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Metabolic Rates of Embryo and Growth of Cobia (Rachycentron canadum) and Mahi (Coryphaena hippurus) Larvae through Early Juvenile Stages

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METABOLIC RATES OF EMBRYO AND GROWTH OF COBIA (*RACHYCENTRON CANADUM*) AND MAHI (*CORYPHAENA HIPPURUS*) LARVAE THROUGH EARLY JUVENILE STAGES

By

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<u>Metabolic Rates of Embryo and Growth of</u>   (May 2014)
<u>Cobia (*Rachycentron canadum*) and Mahi</u>
<u>(*Coryphaena hippurus*) Larvae through</u>
<u>Early Juvenile Stages.</u>

Cobia (*Rachycentron canadum*) and mahi (*Coryphaena hippurus*) are top pelagic predators known for exhibiting rapid rates of development and growth throughout all life stages. Metabolic rates of embryonic stages of both species were studied to determine and compare the relationship between their metabolism and growth during early developmental stages. Metabolic rates (0.246 ± 0.035 µL O_2/egg/hr) and weight-specific metabolic rates (2.562 ± 0.364 µL O_2/mg dry weight/hr) of cobia embryos were not significantly different than metabolic rates (0.345 ± 0.05 µL O_2/egg/hr) and weight-specific metabolic rates (2.199 ± 0.321 µL O_2/mg dry weight/hr) of mahi embryos. Growth rates, length-weight relationship and condition factors were also determined and compared during the first 30 days post-hatching (DPH) of cobia and mahi larvae. Cobia larvae had an absolute growth rate (AGR) in length of 0.901 mm/day and wet weight of 4.2 mg/day. Length and weight specific growth rates (SGR) of cobia larvae were 7.37%/day and 21.7%/d, respectively. Mahi larvae had a length AGR of 0.965 mm/day, weight AGR of 10.57 mg/day, length SGR of 6.3%/day and weight SGR of 23.2%/day. No significant difference was found between cobia and mahi larval growth rates in length (ANCOVA; p<0.05). In weight, however, mahi larvae grew significantly faster than cobia larvae, $F(1, 57) = 29.165$, $p < 0.001$). Cobia and mahi larvae exhibit allometric

growth rates, and their length (x) – weight (Y) relationships are best expressed by the following power equations:

Cobia: $Y = 0.01x^{2.7433}$ $r^2 = 0.9887$ $n = 792$

Mahi: $Y = 0.0024x^{3.4474}$ $r^2 = 0.9808$ $n = 720$

Condition factor (K) for cobia larvae dropped from 1.03 to 0.37, while mahi larvae K value increased from 0.52 to 0.96 during the period studied (30 days post-hatch), when both species had reached juvenile stages.

Metabolic rates and weight-specific metabolic rates of both cobia and mahi embryos were found to be higher than those reported for most other teleosts in the literature. Likewise, larvae of both species exhibit faster rates of development in length and weight than those reported for most other fish, and comparable to those of high performance pelagic species like tunas.

Ad Majoren Dei Gloriam…

To my parents who influenced and inspired me…

To my brother who has always been an example for me…

To Upsilon Sigma Phi where I learned to "hold on"

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CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

Metabolism is the process through which energy and materials are transformed within an organism and exchanged between the organism and its environment. It plays a crucial role in an organism's physiological function such as growth and development (Gillooly et al., 2001; Kearny & White, 2012). It can be generalized that a whole organism's metabolic rate scales with the ¾-power of body mass, also known as "Kleiber's Law" (Kleiber, 1947; Gillooly et al., 2001; Glazier, 2006). Glazier (2006) has found that during the ontogeny of pelagic animals, metabolic rates scale isometrically (1:1), thus being an exception to Kleiber's Law. Pelagic fish eggs and larvae are prone to high rates of mortality due to predation (McGurk, 1987). As a result, eggs and larvae of pelagic fish must have rapid growth rates through those fragile and vulnerable early developmental stages to minimize if not to escape predation. Indeed, isometric scaling is an evolutionary trait in response to an organism's ecological circumstance (Glazier, 2006).

Fast growth rates are an important life strategy for pelagic marine fish larvae. Brill (1996) argues that in order for high performance pelagic fish species to survive as top predators in an energy-depurated environment it must have, among other physiological adaptations, high somatic and gonadal growth rates. Mahi-mahi, *Coryphaena hippurus*, is considered to be among these high performance pelagic predators (Benetti et al. 1995[a]; Brill 1996). It is a non-selective forager preying on flying fish, mackerel, epipelagic cephalopods, and juveniles of larger pelagic fish species like tuna (Bannister, 1976; Oxenford & Hunte, 1999; Olson & Galvan-Magana, 2002). As a

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fast swimming oceanic species it has a worldwide distribution ranging as far north as Georges Bank, Nova Scotia and as far south as Rio de Janeiro, Brazil (Palko, 1982). Benetti (1992) and Benetti et al. (1995[a]) studied the bioenergetics and growth of mahi from embryo to adult. He found that oxygen consumption rates of mahi embryo were considerably higher than those of other tropical and subtropical fishes for which data was available in literature. In addition, he was also able to correlate the increase of oxygen consumption of mahi larvae to its fast growth rates. There is a great deal of variation in reported growth rates of mahi, ranging from as high as 0.5 kg to 16.3 kg in 8 months (Beardsley, 1967) to as low as egg to 4.93 kg in 9.5 months (Benetti et al., 1995[b]). Nonetheless, even the slowest growth rates reported for mahi are still among the fastest of any other fish.

Similarly, cobia (*Rachycentron canadum*) is also an oceanodromous species that is distributed in tropical, subtropical and temperate waters worldwide except in the Eastern Pacific (Shaffer & Nakamura, 1989). Cobia also has pelagic eggs and larvae, and is known to have fast growth rates (Shaffer & Nakamura, 1989; Benetti et al., 2010[a]). However, cobia has evolved different adaptations and strategies, posing interesting challenges to explain growth rates comparable to those of true high performance pelagic species such as mahi and tuna. Unlike its counterparts, cobia can be found in a variety of habitats, encompassing nearshore, offshore, estuarine and occasionally even in rivers (Shaffer & Nakamura, 1989; Kuo & Shao, 1999). Furthermore, cobia has been described as an opportunistic feeder, as gut content examination has revealed the presence of crabs, shrimps, squid and fish - thus associating it with the benthic environment (Shaffer & Nakamura, 1989). In addition, adult cobia has been observed to often rest at the bottom

of tanks in aquaculture settings (personal observation). Despite not being considered a true pelagic or a high performance fish, it can be hypothesized that, during the embryonic and larval stages, cobia will have similar metabolic rates to mahi, since both are exposed to the same environment during early development. Given that metabolism and growth are directly related (Brett and Groves, 1979), the similar fast rates of development and growth for both species should lend further support to this hypothesis.

