2016-12-09

Characterizing Egg Quality and Larval Performance from Captive Mahi-mahi (Coryphaena hippurus) Spawns Over Time

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CHARACTERIZING EGG QUALITY AND LARVAL PERFORMANCE FROM
CAPTIVE MAHI-MAHI (*Coryphaena hippurus*) SPAWNS OVER TIME

By

Steven J. Kloeblen

A THESIS

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Master of Science

Coral Gables, Florida

December 2016

UNIVERSITY OF MIAMI

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Steven J. Kloeblen

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(December 2016)

<u>Characterizing Egg Quality and Larval Performance</u>
<u>from Captive Mahi-mahi (*Coryphaena hippurus*)</u>
<u>Spawns Over Time</u>

Abstract of a thesis at the University of Miami.

Thesis supervised by Professor Daniel D. Benetti.
No. of pages in text. (38)

Mahi-mahi *Coryphaena hippurus*, a high performance pelagic species, is a promising species for aquaculture development and has been used as a model species for oil toxicology and physiology studies. This species has one of the fastest growth rates of any marine teleost and a unique reproductive biology due to the species high spawning frequency and energy allocation into spawning events. These characteristics lend the species to being an excellent model for understanding broodstock nutrition for other high energetic pelagic species in captivity. The reproductive performance of wild-caught mahi-mahi was investigated in this study. Egg morphometries and larval survival were tracked in captivity over time for a 10-week period from the initial capture of the broodstock. Larval quality from subsequent spawns collected over time was quantified using larval survival activity indices (SAIs) which is a metric to assess egg quality. Larval SAIs were maintained and did not significantly decrease ($p < 0.05$) over the time course of this study. A multiple linear regression model based on the elapsed time in captivity of the broodstock, egg diameter, larval SAI at1 dph provided the most accurate prediction of larval SAI at 3 dph ($R^2 = 0.996$ $p < 0.05$) when the larvae began exogeneous feeding. There were strong positive correlations with larval SAIs and the key nutrients:

eicosapentaenoic acid (C20: $5n - 3$ EPA) and related fatty acid ratios, vitamin E, and nearly all amino acids under investigation with the exception of tryptophan, valine, and cystine. This study suggests that larval survival was maintained over time due to the supply of these key nutrients in the broodstock diet. Results indicate selective incorporation of maternally derived vitamin A and amino acids into the eggs over the time course of this study.

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Chapter 1: Introduction

As marine finfish aquaculture continues to expand with the culture of novel

species, one of the major impediments to production is the consistent year-round supply

of high quality, viable eggs of these species. One potential factor limiting this reliable

egg production is improper broodstock nutrition. Improper broodstock nutrition can lead

to poor and infrequent spawns, reproductive dysfunction of broodstock in captivity, and

overall poor egg quality. (Brooks *et al*., 1997; Hauville *et al*., 2015; Lund *et al*., 2007;

Rainuzzo *et al*., 1997; Tocher, 2003). Good egg quality is strongly influenced by the

maternal broodstock diet which is imparted into the developing oocytes through the

process of vitellogenesis (Brooks *et al*., 1997; Tocher, 2003). The diet of the broodstock

is directly reflected in the nutritional composition of the eggs and yolk sac larvae.

Dietary lipids especially *n* -3 series polyunsaturated fatty acids (PUFAs) in the

broodstock diet are crucial to this high quality production of eggs and are one of the

strongest indicators of reproductive performance (Izquierdo *et al*., 2001; Rainuzzo *et al*.,

1997). Essential PUFAs such as docosahexaenoic acid C24:6n-3 (DHA),

eicosapentaenoic acid (EPA) C20:5n-3, and arachidonic acid C20:4n-6 (AA) are crucial

for correct larval ontology and differentiation of undeveloped systems in the larvae (Bell

et al., 1997; Brooks *et al*., 1997; Furuita *et al*., 2003; Izquierdo *et al*., 2001; Lavens *et al*.,

1999; Lund *et al*., 2007; Tocher, 2010; Wiegand, 1996).

Additionally, maternally derived vitamins play a key role in many different

functions in embryonic and larval development. One of the primary roles that vitamins

perform in these important developmental stages are as antioxidants. These antioxidant

vitamins like vitamin A, E, and C protect PUFAs from lipid peroxidation by reactive

oxygen species (ROS) that are volatile and oxidative byproducts of aerobic respiration produced during high periods of metabolic activity that occur in larval development (Hamre, 2011; Palace and Werner, 2006).

In assessing egg quality in any marine broodstock, comparing eggs and related tissues from wild and captive broodstock can identify any nutritional deficiencies that may be in the diet that could be implemented into ameliorating limitations in egg production (Hauville *et al.*, 2015; Migaud *et al.*, 2013). This has been well demonstrated in previous literature with Atlantic cod *Gadus morhua* (Kjørsvik, 1994), striped bass *Morone saxatilis* (Harrell and Woods, 1995) white seabream *Diplodus sargus* (Cejas *et al.*, 2004, 2003), striped trumpeter *Latris lineata* (Morehead *et al.*, 2001), common sole *solea solea* (Lund *et al.*, 2008), Senegalese sole *Solea senegalensis* (Norambuena *et al.*, 2012), and common snook *Centropomus undecimalis* (Hauville *et al.*, 2015) yet the literature is very limited on identifying these deficiencies in high performance pelagic species with notable exceptions with studies on Atlantic bonito *Sarda sarda* (Ortega and Mourente, 2010), Atlantic bluefin tuna *Thunnus thynnus* (Ortega and Mourente, 2010; Pousis et al., 2011) lesser amberjack *Seriola rivoliana* (Saito, 2012), and greater amberjack *Seriola dumerili* (Rodríguez-Barreto et al., 2012; Saito, 2012).

The increased swimming performance and energetics of pelagic species has allowed for the efficient delivery of oxygen and metabolic substrates to the tissues at high rates which has permitted rapid gonadal growth and unmatched egg production in these species (Brill, 1996). Mahi-mahi *Coryphaena hippurus*, an epipelagic circum-tropical species with both sport fishing and commercial importance (Palko *et al.*, 1982), provides an interesting study into assessing egg quality for high energetic species for multiple

reasons. Mahi-mahi are a highly iteroparous fish that become sexually mature at 41.9 cm (fork length (FL)) at 4-5 months post hatch and can spawn 70-180 d yr^{-1} in the wild producing a batch fecundity ranging from 20,000-620,000 eggs spawn^{-1} (McBride *et al.*, 2012). In addition to the high frequency of spawning and fecundity, mahi-mahi have the one of fastest growth rates of any fish species with a specific growth rate (SGR) of a sexual mature fish at 1.3 % day^{-1} (Stieglitz *et al.*, 2016a *in review*).   The energy allocation to each spawning event is extraordinary with a captive female donating 5 % of its bodyweight spawn^{-1} (Kraul, 1989).   With this very high allocation of energy into each spawning event, the transfer of maternal dietary nutrients into the eggs would therefore be assumed to be instantaneous for an income spawner like mahi-mahi but remains unknown. It has been shown in the lower energetic sub-topical species red drum *Sciaenops ocellatus* that dietary shifts in the maternal diet were reflected rapidly in the nutritional composition of the eggs at 2-16 days post dietary shift (Fuiman and Faulk, 2013; McBride et al., 2015).   In assessing the egg quality and subsequent larval performance for other high energetic and commercial important pelagic species, mahi-mahi are an excellent model species due to the species unique reproductive biology and high nutritional requirements needed for consistent egg production.

