Supplementing Broodstock and Larval Diets for Florida Pompano *Trachinotus carolinus* With Taurine to Improve Egg, Larval, and Weaned Juvenile Quality

by

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Abstract

Florida Pompano have been identified as an ideal species for aquaculture in America. As with many cultured marine finfish species there is a production bottleneck between egg and weaned juveniles. Florida Pompano have the potential to produce up to 1.5 million eggs but it is not uncommon to have survivability of 5% to 15% from eggs to weaned juveniles. Recent research shows that amino acids supplemented in broodstock diets were passed onto the eggs. Taurine, a 2-aminoethanesulfonic acid, is a common organic compound and has been suggested to help enhance egg quality. To evaluate the potential of taurine supplementation for Florida Pompano broodstock and larvae, a 2x2 factorial experiment was conducted, where two group of adult fish received formulated gel diets with or without taurine supplementation, and the resulting larvae were divided to receive taurine-supplemented or un-supplemented live prey.

Broodstock received experimental diets three times a day for 3 weeks prior to spawning, while the larvae were raised on an otherwise standard protocol based on rotifers and *Artemia* enriched with a commercial emulsion and weaned on a dry feed at 15 days-post-hatch when the trial was terminated. Results show that the supplementation of the broodstock diets with taurine had a beneficial effect in terms of egg yolk and oil globule size which are indicators of good egg quality. The results from the 15 dph larvae show that there isn't a significant interaction between the treatments when it comes to the lengths of the larvae. However, there is an interaction between the treatments for the weights of the larvae. The data supports recommendations of supplementing broodstock feeds while not supplementing the live prey for the larvae.

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1 Corinthians 13:4-13

Introduction

Florida Pompano Biology

Florida pompano, *Trachinotus carolinus*, are a highly-favored fish for anglers, aquaculturists, and chefs all along the Americas and Caribbean. Florida pompano are members of the Carangidae (jack) family and the genus *Trachinotus* contains 20 different known species. The genus *Trachinotus* includes the Atlantic permit (*T. falcatus*) and palometa (*T. goodie*), which can be found in the same habitat and are often mistaken as Florida pompano. The silver pompano (*T. blochii*) is a high-value finfish that is heavily cultured in the Philippines and Indo-Pacific region.

The Florida pompano is an oval shaped fish with a compressed body, short snout, and inferior mouth. They can grow up to 63 centimeters (25 inches) in length and 4 kilograms (9 pounds) in weight but it is most common to see a range between 25 to 40 cm (~10 to 16 in) and 0.5 to 1 kg (1 to 2 lbs) (Gilbert 1986, Main *et al.* 2007). Its coloration can vary from blue-greenish silver to silver with yellow pigmentation along the ventral portion of the body. The scales are small and cycloid and the lateral line of the Florida pompano arches up to the midpoint of the second dorsal fin and then straightens out toward the caudal fin. The first dorsal fins have 6 spines that are close to the body followed by a second dorsal fin with 22 to 27 soft rays that extend almost to the peduncle. The anal fin has 20 to 24 rays and originates behind the dorsal fin. They have a deeply forked tail and a smooth caudle peduncle that lacks scutes and finlets.

Florida pompano are considered a warm-water fish and are normally found in waters ranging from 25 to 32ºC but juveniles have been known to thrive in 34ºC waters (Main et al. 2007). They are a euryhaline species that can tolerate a wide range of salinities and low levels of dissolved oxygen (≥4 mg/L) (Main et al. 2007). Florida pompano have been found in estuaries and bays but most are spotted along the coastal beaches in higher salinity waters. Florida pompano are highly migratory and can be found from Massachusetts down to Brazil and include the Gulf of Mexico and the coastal regions of Central and South America. The Florida coast is known to have the highest abundance of pompano and accounts for almost 90% of the pompano harvested in the United States of America. During their migration, they travel in schools along the beaches grazing on polychaetes, mollusks, crustaceans, and small fish that get washed out in the rip currents. Florida pompano have very small, conical teeth as juveniles but lack teeth as adults (Gilbert 1986). They have 8 to 14 gill rakers on the lower limb of the gill arch and large pharyngeal plates that it uses to crush its food (Gilbert 1986).

Aquaculture of Florida Pompano

The production of Florida pompano (and all other species of pompano) in the US remains very limited when compared to the Asian market. FAO (2016) announced that in 2014 a total of 119,450 tons of all pompano species were harvested globally with Asia producing 110,258 tons of “Asian pompano.” The aquaculture production of Florida pompano in the United States is a non-commercial entity and most Florida pompano for sale on the market in the United States are typically wild caught (FAO 2016). The silver pompano, *T. blochii,* is the most well-known species

of pompano in the Asian aquaculture community and accounts for a majority of the harvested species. The silver pompano of Asia are grown in open sea cages, brackish-water cages, and ponds (Main et al. 2007, Watanabe and Main 1995). They have well established protocols and the commercial farming is being done by small farmers and private companies.

The demand for Florida pompano in the United States is currently much higher than the supply and a clear majority of the Florida pompano supplied is from wild caught fisheries (FAO 2016). The culture of Florida pompano in the US remains more in the pilot or small farm scale as opposed to large, commercial operations and most Florida pompano cultured in the US are for research and private purposes (FAO 2016). Market sized pompano are generally over 1 kg and most suppliers prefer an eviscerated whole fish with head on (FAO 2016). The ex-vessel value of Florida pompano ranges between $8.88 to $11.11 (USD) per kg., with a wholesale value between $13.33 to $17.77 (USD) per kg and a market value of $20.00 to $31.11 (USD) per kg (FAO 2016). Recent developments, including the ability to culture Florida pompano in low-salinity ponds, have helped increase the popularity of Florida pompano culturing but it is still not well known and practiced (Weirich *et al.* 2009).

Reproduction and Development

Typically, male Florida pompano mature during their first year. Female pompano can mature during their first year but it is more common to see them reach sexual maturity after their 2nd and 3rd year (Main et al 2007). Spawning in the wild has never been observed but there is evidence that spawning is strongly tied to water temperatures (Gilbert 1986, Main et al 2007).

It is believed that during April and October, when water temperatures are over 23ºC, the pompano head offshore to spawn. Finucane (1969) discovered small larvae (3.0 to 4.5 mm long) 24 km offshore in Florida while performing plankton tows, giving strong evidence for offshore spawning. Finucane (1969) also found larvae in surface waters of the Gulf Stream which possibly accounts for their wide range along the Atlantic coast.

Female fecundity is estimated to range from 100,000 to 800,000 eggs per spawning season with most pompano having a fecundity around 300,000 (Gilbert 1986, Main et al. 2007). The spawning behavior has never been documented in the wild but Kloth (1980) observed two Florida pompano spawning in a tank. One female slowly swam around the bottom of the tank and rose to the middle of the column slowly. One male followed her and she remained stationary while the male positioned himself underneath her. After 15 seconds, they returned to the bottom of the tank and eggs were seen floating on the surface shortly after.