1.2 PREVIOUS STUDIES

Because of its extraordinary growth rates and potential for commercial aquaculture, cobia has become a well-studied and well-documented species during the last decade. With its high fecundity coupled with the ability to be conditioned to volitionally spawn year-round through adequate nutrition and environmental cues such as light and temperature, cobia enormous potential for domestication has been recognized (Liao et al., 2004; Benetti et al., 2007, 2010[a]; Stieglitz et al., 2012). Each spawning event would normally produce >1 million eggs with fertilization rates ranging from 80-91% and hatching rates of 70-86% within 21-37 hours (Liao et al., 2004, Faulk & Holt, 2008; Stieglitz et al., 2012). Each female can spawn every other week (personal observation). Larval and juvenile rearing protocols which encompass culture systems (ponds, flow-through conical tanks and recirculating raceways), feed, feeding rate, water exchange, prophylaxis and use of probiotics continue to improve, thus resulting in more efficient and higher production yields while becoming more environmentally sound (Liao et al., 2004; Faulk et al., 2007; Zink, 2010; Benetti et al., 2008, 2010[a]). Furthermore, with a growing interest in expanding cobia culture inland as well as improving shipping protocols, trials have been conducted on growth and survival of juvenile cobia at varying

salinities, temperatures and stocking densities. The results of these studies indicate that it is possible to grow-out juveniles at salinities as low as 5 parts per thousand (ppt) as well as hold and ship them at 12 ppt with densities above 10 kg/m^3 without risking higher mortalities (Atwood et al., 2004; Resley et al., 2006; Stieglitz, 2010). However, temperature below 18°C show that juveniles cease to feed and mortalities start occurring below 13 °C (Atwood et al., 2004; McDonald & Bumguardner, 2010).

In terms of the grow-out phase, much success has also been achieved worldwide. The most common culture system for cobia grow-out is floating near-shore cages where fish have been shown to reach market size of 6-8 kg in approximately 12 months (Liao *et al*., 2004; Sun *et al*., 2006; Benetti et al. 2010[b]). In addition to sea cages, similar results have also been observed both in ponds (Kaiser & Holt, 2005) and in offshore submerged cages (Benetti et al. 2006, 2010[b]). Indeed, cobia has been shown to be eurythermal and euryhaline species, which can adapt to a variety of environments including hypersaline (55 ppt) waters in the United Arab Emirates (Yousif *et al*., 2009).

Despite the rapid progress made in the last 20 years, there remain issues in cobia culture. These include diseases, environmental conditions (i.e. temperature and stocking densities), transport, nutrition - all of which affecting survival, growth and overall production efficiency (Liao et al., 2004; Kaiser & Holt, 2005; Benetti et al., 2010[b]). Recently, several studies have looked into energetics to improve the nutritional and hatchery rearing protocols for juvenile cobia. Sun et al. (2006[a]), found that the partial energy budget of juvenile cobia at satiation ration was $100C = 7F + 76(U+R) + 17\,G$; where C is energy intake, F is energy lost in faeces, G energy retained as growth, and U

& *R* were excretion and metabolism. In a second paper, Sun et al. (2006[b]) described how temperature affects juvenile cobia metabolism and that optimal temperature for growth is between 27-29°C. Feeley et al. (2007) determined mean oxygen consumption rates and total ammonia nitrogen excretion rates in 18-82 days post hatched (dph) hatchery-reared juvenile cobias. They found that juvenile cobia had relatively high energetic rates, which may allow rapid development into pre-adults. More recently, however, Watson and Holt (2010) found that rapid growth in early juvenile cobia (24 dph) was because of high food consumption rates coupled with high feed efficiency. The energy budget they constructed for the juveniles had a range of $42\text{-}49R + 27\text{-}19G + 34F$; where *R* was metabolism, *G* was growth and *F* was faeces.

Existing studies on cobia energetics are currently limited to juveniles and to improve its culture there is a need to determine basic energetics of egg and larval stages. Rapid morphological and physiological changes occur during the early developmental stages of fishes. It is also during these stages where high mortality rates take place (Houde, 1994). In addition, it has been suggested that food availability and predation are key biological variables that affect survival of fishes in their early developmental stages (Houde, 1997). In an aquaculture setting, there are no predators but the production of live feed for first feeding larvae can be costly and may lead to disease and deterioration of water quality (Rice et al., 1994; Benetti et al., 2008). A better understanding of the bioenergetics of cobia eggs and larvae underpinning rearing protocols will enhance the scientific knowledge of their nutritional and environmental requirements, leading to better practices that shall enhance their growth and survival rates and overall hatchery

husbandry practices. Furthermore, the results of bioenergetics studies during egg and larval stages will augment previous research on later stages of cobia energetics.

1.3 OBJECTIVES

This research has 2 main objectives:

1) To determine the metabolic rates of cobia embryos and mahi and compare the results with those of other marine species. This will enhance knowledge and contribute original data to the field. Limited data on metabolic rates of embryos of tropical and subtropical marine fish species are available (e.g. Lasker & Theilacker, 1962; Houde & Schekter, 1983; Walsh et al., 1989; Swanson, 1996; Benetti, 1992). Most studies focused on temperate species (Davenport & Looning, 1980; Ronnestad et al., 1998; Ronnestad et al. 1992) or later stages (Benetti et al., 1995; Brill, 1996; Alvarez & Nicieza, 2005) or a combination of both (Metcalfe et al., 1995; Cook et al., 2000; Peck & Buckley, 2008).

2) To determine and compare growth rates of cobia and mahi larvae in both length and weight through the early juvenile stages. These will be compared to growth rates other larval marine teleost species available in the literature to corroborate the relationship between metabolism and growth during early developmental stages and investigate whether metabolism of embryos could potentially be used as a predictor of growth rates and development. Furthermore, length-weight relationships and condition factors will be determined, aiming at providing the bases for setting standards for larval rearing of cobia and mahi and assisting in identifying stages of these species in field collection and ichthyoplankton studies.

In a similar paper, Brett & Groves (1979) predicted that bioenergetic studies would be essential input parameters for future progress in aquaculture. Indeed, since then, aquaculture's exponential growth has been based on increasing knowledge of metabolism and energetics to determine and meet the nutritional and environmental requirements of the target species. Metabolic and growth rates are integral parts of an energy budget. Quantifying the embryonic metabolism as well as larval growth rates of cobia and mahi will enhance scientific knowledge in the field and help improve protocols for incubation and larval rearing of these iconic species.

CHAPTER 2: MATERIALS AND METHODS

2.1 BROODSTOCK AND EGGS

All eggs used in the trials were obtained from the University of Miami Experimental Hatchery (UMEH). Mahi and cobia broodstock are maintained, respectively, in 50 m^3 and 80 m^3 fiberglass tanks. Both tanks are independent Recirculating Aquaculture Systems (RAS) as described by Benetti et al. (2008[b]). Cobia broodstock are F1 generation bred in captivity as part of a UMEH selective breeding program for this species. The mahi broodstock were caught from the wild and have been kept in captivity for almost a year routinely spawning volitionally in captivity. Conditioning of the broodstock fish of both species require optimized management to meet their nutritional and environmental parameters. It encompasses but it's not restricted to maintaining ideal water quality parameters, adequate feeds and feeding schedule. Fish were fed sardine and squid at 2-5% of the total biomass of the respective tanks 4-6 times a week. The feed is supplemented twice weekly with vitamins. Once conditioned and all of their biotic and abiotic required conditions are met, broodstock of both species spawn volitionally when water temperature is maintained at ~26°C - 30°C. Details of broodstock management and spawning at UMEH are provided by Benetti et al (2007; 2008[b]) and Stieglitz et al. (2012).

Eggs were collected a few hours after spawning events and maintained in either 1000-L or 275-L conical incubator tanks. The eggs were stocked in the tanks at a density of 300-500 eggs/L and a one hour formalin treatment (100 ppm) was given immediately

after stocking. The water exchange rate was approximately 500% per day, and aeration and pure oxygen were provided to maintain them in movement in the water column and D.O. levels at or above saturation levels (6-10 mg/L at 26-30°C) throughout the incubation period, which lasted between 24-36 hours within the temperature range provided.