Though technology for domestication of mahi-mahi first began in 1980s (Hagood et al., 1981; Kraul, 1989; Szyper et al., 1984), it remains to this date not a commercially viable species for culture. At the University of Miami Experimental Hatchery (UMEH), mahi-mahi have been cultured to be used as a successful model species for physiology and environmental toxicology studies to understand the effects of open ocean oil spills on species in the pelagic environment (Alloy *et al.*, 2016; Edmunds *et al.*, 2015; Esbaugh *et*

al., 2016; Mager *et al*., 2014; Stieglitz *et al*., 2016b; Xu *et al*., 2016). The reliance of high quality eggs from year-round volitional spawns of wild-caught mahi-mahi for these studies is essential. Understanding and tracking the egg quality of this species in captivity in comparison to its wild conspecifics gives insight into understanding examining reproductive performance of this species in captivity. The examination of egg quality in mahi-mahi will also help to implement broodstock management strategies in order to maintain optimal reproductive performance of captive mahi-mahi.

The aim of this study was to characterize the larval survival from spawns of wild-caught mahi-mahi over time to assess performance of the broodstock in captivity.

Chapter 2: Materials and Methods

Wild broodstock used in this study were caught in the Straits of Florida off the coast of Miami in July, 2016. Methods for this study on the capture, transport, acclimation, continuous spawning of captive mahi-mahi broodstock are described in further detail in Stieglitz *et al.* (2016a) *in review*.

The first spawn of this study was collected 1-day post capture which is typical for mahi-mahi. This spawn was result of prior stress and handling from capture causing corticosteroids to act on the hypothalamic-pituitary-gonadal axis which triggers ovulation and spawning in fish (Billard et al., 1981). This spawn was collected using a 500 μm mesh standpipe in the quarantine tank. Subsequent fertilized spawns were non-invasively collected in the egg collector of the maturation tank being supplied by surface skimmed water. The egg collector was supplied by light aeration with an aeration ring around 500 μm mesh standpipe to keep the eggs in suspension. Spawning occurred volitionally at a tank temperature held at 26.5 °C on a natural photoperiod and females spawned naturally every other day with multiple females spawning asynchronically on opposing days and the male being able to spawn daily on consecutive days. Eggs were collected 2-8 hours post fertilization and the stage of development and quality of eggs were determined using Motic SMZ- 186 series stereo zoom microscope. For each spawn collected for analysis, a subsample of eggs was collected from the maturation tank egg collector and transferred into a pre-oxygenated 5-gallon bucket that had UV treated seawater at the same temperature as the maturation tank temperature. Eggs were given a prophylactic 100 ppm formalin (Formacide- B, 37 % formaldehyde solution) for 1 hour with 300 % rinse using 0.35 μm filtered UV treated seawater for 30 minutes (Mager et al., 2014; Stieglitz

et al., 2012). Treated eggs were stocked in a replicated PELEC system filled with 0.35

µm filtered UV treated seawater. The design and functioning of PELEC system is

described in further detail in (Stieglitz *et al.*, 2016c). For each replicated tube in the

PELEC system (n = 4), 40 eggs were stocked in 1.8 L PELEC cone system using a large-

bore Pasteur pipette. Environmental conditions for the PELEC systems were held at 26.5

° C with a 16:8 photoperiod light dark. Post hatch, daily mortality was measured in each

tube until the all larvae had died out due to starvation. From the daily mortality, a larval

survival activity index (SAI) (Shimma and Tsujigado, 1981) was implemented, using the

equation SAI $= \frac{1}{N}\sum_{i=1}^{k}(N - h_i) \times i$ where N equals the total number of larvae supplied,

h_i is the cumulative mortality by the *i*th day, and *k* is the number of days elapsed until all

the larvae have died from starvation (Furuita *et al.*, 2000). In this study, SAI metric was

performed for larval survival at 1 day post hatch (dph) and 3 dph. At 3 dph, the yolk-sac

is consumed and mahi-mahi larvae are competent to begin exogeneous feeding

(Ostrowski and Divakaran, 1991). Water parameters (temperature, dissolved oxygen,

salinity, pH) were measured initially and at the end of each replicated trial. Total

ammonia (NH_4^+) using the indophenol blue method described by (Ivančič and Degobbis,

1984) at the end of each replicated trial. In addition to the subsample of eggs used in

PELEC system, a sample of eggs (n = 90) were collected and the egg diameters and egg

oil globule diameters measured using a Leica CME microscope equipped with an ocular

micrometer at 40 X magnification. Lastly, for each spawn collected a sample of eggs

was strained to remove excess water and placed into Whirl-Pak® 532 mL bag (eNasco).

The sample of eggs was flash frozen in liquid nitrogen. Samples were stored in – 80 ° C

freezer for future nutritional analysis. Spawns were collected twice week based on the

availability of broodstock spawning for a 10-week period from when the broodstock were initially caught.

Additionally, the nutritional composition of gonads from both wild caught female mahi-mahi (WG) (n =9) and captive female broodstock (CG) (n =4) were analyzed. Gonads were pooled together respectively and placed Whirl-Pak® 532 mL bags (eNasco). Samples were flash frozen in liquid nitrogen and were stored in – 80 ° C freezer for future analysis.

Nutritional analysis was performed by Eurofins Scientific, Inc. Nutritional Analysis Center (Des Moines, Iowa, US). For each egg (n = 4) and gonad sample (n = 2) analyzed, a complete fatty acid profile, phospholipid profile, vitamin E, A, and C, and amino acid profile were performed.

For the nutritional analysis, a complete fatty acid analysis was performed on each sample using gas liquid chromatography described in Metcalfe and Schmitz (1961).

A phospholipid profile for phosphatic acid (PA), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), 3-sn-lysophosphatidylcholine (LPC), and phosphatidylserine (PS) was performed. These phospholipids were analyzed using high performance liquid chromatography (HPLC) further described in Patton *et al.* (1982).

A total vitamin E analysis was performed by HPLC and fluorometric detection described in Desai (1984). Vitamin A and provitamin A carotenoids analyses were performed each sample by using saponification of the samples followed HPLC with fluorometric and UV detection described in Furr (2004). Vitamin C (ascorbic acid) was analyzed for each sample by oxidizing ascorbic acid with activated charcoal and reacting

the oxidative form with o-phenylenediamine and measure fluorescence intensity as described in detail in Wu *et al*. (2003).

Lastly for each sample analyzed, a complete amino acid profile was performed. The amino acid tryptophan was analyzed using alkaline hydrolysis and reverse phase liquid chromatography. Methods are further described in detail in Spies (1967). Cystine and methionine were analyzed whether the amino acids were bound or free form and were quantitated by the performic acid oxidation-hydrolysis-OPA procedure described in Lee and Drescher (1979). The remaining 16 amino acids were analyzed using cation-exchange liquid chromatography described in Kaiser *et al*. (1974).

Statistical analysis was performed using IBM® SPSS® Statistics Version 22. Empirical data for the egg morphometries were analyzed using one-way Welch ANOVA followed by a Games-Howell post hoc test with 95 % confidence. Empirical data for survival activity indices was analyzed using one-way ANOVA followed by Tukey's post hoc test with 95 % confidence. Multiple linear regression was performed on all empirical data. All results were presented as means ± SEM. Any nutritional changes in the egg composition were correlated using Pearson's moment correlations with empirical data (*p* < 0.05).