Florida pompano eggs can range in size from .85 to 1.0 millimeter in diameter and they typically contain a single oil globule and a yolk (Main et al. 2007). Approximately 36 hours after fertilization the egg will hatch. Newly hatched Florida pompano larvae are about 2.0 millimeters in length and are underdeveloped (Hoff et al. 1978a). The larvae depend on the oil and yolk reserves for the first 2 to 3 days of their life while they develop pigmented eyes, mouth parts, and a rudimentary digestive tract. After developing the means to consume prey, pompano consume a mixture of copepods, *Artemia*, rotifers, and other plankton (Hoff et. al. 1978a, Main et al. 2007). The larvae spend their first month of life at sea and then head to the shore where they congregate along the shoreline of low-energy beaches (Gilbert 1986). Once the water

temperature along the shoreline reaches 19ºC most juveniles leave the shoreline and head towards deeper, warmer waters (Gilbert 1986).

In the hatchery setting, Florida pompano are held in large, recirculating tanks with temperature controlled water and lights with photo-thermal regimes to mimic seasons (Main et. al 2007). The optimal sex ratio for Florida pompano in a tank is 1 male:1 female (Hoff et al. 1978, Main et al 2007). To induce oocyte development, they are held at 28ºC for approximately 8 weeks (Hoff *et al.* 1978). They are then sampled to determine oocyte maturation and then injected with a spawn-inducing hormone (e.g. GnRHa). After 32 to 48 hours the pompano are either strip spawned or allowed to spawn in the tanks volitionally (Hoff et al. 1978, Main et al. 2007).

The foremost bottleneck when it comes to Florida pompano aquaculture production is successfully spawning and raising quality, weaned juvenile fish. Florida pompano are notorious for having variable fertilization and survival rates in the hatchery setting (Main et al. 2007). It is not untypical to see spawns with 10% fertilization and survival rates well below 25%. The variability in spawning and egg quality has raised many questions on how to better enhance protocols for both broodstock maintenance and larval culture. Nutrition is one of the most important factors of animal husbandry and reproduction and there is an evident need for advancements and established protocols to help keep up with the advancements of aquaculture and to increase aquaculture potential.

Florida Pompano Nutrition

Broodstock

Proper broodstock nutrition is vital for having healthy eggs and larvae. There has been much research on the advancement and development of proper broodstock feeding practices. Poor broodstock feeding practices and low nutritional feeds can greatly affect fecundity, spawn quality, fertilization rate, and overall larval quality (Fernandez-Palacios et al. 2011). Most governmental and research hatcheries feed the broodstock a diet consisting of frozen fish and invertebrates that mimic the natural prey of the cultured fish (FAO 2016).

Diets that are high in protein and lipids are typically used for feeding broodstock and broodstock nutrition research has focused heavily on the importance of highly-unsaturated fatty acids (HUFA). There have been numerous studies that have shown that reproductive performance and egg and larval quality are related to the inclusion of HUFAs in marine finfish diets (Bruce *et al.* 1999, Zakeri *et al.* 2011, Fernández-Palacios *et al.* 2011). Studies show that the enrichment of broodstock diets with HUFA's increased survival rates and fecundity but it did not alleviate the variability of survival rates and egg quality. The eggs from the enriched broodstock's diet did have an increase in the concentration of HUFA, showing that it is possible to influence the composition of the eggs by diet manipulation. The increase in survival, fecundity, and HUFA concentration in eggs is a promising sign and has led to further research with enrichments for broodstock diets. However, there are many nutrients other than HUFA's that can influence reproductive and larval quality, such as amino acids.

Amino acids are critical in the growth and development of fish (Wilson and Halver 1986). Fish consume proteins that are digested and broken down into free amino acids which are then distributed throughout the body to form new proteins (Wilson and Halver 1986). Additionally,

amino acids have been shown to regulate metabolic pathways that are critical to growth, reproduction, maintenance, and immune response (Li et al. 2008). Florida pompano have pelagic eggs and it is noted that in pelagic eggs amino acids are heavily utilized as a metabolic fuel during the embryonic stage (Moran *et al.* 2007, Cruzado *et al.* 2013). In theory, an increase in protein or free amino acids (FAA) in the diet of the broodstock should improve egg quality.

A study performed by Hastey *et al.* (2015) attempted to increase the overall FAA concentration in eggs of red snapper *Lutjanus campechanus* by supplementing the broodstock snapper with an injection of a mix of FAA to determine the effect FAA concentrations had on eggs and hatchlings. The mixture was a cocktail consisting of 25% valine, 25% leucine, 25% isoleucine, and 25% lysine. The study showed that the broodstock injected with the FAAs has larger eggs in terms of weight, diameter and oil globule. In the same study performed by Hastey et al (2013), the FAA treated broodstock's larvae had a larger yolk sac and oil globule than compared to the control. The researchers did note that the injection of FAA into the red snapper prior to ovulation did little to increase the actual FAA concentrations in the eggs but it did result in a greater rate of FAA utilization, prolonging the yolk-sac and oil globule reserves. These studies show that broodstock fish with higher concentrations of FAA were successful in passing more FAA into the egg and subsequent larvae thus giving the eggs and larvae more FAA to use during their development.

Taurine and Broodstock Nutrition

These findings paved the way for more research on the requirements of amino acids in broodstock fish and the supplementation of broodstock diets. Recently, taurine has been identified as an essential amino acid for finfish and research on the effects of taurine on broodstock reproduction has been raised. Taurine is a beta sulfonic amino acid that is found in nature and is also the most abundant free amino acid in fish tissues. Taurine is synthesized from methionine after it has been oxidized to cysteine (Takagi *et al.* 2008). Biosynthesis of taurine varies among fish species and certain commercially relevant species (like Florida pompano) cannot metabolically synthesize taurine and must rely on dietary intake sources for their physiological processes (Rossi and Davis 2012). Studies have found that taurine is used in fat digestion, anti-oxidative defense, cellular osmoregulation, and the development of neural systems including the visual and muscular systems (Fang *et al.* 2002, Omura and Inagaki 2000). Taurine deficiencies in fish have been known to decrease oocyte maturation, promote green liver syndrome, and lower hematocrit levels (Matsunari *et al.* 2006, Rhodes 2011, Salze and Davis 2015). In 2015, Salze and Davis performed a dose-response study for taurine and Florida pompano and proposed that the requirement for dietary taurine was 0.54-0.65% thus confirming a dietary need for taurine in Florida pompano and raises the question of efficacy of broodstock diets.