2.2 OBJECTIVE 1: METABOLIC RATES OF COBIA AND MAHI EMBRYOS

2.2.1 Embryo Respirometry

The fertilized eggs were allowed to develop in the incubators and were collected for respirometry trials a few hours before hatching. Oxygen consumption rates of fertilized mahi and cobia embryos were conducted in a closed respirometry system (Fig. 1). The system was composed of a vertical micro glass chamber (Loligo Systems) connected to a Fibox 3 temperature-compensated oxygen meter (PreSens GmbH, Germany) by a fiber-optic oxygen mini-sensor with measurement range capability of 1ppb up to 45 ppm dissolved oxygen. A glass coated stir bar was placed in the bottom of the chamber to ensure the water did not become stagnant and a steel mesh separated the stir bar from the egg. With both stir bar and steel mesh, the chamber held 0.618ml of water. Prior to each trial, the Fibox 3 oxygen meter was calibrated according to its manual and barometric pressure and temperature entered. The Fibox 3 oxygen meter recorded oxygen measurements in the chamber as percent saturation and measurements were taken every 30 seconds. Respirometry trials lasted approximately 90 minutes or until oxygen saturation in the chamber dropped by 15% (i.e. from 100% to 85% O_2 saturation), which is well above the 70% level determined for respiratory distress in mahi

by Benetti (1992). Each respirometry trial measured the oxygen consumption of an individual egg. At the end of 1-3 trials, a blank (no egg) chamber trial was run to test for background oxygen consumption, which was eventually subtracted to correct for any background oxygen uptake by the system. Twelve respirometry trials were conducted with cobia, using eggs from 6 different spawning events (Feb. 25 & 27, Mar. 4, June 20, July 25 & 26). Twelve respirometry trials were also conducted with mahi, using eggs from 5 different spawning events (Feb. 23 & 24, Mar. 6, Sept. 20 & 22). All trials were conducted at 26°C.
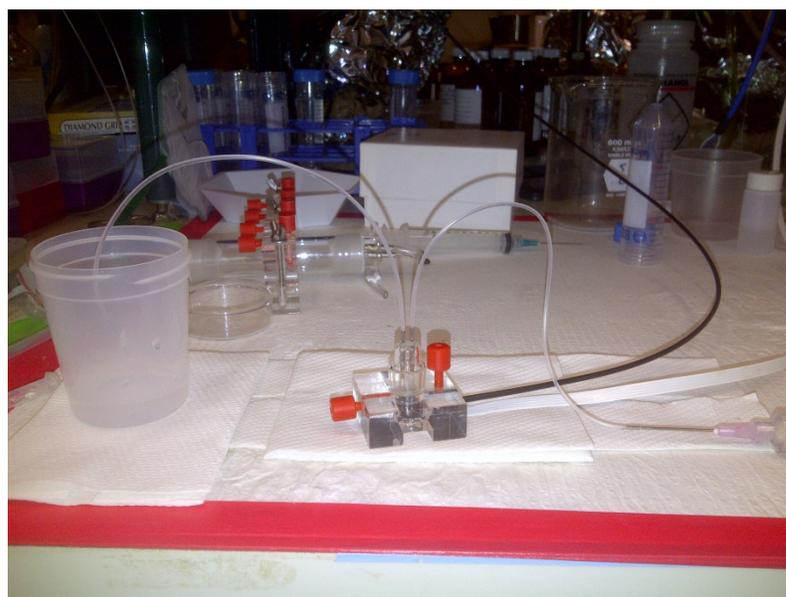
Figure 1. Closed respirometry system; vertical micro glass chamber (Loligo® Systems, Denmark) and fiber-optic oxygen mini-sensor.

Mean metabolic rates of 12 embryos from each species are reported as µL O_2/egg/hr. Mean weight-specific metabolic rates accounted for the mean dry weights of embryos for each species and are reported as µL O_2/mg dry weight/hr. Methods for sampling embryo dry weights were adopted from Lasker & Theilacker (1962), Walsh *et al* (1989) and Swanson (1996). Three samples of 100 mahi and 100 cobia embryos were isolated and rinsed with distilled water. The embryos were then placed on a microscope

slide and oven dried at 65°F for 24 hours. After drying the eggs were placed on a tared weighing dish and weighed. Individual egg dry weights were derived for each sample of the 100 dried embryos, and the mean was computed from the three samples.

2.2.2 Statistical Analysis of Metabolic Rates

An independent T-test was done to test for significance between both mean metabolic rates and weight-specific metabolic rates of cobia and mahi embryos. Prior to the actual test, the data was examined to ensure that all assumptions of the T-test were satisfied. The statistical package used was SPSS v. 20.0.0 (SPSS, Inc., Chicago IL.).

2.3 OBJECTIVE 2: GROWTH RATES, LENGTH-WEIGHT RELATIONSHIP AND CONDITION FACTOR OF COBIA AND MAHI LARVAE

2.3.1 Larval Rearing

At 1 Day Post Hatch (DPH), the larvae were stocked in identical 12,000-L conical larval rearing tanks. Stocking densities for each larval rearing tank was 10 larvae per liter. The tanks were not equipped with a chiller/heater and temperatures were subject to ambient seawater temperatures for the duration of the trials. Tank water temperatures ranged from 22.6-26°C with a mean of 24.6°C. Larval rearing protocols for feeding (food type, feed rate, etc.), water exchange, and management were those incorporated as standard protocol at the UMEH as detailed described by Benetti et al. (2008).

2.3.2 Growth Parameters

The growth parameters measured were wet weight and standard (notochord) length. Both were monitored daily from 1 DPH to 30 DPH (February 26 – March 26, 2012) for both species. At the end of each day, an hour after final feeding (~6pm), 24

larvae were randomly sampled from each larval rearing tank. Initially, notochord/standard lengths were measured and determined by taking pictures of the larvae using a Moticam 1000 camera mounted on a microscope and using its software Motic Images Plus 2.0 (Speed Fair Co., Ltd.) (Fig. 2). At 11 DPH, the larvae were large enough to be measured using a digital caliper (TruePower 150mm Vernier Caliper). Weights were measured using a Mettler Toledo scale AX 105 Delta Range® (Mettler Toledo, LLC, Columbus, OH) capable of measuring up to 10^{-5}g. Each larva was blotted dry with Kimwipes® (Kimberly-Clark Worldwide, Inc.) and placed on the scale with a pre-weighed weighing dish.

Figure 2. Larval (1DPH) cobia (left) and mahi (right) measured under a microscope using Motic Images Plus 2.0 (Speed Fair Co., Ltd.) software.

2.3.3 AGR, SGR, Length-weight Relationship, and Condition Factor Equations

Larval growth rates are presented as absolute growth rate (AGR) expressed as mm/day, and specific growth rate (SGR) as %/day. AGR and SGR were calculated using the following formulas (Hopkins 1992):

$$AGR = (L_f - L_i)/\Delta t \text{ or } (W_f - W_i)/\Delta t$$

$$SGR = 100(Ln\ L_f - Ln\ L_i)/\Delta t, \text{ or } 100(LnW_f - LnW_i)/\Delta t$$

Where: L_f and L_i are the mean final and initial lengths (mm) respectively, $W_f - W_i$ are mean final and initial weights respectively and Δt duration (number of days) of the experiment.

The length-weight relationship of larval cobia and mahi were expressed using the equation (Busacker et al., 1990; Wootton. 1998):

$$W = aL^b,$$

Where W = weight in milligrams, L = length in millimeters, a is a constant, and b is an exponent

Condition factors were calculated using the formula (Deloach, 2005):

$$K = W (100) / SL^3$$

Where K = the condition coefficient, W = weight in milligrams, and SL = standard length in millimeters.

