Advances in Understanding of Taurine Functions in Fishes Across Species and Life Stages

Guillaume P. Salze[1], D. Allen Davis[1], Matthew Resley[2], Nicole Rhody[2], Kevan Maine[2], Kevin Stuart[3], Mark Drawbridge[3]

[1] School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University, Auburn, AL

[2] Mote Marine Laboratory, Center for Aquaculture Research and Development, Sarasota, FL, [3] Hubbs-SeaWorld Research Institute, San Diego, CA, USA

Abstract

Taurine is now widely recognized as an essential nutrient in many teleost species, and during the past decade investigations have focused on determining quantitative requirement levels and physiological and metabolic responses to dietary taurine. Although the current state of knowledge is biased toward high-value marine carnivorous species, evidence points to functional differences among species (e.g., bile salt conjugation, osmoregulation, membrane stability). Prediction of the qualitative or quantitative requirement based on ecological boundaries is difficult, although trophic level seems to be a better predictor even if several exceptions exist. Thus caution must be exerted when assuming the qualitative or quantitative taurine requirement in a given species. Additionally, a number of studies highlight changes in the quantitative requirement between life stages, particularly in larval stages. If knowledge of taurine functions and potential technological uses in larval stages is limited compared to juvenile stages, it is even scarcer in reproducing broodstocks. Consequently, the first part of this paper reviews the current understanding of the species- and life stage-dependent differences in in taurine function and requirement levels. In a second part, initial experimental results obtained in California yellowtail *Seriola lalandi* and Florida pompano *Trachinotus carolinus* broodstocks are presented. While the crucial importance of essential fatty acid in egg quality and overall reproduction performances needs no additional proof, results highlight the importance of proteins as well. In this context, not only were the total amount of protein and amino acid levels correlated with hatching success, but results also suggest the relationship between urea cycle and survival to 1st feeding in the newly hatched larvae.

Keywords: Taurine function, *Seriola lalandi, Trachinotus carolin*

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1. Introduction

Taurine had largely been considered a dispensable amino acid in teleosts until evidence accumulated in the late 1990s and early 2000s that this β-amino acid was indeed an indispensable nutrient for many species. Initial studies investigated the reasons explaining the superior performances of fish larvae when fed wild copepods compared to those fed traditionally-enriched rotifers and *Artemia* (Conceicao *et al*., 1997; Shields *et al*., 1999), and identifying the taurine content as a major difference between the two types of prey (Aragao *et al*., 2004; Helland *et al*., 2003). In the following years, the essentiality of taurine was further demonstrated when taurine supplementation to otherwise taurine-poor feeds successfully restored growth and survival in larvae and juveniles (Chatzifotis *et al*., 2008; Kim *et al*., 2005; Lunger *et al*., 2007; Rossi Jr and Davis, 2012; Salze *et al*., 2011; Salze *et al*., 2012a).

Concerns in environmental and economic sustainability of aquaculture feeds have been driving the vast research effort toward the replacement of fishmeal with other sources of protein. However, taurine is found in relatively large quantities in fishmeal (provided adequate ingredient processing), while mostly-used alternatives such as soybean products are practically devoid of it. Consequently, taurine must be supplemented to the diet when low-taurine ingredients are used; possible source include krill meal, other animal proteins such as poultry by-product meal, or crystal taurine. Knowledge of the taurine requirement's existence allowed the further reduction of fishmeal and other animal proteins sources as dietary ingredients, thereby improving the sustainability of fish feeds.

Differences in taurine requirement among species is evident (Salze and Davis, 2015). Most of the taurine-related studies have been conducted in marine species living in warm waters, although some have focused on cold and/or freshwater species. Some

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evidence also points to changes in requirement according to life stages. This study will first review the available information regarding differences in taurine requirement among species and life stage. Information regarding the latter is currently limited, hence in a second part experimental data pertaining to taurine supplementation in broodstock diet will be presented.

1 Essentiality & ecology – Prediction of requirement in juveniles

The quantitative or qualitative requirement for taurine has been evaluated in a number of species (Salze and Davis, 2015). Many marine carnivores were found to require taurine in their diet, in contrast with most freshwater species. However, the essentiality of taurine does not reliably follow broad, ecological categories, such as salinity (e.g., common carp vs. channel catfish vs. white seabass) or temperature (e.g., red tilapia vs. Florida pompano) gradients. Figure 1 shows that the taurine requirement does not exactly follow trophic level either: while a cluster of species seems to follow a linear relationship between trophic level and quantitative taurine requirement, other species markedly depart from this paradigm, e.g., Nile tilapia and channel catfish.