Matsunari et al. (2006) assessed the interaction of taurine supplementation in broodstock diets for yellowtail *Seriola quinqueradiata*. The treatment group that had 0% taurine did not have a successful spawn but the groups with 0.5% and 1.0% taurine supplemented diets had successful spawns, with the 1.0% group having the best results. The study also found that ovarian

maturation was much quicker as the taurine concentrations increased. They concluded that taurine does have a positive effect on the spawning performance but more research is needed to determine the suitable level of taurine for yellowtail broodstock diet.

The results from research on taurine and brooodstock diets has been very promising. However, current research on supplementation of broodstock diets with taurine is very limiting and only performed on a small number of fish species. With the discovery of differing nutritional requirements of taurine for different marine finfish species, the need for more research on taurine and its effects on broodstock, mainly reproduction and maintenance, is warranted.

Larval Nutrition

The first 18 to 25 days of Florida pompano larvae's life are the most important and difficult. When they are first hatched, they do not have a fully formed digestive tract and are completely reliant on their yolk reserves which can last up to 4 days post hatch at a water temperature above 24ºC (Riley *et al.* 2009). The yolk provides nutrients and energy for the morphological and cellular changes until the larvae have developed the means to consume and digest prey, usually after 2 to 3 days post hatch. Exhaustion of the yolk reserve, food deprivation, and low nutritional value of first feeds can lead to massive mortalities (Gopakumar *et al.* 2013).

Copepods are the most common prey item for larval Florida pompano in the wild (Izquierdo *et al.* 2001, Fernández-Palacios et al. 2011). Copepods provide a wide range of nutritional benefits for the larvae but their mass production in a commercial hatchery setting is difficult. Rotifers and *Artemia* have been identified as an efficient replacement for copepods and can be produced efficiently in a hatchery setting. Rotifers (*Brachionus plicatilis*) are usually the

first food introduced to marine larval fish. Compared to copepods, rotifers contain a low fraction of soluble protein (6.0 – 9.0% of dry weight for rotifers, 32.0 – 54.0% of dry weight for copepods) and an unfavorable essential fatty acid profile (Watanabe *et al.* 1983, Aragão *et al.* 2004, Srivastava *et al.* 2006). Since rotifers have such low nutritional value, the use of commercial enrichments has garnered much support. These enrichments typically include a wide range of nutritional components needed for larval development and include fatty acids, carbohydrates, sterols, proteins, and vitamins. In a study by Srivastava et al. (2006), rotifers were fed diets with varying amounts of amino acids, taurine not included. They noticed a small but statistical difference in some amino acid concentrations but not every amino acid they supplemented. In another study by Aragão et al. (2004) they were able to significantly increase the overall amount of protein and free amino acids in the rotifers by giving them different commercial enrichment products. These studies confirm that it is possible to change the nutritional composition of rotifers with proper enrichment protocols. In theory, the more favorable nutritional composition of the rotifers will be passed onto the larval fish.

The next step in larval nutrition is the introduction of *Artemia* (*Artemia parthenogenetica*). Just like rotifers, *Artemia* contain low levels of protein (5.0-12.0% dry weight) and a small FAA pool when compared to copepods, which can be increased through enrichment protocols (Aragão et al. 2004, Watanabe et al. 1983). Aragão et al. (2004) performed an experiment on *Artemia* and were successful in increasing the amount of free amino acids in the *Artemia* by using the same commercial enrichments they used on the rotifers.

Taurine and Larval Nutrition

As discussed earlier, taurine has been identified as an essential nutrient in a number of marine finfish including Florida pompano. Salze *et al.* (2016) identified a correlation between the depletion of taurine and a decrease in the oxidative capacity of mitochondria in the liver, suggesting that taurine is involved in mitochondrial function. Taurine is most commonly in the free-form but it has been shown to bind and modify some mitochondrial tRNA at the wobble position of the anticodon which enables precise codon-anticodon pairing (Suzuki *et al.* 2002). A lack of taurine bound to mitochondrial tRNA leads to errors in the translation of proteins that are encoded by the mitochondrial genome, including the electron transport chain enzymes, that could cause superoxide generation and oxidative stress (Suzuki et al. 2002). Developing larvae have a high energy demand and a defect in the production of ATP would severely impede development and likely induce mortality. Therefore, taurine may be beneficial to Florida pompano larvae as well as to the embryo.

The taurine concentration of rotifers and *Artemia* are much lower than that of wild plankton. Rotifers and *Artemia* have a taurine content of approximately 80 – 180 mg/100g (dry basis) and 600 – 700 mg/100g (dry basis) respectively, which is significantly lower than the content found in wild plankton (1200 mg/ 100g dry basis) (Takeuchi 2001). Consequently, larval and juvenile fish grown in a laboratory had significantly lower concentrations of taurine in the whole-body analysis than that of wild caught fish (Matsunari *et al.* 2003). This finding confirms that that there is a need for improvement in larval rearing diets.

The taurine content of fertilized eggs does not decrease during embryonic development but instead the concentration of taurine decreases quickly during the rotifer feeding period (Matsunari *et al.* 2013). In a study by Matsunari et al. (2013), they enriched rotifers with varying levels of taurine (0 – 800 mg/L) for 12 hours to feed out to larval amberjack (*Seriola dumerili*). At the end of the experiment, the amberjack in the 800 mg/L group were significantly larger than those of the fish in the other treatments and had a higher concentration of taurine in their whole body composition. The survival rates between the treatment groups were not significantly different but a minor increase in survival was noted as the taurine concentration increased. However, a study by Rotman *et al.* (2017) found a significant increase in survival when they fed California yellowtail (*Seriola lalandi*) taurine-enriched rotifers. They also found a significant difference between the notochord lengths, helping cement the findings from the previous research that taurine-enriched rotifers have an advantageous effect on overall growth. These results show that taurine enrichment of rotifers can help increase the growth rates and concentration of taurine for amberjack.

Another study by Salze et al. (2012) fed larval cobia (*R. canadum*) taurine enriched live feeds, this time including rotifers and *Artemia*. They found that the larval cobia fed taurine enriched rotifers and *Artemia* had heightened enzymatic activities, including heightened amylase and trypsin activities, in the early larval stages. The heightened enzymatic activities can help enhance nutrient availability, possibly explaining the improved development, growth, and survival rates of larval fish fed a taurine enriched live food.

The need for established protocols for the taurine requirement for larvae is ever present and more research is needed to determine the levels of taurine required for successful larval culture. The recent approval of taurine for supplementation of fish feeds has caused more interest into performing research on varying species of fish to determine their dietary taurine requirements.

Objective

The objective of this study is to determine the effect taurine has on reproduction and larval development and to evaluate the interaction between taurine supplementation at the broodstock and larval levels. These finding can help improve on hatchery techniques and protocols for broodstock and larval Florida pompano. In addition, this study can help enhance protocols for enrichment of broodstock diets and live feeds with amino acids.