2.3.4 Statistical Analysis of Growth Rates

Regression analysis was used to determine significance of growth of each species throughout the trails. Mean growth increments were natural log transformed and the resulting linear regressions were tested by ANCOVA. Regression analysis was done on Microsoft Excel® 2010 (v. 14) and ANCOVA using SPSS v. 20.0.0 (SPSS, Inc., Chicago IL.).

CHAPTER 3: RESULTS

3.1 OBJECTIVE 1: METABOLIC RATES OF COBIA AND MAHI EMBRYOS

The oxygen consumption trials of 12 cobia embryos resulted in a mean metabolic rate (μL O_2/egg/hr.) of 0.246 ± 0.035 (S.E.), while the 12 mahi trials resulted in a mean of 0.345 ± 0.05 (S.E.) (Fig. 3). An Independent sample T-test was run to compare the two means and no significant difference was found ($t(22)= -1.619$, $p = 0.120$). The mean dry weight-specific metabolic rate (μL O_2/mg dry weight/hr.) of cobia was 2.562 ± 0.364 (S.E.) and similar to that of mahi at 2.199 ± 0.321 (S.E.) (Fig. 4). An independent sample T-test found no significant difference ($t(22) = 0.749$, $p=0.426$) between the two species.
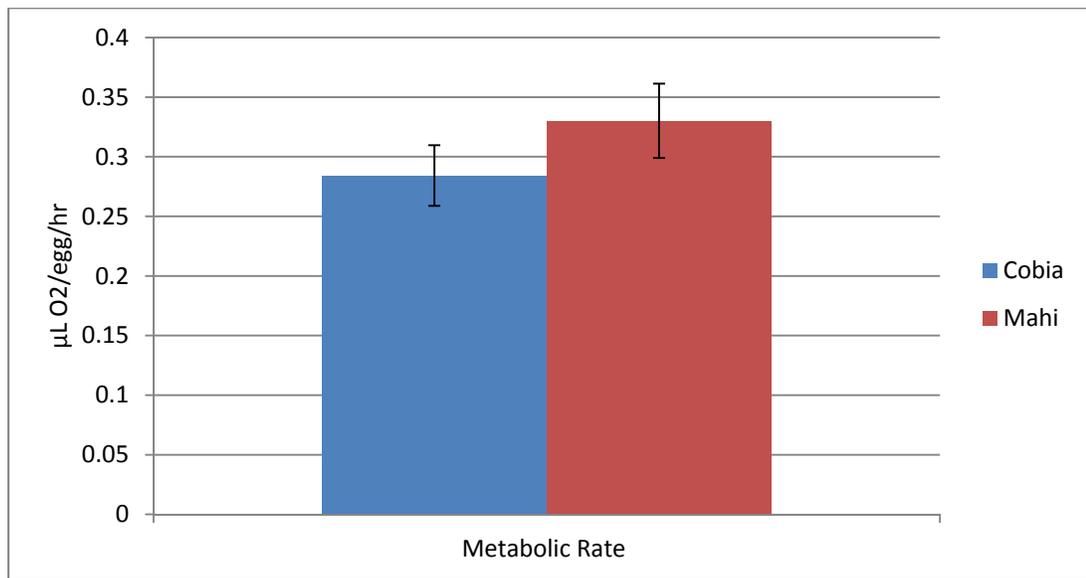
Figure 3. Mean Metabolic Rate (μL O_2/egg/hr.) of Cobia (0.246 ± 0.035 S.E.) is not significantly different from Mahi (0.345 ± 0.05 S.E.).
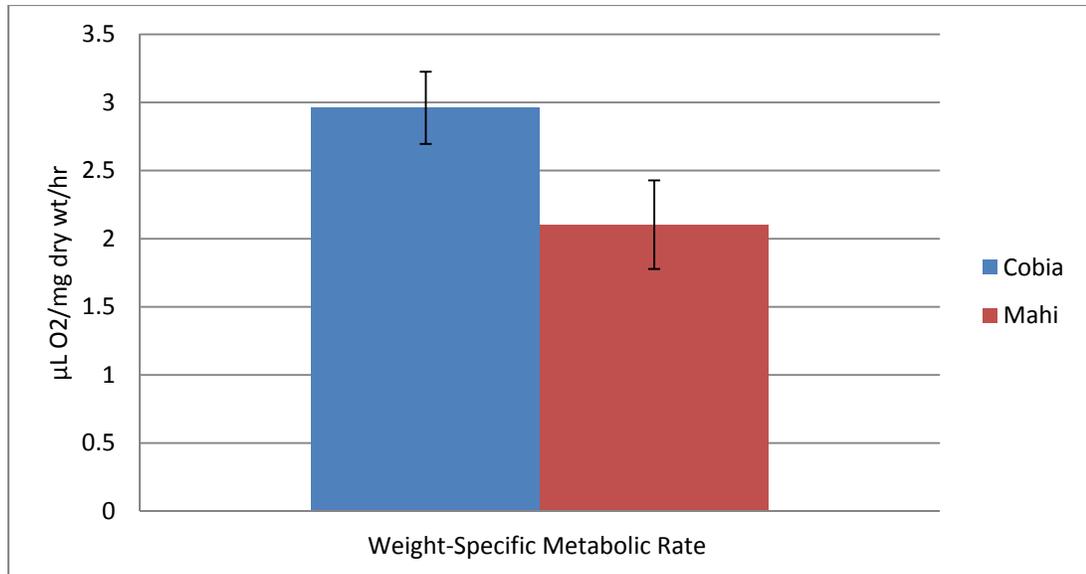
Figure 4. Mean weight-specific metabolic rates (μL O_2/mg dry weight/hr) of cobia (2.562 ± 0.364 S.E.) and mahi (2.199 ± 0.321 S.E.) were statistically similar.

3.2 OBJECTIVE 2: GROWTH RATES, LENGTH-WEIGHT RELATIONSHIP AND CONDITION FACTOR OF COBIA AND MAHI LARVAE

Both mahi and cobia larvae showed relatively fast growth over the 30 day rearing trial (Figs. 5 and 6). Growth in length and weight of the larvae were best fit by the following exponential models [x = time in days (DPH)]:

Length (Y): Cobia $Y = 3.5259e^{0.0741x}$ $r^2 = 0.9962$ n = 792

Mahi $Y = 4.1203e^{0.0646x}$ $r^2 = 0.9863$ n = 720

Weight (Y): Cobia $Y = 0.3253e^{0.2035x}$ $r^2 = 0.9895$ n = 743

Mahi $Y = 0.3119e^{0.2272x}$ $r^2 = 0.9887$ n = 661
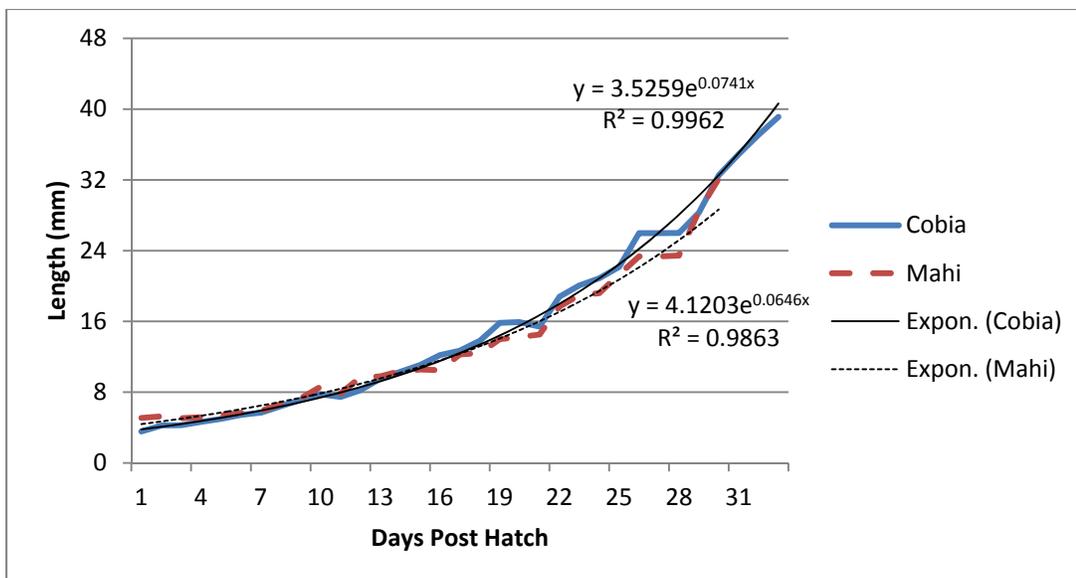
Figure 5. Mean daily length (mm) increments of cobia and mahi larvae.
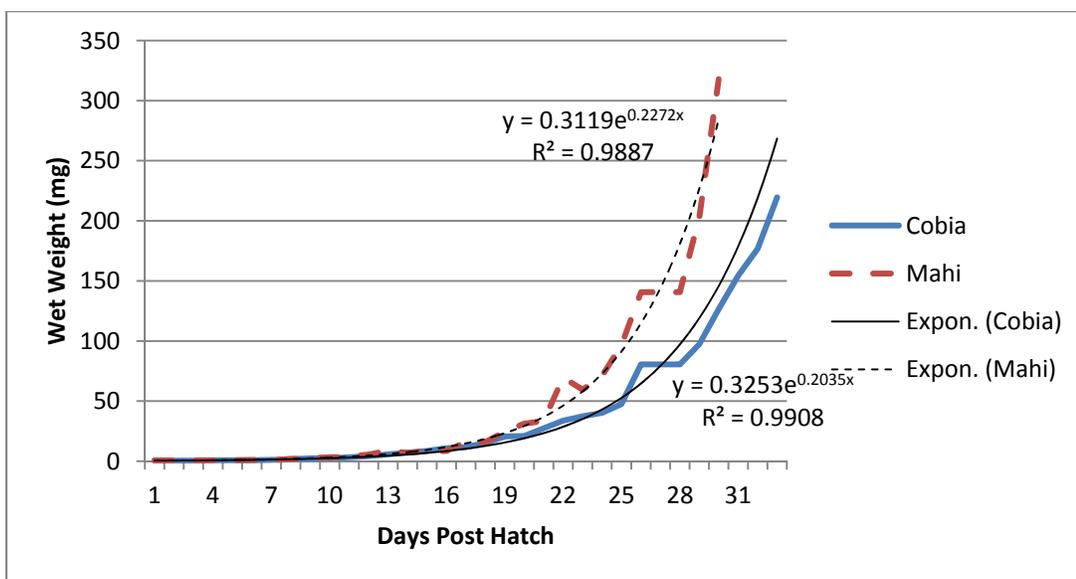
Figure 6. Mean daily wet weight (g) increments of cobia and mahi larvae.

Cobia larvae had a mean standard length (SL) of 3.57 ± 0.26 (S.D.) mm and a mean

wet weight (WW) of 0.47 ± 0.07 (S.D.) mg at 1DPH. By the end of the trial at 30 DPH,

when cobia larvae had metamorphosed into early juvenile stage, it had grown to $32.52 \pm$

0.64 (S.D.) mm in length and 126.56 ± 7.29 (S.D.) mg in weight, resulting in absolute

growth rate (AGR) in length of 0.901 mm/day and weight of 4.2 mg/day. It exhibited

length specific growth rate (SGR) of 7.37%/day and weight SGR of 21.7%/d. Mahi larvae started with a mean SL of 5.09 ± 0.17 (S.D.) mm and a mean WW of 0.69 ± 0.12 (S.D.) mg at 1 DPH and, at 30 days post hatch (DPH), had grown to 32.13 ± 4.51 (S.D.) mm in length and 317.85 ± 129.44 (S.D.) mg in weight. Mahi larval length AGR was 0.965 mm/day, weight AGR of 10.57 mg/day, with length SGR of 6.3%/day and weight SGR of 23.2%/d. No significant difference was found between growth rates in length of cobia and mahi larvae through the early juvenile stages (ANCOVA; $p<0.05$). In weight, mahi larvae grew significantly faster than cobia larvae, $F(1, 57) = 29.165$, $p < 0.001$).

Length (X) –weight (Y) relationship of cobia and mahi larvae from 1 DPH to 30 DPH are demonstrated in Fig. 7 and best expressed by the following power equations:

Cobia $\quad\quad\quad Y = 0.01x^{2.7433} \quad r^2 = 0.9887 \quad\quad\quad$ n= 792

Mahi $\quad\quad\quad\; Y = 0.0024x^{3.4474} \quad r^2 = 0.9808 \quad\quad\quad$ n =720

The initial condition factor (K) for cobia larvae was 1.03 and dropped to 0.37. For mahi larvae, the initial K value was 0.52 and increased to 0.96 at the end of the experiment. Results clearly show that, at any given age, cobia larvae are more streamlined than mahi larvae.
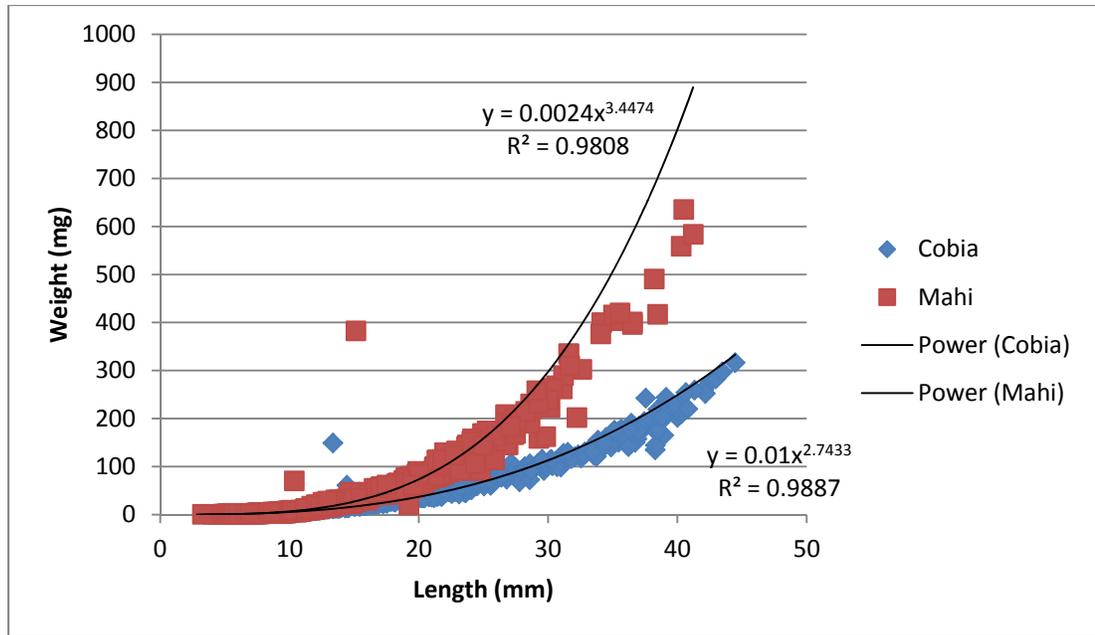
Figure 7. Length-weight relationships for larval cobia and mahi.