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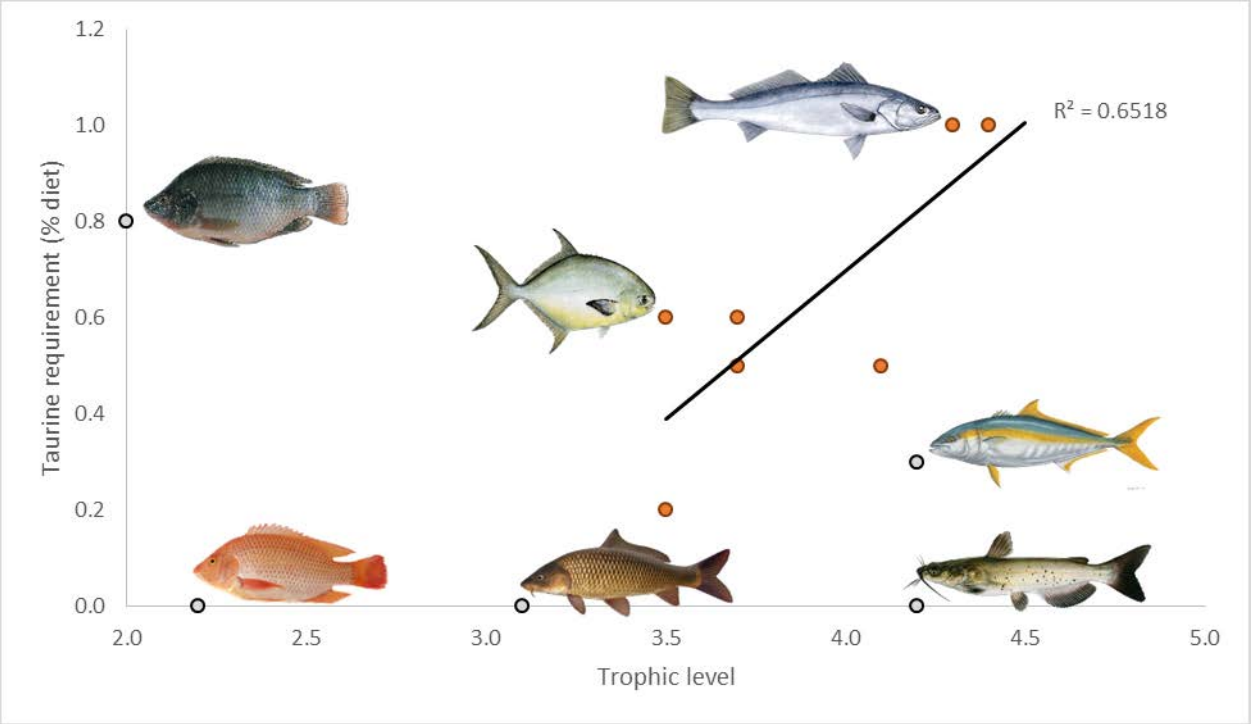

Figure 1: Correlation between taurine quantitative requirement and trophic level in teleosts

This shows that one should be cautious when predicting the essentiality of taurine based on ecological and environmental characteristics.

2 Taurine function among species

Many physiological functions have been attributed to taurine in mammals. However, knowledge of taurine physiology and metabolism in teleost is limited, and to which extent these functions are conserved between mammals and teleosts has not yet been studied in depth. Functional differences between teleost species are possible. Indeed, taurine would not be expected to be found in the diet of an animal to which taurine is not essential, and therefore may have different functions. For example, in contrast with most

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other teleosts, the bile of cyprinids such as carp and zebrafish is mostly made of bile alcohols, which conjugate with sulfate instead of taurine or taurine derivative. Therefore, taurine does not participate in the solubilization of lipids for digestion in these species. Other interspecific differences may be found as the functions of taurine in fish are further investigated.

Depending on their natural environment, different species are exposed to changes in salinity of various magnitude and frequency; hence their need to cope with changes in environment osmolality is different. In carp and tilapia expression of the taurine transporter is also significantly upregulated in response to increase is osmolality, but an increase in cellular taurine was observed (Takeuchi *et al*., 2000a; Takeuchi *et al*., 2000b) concomitantly with a decrease in plasma taurine (Assem and Hankf, 1979), thereby strongly suggesting that taurine is a major osmolytes in these species. Moreover, dietary taurine supplementation was shown beneficial for acute hypoxia tolerance in carp through a reduction in hemolysis rate (Yang *et al*., 2013). When subjected to hyperosmotic stress, salmon branchial cell also upregulated the expression of the taurine transporter, suggesting a role of taurine in osmoregulation in this species. However, taurine supplementation does not result in improved survival despite being cultured on taurine-free media. It is possible that other osmolytes present in the culture media (e.g., inositol) fulfilled the osmoregulation need in the absence of taurine (Zarate and Bradley, 2007), suggesting that taurine is not an osmolyte in salmon, or that other osmolytes may be preferred. Other examples are seen in marine species such as yellowtail *Seriola quinqueradiata* (Takagi *et al*., 2006), where hemolytic anemia was observed in taurine-deficient fish, along with reduced serum osmolality and osmotic resistance of erythrocytes. In contrast, red seabream *Pagrus major* does not exhibit such decrease in hematocrit in response to low dietary taurine intake (Takagi *et al*., 2011), nor does Florida pompano (Salze *et al*., Aquaculture, Submitted). Finally, tissue taurine levels did not change in banded killifish *Fundulus diaphanous*, despite exposing individuals to freshwater or full-strength sea water (Ahokas and Sorg,

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1977). Such differences suggest that taurine in not (or is less) involved in osmoregulation depending on the species. Similarly to the quantitative requirement, these differences are not aligned with broad ecological characteristics.

Another area of discrepancies in the roles of taurine among teleosts is seen in the changes in nutrient utilization and deposition with the dietary levels of taurine (Salze and Davis, 2015). Overall, there is a general positive trend of increased body lipid content with levels of dietary taurine in cobia (Lunger *et al*., 2007) Florida pompano (Salze *et al*., 2014), and turbot (Qi *et al*., 2012; Yun *et al*., 2012). However, the opposite trend have been observed in different species such as Atlantic salmon (Espe *et al*., 2012a; Espe *et al*., 2012b) and rodents (Tsuboyama-Kasaoka *et al*., 2006).