Materials and Methods

Facilities

This research was performed at Mote Marine Lab's Aquaculture Park (MAP) in Sarasota, Florida. MAP is a privately-funded research organization dedicated to the advancement of aquaculture and teaching the community about its work. The broodstock pompano were held in two $45m^3$ fiberglass, circular tanks with a volume of approximately 45,000L. The tanks were attached to a recirculating aquaculture system. This system contained a $0.085m^3$ drop filter (Aquaculture Systems Technologies, LLC, New Orleans, LA), a 900L moving bed biofilter, and two 150W High Output Smart Ho UV® units (Emperor Aquatics, Inc®, Pottstown, PA), one on the inflow to the tank and one on the outflow of the tank. A protein skimmer was also attached to the tanks and a 126,000-BTU heater/chiller (AquaCal AutoPilot, Inc., St. Petersburg, FL). Lighting for the tanks was controlled by a Solar 1000 series professional dimmer (BlueLine Aquatics, San Antonio, TX) to achieve sunrise and sunset patterns of the Florida pompano's natural habitat. Each tank had four 10,000ºK Coralife® bulbs. Aeration was provided by a 5 horsepower Sweetwater® regenerative blower (Pentair Aquatic Eco-Systems®, Cary, NC) and an emergency oxygen bottle was set up in the broodstock room in case of a low dissolved oxygen situation, but it was never used. The Florida pompano broodstock were a mix of wild and laboratory raised fish.

The sex ratio for the control tank was 19F:18M and 16F:12M for the taurine tank. An egg collector was attached to the broodstock tanks which was a 55-gallon drum that has a piece of PVC pipe inserted into the top portion of the drum and a net to catch the eggs. The PVC pipe was cut in half to form a trough and extended into the broodstock tank, skimming the surface. The floating eggs were caught by the PVC pipe and flowed down to the egg collector.

The larval rearing tanks consisted of 24 black, circular, fiberglass tanks that were 2-ft in diameter and 2-ft deep with a flat bottom and were kept at a volume of approximately 150L. The tanks were attached to a recirculation system that mimics that of the broodstock system. Lighting for the larval tanks was provided by two 10,000ºK Coralife bulbs that were suspended above the tanks. Each tank "shared" their light source with 3 other tanks for a total of 6 light fixtures in the system. Soft aeration was provided to the tanks around the stand pipe to ensure larvae did not get stuck to the screen on the stand pipe. Emergency oxygen was set up in case of a low dissolved oxygen situation but never used.

The broodstock tanks were maintained at a temperature between 27.6 $\pm$ 0.8ºC during the spawning trials and salinity stayed relatively constant (34 $\pm$ 0.32ppt). Dissolved oxygen (D.O.) was between 4.2 and 12 mg/L and pH was maintained between 7.5 and 8.2 using sodium bicarbonate to adjust when the pH was too low. The water in the larval tanks was held at 26.7$\pm$ 0.5ºC, had a D.O. of 4.8 $\pm$ .64 mg/L, a salinity of 36.2 $\pm$ 1.94 ppt, and a pH of 8.3 $\pm$ .12. All water quality was measured daily with a YSI Pro Plus meter (YSI Inc., Yellow Springs, OH). Water chemistry was assessed once a week and the tanks were maintained within the following limits: total ammonia nitrogen <0.5 mg/L, total nitrate-N <50 mg/L, and total nitrite-N <1.0mg/L. All of the broodstock

and larval tanks never exceeded these limits and water exchanges were limited to 20% exchanges once a week for only the broodstock tanks and water was added to the larval system to compensate for evaporation with no water changes performed during this trial. All salt water was made artificially using Instant Ocean ® salt and fresh, well water. Newly made water and water from the tanks were sent through a carbon filter and ultra violet filters, then held in three large, 5,000L circular tanks with a fluidized bed biological filter. After 24 hours in the fluidized bed, the water was sent into one of three 100,000L storage tanks.

Experimental Procedures

The experiment was set up in two levels and the experimental design is shown in Figure 1. The first level was the broodstock trials that consists of a control population and a taurine enriched population. Once the eggs hatched, the spawns were split between four treatments; control broodstock-control larvae (CBCL), control broodstock-taurine larvae (CBTL), taurine broodstock-control larvae (TBCL), and taurine broodstock-taurine larvae (TBTL).

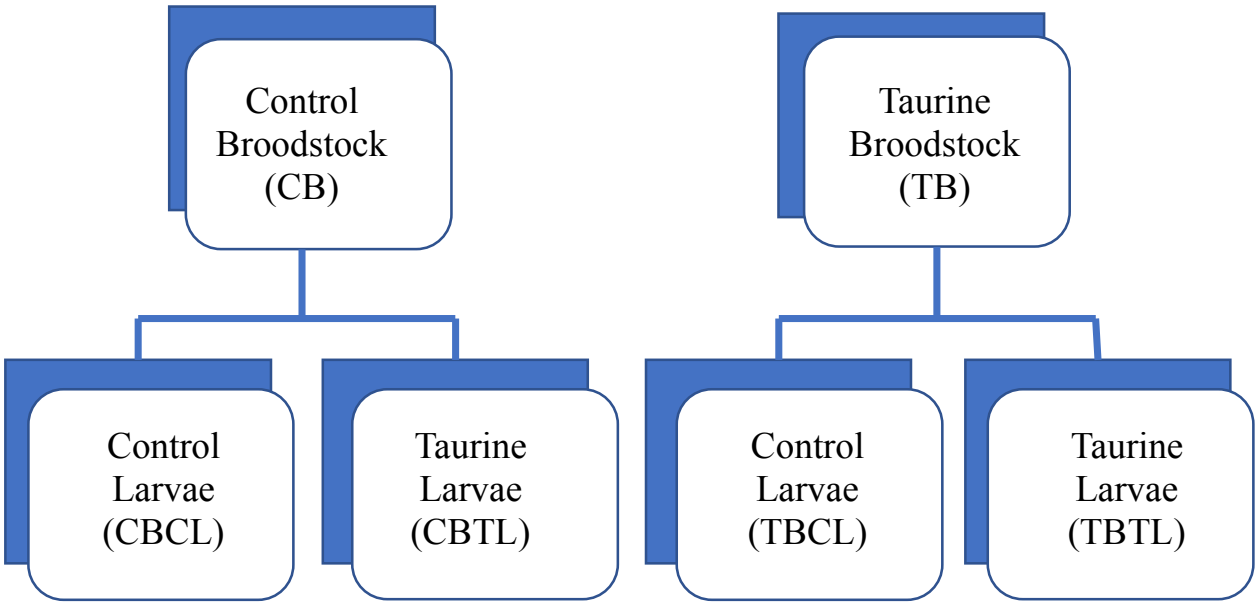

Figure 1- Conceptual diagram of the 2x2 factorial experimental design

Broodstock

Three weeks before spawn, the Florida pompano were switched from a normal diet of 50% shrimp and 50% squid to an experimental gel diet fed at a rate of 6% their body weight. The gel diet was made for the broodstock pompano and consisted of squid, shrimp, fish (Spanish sardine *Sardinella aurita*), menhaden fish oil, de-ionized water, and food-grade gelatin. The formulation of the diets can be found in Table 1. Crystalline taurine (MP Biochemicals, Inc., Solon, OH, USA) was added to the taurine-group's gel at a 5% concentration. The broodstock fish were then fed the gel diet for three weeks and then sampled for oocyte maturation.