Chapter 3: Results

3.1 Water Parameters

The Water chemistry parameters remained consistent throughout the study (see table 1) with increase in salinity from the initial PELEC reading of 32.93 ± 0.28 ppt to a final PELEC reading of 32.37 ± 0.29. The final PELEC total ammonia (NH_4^+) was 0.03 ± 0.02 mg L^{-1}.

3.2 Empirical Results

(3.2a) Egg Morphometries

Egg diameters and egg oil globule diameters for spawns over time are presented in table 2. Egg diameter and egg oil globule diameter were significantly different ($p < 0.05$) over time, respectively. Both egg diameters and egg oil globule diameters had the lowest values for days 1, 19, 23 (1236.51 ± 3.35 µm, 1357.10 ± 3.24 µm, and 1371.79 ± 3.71 µm and 256.91 ± 1.32, 274.12 ± 1.96 µm, and 266.11 ± 1.89 µm, respectively). Egg diameter was the highest on day 39 at a value of 1594.88 ± 5.95 µm and egg oil globule diameter was the highest on day 47 with a value of 307.05 ± 1.66 µm.

(3.2b) Larval Survival Activity Indices

The larval SAIs based on larval survival at 1 dph and 3 dph were both significantly different at 1 day from all other SAIs time periods ($p < 0.05$) but no later time periods in the time course of this study were significantly different from each other (see Fig. 2). A multiple linear regression was performed on the empirical data gathered in this study. In this multiple linear regression model, the independent variables of elapsed time in captivity, egg diameter, larval SAI at 1 dph significantly predicted larval SAI at 3 dph ($R^2 = 0.996$, $p < 0.05$). The independent variable egg oil globule diameter

over time course of this study did not significantly added to the prediction of larval SAI at 3 dph for this regression model. Regressions coefficients and standard errors for this linear regression model can be found in Table 2.

3.3 Gonad Analysis

In comparing the two pooled gonad samples: gonads of wild caught mahi-mahi females (WG) (n = 9) and gonads from captive broodstock (CG) (n = 4), there were suggestive differences between the two pooled samples though not significant different. Results of the vitamin composition of pooled gonads are represented in Table 4. In assessing the compositional differences between several vitamins, there was a higher level of retinol in the CG in comparison to WG with 397 IU 100 g^{-1} and 174 IU 100 g^{-1}, respectively. There were higher levels in α- tocopherol in the CG with an α-tocopherol level of 2.91 mg 100 g^{-1} while the WG had 1.63 mg 100 g^{-1}. Conversely, there was 6.27 mg 100 g^{-1} per sample of ascorbic acid in the CG while the WG had 14.5 mg 100 g^{-1}. The composition of fatty acids in the WG and CG are represented in Table 5. In comparing the composition of the two pooled gonads, there was lower levels of DHA (0.80 % of sample) in the CG compared to the WG (0.94 % of sample). In addition, there were lower levels of AA in the CG (0.07 % of sample) compared to the WG (0.13 % of sample) while there were higher levels of EPA in the CG (0.32 % of sample) compared to the WG (0.14 % of sample). Due to this, the ratio of EPA/AA in the CG was higher than the WG and DHA/EPA was lower in the CG than the WG. There were higher levels of total saturated fats in the WG (1.25 % of sample) while the levels of saturated fats in the CG was 0.84 % of sample. There were also lower levels of PUFAs in the CG compared to the WG but higher levels of MUFAs in the CG. There were no differences between

the phospholipid composition of the WG and CG for phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylinositol, and lyso-phosphatidylcholine. There were higher levels of phosphatic acid in the WG mahi-mahi (< 8000 ppm) than the CG (< 5000 ppm). The composition of the amino acids in the gonads of the WG and CG in Table 6. There were no suggestive differences for any amino acids between the two gonad compositions.

3.4 Nutritional Composition of Eggs Over Time

Pearson product-moment correlations were performed on different nutrients to larval SAIs over time to assess any correlation. Results for fatty acid correlations, vitamin E, and amino acid correlations to SAIs over time at 1 and 3 dph represented in Fig. 4-6 and Table 7. In correlating these nutrients with SAIs over time, there were statistically significant positive correlations between SAIs over time for larval survival at 1 and 3 dph and EPA concentrations in the eggs, $r(14) = 0.964$ and $r(14) = 0.919$ ($p < 0.05$), respectively (see figure 3). Consequently, there was also a statistically significant positive correlation between SAIs at 1 and 3 dph and the EPA/AA relative ratios over the time course study with coefficients of $r(14) = 0.898$ and $r(14) = 0.872$ ($p < 0.05$) for SAIs at 1 dph and 3 dph, respectively (see figure 4). There was strong negative correlation between SAIs over time and DHA/EPA concentration in the eggs $r(14) = -0.996$ and $r(14) = -0.980$ ($p < 0.05$) for larval SAI at 1 and 3 dph, respectively (see figure 5). There was a strong positive correlation with vitamin E concentrations (mg 100 g^{-1}) in the eggs and larval SAIs at 1 and 3 dph during the time course of this study, generating coefficients of $r(14) = 0.904$ and $r(14) = 0.940$ ($p < 0.05$) for SAIs at 1 and 3 dph, respectively (see figure 6). There was no correlation with larval SAIs to vitamin A,

ascorbic acid, and AA concentrations in the eggs but there was a negative correlation with DHA levels with only larval SAI at 3 dph with correlation coefficient of $r(14) = -0.561$. There was also no correlation with larval SAI over time with any of phospholipid concentrations in the eggs. There were statistically significant positive correlations between nearly all amino acids under investigation except tryptophan, valine, and cystine (see Table 7).

Chapter 4: Discussion

Results from this study illustrated a strong relationship between the egg

morphometries and larval survival with time in captivity and numerous suggestive

correlations between larval survival and the key nutrients: EPA, vitamin E, and amino

acids supplied in the broodstock diet. Some of these nutritional correlations are seen in

differences between nutritional composition of the WG and CG.

Interestingly, there was no statistically significant inverse relationship with egg

diameter and salinity ($R^2 = 0.384$, $p < 0.05$) during this trial which has been seen in

Baltic cod *Gadus morhua* (Vallin and Nissling, 2000) and European flounder

Pleuronectes flesus (Solemdal, 1967). There was a significant relationship over time

course of this study between elapsed time in captivity of the broodstock, egg diameter,

larval SAI at 1 dph with larval SAI at 3 dph. This indicated that there was a linkage

between egg diameter size, hatch rate, and larval survival in mahi-mahi that which has

been reported in Atlantic cod (Knutsen and Tilseth, 1985; Marteinsdottir and Steinarsson,

1998). For many fish species, the size of the egg may be an indicator of the reproductive

success of the broodstock with larger eggs having with more metabolic reserves

(Berkeley *et al.*, 2004; Lund *et al.*, 2008). This is supported with evidence in common

sole eggs that shows the larger in diameter the eggs were the more yolk reserves were

available to the larvae; additionally, the larvae were also subsequently longer in length

with deeper myotomes which could give a selective advantage that favors larger eggs