Taurine has long been reported to have hypoglycemic and properties (Das *et al*., 2012; Goldberg and Jefferies, 1946; Huxtable, 1992; Ito *et al*., 2012; Kim *et al*., 2007). In mammals the relationship between taurine and glucose and lipid metabolism is particularly complex and involves many interacting factors, all of which could explain interspecific differences observed. Although taurine has been found to directly bind with the insulin receptor (Maturo and Kulakowski, 1988), affinity is about 1% of typical extracellular concentrations, thus questioning such mechanism of action. Rather, the insulin-like action of taurine seem mediated by a pancreatic stimulation to liberate insulin it has been suggested that taurine exerts its hypoglycemic action through cAMP and protein kinase signaling pathways (Ribeiro *et al*., 2010). In fish, only a few studies investigated the effects of taurine on the glucose and lipid intermediary metabolism. In Atlantic salmon taurine supplementation of a plant-based diet decreased lipid deposition without significantly reducing final live weight compared to the same plant-based diet unsupplemented with taurine with results suggesting an increase in S-adenosyl methionine (SAM) and liver polyamine concentrations (Espe *et al*., 2012a), which may explain the decrease in lipid accumulation (Jell *et al*., 2007). In contrast, the hepatic lipid content totoaba does not

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decrease when fed taurine-supplemented, plant-based diets, not is there any influence on the activities of fatty acid synthetase or Malic enzyme (Bañuelos-Vargas *et al*., 2014). Rather, the activities of glycolytic hexokinase and gluconeogenesis fructose 1,6-bisphosphatase are significantly increased. This supports the existence of interspecific differences in the way that dietary taurine mediates metabolism in teleosts.

3 Function across life stages

Taurine is sometimes referred to as a conditionally required nutrient. This terminology should not be applied relative to ingredients used in feed formulations is incorrect: while using various ingredients may affect the bioavailability of a given nutrient, it is not likely to affect the requirement itself, i.e. the amount of nutrient needed to achieve a metabolic target. For instance, methionine is not conditionally required when feeding soybean-based diets; simply, soybean-based diets tend to be deficient in methionine, and must be supplemented using synthetic methionine or other, methionine-rich ingredients. The same applies to taurine, as it does for any other essential nutrients.

However, some studies have investigated the changes in taurine requirement with life stages / size, which would then constitute a conditional requirement. In turbot *P. maxima*, benefits of taurine dietary supplementation was 0.64% dietary taurine for 165.9g individuals, compared to 1.15% in 6.3g individuals, thus strongly indicating that the quantitative requirement in this species decreased as the fish grew in size (Qi *et al*., 2012). Moreover, feed intake increased in response to increasing dietary taurine in both sizes, but feed efficiency was improved only in the smaller fish. This suggests that taurine acted mostly as an attractant in the bigger turbot, but had a more complex effect in the smaller fishes.

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In Atlantic salmon parr (2g), dietary taurine caused an increase in polyamine synthesis, which implies an increase in S-adenosyl methionine (SAM) as methyl donor (Espe *et al*., 2012a). However, hepatic cells isolated from 1.4kg Atlantic salmon showed no changes in methylation capacity in response to taurine supplementation in the culture medium while also significantly reducing apoptosis (Espe and Holen, 2013). Though the essentiality of taurine in salmon remains to be clearly established, these data indicate that life stage is an important factor to consider when attempting to answer this question. The functional changes of taurine with life stages can be seen during major ontogenic milestones, such as during smoltification in Atlantic salmon. Indeed, expression of the taurine transporter gene *TauT* was decreased by dietary taurine in smolt but not parr held in sea water (Zarate and Bradley, 2007), thereby suggesting that taurine becomes more important as the fish adapts its physiology to cope with increasing salinity. Similarly, *TauT* expression was positively correlated with metamorphosis stage in Senegalese sole, suggesting an important role of taurine in this crucial developmental process (Pinto *et al*., 2011). In juvenile, *TauT* is particularly expressed in the hindgut (likely for enterohepatic recirculation) as well as in the stomach. It is not clear at which point during larval development *TauT* expression starts. It is quite possible however that it occurs prior to the completion of the acidic pepsin digestion capacity: dietary taurine supplementation significantly improved morphological and enzymatic development in larval cobia in the earliest phase (Salze *et al*., 2011; Salze *et al*., 2012a; Salze *et al*., 2012b), indicating that dietary taurine must be sensed and detected by the organism in order to produce such effect.

This overview of the current taurine literature clearly shows the limitations in our understanding of taurine function within and among teleost species, as well as among life stages; additional research is necessary to elucidate both the similarities and differences. Even among commercially-relevant aquaculture species, knowledge remains fragmented as the majority of studies were conducted on juvenile animals, and only a few performed with larvae. To the best of our knowledge there is to date only one study reporting the effects of

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taurine supplementation in sexually maturing individuals on reproductive performances (Matsunari *et al*., 2006). In this study, yellowtail *S. quinqueradiata* broodstock were fed 3 diets with graded levels of taurine: the group receiving taurine-unsupplemented feed did not spawn, and improved egg quality was observed in the group fed the highest taurine level. Acknowledging both the promising results and the dire need for additional information in this area, we conducted two studies concerned with the nutritional taurine status in broodstock and its effect onto reproductive output. The first study was an *a posteriori* analysis of historical egg samples from California yellowtail *Seriola lalandi*, matched with the performances of the resulting larvae. In the second study Florida pompano (*Trachinotus carolinus*) broodstock were fed diets that were supplemented or unsupplemented with taurine and the resulting egg quality was observed. These preliminary data provide initial information to better focus future studies on the subject.

