Characterizing egg quality and larval performance from captive mahi-mahi Coryphaena hippurus (Linnaeus, 1758) spawns over time

Steven Kloeblen | John D Stieglitz | Jorge A Suarez | Martin Grosell | Daniel D Benetti

Abstract
Mahi-mahi Coryphaena hippurus 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 its high spawning frequency and reproductive energy allocation. These characteristics lend the species to being an excellent model for understanding broodstock nutrition for other high energetic pelagic species. In this study, egg morphometrics and larval survival were tracked over a 10-week period from the initial capture of wild mahi-mahi broodstock. Larval quality from subsequent spawns collected over time was quantified using larval survival activity indices (SAIs) as a metric to assess egg quality. Larval SAIs were maintained and did not significantly decrease ($p < .05$) over the time course of this study. A multiple linear regression based on the elapsed time in captivity of the broodstock, egg diameter and larval SAI at 1 dph provided the most accurate prediction of larval SAI at 3 dph ($R^2 = 0.996$ $p < .05$). There were strong positive correlations with larval SAIs at 1 and 3 dph and the key nutrients: eicosapentaenoic acid (C20: 5n-3, EPA) and related fatty acid ratios, vitamin E and nearly all amino acids under investigation. This study demonstrated that larval survival was maintained over time due to the supply of these key nutrients in the broodstock diet.

KEYWORDS
broodstock, egg quality, mahi-mahi, survival activity index, vitellogenesis

1 INTRODUCTION
As marine finfish aquaculture continues to expand with the culture of novel species, one of the major impediments to production of these species is the consistent year-round supply of high quality, viable eggs. 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, Tyler & Sumpter, 1997; Hauguel et al., 2015; Lund, Steenfeldt & Hansen, 2007; Rainuzzo, Reitan & Olsen, 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 vital to this production of high-quality eggs and are one of the strongest indicators of reproductive performance (Izquierdo, Fernández-Palacios & Tacon, 2001; Rainuzzo et al., 1997). Essential PUFAs such as
docosahexaenoic acid C22:6n-3 (DHA), eicosapentaenoic acid (EPA) C20:5n-3 and arachidonic acid C20:4n-6 (AA) are crucial for correct larval ontogeny and differentiation of undeveloped systems in the larvae (Bell, Farndale, Bruce, Navas & Carillo, 1997; Brooks et al., 1997; Furuita, Yamamoto, Shima, Suzuki & Takeuchi, 2003; Izquierdo et al., 2001; Lavens et al., 1999; Lund et al., 2007; Tocher, 2010; Wiegang, 1996).

In addition, 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 such as vitamin A, E and C protect PUFAs from lipid peroxidation by reactive oxygen species (ROS) which are volatile, oxidative byproducts of aerobic respiration produced during high periods of metabolic activity that occur in larval development (Hamre, 2011; Palace & Werner, 2006).

In assessing egg quality in the broodstock of any marine finfish, comparing eggs and related tissues from wild and captive broodstock can identify any nutritional deficiencies that may be present in the diet which 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 (Linnaeus, 1758; Kjørsvik, 1994), striped bass Morone saxatilis (Walbaum, 1792; Harrell & Woods, 1995), striped trumpeter Latrips lineota (Bloch and Schneider, 1801; Morehead, Hart, Dunstan, Brown & Pankhurst, 2001), white seabream Diplodus sargus (Linnaeus, 1758; Cejas et al., 2003, 2004), common sole Solea solea (Linnaeus, 1758; Lund, Steenfeldt, Suhr & Hansen, 2008), Senegalese sole Solea senegalensis (Kaup, 1858; Norambuena, Estvez, Bell, Carazo & Duncan, 2012) and common snoek Centropomus undecimalis (Bloch, 1792; Hauville et al., 2015) yet the literature is very limited on identifying these deficiencies in high-performance pelagic species with notable exceptions including studies on Atlantic bonito Sarda sarda (Bloch, 1793; Ortega & Mourente, 2010), Atlantic bluefin tuna Thunnus thynnus (Linnaeus, 1758; Ortega & Mourente, 2010; Pousis et al., 2011), lesser amberjack Seriola rivoliana (Valenciennes, 1833; Saito, 2012) and greater amberjack Seriola dumerili (A. Risso, 1810; Rodriguez-Barreto et al., 2012; Saito, 2012).

The increased swimming performance and energetics of pelagic species have 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 (Linnaeus, 1758), an epipelagic circum-tropical species with both sport fishing and commercial importance (Palko, Beardsley & Richards, 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 ~40 cm fork length (FL) at 4–5 months posthatch in the wild (McBride, Snodgrass, Adams, Rider & Colvocoreses, 2012) while Stieglitz, Mager, Hoenig, Alloy, et al. (2016) reported that F1 (first-generation) mahi-mahi reached sexual maturity in captivity at ~30 cm FL at 4 months posthatch. Mahi-mahi can also spawn 70–180 days/year in the wild producing a batch fecundity ranging from 20,000 to 620,000 eggs/spawn (McBride et al., 2012). In addition to the high spawning frequency and fecundity, mahi-mahi have one of the fastest growth rates of any fish species with a specific growth rate (SGR) of a sexually mature wild-caught mahi-mahi at 1.3% per day (Stieglitz et al., 2017) while Benetti, Iversen and Ostrowski (1995) reported a SGR with F1 mahi-mahi broodstock of 4.3% per day throughout the juvenile stage up to the onset of sexual maturity. The energy allocation into each spawning event is extraordinary with a captive female donating 5% of its bodyweight per spawn (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 (McBride et al., 2015). It has been shown in the lower energetic sub-topical species red drum Sciaenops ocellatus (Linnaeus, 1766) that dietary shifts in the maternal diet were reflected rapidly in the nutritional composition of the eggs at 2–16 days post-diietary shift (Fuiman & Faulk, 2013).