(Baynes and Howell, 1996). Smaller egg diameter and low larval survival at day 1 of this

study could be largely attributed to the stress of capture and handling of the broodstock

possibly causing elevated levels of corticosteroids to act on induction of

spawning and limit the process of vitellogenesis (Billard et al., 1981). Elevated cortisol due to capture and handling stress can act on the hypothalamic-pituitary-gonadal axis to stimulate ovulation and spawning (Stieglitz *et al.*, 2016a *in review*) as seen on day 1 of this study. In addition, elevated maternal cortisol levels have been shown to produce egg smaller in diameter in rainbow trout *Oncorhynchus mykiss* (Campbell *et al.*, 1992). Cortisol can be maternally transferred to the eggs through the process of vitellogenesis (Brooks *et al.*, 1997). Elevated levels of maternal cortisol in the oocytes can have deleterious effects on the developing embryos and larvae. Corticosteroid binding proteins (CBP) in the surrounding follicle cells of oocytes act as a regulatory mechanism for preventing maternal cortisol from entering into the oocytes but this system can become saturated and overwhelmed (Schreck *et al.*, 2001). Cortisol can also severely downregulate the process of vitellogenesis. The liver is the site for vitellogenin synthesis which is initiated by estrogen-dependent mechanism. Cortisol can strongly inhibit the estrogen receptors in the liver at transcription and therefore decrease plasma vitellogenin levels (Lethimonier *et al.*, 2000). For an income spawner like mahi-mahi in which vitellogenic transfer of maternal nutrients onto the eggs could be instantaneous, the elevated stress and handling from the capture of broodstock that was exhibited on day 1 of this study could explain lower egg diameter and low hatch rates due to inhibition of vitellogenesis and malformations of the eggs.

During this study, the larval SAIs at both 1 and 3 dph generally increased after day 1 and leveled off with survival in following time course of this study. For a high-performance species like mahi-mahi, with a high-energy allocation into each spawning event possible deficiencies in the broodstock diet would be expected to have caused a

rapid decrease in the larval survival over time. However, in this study there was a

general maintenance in larval survival which did not significantly decrease over time.

Further evidence of increased spawning performance with time in captivity over

spawning seasons has also been shown in cobia *Rachycentron canadum* (Nguyen *et al.*,

2012).

Evidence from this study suggests a strong correlation between several key

nutrients with the replicated larval SAIs over time which was displayed in the different

composition of the gonads of wild and captive broodstock. There was a strong

correlation between EPA concentrations and larval SAIs for 1 and 3 dph over time. EPA

is known to be a potent inhibitor AA derived metabolic pathways such as the production

of eicosanoids which earlier stated are crucial for steroid production to be used in

endocrine, hypothalamic, immune, and reproductive functions (Rainuzzo *et al.*, 1997;

Tocher, 2010). Lower absolute levels of AA as well as a higher ratio of EPA/AA have

been associated in captive broodstock as seen in common sole (Lund *et al.*, 2008),

Senegalese sole (Norambuena *et al.*, 2012), white sea bream (Cejas *et al.*, 2004, 2003),

common snook (Hauville *et al.*, 2015), and greater amberjack (Rodríguez-Barreto *et al.*,

2012). The results suggest that elevated levels of the EPA and higher ratio of EPA/AA

did not affect the performance of the larval survival over time. Similar results were

shown in the cobia over two breeding season in Faulk and Holt (2008) with no

statistically different changes in hatch rates and higher levels of EPA and relative ratios

of EPA/AA in the second season of the study. The elevated EPA/AA ratio was also

conserved in the nutritional composition of gonads of the captive broodstock (4.57)

compared to gonads of the wild mahi-mahi (1.08) adding evidence that eggs and

composition of gonads reflected the diet in captivity. Elevated levels of EPA might

possibly come from excessive amounts of AA in the broodstock diet which has been

shown in Japanese olive flounder *Paralichthys olivaceus* to be converted into EPA

(Furuita *et al*., 2003). DHA was also observed to be lower over time, though not

statistically significant correlated to larval survival. Relative ratio of DHA/EPA was

strongly negatively correlated with larval SAIs at 1 and 3 dph. DHA is vitally important

to larval ontology and correct development. DHA is especially in important as precursor

for many different types of phospholipids used in the development of neural and retinal

tissue and is crucially relevant in the case of rapid growing larvae like mahi-mahi which

have a high percentage of neural tissue relative to the larval body mass (Bell *et al*., 1997).

Deficiencies in DHA have been linked vision impairment in herring *Clupea harengus* L.

(Bell *et al*., 1995b) and the inability for juveniles to school properly shown in yellowtail

Seriola quinqeradiata (Ishizaki *et al*., 2001). DHA and EPA compete for the formation

of structural phospholipids with DHA being more biologically favored and selectively

incorporated at higher rates than EPA into polar lipids of yolk during vitellogenesis

(Wiegand, 1996). Proper DHA/EPA ratios are also important for the correct embryo

ontology needed for hatching (Bell *et al*., 1997). Interestingly, the results of this study

suggest that possible reduced DHA levels seen in captivity did not illicit a negative effect

on larval survival in mahi-mahi.

     The results suggest that vitamin E was strongly correlated to larval survival in this

study. Vitamin E acts an antioxidant in its primarily role to prevent lipid peroxidation

from ROS during high periods of metabolic activity such as embryonic development

(Palace and Werner, 2006). One tocopherol molecule has the ability to protect up to

1000 lipid molecules from peroxidation with α-tocopherol being the most biopotent and therefore is selectively favored for incorporation into developing oocytes (Hamre, 2011; Liebler, 1993; Tokuda *et al.*, 2000). It has been shown in gilthead seabream *Sparus aurata* that elevated levels of PUFAs with insufficient levels of vitamin E causes yolk sac hypertrophy, oxidative type lesions, and larval mortality (Fernandez-Palacios *et al.*, 1998). It could be argued that for mahi-mahi which has one of the fastest embryonic metabolic rates of any fish (Pasparakis *et al.*, 2016), that an antioxidant such as vitamin E would take precedence in incorporation into developing oocytes to protect against lipid peroxidation. Vitamin E was conserved at the CG (2.91 mg 100 g^{-1}) in comparison to the WG (1.63 mg 100 g^{-1}) alluding to a diet in captivity that is high in this particular antioxidant. Vitamin C which increases the biopotency of vitamin E and is also a potent antioxidant (Palace and Werner, 2006) was not correlated with larval survival and remained at lower levels in the CG in comparison to the WG. Vitamin A as retinol which has a secondary role as an antioxidant was shown to be suggestively higher in the CG (397 IU 100 g^{-1}) of mahi-mahi than in the WG (197 IU 100 g^{-1}) but interestingly incorporated into the eggs at 13 times less than levels present in gonads. Similarly, it was shown in Japanese olive flounder where excessive levels of vitamin A supplied in the broodstock diet over different incremental treatments were also not incorporated into the eggs at proportional rates in comparison to the levels of vitamin A esters found in the liver (Furuita *et al.*, 2001). This suggests a maternal control for incorporation of vitamin A into the eggs due to hypervitaminosis of this particular vitamin. This same mechanism of control and reallocation of vitamin A could be occurring in mahi-mahi.