CHAPTER 4: DISCUSSION

4.1 OBJECTIVE 1: METABOLIC RATES OF COBIA AND MAHI EMBRYOS

Although there was a difference in egg sizes, the results of this study revealed that both metabolic rates and weight-specific metabolic rates of cobia eggs were similar to those of mahi eggs. In this study, the mahi eggs had a mean diameter of 1.59 mm, mean wet weight of 1.9 mg and a mean dry weight of 0.157 mg. Mahi egg dry weight density was 0.075 mg/μL. Conversely, cobia eggs had a mean diameter of 1.29 mm, mean wet weight of 1.1 mg and a mean dry weight of .096 mg. Cobia egg dry weight density was 0.085 mg/μL. As such, cobia eggs were smaller but more dense and therefore have more metabolically active tissue, which is one of the ontogenetic factors that influence metabolic rates the most (Rombough, 1988). Despite these differences in egg metrics, embryonic metabolism for the two species was statistically similar.

The standard metabolic rate (SMR) of mahi was determined to be several times higher than salmonids and other fish; likewise, mahi exhibited growth rates that were among the fastest in teleosts (Benetti et al., 1995[a]; Benetti et al., 1995[b]). The results of this study show mean weight-specific metabolic rates of mahi embryos to be slightly lower but comparable to previous measurements reported by Benetti (1992). The difference in measurements can be attributed to a number of factors. Among these are the differences in the methods between the two studies. Benetti (1992) used polarographic electrodes to measure oxygen consumption of 20-50 embryos, whereas the current study measured oxygen consumption of individual eggs using a fiber-optic oxygen minisensor. In contrast to polarographic electrodes, fiber-optic sensors are non-

invasive and do not consume oxygen thus taking more accurate and precise measurements. Another plausible factor is the possible difference in mahi populations/sub-species; Benetti (1992) used mahi from the Hawaiian population while those used in the current study came from a south Atlantic population. To further illustrate this difference, the mean diameter of mahi eggs sourced from Hawaii was 1.3 mm, whereas those used in this study measured 1.6 mm in diameter. Also, the ~ 36 hour incubation time for mahi embryos at UMEH were shorter than the 44-48 hour incubation time in Hawaii. Lastly, as with any experiment, there is always the factor of human error. Indeed, there are notable behavioral differences between mahi from the Hawaiian Islands in the Pacific Ocean and those of the Atlantic Ocean captured off the Straits of Florida (Benetti, personal communication, October 2012). Albeit speculative, evidence gathered in this study suggests that it is highly plausible that these are two sub-species of mahi. The extent of the genetic, morphological, anatomical, phsyisological and ecological differences remains to be investigated.

Mean metabolic rates and mean weight-specific metabolic rates of this study compare well with existing data for tropical and subtropical marine fish species that are available in literature (Table 1). The mean weight-specific metabolic rate of cobia embryos (2.562 ± 0.364 S.E. O_2/mg dry wt/hr) is higher than the 0.05, 1.4, 2.0 and 2.4 O_2/mg dry wt/hr of single pacific sardine (*Sardinops caerulea*), bay anchovy (*Anchoa mithcilli*), lined sole (*Archirus lineatus*) and seabream (*Archosargus rhomboidalis*) respectively (Lasker & Theilacker, 1962; Houde & Schekter, 1983). It is similar to the 2.516 µL O_2/mg dry wt/hr reported for striped mullet (*Mugil* cephalus) but lower than the

2.98 and 3-3.5 µL O$_2$/mg dry wt/hr of the milkfish (*Chanos chanos*) and mahi, respectively (Walsh et al., 1989; Swanson, 1996; Benetti, 1992).

Table 1. Mean metabolic rates and mean weight-specific metabolic rates of embryos of tropical and subtropical marine fish species available in literature and this study.

Author	Species	O2 Respiration Rates	Weight-specific Metabolic Rate
Lasker & Theilacker, 1962	Single Pacific Sardine		0.05 µL O$_2$/mg dry wt/hr (stage IV ~2o hour old)
Houde & Schekter, 1983	Bay anchovy	0.03 µL/egg/hr	1.4 µL O$_2$/mg dry wt/hr
	Lined Sole	0.06 µL/egg/hr	2.0 µL O$_2$/mg dry wt/hr
	Sea bream	0.08 µL/egg/hr	2.4 µL O$_2$/mg dry wt/hr
Walsh et al. 1989	Striped Mullet	0.024 µL/egg/hr (blastulae)	0.323 µL O$_2$/mg dry wt/hr
		0.177 µL/egg/hr (prior to hatch)	2.516 µL O$_2$/mg dry wt/hr
Benetti, 1992	Dolphin Fish	0.262 ± 0.11 & 0.249 ± 0.13 µL/egg/hr (mean)	1.92 ± 0.91 & 1.7 ± 0.86 µL O$_2$/mg dry wt/hr
		0.061-0.063 µL/egg/hr (gastrula -9-10 HAF)	0.42 & 0.45 µL O$_2$/mg dry wt/hr
		0.477 & 0.439 µL/egg/hr (prior to hatch)	3.5 & 3.0 µL O$_2$/mg dry wt/hr
Swanson, 1996	Milkfish	0.06 ± 0.01 µL/egg/hr (blastulae)	0.48 ± 0.01 µL O$_2$/mg dry wt/hr
		0.37 ± 0.01 µL/egg/hr (just before hatch)	2.98 ± 0.15 µL O$_2$/mg dry wt/hr
This Study	Mahi/Dolphin Fish	0.345 ± 0.05 (S.E.) µL/egg/hr (just before hatch)	2.199 ± 0.321 (S.E.) µL O$_2$/mg dry wt/hr
This Study	Cobia	0.246 ± 0.035 (S.E.) µL/egg/hr (just before hatch)	2.562 ± 0.364 (S.E.) µL O$_2$/mg dry wt/hr

Feeley et al. (2007) suggested that the relatively higher metabolic rates of juvenile cobia contribute to its fast growth rates. If so, then it can be presumed that the relatively high weight-specific metabolic rates of cobia embryos also result in faster development and hatching sooner. This is supported by incubation time of cobia and mahi embryos. At the UMEH, incubation time of cobia embryos at temperatures of 27°C is 21-24 hours

while mahi embryos at the same conditions hatch in approximately 36 hours (Benetti et al., 2007; personal observation). In other hatcheries, such as the Oceanic Institute, Anuenue Fisheries Research Center (State of Hawaii) and the Waikiki Aquarium (University of Hawaii), incubation time of mahi embryos ranged from 44 to 48 hours (Benetti, 1992). Even in the wild, cobia embryos hatch faster than mahi embryos. Shaffer and Nakamura (1989) reported cobia embryos hatch within 36 hours while Palko (1982) reports that mahi embryos hatch 60 hours post fertilization. In addition, milkfish and striped mullet embryos which had slightly higher and almost exactly the same weight-specific metabolic rates as cobia have shorter incubation times (~31 hours @ 26°C and ~33 hours @ 24°C respectively) than mahi at similar temperature ranges (Swanson, 1996; Walsh et al., 1989).

Although not significantly different, the slightly higher weight-specific metabolic rates of cobia embryos could be related to the spawning behavior of adults. In an aquaculture setting, where conditions are ideal, a female cobia would spawn every other week whereas a female mahi would spawn every other day and sometimes even daily In addition, cobia spawns will generally produce more than one million eggs per spawning event while mahi produce approximately one to three hundred thousand (Benetti, 1992, 2007; Stieglitz et al., 2012; personal observations). Since cobia spawn less often and a lower number of eggs than mahi (i.e., 1 million eggs in 2 weeks vs. 2.1 million eggs in 2 weeks, respectively), it can be assumed that cobia's fecundity is lower, thus suggesting that it would be advantageous that the embryos develop faster and hatch fast.