4 California yellowtail

4.1 Introduction and methods

California yellowtail, along with other species in the *Seriola* genus, is a species of commercial importance: in 2013, aquaculture production of *Seriola* sp. was about 186k t (FAO, 2015). Hatchery production of California yellowtail is variable and typical survival rates of larvae at weaning range 1-2% to 30-40% (Ma *et al*., 2013; Roo *et al*., 2014; Stuart and Drawbridge, 2011). In an attempt to gain information on the role of taurine and other amino acids in broodstock maturation and gametogenesis, historical samples of fertilized eggs were analyzed for crude protein and amino acid profile. Results were then correlated with husbandry results such as spawn size, fertilization rate, hatching rate, and larval survival at weaning.

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4.1.1 Broodstock collection and holding

Broodstock were collected in 2003 and 2004 off San Diego and Santa Catalina Island, CA. The fish were captured with hook and line and transported by boat to a 555 m^3 net pen at Santa Catalina Island, CA, or directly to the Hubbs Sea World Research Institute (HSWRI) by live haul truck. The fish that were brought directly to HSWRI were introduced to the maturation pool in the winter of 2004, prior to the spring spawning season. All fish were weighed and individually PIT tagged (AVID, Norco, CA) after capture. The fish held at Santa Catalina Island were introduced to the maturation pool in 2008 (Stuart and Drawbridge 2013).

The broodstock were held in a 140 m^3 fiberglass pool (9.1 m diameter x 2.4 m deep) and exposed to shaded natural light and ambient seawater temperatures of 12 to 23°C. The seawater was recirculated at a rate of 1,135 L min^{-1} using an airlift-driven bead filter (0.7 m^3 PolyGeyser Bead Filter, Aquaculture System Technologies, New Orleans, LA). The bead filter performed the critical processes of solids capture and biofiltration. Water supplied to the pool by the airlift flowed by gravity from the top of the pool and a central bottom drain into an egg collector, so that all eggs were collected during the study period. The egg collector measured 1.27 m x 1.14 m x 0.64 m and contained a 500 µm mesh bag to trap the eggs before the water returned to the filter. Makeup water drawn from Mission Bay was sand-filtered and sterilized with ultraviolet light before being supplied to the pool at a rate of 5 – 20 L min^{-1}. Pure oxygen was supplemented as needed to maintain oxygen levels above 7 mg L^{-1} (90 – 100% saturation) during the warm summer months.

As described in Stuart and Drawbridge (2013), the broodstock diet consisted primarily of frozen sardines and squid that were thawed and injected with vitamins. Mackerel and anchovies were used occasionally to further supplement and vary the diet.

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The vitamin pack consisted of a custom premix (1.5% of the total diet), thiamin (0.02% of the total diet), vitamin C (0.5% of the total diet; ROVIMIX® Stay-C 35, DSM Nutritional Products, Basel, CH), lecithin (2.0% of the total diet), fish oil (3.3% of the total diet), and AlgaMac-3050 (10.0% of the total diet; used only during the spawning season; Aqua fauna, Hawthorne, CA). For all years the broodstock were provided a winter (non-spawning) and summer (spawning) ration. The winter ration was generally consistent at 4% body weight $week^{-1}$ based on near satiation feeding during the colder seasons. The summer ration was varied from 6 to 15% during the four year period and was subsequently evaluated relative to the performance of the broodstock.

4.1.2 Egg Collection and Incubation

The egg collector was checked daily at 0800 hours, and any eggs found were collected with a fine mesh aquarium net. Eggs were then placed into an 8.0 L container with aeration prior to volumetric estimation of spawn size using 1.0 L graduated cylinders. Eggs were poured into cylinders, and floating and sinking eggs were allowed to separate for 5 to 10 minutes; only the floating, fertilized eggs were considered viable and used for culture. Percent fertilization was calculated for each spawn as the ratio of the floating versus total eggs produced per spawn multiplied by 100. Following separation viable eggs were disinfected with 100 mg L^{-1} of formalin for one hour prior to stocking.

Eggs were stocked into 1600 L cone-bottom, fiberglass “incubator” tanks for culture. Flow rates were maintained at 3 – 6 turnovers day^{-1}, depending on the developmental stage of the larvae, and mild aeration was provided with air-stones and bubble-ring diffusers. Controlled lighting installed above the incubator tanks provided an illumination of 7,000 – 13,000 lux at the surface. For each spawn, five subsamples of 100 eggs were taken to estimate hatching rates. Subsequently, five subsamples of ten hatched larvae were taken to estimate survival to first feeding.

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4.1.3 Statistical analysis

Direct analysis of the results using multiple regression analysis was not possible due to high degree of collinearity between the levels of amino acids. Consequently a principal component analysis was performed to generate orthogonal variables. Initial variables included spawning year, all amino, crude protein and crude lipid contents of fertilized eggs. Resulting principal components were then regressed against fertilization rate, hatching rate and survival at first feeding. Regressions were significant at $P<0.05$. Quadratic regression was evaluated and deemed significant when the 2^{nd} degree parameter of the polynomial equation was significantly different from zero ($P<0.05$).