This study builds on earlier work of (Ostrowski and Divakaran, 1991) on the amino acid energy substrates and utilization in mahi-mahi eggs and larvae. Amino acids act as an important building blocks for structural proteins and as a secondary energy source. Almost all amino acids showed strong suggestive correlations with larval survival except for tryptophan and valine which remained at a constant level while cystine showed no statistical correlation with the larval SAIs. Ostrowski and Divakaran (1991) found that non-essential amino acids were preferentially used as the main energy substrate during the hatching period for mahi-mahi larvae due to lower oxygen requirements needed to metabolize amino acids in comparison to lipids. This switch from lipid to amino acid utilization minimizes oxygen consumption which may be limiting during this critical period. Additionally, marine finfish larval tail buds at hatching that consist of undifferentiated white muscle that does not utilize lipids very well (Crabtree and Newsholme, 1972). Interestingly, there were no differences between the gonads of the WG and CG with respect to amino acid content but there were very strong correlations with larval survival and almost all amino acids. This indicated that there may be a selective incorporation of different amino acids that can be reflected in the larval survival. In captivity, where the broodstock are fed to satiation every day with a high protein diet, excess amounts of amino acids might be incorporated to into the developing oocytes to give additional energy reserves needed for hatching and larval development as a selective advantage.

Overall, this study elucidated changes in the egg morphometries and larval survival over time. This study found that larval survival was maintained over time in captivity and did not significantly decrease over time despite the high metabolic and

energetic requirements needed for consistent egg production in mahi-mahi. Suggestive

evidence on the nutritional composition of gonads and spawns collected over time in

captivity further illustrated key empirical findings of this study. Key nutrients such as

vitamin E, and amino acids were shown to strongly correlate with larval survival. It was

also found that EPA and its related ratios did not have negative effect on larval survival,

it may be that the levels of this essential fatty acid had not reached inhibitory levels for

this species. Understanding key nutritional requirements for early larval stages of high

energetic species is crucial to improving the future culture of these species. This study

provides the ground work for other future broodstock nutrition studies for high

performance pelagic species

Tables

	Maturation tank	Initial PELEC system	Final PELEC system
Temperature (°C)	27.05 ± 0.39	26.53 ± 0.05	26.75 ± 0.05
Dissolved oxygen (mg/L)	10.15 ± 0.19	6.18 ± 0.04	6.28 ± 0.04
Salinity (ppt)	32.98 ± 0.19	32.93 ± 0.28	35.37 ± 0.29
pH	7.81 ± 0.02	8.04 ± 0.01	8.09 ± 0.01
NH_4^+ (mg/L)	0.14 ± 0.04	†	0.03 ± 0.02

Table 1 Water Parameters of the maturation system, initial PELEC readings, and final PELEC readings. Results presented as mean ± SEM. † represents analysis not being performed.

Table 1

Egg diameter and egg oil globule diameter measurements (µm) of mahi-mahi spawns over time in captivity (n = 90). Superscripts indicate significant difference within a column.

Days Post Broodstock Capture	Egg Diameter (µm)	Egg Oil Globule Diameter (µm)
1	1236.51 ± 3.53^g	256.91 ± 1.32^e
19	1375.10 ± 3.24^f	274.12 ± 1.96^d
23	1371.79 ± 3.71^f	266.11 ± 1.90^d
37	1583.04 ± 3.67^{cd}	303.64 ± 1.67^{ab}
39	1594.88 ± 5.95^a	303.05 ± 3.28^{ab}
45	1532.88 ± 4.49^c	293.26 ± 2.15^c
47	1553.64 ± 4.18^b	307.05 ± 1.66^a
55	1511.67 ± 3.45^d	297.55 ± 1.81^{bc}
57	1471.62 ± 3.50^e	302.16 ± 1.24^{ab}
63	1501.13 ± 3.93^d	297.71 ± 1.60^{bc}
67	1502.02 ± 4.11^d	296.82 ± 1.51^{bc}
71	1518.79 ± 4.36^{cd}	300.67 ± 1.20^{ab}

Variable	B	SE_B	ß
Intercept	17.3	0.997	
Time	0.42	0.003	0.101*
Egg Diameter	-0.01	0.001	0.167*
SAI (1 DPH)	1.66	0.014	1.038*

Table 3 Summary of multiple regression analysis. * Note $p < 0.05$; B = unstardardized regression coefficient; SE_B = standardized regression coefficient; ß = standard coefficient.

Vitamins	Wild Gonad (n = 9)	Captive Gonad (n = 4)
Retinol	174	397
Ascorbic Acid	14.5	6.27
Alpha-Tocopherol	1.63	2.91
Beta-Tocopherol	< 0.100	< 0.100
Gamma-Tocopherol	< 0.100	< 0.100
Delta-Tocopherol	< 0.100	< 0.100
Total Vitamin E (Tocopherols)	1.63	2.91

Table 4 Comparison of the vitamin composition of wild and captive gonads of mahi-mahi. Retinol in IU 100 g^{-1} of sample and ascorbic acid and tocopherols in mg 100 g^{-1} of sample. < 0.100 mg 100 g^{-1} is below the sensitivity of the analysis for tocopherols.

Table 5 Fatty acid profile (% of sample) of wild (n = 9) and captive gonads (n = 4) of mahi-mahi broodstock.

	Wild Gonad	Captive Gonad
14:0	0.05	0.05
15:0	0.02	0.01
16:0	0.76	0.57
17:0	0.05	0.02
18:0	0.31	0.17
$\sum$SFAs[1]	1.25	0.84
16:1n – 7	0.02	0.11
18:1n – 7	0.06	0.09
18:1n – 9	0.30	0.36
$\sum$MUFAs[2]	0.47	0.67
18:2n – 6	0.06	0.05
20:4n – 6	0.13	0.07
20:5n – 3	0.14	0.32
22:6n – 3	0.94	0.80
$\sum$PUFAs[3]	1.47	1.37
$\sum$n – 3[4]	1.15	1.21
$\sum$n – 6[5]	0.29	0.15
Triglycerides	3.40	3.04
Total FAs	3.26	2.91
EPA/AA	1.08	4.57
DHA/EPA	6.71	2.50
n-3/n-6	3.97	8.07

Abrreviations as in Table 1.
[1] includes 12:0
[2] includes 15:1, 20:1n – 9
[3] includes 18:3n – 4
[4] includes 18:3n – 3, 18:4n – 3, 20:3n – 3, 20:4n - 3
[5] includes 18:3n – 6, 20:2n – 6, 20:3n – 6

Table 6 Amino acid profile (% of sample) of wild (n = 9) and captive gonads (n = 4) of mahi-mahi broodstock.

	Wild Gonad (n = 9)	Captive Gonad (n = 4)
Essential		
Arginine	1.23	1.23
Histidine	0.55	0.55
Isoleucine	1.01	1
Leucine	1.6	1.57
Total Lysine	1.51	1.48
Methionine	0.53	0.58
Phenylalanine	0.93	0.93
Threonine	1.02	1.03
Tryptophan	0.27	0.27
Valine	1.29	1.3
Non-essential		
Alanine	1.14	1.12
Aspartic Acid	1.62	1.64
Cystine	0.26	0.27
Glutamic Acid	2.46	2.46
Glycine	0.82	0.85
Proline	1.46	1.51
Serine	1.13	1.15
Tyrosine	0.89	0.89

	SAI (1 DPH)	SAI (3 DPH)
Essential		
Arginine	0.996*	0.973*
Histidine	0.992*	0.962*
Isoleucine	0.996*	0.978*
Leucine	0.998*	0.982*
Total Lysine	0.991*	0.980*
Methionine	0.812*	0.874*
Phenylalanine	0.998*	0.979*
Threonine	0.991*	0.980*
Tryptophan	[b]	[b]
Valine	[b]	[b]
Non-essential		
Alanine	0.993*	0.977*
Aspartic Acid	0.993*	0.970*
Cystine	0.377	0.468
Glutamic Acid	0.997*	0.985*
Glycine	0.995*	0.986*
Proline	0.987*	0.963*
Serine	0.997*	0.975*
Tyrosine	0.997*	0.975*

Table 7 Pearson correlations of amino acids with egg morphometries and larval survival indices over time in captivity. Asterisks denote statistical significance ($p < 0.05$). [b] denotes no change in level of amino acid over time.