To collect the fish the water level was dropped by 70% and the fish were corralled into a makeshift pen. One by one the fish were removed from the pen and anesthetized using tricane methanesulfonate (MS-222) at a concentration of 200mg/L. Once anesthetized, the fish were weighed and measured and a sample of oocytes were taken using a syringe and cannulation tubing. Oocytes were observed under the microscope to determine development and if the oocytes exhibited secondary growth the fish was injected with Ovaplant (Salmon gonadotropin sGnRHa) at a dosage of 50 μg/kg to induce spawning. All males were sampled to determine if milt was flowing and then were released back into the tank without an injection. The milt was then examined for sperm motility using a microscope. After handling the fish they were revived by gill ventilation in the original tank and were released to spawn volitionally.

Table 1- Diet formulations for taurine and non-taurine enriched gel diets for Florida pompano broodstock. Proximate composition of control and taurine gel diets g/100g as is.

	Control Gel Diet	Taurine Gel Diet
Total Feed (g)	5000	5010
Spanish Sardine[1] (g)	960	960
Shrimp[1] (g)	720	720
Squid[1] (g)	720	720
Gelatin[2] (g)	900	900
Water (De-ionized) (g)	1656	1656
Fish Oil[3] (g)	44	44
Taurine[4] (g)	0	10
Proximate Analyses (%, as-is)		
Moisture (%)	71.6	71
Dry matter	28.4	29
Protein (%)	24.1	24.4
Fat (%)	3.39	3.59
Ash (%)	0.59	0.99
Sulfur (%)	0.23	0.26
Taurine as is (%)	0.1	0.25
Taurine dry matter (%)	0.35	0.86

[1]A.P. Bell Seafood Company, Cortez, Florida, USA
[2]PB Gelatins, Davenport, Iowa, USA
[3]Omega Protein, Houston, Texas, USA
[4] MP Biochemicals, Inc., Solon, Ohio, USA

Eggs and Larvae

After the successful spawn event the eggs were collected into the egg collector attached to the tank. The eggs are then taken out of the collector and enumerated. Approximately 10 hours after fertilization, a sample of 100 eggs was taken and photographed under a microscope to determine the size of the oil globule, yolk, and overall egg and then frozen for compositional analysis. The number of viable, fertilized eggs and non-fertilized eggs are collected and enumerated. Eggs were considered viable if they were floating on the surface and all eggs resting on bottom were considered non-viable. Fertilization rates were determined by taking a sample of 100 floating eggs and counting the number of eggs under a microscope that had either a "scar" left from the sperm penetrating the egg or a developing embryo. The formula for the volume of a sphere was used to calculate the volume of the eggs, yolk, and oil globule.

The eggs were stocked directly into the larval rearing tanks at 5,000 eggs per tank. From 2 days post hatch (dph) to 10 dph the water in the tanks was shaded with RotiGrow Nanno (Reed Mariculture Inc., Campbell, CA) at a concentration of 300,000 cells per liter. Once the larvae developed mouth parts and a functional digestive system, about 3 dph, rotifers were introduced. Each larval treatment level had 6 replicates. Rotifers were either enriched with taurine and a commercial enrichment (Algamac 3050 Flake, Bio-Marine Inc., Hawthorne, CA) or just the commercial enrichment for 18 to 24 hours. The rotifers were harvested using a 45-micron mesh bag and given a rinse with fresh salt-water before feeding to remove excess enrichment. At 8 dph, the larvae were fed a mixture of rotifers and *Artemia*. The taurine *Artemia* were enriched in

the same manner as the rotifers with an 18 to 24 hour enrichment period followed by a fresh salt-water rinse.

Representative samples of 50 larvae were taken at hatch, 1, 2, 10, and 15 dph. At the hatch, 1, and 2 days post hatch the larvae's total length, weight, yolk-sac, and oil globule were measured. Afterwards, only the weight and length were measured. The fish that were sampled were then frozen and saved for compositional analysis to determine concentrations of amino acids, specifically taurine.

Statistical Analysis

All statistical calculations were performed on Statistical Analysis Software (SAS) (V9.1 SAS Institute, Cary, NC). Broodstock measurements were analyzed using a t-test to determine the statistical significance amongst the treatments. Larval measurements were analyzed using a two-way analysis of variance (ANOVA). When differences were indicated (considered statistically significant at $P \leq 0.05$) in the broodstock treatments, Tukey's Studentized range test was used to separate significantly different means.

Results

Broodstock Trials

Mature, female Florida pompano were injected with Ovaplant® on May 17th, 2016 and both tanks spawned the next night around 11 pm. Each tank had three spawn events between May 18th to May 20th, 2016. The total number of eggs, total number of viable eggs, fertilization and hatch rates are listed in Table 2. Over the three-day spawn period the control tanks had an average, per spawn event, of 197,200 eggs, 99,167 viable eggs, a fertilization rate of 50.3%, and a hatch rate of 72.7%. The taurine tank averaged, per spawn event, 201,733 eggs, 88,400 viable eggs, a 43.8% fertilization rate, and a hatch rate of 71.3%.

The results for the eggs are listed in Table 3. The control eggs had an average volume of 0.4187 mm^3, an average yolk volume of 0.1907 mm^3, and an average oil volume of 0.001836 mm^3. The taurine eggs had an average volume of 0.4166 mm^3, an average yolk volume of 0.1996 mm^3, and an average oil volume of 0.008578 mm^3. The composition of the eggs and post-hatch larvae can be found in Table 4. The concentration of taurine in the eggs were identical (0.04 mg) between the two treatments. The concentration of taurine in the post-hatch larvae was also identical (0.65 mg) between the two treatments.

Table 2- Total number of eggs, total number of viable eggs, average fertilization rate and average hatch rates between three spawning events.

n=3	Total Number of Eggs	Total Number of Viable Eggs	Avg. Fertilization Rate	Avg. Hatch Rate
Control	591,600	297,500	50.3%	72.7%
Taurine	605,200	265,200	43.8%	71.3%

Table 3- Average egg volume, yolk volume, and oil globule volume for control and taurine treated broodstock diets. Means come from a sample of 50 eggs per treatment.