4.2 Results and discussion

The first three principal components, explaining 92.18% of the data variability were kept for subsequent analysis. Component patterns are illustrated by the vector plots in

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. Most of the amino acids and crude protein content of the egg heavily loaded on the 1st component, while hydroxylysine and ornithine loaded mostly on component 2. Spawning year loaded mostly on component 3, and egg crude lipid loaded almost equally on component 1 and 3.

Regression analysis on the components reveals that hatching rate is quadratically correlated with component 1 (Figure 3).This suggests that hatching rate is correlated with the egg protein content, and that hatching rates tend to increase quickly before stabilizing as egg protein content increases. Similar correlation has been found in angelfish *Pterophyllum scalare* (Shelar *et al*., 2014), where adults fed 52% dietary protein produced eggs with higher protein content and had improved reproductive performances, including relative fecundity rate, fertilization rate, and hatching rate. Conversely, no such relationship was found in turbot *Psetta maxima*: no correlations were found between the composition of the eggs and fertilization or hatching rates (Jia *et al*., 2014). Although best performances were observed in the middle of the reproductive season where content in some essential and non-essential amino acids was increased, the performance parameters were correlated with levels of specific fatty acids rather than amino acids. It remains unclear whether the discrepancy among these two species and California yellowtail may be explained by their different spawning strategy (pelagic vs. substrate spawners), environmental conditions (e.g., water temperature), or other factors.

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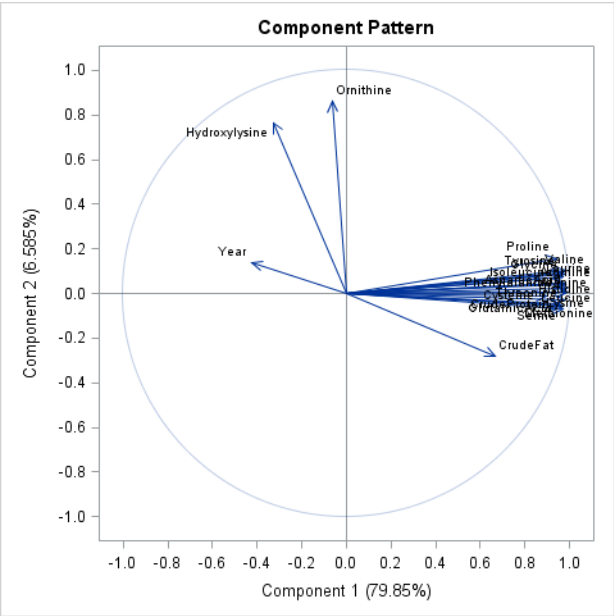

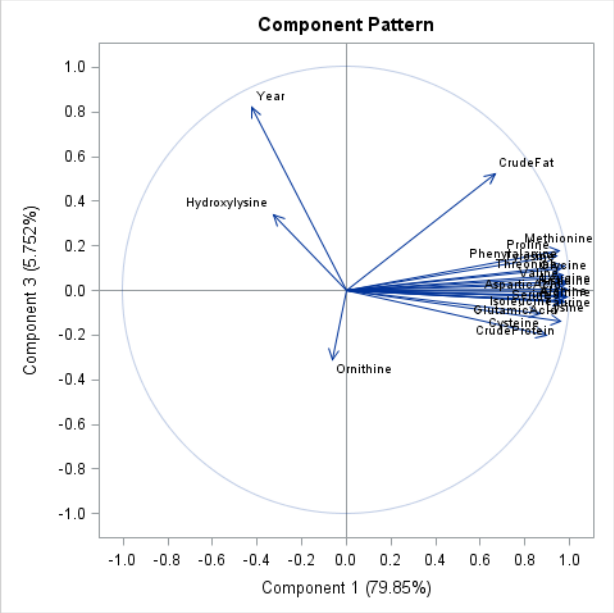

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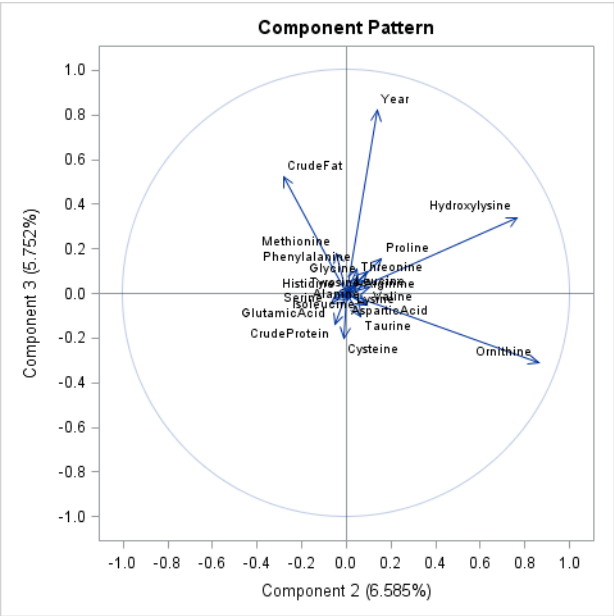

Figure 2: Vector plots illustrating the variable loadings onto the first three principal components

The fertilization rate was negatively correlated with component 3 (Figure 5), on which egg crude lipid content partly loaded together with the spawning year. Therefore this result may reflect genetic variability and/or aging of the broodstock as spawning pairs differed and aged over the years, negatively affecting egg fertilization.