Figures
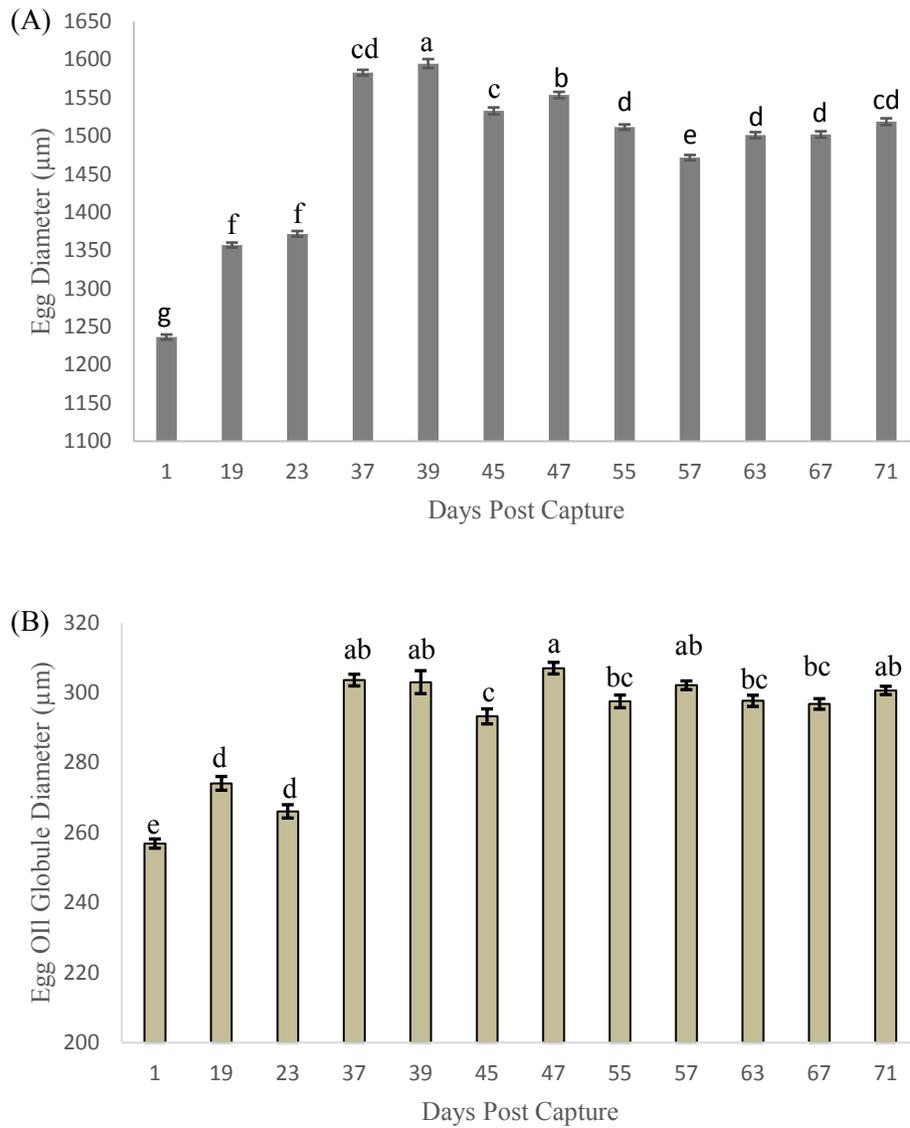
Figure 1 Egg morphometries of captive mahi-mahi spawns over time (n = 90). (A) Egg diameter (μm) of captive mahi-mahi spawns over time. Lettering indicates significant difference (P< 0.05). (B) Egg globule diameters (μm) of captive mahi-mahi spawns over time.
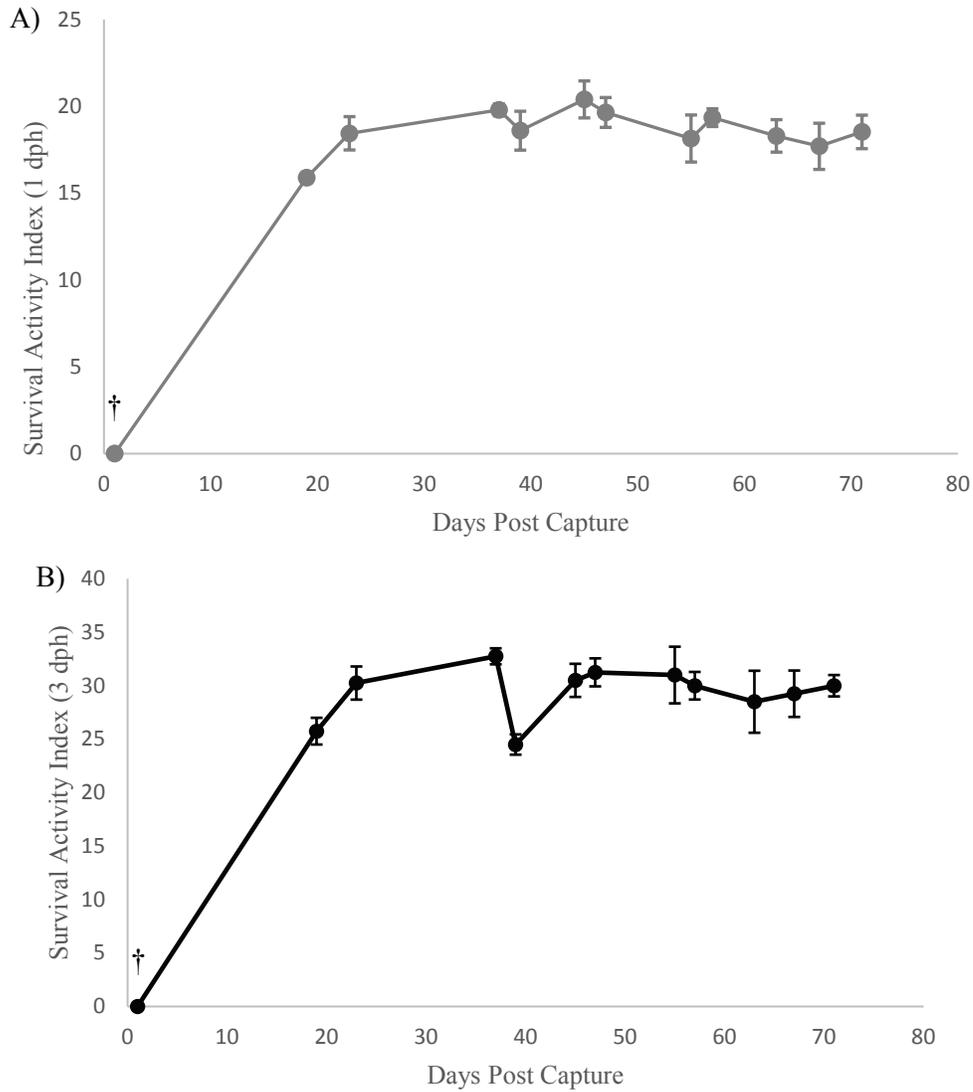
Figure 2 Larval survival activity indices (n = 4) based on larval survival at A) 1 dph and B) 3 dph over time from captivity mahi-mahi spawns. Lettering indicates statistically significance (P < 0.05).
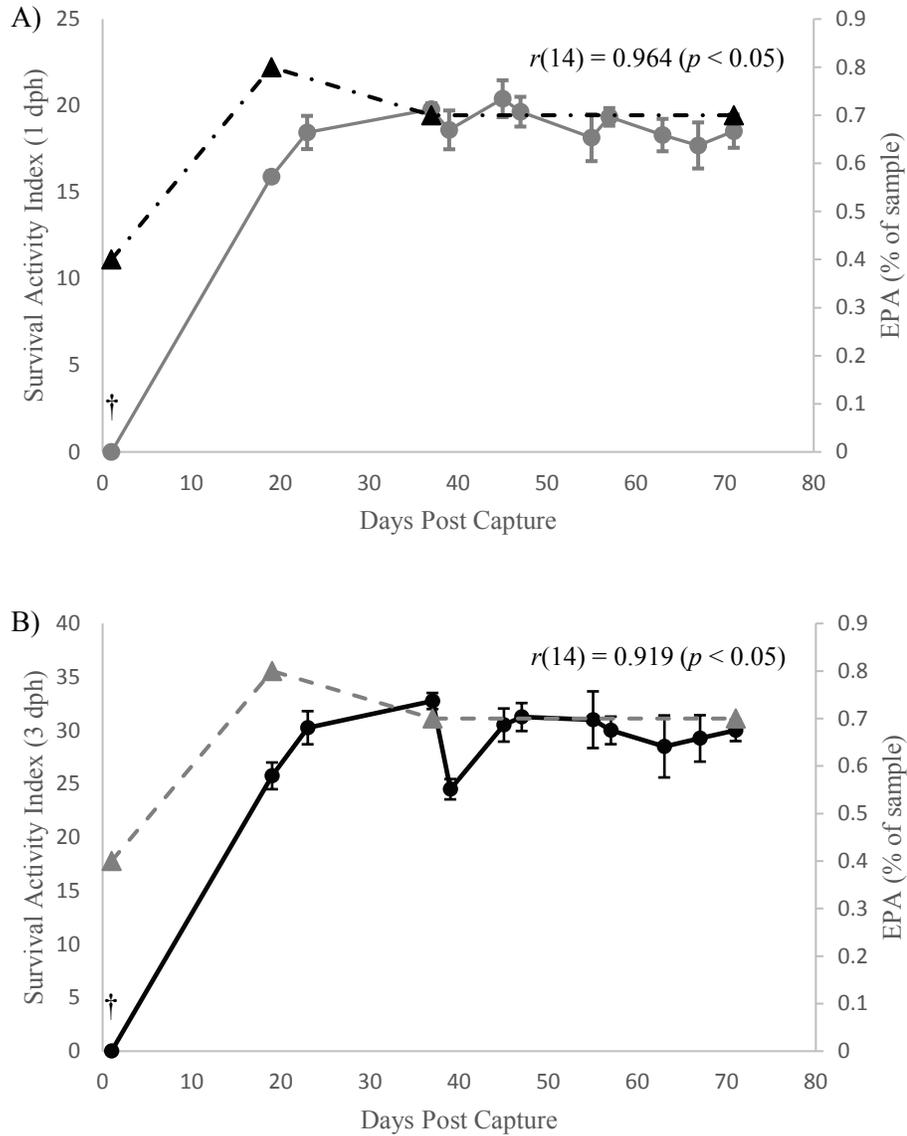