	Eggs	
	Control	Taurine
Avg. Egg Volume (mm^3)	.4187	.4166
Avg. Yolk Volume (mm^3)	.1907	.1996
Avg. Oil Volume (mm^3)	.007127	.008578

Table 4- Total concentration of taurine in eggs, post-hatch larvae, and 15 dph larvae.

	Eggs (n=3)	Post-hatch Larvae (n=3)	15dph Larvae (n=6)	
Control	0.04	0.65	CBCL	0.21[a]
			CBTL	0.31[ab]
Taurine	0.04	0.65	TBCL	0.28[ab]
			TBTL	0.37[b]

Larval Trials

At 15 dph the larval Florida pompano were successfully weaned onto a dry diet and the trial was terminated. The results for the larvae are listed in Table 4. The taurine broodstock-taurine larvae (TBTL), taurine broodstock-control larvae (TBCL), control broodstock-control larvae (CBCL), and control broodstock-taurine larvae (CBTL) had taurine concentrations of 0.37 mg, 0.28 mg, 0.21 mg, and 0.31 mg respectively. There was no significant difference between the CBTL and TBCL groups but there were significant differences between the rest of the treatments. The means are displayed in Table 2.

The length and weights were recorded and analyzed and can be found in Table 6. An interaction plot for lengths and weights can be found in Figures 2 and 3. The CBCL larvae had a length of 9.66 ± 1.52 mm, CBTL lengths were 9.07 ± 1.20 mm, TBCL lengths were 9.87 ± 1.29 mm, and the TBTL lengths were 8.92 ± 1.30 mm. The CBCL larvae had weights of 0.0133 ± 0.0055 g, CBTL weights were 0.0124 ± 0.0056 g, TBCL weights were 0.0153 ± 0.0055 g, and TBTL weights were 0.0124 ± 0.0054 g. Survival rates were 5.5% for BCLC, 11% for BCLT, 6% for BTLC, and 9% for BTLT. The survival rates were found not significant and can be found in Figure 4.

Table 5- Average length and weight for all four levels of treatments in the larval grow-out trials (n=6).

Broodstock Treatment	Larval Treatment	Length (mm)	Weight (g)
Control	Control	9.658 ± 1.515	0.0133 ± 0.0055
	Taurine	9.067 ± 1.193	0.0124 ± 0.0056
Taurine	Control	9.867 ± 1.289	0.0153 ± 0.0055
	Taurine	8.917 ± 1.294	0.0124 ± 0.0054

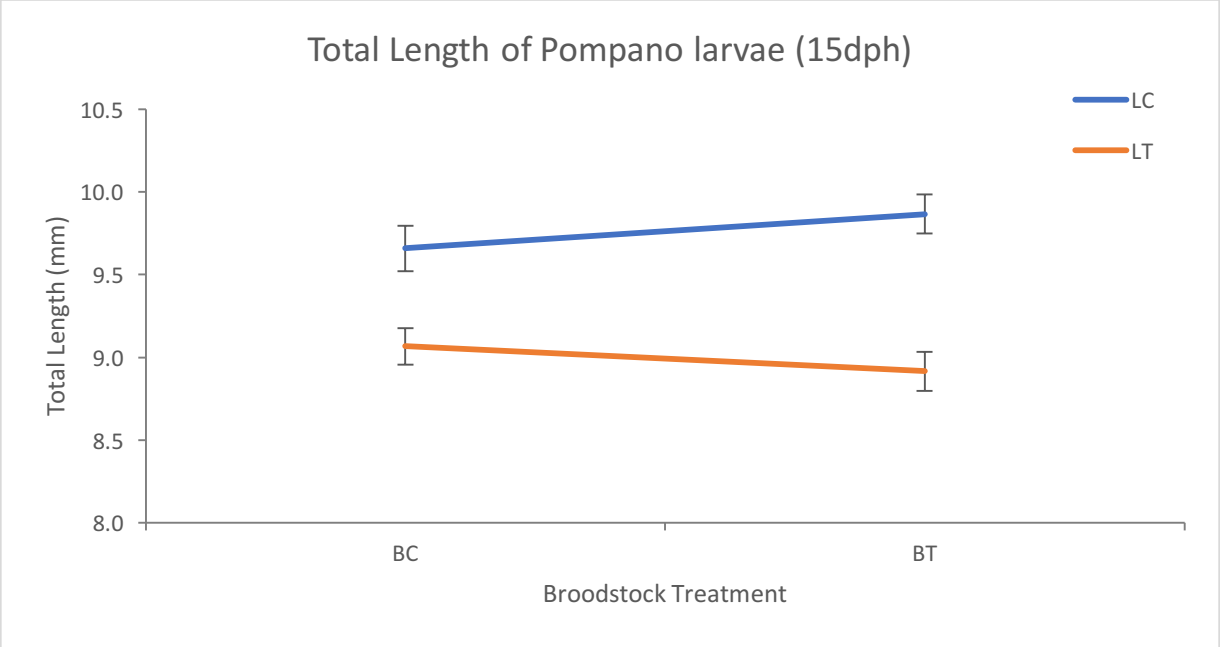

Figure 2- Interaction graph for 15 dph larvae lengths. Results are from a sample of 50 larvae per treatment. P-values are; Interaction P-value: 0.1404, Broodstock P-value: 0.81, Larval P-value: <0.0001.