Finally, survival at first feeding is negatively correlated with component 2 (Figure 4), where hydroxylysine and ornithine mostly loaded. The former is an important constituent of collagen, while the latter is used during urea production. It is known that amino acids are particularly used as metabolic fuel during embryogenesis and hatching (Cruzado *et al*., 2013; Moran *et al*., 2007), and this may point to suboptimal protein metabolism and nitrogen waste toxicity. Teleosts excrete nitrogen mostly in the form of ammonia (80 to 85%) while the remainder is excreted as urea. This is in contrast with terrestrial animals, which must convert ammonia to urea before it can be excreted; indeed

urea is far less toxic and can be temporarily stored in the urinary bladder. Because fish live in water, ammonia can be continuously excreted through the gills, thereby negating for the most part the need and associated energetic cost of conversion to urea. Nevertheless, the genes coding for the enzymes of the urea cycle are present in most but not all teleost species, though their activities remain barely detectable in juvenile and adult fish while being distinctively high during the first few days after hatching (Chadwick and Wright, 1999; Wright *et al*., 1995). The metabolism of yolk nutrient reserves leads to a significant production of cytotoxic ammonia, which must be disposed of. The egg chorion surrounding the embryo is characterized by a relatively low permeability; while ammonia slightly permeates, urea transport requires a specific carrier protein (Levine *et al*., 1973). Results in embryo and larvae of rainbow trout and Atlantic cod indicate that in spite of a detectable ammonia excretion, levels of both ammonia and urea still increase inside the developing egg. Trout eggs lack the urea specific transporter, and urea is excreted only after hatching (Wright *et al*., 1995). Although the urea concentration gradient between the animal and the water is highest at hatching, rates of excretion are low and sharply increase as the gills develop. Taken together, this suggests the urea cycle is a temporary but critical mechanism of ammonia detoxification during the early stages of life in teleosts. Therefore, the negative relationship between survival at 1st feeding and ornithine levels suggests a failure of this detoxification system, leading to increased mortality.

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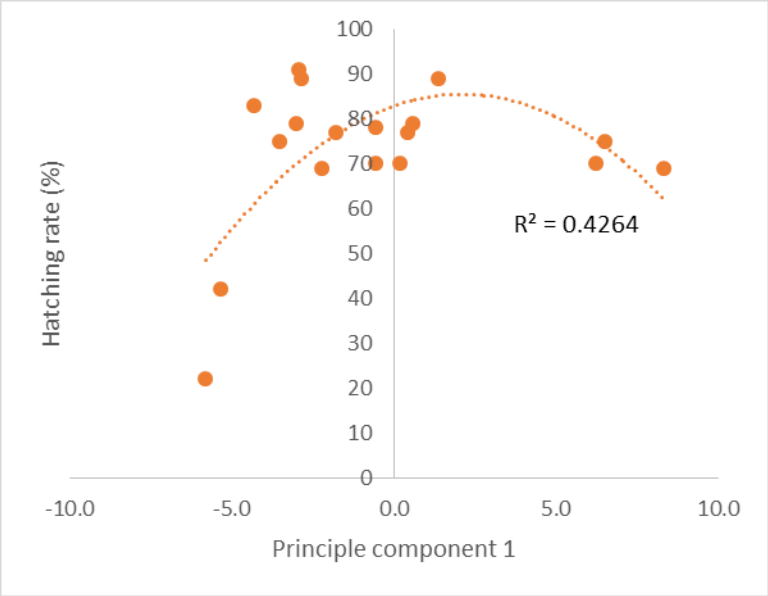

Figure 3: Regression of hatching rate in California yellowtail oocyte with principal component 1

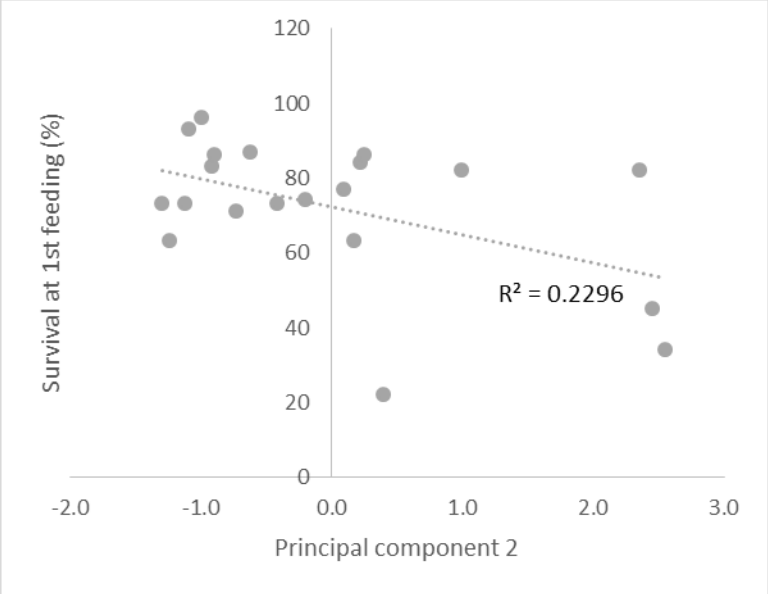

Figure 4: Regression of survival at 1st feeding in California yellowtail larvae with principal component 2