Figure 3 Correlations of larval SAI based on larval survival at A) 1 dph and B) 3 dph with EPA (% of sample) from spawns over time in captivity. Statistical significance indicated by lettering ($p < 0.05$).
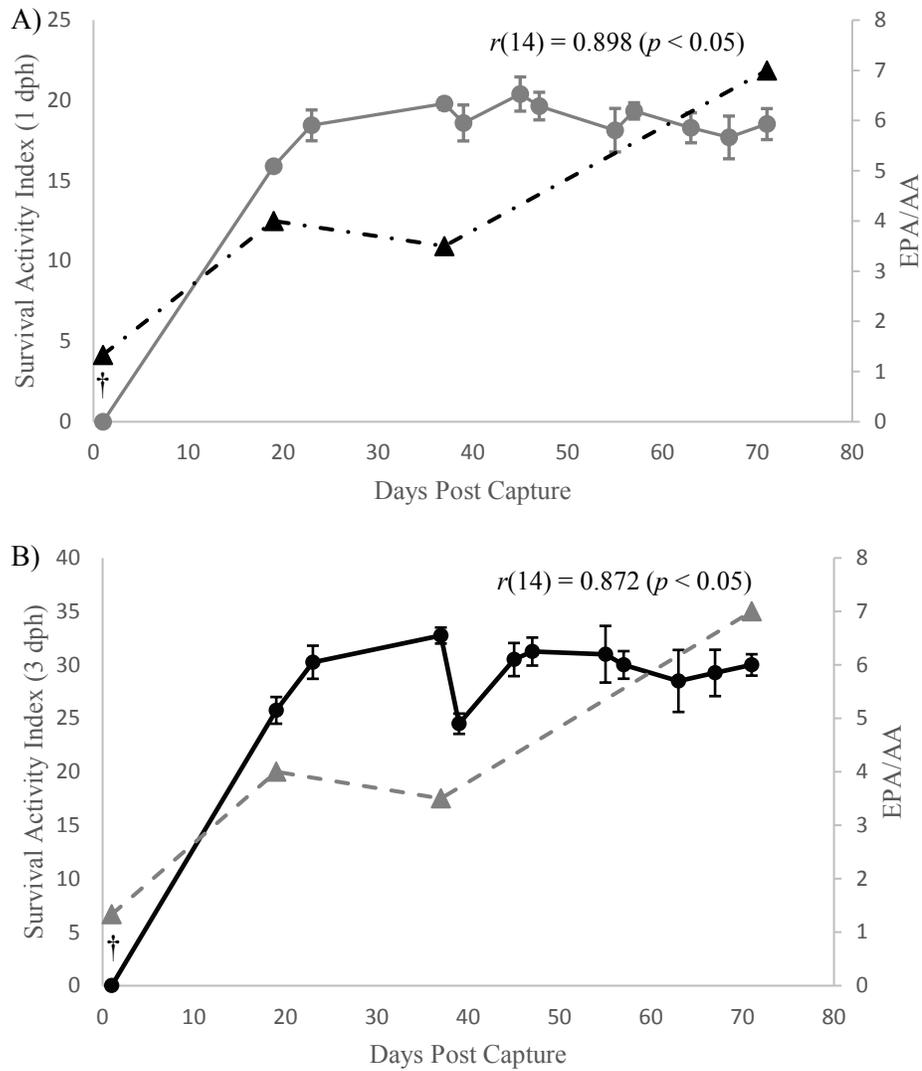
Figure 4 Correlations of larval SAI based on larval survival at A) 1 dph and B) 3 dph with EPA/AA relative ratios from spawns over time in captivity. Statistical significance indicated by lettering ($p < 0.05$).
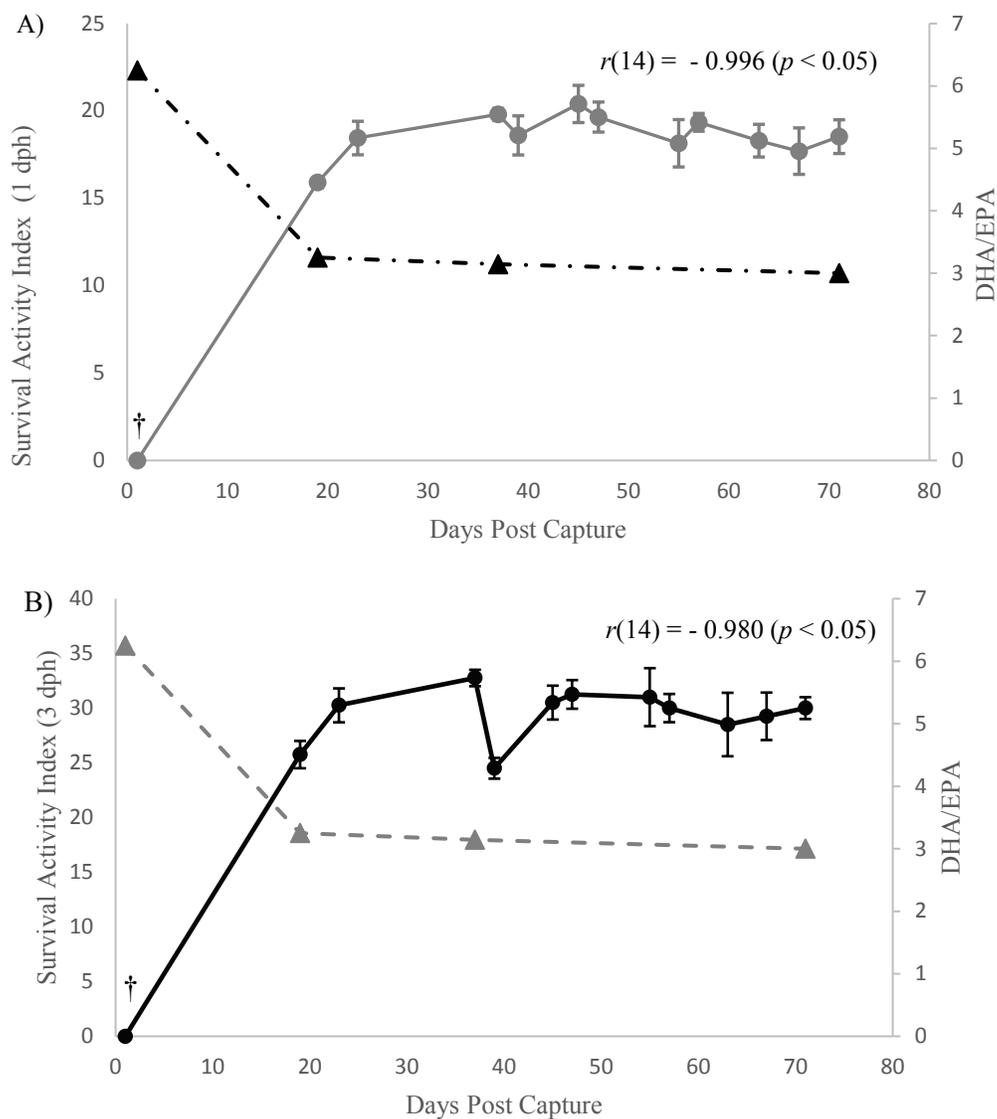
Figure 5 Correlations of larval SAI based on larval survival at A) 1 dph and B) 3 dph with DHA/EPA relative ratios from spawns in captivity. Statistical significance indicated by lettering ($p < 0.05$).