Given the limited amount of data available in literature on early developmental stages of such ecologically and economically important species, there are many

possibilities for future research. Based on this study, it would be interesting to see how metabolic rates of embryos of various species would correlate to incubation time as well as spawning behavior of the adults. Vetter et al. (1983) reports that embryos of red drum (*Sciaenops ocellata*), another pelagic species, will hatch within 19 hours given the right temperatures. Unfortunately there was no mention of oxygen consumption rates. This could have further supported or contradicted the isometric ontogenetic metabolic scaling in pelagic animals of Glazier (2006). More data is needed to determine if isometric ontogenetic metabolic scaling also exists outside the pelagic realm.

Correlating metabolic rates would also provide a clearer picture of the extent to which metabolic rates influence the ontogeny of marine teleost embryos. In juvenile Atlantic salmon, Metcalf et al. (1995) reported a positive correlation between standard metabolic rates (SMR) of individuals and their dominance. The more dominant individuals have preferential access to resources, become sexually mature at a younger age and have higher survival than those of lower rank. Interestingly, larvae of both species are highly cannibalistic, particularly between 15 and 30 DPH. When stocked together in the same tank at same age (DPH) and stocking densities, the "bulkier" mahi larvae are more aggressive and eventually prevailed with regards to dominance and survival over the more streamlined cobia larvae (personal observation). For juvenile cobia, Feeley et al. (2007) suggested that high metabolic rates and the high urea excretion rates could be the reason for its exceptional growth rates. Then again, there are studies that also show that the relationship between SMR and growth is mainly determined by the variability of the environment (Alvarez & Nicieza, 2005). Similar information for embryonic stages could be essential input for a better understanding of the physiology of specific species how

they interact within the broader framework of ecosystem energetics. Furthermore, this could also help in developing better management practices in aquaculture hatcheries.

4.2 OBJECTIVE 2: GROWTH RATES, LENGTH-WEIGHT RELATIONSHIPS AND CONDITION FACTOR OF COBIA AND MAHI LARVAE

The results of this study support as well as fill in information gaps on the existing knowledge on the basic biology of cobia and mahi during early developmental stages. When compared to other species such as snapper (Lutjanidae), groupers (Serranidae), Pompanos (Carangidae), or snooks (Centropomidae), red drum (Sciaenidae) or flounder (Paralichthydae) both cobia and mahi exhibit extraordinary growth rates, at least in hatchery conditions. Indeed, their growth and development can be an order of magnitude faster than those of other typical perciform teleosts (Benetti, personal communication, July 2012). By the end of the 30-day trial, mean lengths of cobia and mahi were almost identical, 32.52 mm and 32.13 mm respectively, as shown in Fig. 5. Absolute growth rates (AGR) were also similar at 0.965 mm/d for cobia and 0.901 mm/d for mahi. However, since initial mean larval length of cobia (3.57 mm) was slightly smaller than mahi (5.09 mm), its length specific growth rate (SGR) was higher at 7.37% and 6.13%, respectively. The length AGR and SGR values attained by cobia and mahi in this study are among the highest compared to other tropical/subtropical aquaculture species (Table 2).

The larval cobia in this study had the highest length AGR (0.965 mm/d), even higher than the true pelagics like mahi (this study, Benetti, 1992) and Pacific bluefin tuna (Sawada et al. 2005). Apart from cobia, larval mahi length AGR of 0.901 mm/d in this study was only lower than the 0.925 mm/d of greater amberjack, Seriola dumerili

(Papandroulakis, 2005). Regarding SGR in length, only the values reported by Benetti (1992) for mahi and Sawada et al. (2005) for Pacific bluefin tuna (Sawada et al., 2005) were higher than the 7.37%/d exhibited by cobia larvae in this study. Interestingly, the 6.13%/d length SGR of the mahi larvae in this study was lower than the 8.78%/d previously reported for mahi (Benetti, 1992), 7.63%/d of Pacific bluefin tuna (Sawada et al., 2005), 7.24%/d of milkfish (Martinez et al., 2006) and 6.57%/d of greater amberjack (Papandroulakis, 2005). Given the multiple variables that influence growth, it is difficult to determine why this was the case. However, it can be speculated that, for the larval mahi in this study and those of Benetti (1992), the disparity could be limited to a few most likely variables. Among the most likely are equipment, methods and human error. Another likely cause would be genetics; the eggs that hatched the larvae used by Benetti (1992) were from the Pacific Ocean and had a diameter of 1.35-1.4 mm whereas the eggs of the mahi larvae used in this study had a diameter of 1.59 mm and were from broodstock from the Atlantic. Lastly, and rather importantly, differences in temperatures of the rearing conditions is a limiting factor for growth (Busacker et al., 1990). The temperatures used by Benetti (1992) for larval rearing was 26 °C while in this study, temperatures ranged from 22.6 – 26 °C and a mean of 24.6 °C. It is worth noting that a mean temperature of 24.6 °C is on the lower end of the optimal range for growing cobia at UMEH (Benetti et al., 2008). Previous cobia larval rearing runs with higher temperatures have produced larvae to juveniles with length AGR greater than 1 (unpublished data).

Table 2. Length AGR and SGR of some tropical/subtropical marine aquaculture species.

Species & Author	Egg Diameter (mm)	Lt @ Hatch (mm)	Rearing Conditions (salinity & temperature)	Time (dph)	AGR Lt (mm/d)	SGR Lt (%/day)
Thick lipped grey mullet; Khemis el al, 2006	4 days incubation @ 16°C	3.7 mm TL	37.5‰ & 20-24.2°C	71 days	0.217	3.5
Fat snook; Cerqueira & Tsuzuki, 2009	0.67 ± .007 mm	1.85 ± 0.08 mm TL	26 ± 1°C	40 days	0.204	4.22
Atlantic red porgy; Morris et al, 2008	1.0 ± 0.05 mm; hatches ~48 hours @ 19°C		34‰ & 19-22°C	35 days	0.25	3.58
Dusky grouper; Russo et al., 2009	0.85 ± 0.04 mm; hatches in 30 hours @ 23°C	1.52 ± 0.066 mm TL	35‰ & 25°C	32 days	0.261	4.43
Grey mullet; Kuo et al, 1973	0.93 mm; hatches in 36-38 hours @ 24°C or 48-50 hours @ 22°C	2.63 mm TL	32‰ & 22°C	42 days	0.359	4.54
Yellowtail kingfish; Chen et al, 2006	1.19 – 1.27 mm; hatches in 40 hours @ 18-20°C	4.30 mm SL/NL	38‰ & 24 ± 1.3°C	36 days	0.51	4.6
Milkfish; Martinez et al, 2006 & FAO	1.1-1.25 mm; hatches in 24-36 hours	3.5 mm	30-34‰ & 26-30° C	21 days	0.595	7.24
Mahi; Benetti, 1992	1.35-1.4 mm; hatches in 44-48 hours	3.7 mm SL/NL	26°C	18 days	0.711	8.78
Pacific Bluefin Tuna; Sawada et al, 2005	1.01 mm	3.08 mm TL	23.3-26°C	30 days	0.909	7.63
Greater amberjack; Papandroulakis et al, 2005	1.03 ± 0.02 mm; hatches in 30-34 hours @ 23.5 ± 1°C	2.88 ± 0.22 mm TL	21 - 23.5°C	40 days	0.925	6.57
Mahi; This Study	1.59 mm; hatches ~36 hours @ 27°C	3.7 mm SL/NL	24.6°C	30 days	0.901	6.13
Cobia; This Study	1.29 mm; hatches in 21-24 hours @ 27°C	2.6-3.2 mm SL/NL	24.6°C	30 days	0.965	7.37

In contrast to length measurements, weight-specific growth rates of larval mahi were found to be significantly faster than cobia. Although both species grew very fast in weight over the 30-day trial, mahi larvae exhibited a weight AGR of 10.57 mg/d while cobia has a less than half weight AGR of 4.2 mg/d. The difference between weight SGR of mahi and cobia larvae were not as drastic, 0.232 %/d and 0.217 %/d respectively. Results corroborate that cobia larvae are more streamlined than mahi larvae. This is clearly indicated by the exponent value of their length-weight relationships (2.74 for cobia vs. 3.44 for mahi).