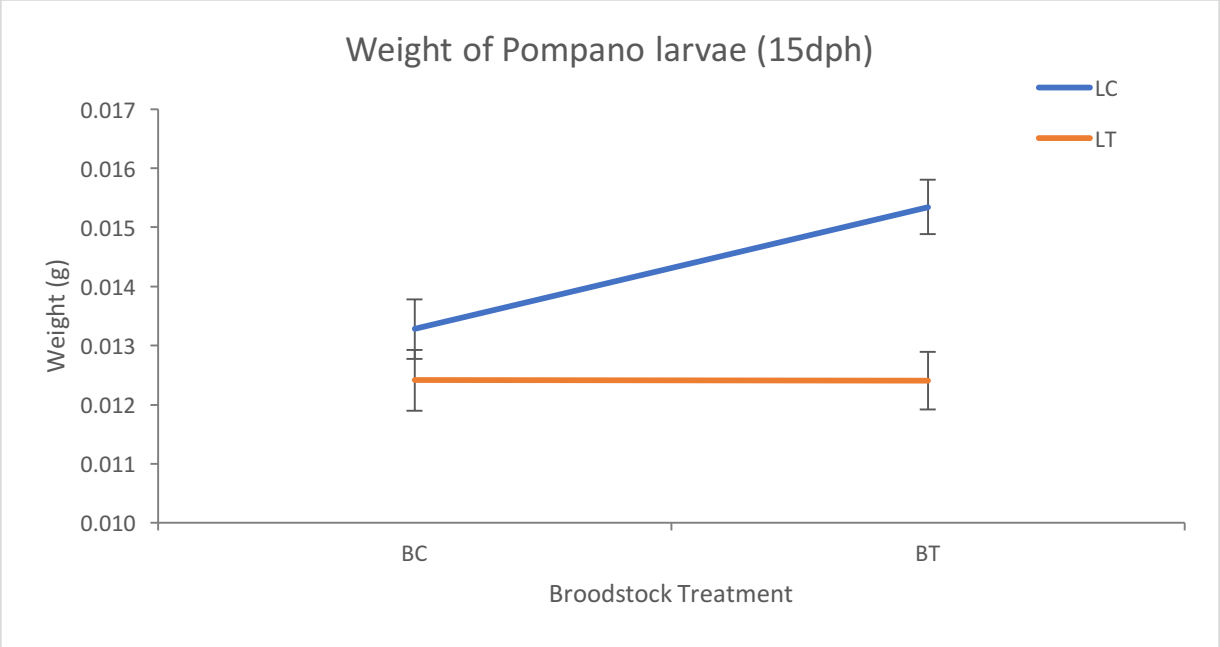

Figure 3- Interaction graph for 15 dph larvae weights. Results are from a sample of 50 larvae per treatment. P-values are; Interaction P-value: 0.0356, Broodstock P-value: 0.0377, Larval P-value: 0.0001.

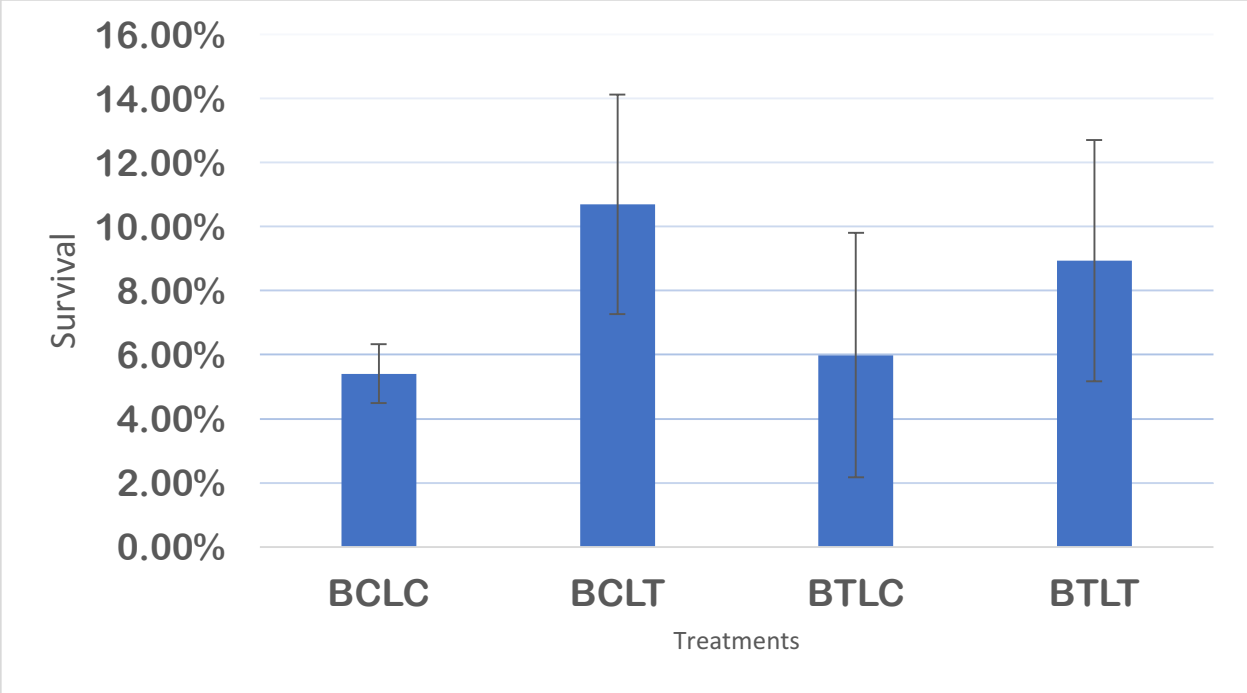

Figure 4- Survival results from Florida pompano. No significant difference between treatment groups (n=6). BCLC treatment had one tank with zero fish after 7 dph.

Discussion and Conclusion

The variability in the data and in this trial is testament to the problems that face aquaculture and hatchery production. Nutrition of broodstock and larval fish has become a major focal point for both commercial and governmental hatcheries. The advancements in aquaculture nutrition leads to changes in protocols and without proper understanding, problems can arise. Nutrition is one of the most significant aspects of reproduction and larval development and the need for more research is ever-present.

Results from the broodstock portion of this study suggest that taurine enrichment of the diets does not significantly increase egg production, fertilization, or hatch rates (Table 2). These results are contradictory to the results from Matsunari et al. (2006) in which they experienced an increase in spawning success, total egg numbers, fertilization rate, and hatch rate for yellowtail (*Seriola quinqueradiata*) as the taurine concentration in the feed increased. An explanation for the results from the current study on Florida pompano could be indicative that taurine is not critical for oocyte maturation, fertilization, and hatch rates in this species. Another possible explanation could be that in the same study by Matsunari et al. (2006) they fed the experimental diets to the broodstock yellowtail for 6 months. In this study, the Florida pompano were fed the experimental diets for a total of three weeks. Extending the feeding period to a longer period of time could help ensure that the control broodstock Florida pompano have completely diminished their taurine reserves. This could possibly explain how there wasn't a significant difference between the two treatment groups in terms of spawning performance and hatch rates. Further

research with Florida pompano broodstock and taurine, along with a longer feeding period, could help confirm or deny our findings.

The concentration of yolk and oil in the taurine enriched group was significantly higher than the control group, while the overall egg size was larger in the control group (Table 3). The study by Matsunari et al. (2006) had similar results with the yellowtail broodstock. Yolk size was not calculated by Matsunari et al. (2006) but overall oil globule size was higher in the taurine group and the overall egg size was smaller in the taurine group. The increase in the oil globule could be attributed to the stimulating effect taurine has on enhancing protein synthesis and deposit (Li *et al.* 2016). The decrease in egg size is hard to explain; with an overall increase in oil globule and yolk size, it is expected that the egg size should also increase. Genetics and water temperature could be a possible explanation. Florida pompano oocytes mature at a temperature around 24-26ºC and any variation in the tanks could cause a decrease or increase in oocyte maturation and size (Matsunari et al. 2006, Hoff et al. 1978). With more yolk and oil reserves, the post-hatch taurine larval fish would have more energy to fully develop mouth parts and a functional digestive system. However, the size of the yolk sac is not a true indicator of quality. Unlike the oil globule, yolk contains water and a larger yolk does not necessarily correlate with nutrient density. Looking at the composition of the yolk on a single egg basis is almost impossible and very expensive, thus making it difficult to determine what a bigger yolk entails. More research on how taurine concentrations effect overall egg quality for Florida pompano is needed to further explain our findings.