Figure 6 Correlations of larval SAI based on larval survival at A) 1 dph and B) 3 dph with Vitamin E (mg 100 g^{-1}). Statistical significance indicated by lettering ($p < 0.05$).

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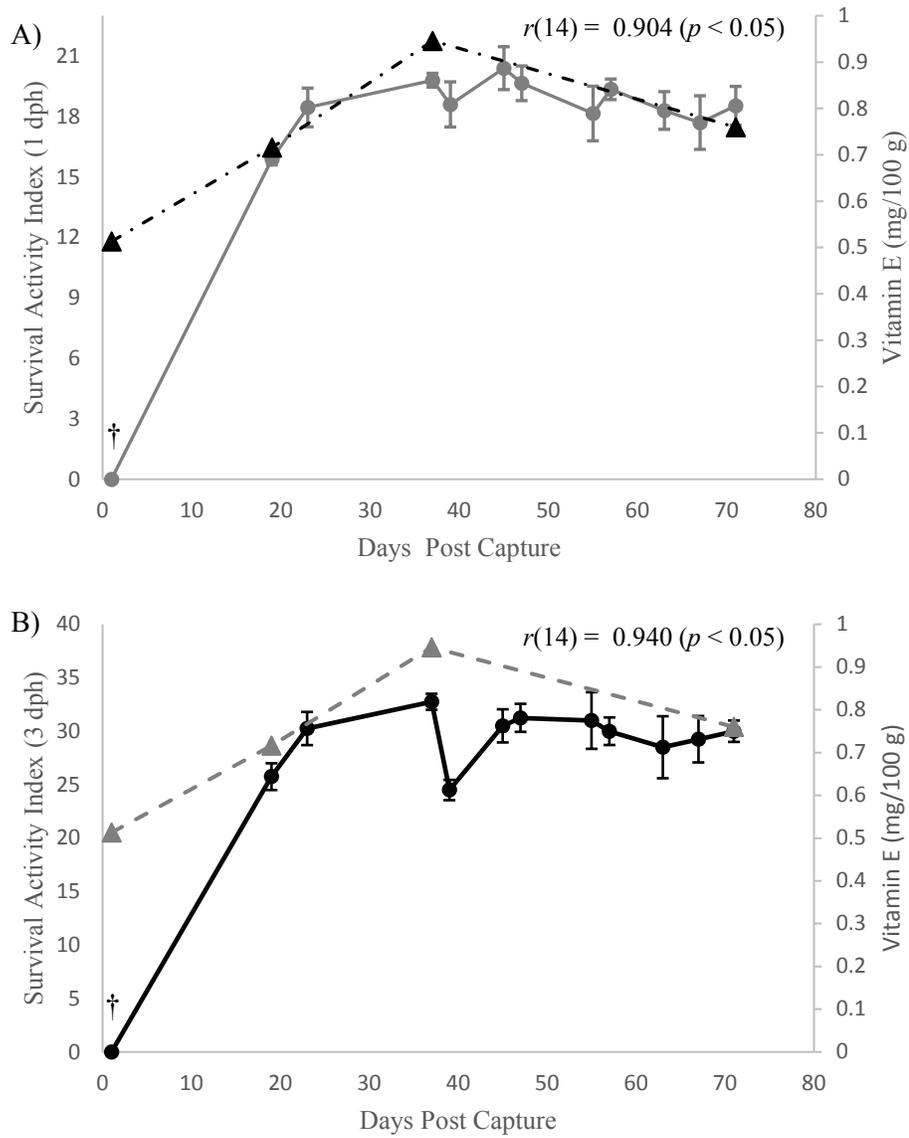
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