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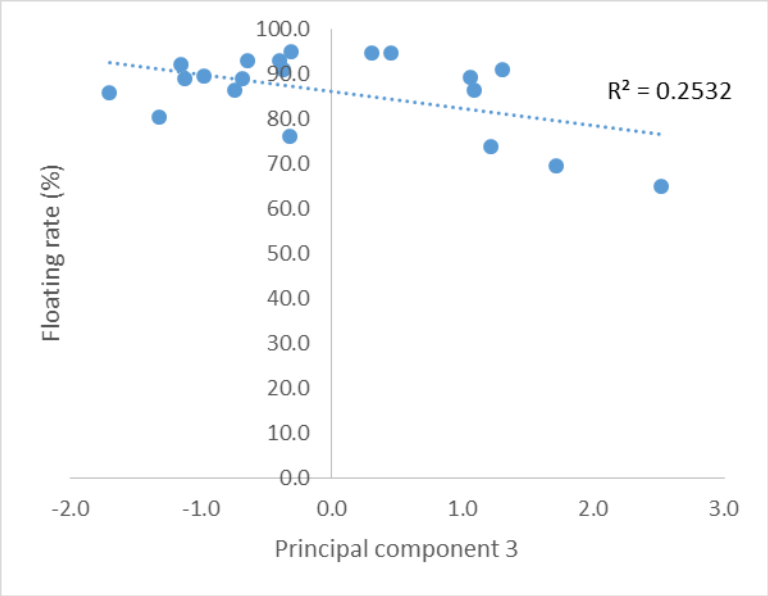

Figure 5: Regression of fertilization rate in California yellowtail oocytes with principal component 3

5 Florida pompano

5.1 Introduction and methods

Currently there is no commercial aquaculture production of Florida pompano. Some reports suggest that farms based in the Dominican Republic and Panama have now ceased their activities. Several research and pilot-scale operations are found in several countries including the United States; however, all commercial production is achieved through fisheries harvesting, which totaled 350 tonnes globally in 2013 (FAO, 2015). Nevertheless, Florida pompano combines highly desirable traits that makes it an excellent candidate for commercial operations, and has been widely recognized as such for years (Lazo *et al*., 1998; Main *et al*., 2007).

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5.1.1 System design and broodstock maintenance

Pompano broodstock were maintained in 25m^3 tanks (4.57 m diameter) equipped with an egg collector with a 500µm mesh bag, mechanical and biological filtration, a protein skimmer, and two 150 W UV units. Water quality was monitored daily, including dissolved oxygen (DO), salinity (ppt), pH, and temperature (°C). Optimal conditions for each of these variables were as follows: DO: 4.0 - 9.0 mg/l, Salinity: 35.0 ± 1 ppt, pH: 7.5 - 8.5, and Temperature: 27 ± 1 °C. Water chemistry was monitored weekly. Measurements taken were total ammonia nitrogen (TAN) maintained at <0.5ppm, nitrite nitrogen (NO_2-N) at < 1.0ppm, and nitrate nitrogen (NO_3-N) at <50ppm.

5.1.2 Maintenance and experimental feeds

The pompano broodstock population was composed of wild-caught and F1 fish. All were individually implanted with a PIT tag. Initially all fish were maintained on a diet of thread herring (40%), shrimp (30%), and squid (30%), and were fed close to satiation (4-5% of the tank biomass) daily, adjusting as necessary. Because of their high metabolisms and small guts, the fish were fed this amount over three daily feedings. Three weeks prior to spawning, experimental feeds were introduced and fed according to the same protocol as the maintenance regimen. Experimental diets were based on a blend of seafood (same proportions as maintenance diet) and gelatin, supplemented or not with taurine (

Table 1). The formulation was designed to approach the maintenance diet without compromising feed intake or physical characteristics of the pellets. Pellets were prepared by coarsely dicing the seafood and mixing with the rest of the ingredients. Gelatin was dissolved in warm water and added to the ingredient mix. The preparation was then poured into a large, shallow container and left to cool in the refrigerator before being cut into bite-

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size cubes. Diets were kept at 4°C until fed to the fish. These diets were well accepted by the fish, and fed for three weeks prior to spawning.

Table 1: Formulation and basic composition of the experimental diets

		Maintenance	Control	Taurine
Formulation (% as-is)				
Herring		40.00	19.20	19.16
Shrimp		30.00	14.40	14.37
Squid		30.00	14.40	14.37
Gelatin		-	18.00	17.96
water		-	33.12	33.05
fish oil		-	0.88	0.88
Taurine		-	0.00	0.20
Composition (calculated)				
Dry matter	(%)	19.72	27.3	27.2
Crude protein	(%, dry)	73.6	84.3	84.3
Crude lipid	(%, dry)	8.1	6.0	6.0
Taurine	(%, dry)	0.88	0.31	1.04

5.1.3 Maturation and spawning protocols

The maturation was controlled by manipulating photoperiod and water temperature in order to emulate Spring conditions in West coast Florida (13hr light:11hr dark, 27 ± 1.0°C). For resting we used winter conditions (11.5hr light: 12.5 dark, 22°C).

Spawning was stimulated by hormonal implants. To easily sample the fish and minimize stress, the tank volume was reduced by two thirds and the fish were corralled in a small area of the tank. The pompano were then removed one fish at a time, and placed in an anesthetic tank with 300ppm of buffered tricaine methanesulfonate (MS-222). When the

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fish were anesthetized, they were identified with their PIT tag, weighed, and measured for fork length. Males were sampled by applying light pressure to each side of the abdomen: assessments were made by recording if milt was expressed, and if it was, if the fish had a mild or heavy flow. Females were cannulated using an 8fr premature infant feeding tube mounted on a 10ml syringe. A few oocytes were removed and immediately placed on a slide with a small amount of salt water to assess their maturation stage using light microscopy. Females were considered mature when their oocytes were staged at or beyond the secondary growth final growth (SGfg) stage of oocyte maturation. Mature females were then implanted with ≥50µg/kg of Ovaplant® (sGnRHa). Since Ovaplant is only available in doses of 75 or 150µg, females weighing less than 1500, 3000, or more than 3000g were implanted with 75, 150, or 225µg, respectively. The fish were then revived and placed back in the main portion of the tank.