Taurine concentrations in eggs between the two treatment groups were identical (Table 3). Matsunari et al. (2006) also reported that the concentration of taurine in the eggs was not significantly different amongst the treatments. Even though the findings by Matsunari et al. (2006) converge with ours, they still experienced a small but non-significant difference in the taurine concentration of the eggs. As mentioned earlier, Matsunari et al. (2006) fed their yellowtail broodstock for a total of six months before spawning and this study fed the Florida pompano the experimental diets for a total of three weeks. This shortened feeding time could be a factor in the taurine concentration of the eggs. Three weeks might not be long enough to allow the depletion of taurine in the control Florida pompano and an extended feeding period could help alleviate that problem. The taurine dosage for the study could be another issue for the lack of taurine found in the eggs and post-hatch larvae. Even though the proposed recommended requirement for dietary taurine is between 0.54 – 0.65%, the amount of taurine required by the fish could be different as the fish initiates sexual maturation. With more growth during the first three years of a fish's life more energy would need to be produced to keep up with the physiological energy demands. Determining how the requirement for taurine changes as the fish changes its metabolic priority from growth to reproduction could help propose a new recommended dietary taurine range that is more specific to the life stage of the fish.

At 15 days post hatch the larvae were successfully weaned onto a dry food only diet and the experiment was terminated. The larvae fed the taurine enriched rotifers and *Artemia* had smaller lengths and weights than the control larvae (Table 4). Interestingly, the taurine enriched broodstock and control larvae (TBCL) out grew all study groups while the taurine enriched

broodstock and larval (TBTL) diets were the smallest (Table 4). This result is contradictory to what Salze *et al.* (2011) found with larval cobia (*Rachycentron canadum*) fed taurine enriched rotifers in which they had more growth than compared to the control. With the results from Salze *et al.* (2011) and this research, one could determine that taurine supplementation of live feeds for Florida pompano are not warranted and could be potentially detrimental to the growth of the larvae. However, more research is needed to help determine the role of taurine in the development of post hatch Florida pompano larvae.

When looking at the interaction plot for the lengths, there was no significant interaction (P-value: 0.1404) between the treatment levels (Figure 2). However, there is a significant effect of taurine supplementation at the larval level (P-value: <0.0001), with control larvae being longer than the taurine-supplemented larvae. Therefore, taurine supplementation for larvae is not needed to increase the overall length of the larvae, no matter of the broodstock treatment.

The interaction plot for the weights in Figure 3 show that there is a significant interaction between the treatment levels (P-value: 0.0356), hence we can look at the graph as an If-Then statement to determine whether or not taurine supplementation is needed on either levels. Broodstock supplementation with taurine had a positive effect on larval weight only if larvae did not receive supplementation. Therefore the data supports recommendations of supplementing broodstock feeds while not supplementing live prey for larvae.

Survival after 15 days post hatch was highly variable, low and not significantly different among the treatments (Figure 2). The taurine enriched larval diets from both broodstock treatments seem to have had better survival than the control larval diets. In the study by

Matsunari et al. (2013) they found that taurine enrichment of larval diets had no significant effect on the survivability of amberjack *S. dumerili*. The findings from Matsunari et al. (2013) and this current study on Florida pompano could be indicative that taurine supplementation of live feeds does not have a significant effect on the survivability of the larvae. However, the increased survivability from the treatment groups that had taurine enriched live feeds is an interesting result. In the study by Salze *et al.* (2012) they determined that feeding larval cobia taurine enriched live feeds increased the enzymatic activity of trypsin and amylase in the early larval stages. These heightened enzymatic activities could lead to an increase in nutrient availability and could be an explanation for the increase in survival for the taurine group. In another study performed by Salze et al. (2016) on Florida pompano and taurine they theorized that taurine deficiencies could cause a linear decrease in cytochrome c oxidase (COX) specific activity in the liver. As mentioned earlier, taurine has been identified to covalently bind to and modify mitochondrial tRNA. This binding and modification enables precise codon-anticodon pairing and a lack of the modification increases the errors in translation of proteins encoded by the mitochondrial genome, i.e. the enzymes of the electron transport chain. A defect in the electron transport chain causes superoxide generation and the ensuing oxidative stress, which could be a possible explanation for the low survivability between the larval control groups.

During this trial, one tank from the BCLC treatment group has zero live fish after 7 dph. This tank did not have the same hatch rates as the other tanks and had less than 100 larvae in it after hatch. As we believe this was not related to dietary treatment, this tank was not included in data analysis. Water quality parameters and feedings were normal and the same as the other

tanks. The true reason why this tank “crashed” will never be known but it is another example of how variable larval culture is for Florida pompano.

The concentration of taurine in the 15 days post hatch juveniles was highest in the TBTL treatment group (0.37 mg/g dry matter) and lowest in the CBCL treatment group (0.21 mg/g dry matter), which was expected. There was a significant difference in the concentration of taurine in the juveniles between the two treatments, TBTL and CBCL. These results suggest that amino acid supplementation of larval diets, particularly taurine, will effectively increase the overall concentration of amino acids in the fish.

In conclusion, taurine supplementation of broodstock diets has no significant effect on the size of the eggs but it does have a significant effect on the amount of yolk and oil inside of the egg therefore increasing the overall quality of the egg. The larval portion of this study showed that while supplementation of live feeds with taurine increased the overall survivability it had a negative effect on the overall size and weight of the larval Florida pompano. Larval diet supplementation did have an increase in survival, however, the growth of the Florida pompano larvae was negatively affected by taurine supplementation of live feeds.

It is recommended that the supplementation of taurine for larvae is not needed to increase the overall weight of the larvae treatment. In terms of length, it is not recommended to supplement your larval diets with taurine. If the larval feeds are enriched with taurine then it is recommended that the broodstock diets are not enriched. However, if the larval feeds are not enriched then it is recommended that the broodstock diets be enriched with taurine to increase the weight of the larvae.

More research into the exact role of taurine in the growth, metabolism, oocyte maturation, and survival could potentially help aquaculturists understand the importance of proper fish nutrition in a hatchery setting. The need to develop proper nutritional requirements for taurine and other amino acids in broodstock and larval diets could help alleviate these issues. Without proper knowledge of the nutritional requirement for broodstock and larval fish, one cannot expect to successfully and efficiently produce fish for commercial and recreational purposes.

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