (1993). Ammonia and urea metabolism and excretion [in fish]. In *The Physiology of Fishes* (Evans, D. H., ed.), pp. 379–426. Boca Raton, FL: CRC Press.

- Wood, C. M. (2001). The influence of feeding, exercise and temperature on nitrogen metabolism and excretion. In *Fish Physiology*, Vol. 20 (Anderson, P. M. & Wright, P. A., eds), pp. 201–238. Orlando, FL: Academic Press.
- Wood, C. M., Hopkins, T. E., Hogstrand, C. & Walsh, P. J. (1995). Pulsatile urea excretion in the ureagenic toadfish *Opsanus beta*: an analysis of rates and routes. *Journal of Experimental Biology* **198**, 1729–1741.
- Wood, C. M., Hopkins, T. E. & Walsh, P. J. (1997). Pulsatile urea excretion in the toadfish (*Opsanus beta*) is due to a pulsatile excretion mechanism, not a pulsatile production mechanism. *Journal of Experimental Biology* **200**, 1039–1046.
- Wood, C. M., Gilmour, K. M., Perry, S. F., Pärt, P., Laurant, P. & Walsh, P. J. (1998). Pulsatile urea excretion in gulf toadfish: evidence for activation of a specific facilitated diffusion transport system. *Journal of Experimental Biology* **201**, 805–817.
- Wright, P. A., Felskie, A. K. & Anderson, P. M. (1995). Induction of the ornithine-urea cycle enzymes and nitrogen metabolism and excretion in the rainbow trout (*Oncorhynchus mykiss*) during life stages. *Journal of Experimental Biology* **198**, 127–135.

### Electronic Reference

- Froese, R. & Pauly, D. (Eds). (2003). *FishBase*. Available at <http://www.fishbase.org>