One likely reason for the mahi larvae's faster growth increment in wet weight and higher variability was because, towards the end of the trial, the larval fish density in the mahi larval rearing tank was distinctively lower than that in the cobia larval rearing tank. Despite all efforts to try to control and limit variability in rearing conditions, high mortalities were experienced towards the last 10 days of the experiment. The mean weight increment graph (Fig. 6), reflects how mean weights of the two species were very similar at the start. However the mahi mean weights started deviating at around 22 DPH, immediately following the onset of a period when high mortalities occurred. Aside from cannibalism, other causes of these mortalities could not be determined with certainty. However, it can be speculated that there may be natural (i.e. genetics) and environmental (i.e., high stocking density) causes. Cannibalism is prevalent throughout late larval and early juveniles in both species. Mahi has been observed to be very aggressive and cannibalistic in aquaculture conditions (Benetti, 1992; personal observation) and though some larvae may escape being eaten, many are left injured (i.e. chewed-off tails) and eventually succumb. This cannibalistic behavior is most likely exacerbated in high

densities commonly found in aquaculture tanks but also occurs in the wild as reported by Bannister (1976) and Oxenford & Hunte (1999).

The difference in densities could influence growth by affecting social interactions and water quality (Buscker et al., 1990). For example, Metclaf et al. (1995) found a strong relationship between individual status and standard metabolic rate (SMR) in juvenile Atlantic salmon. Individuals with higher SMR were more dominant and have better access to food. Intuitively, an already dominant individual get more food and grows faster and continues its dominance as it grows. Also since both larval tanks were being fed ad libitum, a less dense tank would most likely have better water quality. The more larvae there are in a tank the more metabolites like ammonia and nitrite are produced. Although both tanks were flow-through systems with high flushing rates, acute toxicity of ammonia and nitrite can lead to loss of appetite in juvenile cobia (Rodrigues et al., 2007).

In fisheries biology, length-weight relationships are used as indicators to determine the condition of fish populations. The plumpness of individuals are said to be reflective of favorable environmental conditions while thinner fish indicate less than ideal conditions (Blackwell et al., 2000). Furthermore, a length-weight regression coefficient equal to 3 is said to have isometric growth, where increase in length is in equal proportion with body weight for constant specific gravity. Fish with regression coefficients less than or greater than 3 are said to have allometric growth (Gayanilo & Pauly, 1997). The length-weight coefficients of larval mahi and cobia fall within the expected values of 2.5 and 3.5 (Busacker et al., 1990). Both larval species expressed allometric growth in their larval stages. Mahi larvae had a regression coefficient value of

3.4474 indicating that it increased in weight faster than the cube of its length, while cobia had a more streamline approach with a regression coefficient value of 2.7443.

The condition factor (K) of a fish reflects an individual's well-being during observed growth (Busacker et al., 1990; Abowei, 2010). Condition factor for both larval cobia and mahi fluctuated from start to end of the growth trial. The K for cobia at 1 DPH was 1.03 and decreased to 0.37 at the end of the trial. Mahi on the other hand, started with 0.52 and nearly doubled to 0.96 in the end. The less than 3 coefficient value of larval cobia and decrease of K from start to end of the trials could be related to its high rates of nitrogen excretion. Feely et al. (2007) reports that endogenous ammonia excretion rates for juvenile cobia are an order of magnitude higher than mahi and other larger temperate juveniles. Additionally, more than half of the nitrogenous waste of cobia is urea which is metabolically more costly to produce than the equivalent amount of ammonia. Assuming this is the case, then this study also supports the findings of Sun et al. (2006) and Watson & Holt (2010), that the high growth rates of cobia juveniles is due to high consumption rates and high feed efficiencies.

CHAPTER 5: CONCLUSION

Within the last decade, cobia has moved from a candidate species with aquaculture potential to being a popular species farmed in Asia, the Americas, and the Middle East. It is likely that the main reason for the successful aquaculture development for cobia is its extraordinary growth rates. Other marine teleosts with rapid growth rates are the high performance true pelagics like mahi and tuna. Brill (1996) argued that in order for high performance fish like tunas, swordfish and mahi to survive as top predators in the oligotrophic pelagic environment these fish species must have, among other physiological adaptations, very high growth rates. Benetti (1992) and Benetti et al (1995) showed that high metabolic rates and high metabolic efficiency of mahi throughout its life stages resulted in its fast growth rates. Similarly, Feeley et al. (2007) showed that metabolic rates of cobia were similar to those of mahi at the same juvenile stage. Therefore, metabolic rates at the embryonic stage and growth rates at the larval stage should also be similar for cobia and mahi.

The results of this study indicate that cobia has embryonic metabolic rates as well as growth rates similar to those of high performance fish such as mahi and even tuna. Mean metabolic rates and weight-specific metabolic rates of cobia embryos were not significantly different than those of mahi embryos. Furthermore, weight-specific metabolic rates of cobia embryos were higher than those of most other tropical and subtropical marine teleost species available in literature. Similarly, in terms of length, cobia larval growth rates were not significantly different from those of mahi larvae. In terms of weight, however, mahi larvae grew significantly faster than cobia larvae. Nonetheless, lengthwise, cobia larvae exhibit the growth potential of a high performance

fish like mahi. Furthermore, it is likely that the <3 coefficient value of the length-weight relationship of cobia larvae as well as the decrease in condition factor throughout early development all the way to the juvenile stage at 30 DPH could be attributed to the higher than usual excretion of endogenous waste of cobia as suggested by Feeley et al. (2007).

The high weight-specific metabolic rates of cobia embryos could be a life strategy to develop and hatch rapidly since adult cobia spawn less frequently than mahi. Further research on metabolic rates of other species could be beneficial in enhancing the understanding of the role metabolic rates play in the ontogeny of marine teleost embryos. Results of this study corroborated the relationship between metabolism and growth during early developmental stages of these two species of great ecological and economic importance and paved the way to investigate whether metabolism of embryos could be used as a predictor of growth rates and development of other fish species throughout all life stages. Furthermore, length-weight relationships and condition factors were determined, providing basic scientific information for setting standards in larval rearing of cobia and mahi.

Also, future studies should focus on developing a complete energy budget for embryo and larval stages of cobia. Aside from the data presented in this study, there is no existing information to compare wet weight increments, length-weight correlations and condition factors for larval cobia and mahi and other commercially important aquaculture species. Such information would be vital in improving and developing current rearing protocols and best management practices for cobia and mahi hatcheries worldwide. Finally, data and results of this study will contribute to enhance the scientific knowledge

on the physiology of cobia and mahi during early developmental stages, paving the way

for developing improved hatchery protocols for the species.

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