5.1.4 Egg collection

After receiving the hormonal implant, the fish were allowed to spawn volitionally. The egg collector was monitored by checking the bag and the tank for eggs every 2 hours, in order to minimize disturbance of the spawning behavior. Once eggs were found, water samples were taken every hour to ensure that the totality of the spawn has been collected. The 500µm bag was then emptied into a hatching cone filled to a known volume of aerated seawater. When the eggs reached the blastula stage (~6hr post-fertilization), three, 10ml aliquots were taken to determine the total number of eggs and fertilization rate. The aeration was then turned off to allow the settling and removal of unfertilized eggs, and floating viable eggs were cleaned on a 500µm sieve with clean salty water prior to being placed in a hatcher. Hatching occurred between 24-26hr post-fertilization, at which point the larvae were counted to determine the hatching rate. Samples of eggs and newly hatched

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larvae were taken, de-watered, and frozen at -80^{o}C pending dry matter, crude protein, and amino acid analyses.

5.1.5 Calculations and statistical analysis

The amount of sample was insufficient to measure lipid content of eggs and larvae. Consequently, the amount of lipid was estimated by assuming that both eggs and larvae contained negligible amounts of carbohydrates. Then lipids may be calculated by subtracting the moisture and protein content from 100%. Composition data of the egg and larvae were analyzed by 2-way ANOVA with developmental stage (egg or larvae) and treatment (control or taurine) as main effects. Results were considered significant when $P<0.05$.

5.2 Results and discussion

One spawn was obtained in each dietary treatment group: the absolute number of fertilized egg were close between the two treatments; however the taurine group spawned fewer eggs, leading to a much increased fertilization rate (Table 2).

Table 2: Spawn results from Florida pompano fed a control or taurine-supplemented diet for 3 weeks

	Taurine	**Control**
Total eggs collected	102,000	299,200
Total Fertilized Eggs	54,400	45,900
Percent Fertilization	53.5%	15.4%

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Table 3: Taurine and proximate composition (% as-is) of Florida pompano eggs and larvae from broodstock fed a control or taurine-supplemented diet for 3 weeks

	Taurine (% as-is)	**Crude Protein (% as-is)**	**Moisture (% as-is)**	**Lipid (calculated, % as-is)**	**Crude protein/Lipid**
Egg					
Control	0.05±0.01	5.02±0.71	92.33±1.15	2.65±0.45	1.91±0.12
Tau	0.07±0.01	6.85±1.18	89.95±1.82	3.20±0.66	2.15±0.12
Larvae					
Control	0.12±0.04	8.18±2.36	85.82±4.28	6.00±1.92	1.37±0.06
Tau	0.21±0.03	14.55±1.15	79.52±2.39	5.93±1.55	2.56±0.67
P-values (2-way ANOVA)					
Developmental stage	<0.0001	<0.0001	<0.0001	0.0008	0.7162
Treatment	0.0017	0.0005	0.0122	0.6637	0.0017
Interaction	0.0452	0.0132	0.1759	0.6392	0.0199

Value are averages ± SD with n=4 for egg samples, n=3 for larvae samples. Lipid content = 100-moisture-crude protein.

Proximate composition of the eggs and larvae are shown in

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Table 3. There was a significant increase in taurine content in both eggs and larvae, and the significant interaction indicates that the difference between treatments was greater in larvae. This shows that three weeks of feeding the broodstock with the experimental diets was sufficient to impact the composition of the egg and newly hatched larvae. Such rapid deposition of free amino acids in the eggs has been observed in red snapper *Lutjanus campechanus* broodstock injected with supplemental amino acids along with HCG injection to stimulate spawning (Hastey *et al*., 2015). Additionally, and although the pompano experimental diets were isonitrogenous and isolipidic, the taurine supplementation caused a significant increase in crude protein in both eggs and larvae at the expense of moisture. However, there was no change in total lipid content in response to taurine supplementation. This contrasts with the aforementioned red snapper study, where the oil globule diameter was significantly larger in amino acid-injected fish than in sham-injected fish (Hastey *et al*., 2015). The relative changes in crude protein and lipid contents result in a crude protein/lipid ratio to significantly decrease from egg to larvae in the control group whereas it remains relatively stable in the taurine-supplemented group. This suggests that in Florida pompano broodstock, dietary taurine signals for an increase in protein deposition and nutrient density in the oocytes, as well as different nutrient utilization as was also seen in red snapper (Hastey *et al*., 2015).

Unfortunately correlation with egg quality parameters was not possible due to the limited number of spawns and the very low fertilization rate in the control group. The trade-off between number of eggs produced and fertilization rates resulted in a similar number of fertilized eggs produced by each group. However, if the taurine-supplemented eggs and larvae are more nutrient-dense, it is reasonable to hypothesize that these stand better chances of surviving throughout the larval development stages than their control counterpart. This hypothesis is currently begin tested as the experiment is repeated with the same diets, and the resulted larvae will be cultured until completion of metamorphosis and weaning onto a dry larval diet.

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