Although technology for domestication of mahi-mahi first began in 1980s (Hagood, Rothwelly, Swafford & Tosaki, 1981; Kraul, 1989; Szypier, Bourke & Conquest, 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; Nelson et al., 2016; Pasparakis, Mager, Stieglitz, Benetti & Grosell, 2016; Stieglitz, Mager, Hoenig, Alloy, et al., 2016; Sweet et al., 2016; Xu et al., 2016). The reliance on the production 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 the 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 the 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.

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 and continuous spawning of captive mahi-mahi broodstock are described in further detail in Stieglitz et al. (2017). Captured broodstock were fed a natural diet of whole and chopped Spanish sardines (Sardinella aurita) and squid (Loligo opalescens) to satiation every day. The broodstock were also fed a dietary supplement comprised of Madmac-MS (Aquafuana
Bio-Marine, Inc., Hawthorne, CA, USA) once per week at 10% of the food weight per day and additionally vitamin capsules were fed once per week at 1% of the food weight per day. The nutritional composition of the natural diet and dietary supplements is further described in Stieglitz et al. (2017).

The first spawn of this study was collected 1-day postcapture which is typical for mahi-mahi captured for previous studies at UMEH. This spawn was collected using a 500 µm mesh standpipe in a quarantine tank. Subsequent fertilized spawns were non-invasively collected in the egg collector of an 80,000 L maturation tank being supplied by surface skimmed water. The egg collector was equipped with an aeration ring around a 500 µm mesh standpipe to keep the eggs in suspension. Spawning occurred volitionally at a sex ratio of 1 male:8 females with the tank temperature maintained at 26.5°C on a natural photoperiod (14 L:10 D). Female broodstock spawned naturally every other day with multiple females spawning asynchronously on opposing days and the male being able to spawn daily on consecutive days. Eggs were collected 2–8 hr 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. Eggs were given a prophylactic 100 ppm formalin (Formacide- B, 37% formaldehyde solution) for 1 hr with 300% rinse using 0.35 µm filtered UV-treated seawater for 30 min (Mager et al., 2014; Stieglitz et al., 2012). Treated eggs were stocked in a replicated pelagic embryo-larval exposure chamber (PELEC) system, with each 1.8 L chamber filled with 0.35 µm filtered UV-treated seawater. The design and functioning of the PELEC system is described in further detail in Stieglitz, Mager, Hoenig, Benetti and Grosell (2016). For each replicated chamber in the PELEC system (n = 4), 40 viable eggs were stocked using a large-bore Pasteur pipette. Environmental conditions for the PELEC systems were maintained at 26.5°C with a 16L:8D photoperiod. Hatching occurred ~30 hr postfertilization at these conditions. Posthatch, daily mortality was measured in each chamber of the PELEC system until all the larvae had died out due to starvation. From the daily mortality, a larval survival activity index (SAI; Shimma & Tsujigado, 1981) was implemented, using the equation

\[
SAI = \frac{1}{N} \sum_{i=1}^{N-1} (N - h_i) \times i
\]

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, Tanaka, Yamamoto, Shiraishi & Takeuchi, 2000). In this study, the SAI metric was performed for larval survival at 1 day posthatch (dph) and 3 dph. At 3 dph, mahi-mahi larvae have consumed their yolk sac and are competent to begin exogenous feeding (Ostrowski & Divakaran, 1991). Water parameters (temperature, dissolved oxygen, salinity, pH) were measured initially and at the end of each replicated trial. Total ammonia (\(\text{NH}_4^+\)) using the indophenol blue method described by Ivancic and Degobbis (1984) was measured at the end of each replicated trial. In addition to the subsample of eggs used in the PELEC system, a sample of eggs (\(n = 90\)) was collected and the egg diameters and egg oil globule diameters were measured using a Leica CME microscope equipped with an ocular micrometre at 40× 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 a week based on the availability of broodstock spawning for a 10-week period from when the broodstock were initially caught.

In addition, the nutritional composition of gonads from both wild-caught female mahi-mahi (WG) and captive female broodstock (CG) was analysed. Gonads from females used in this study all came from the same cohort of wild fish that were initially captured at the beginning of this study. WG (\(n = 1\)) were comprised of the gonads from 9 wild-caught females while CG (\(n = 1\)) were comprised of gonads from 4 females that were euthanized at the end of this study. These samples were placed into Whirl-Pak® 532 ml bags (eNasco), flash frozen in liquid nitrogen and were stored in a −80°C freezer for future analysis. Nutritional analyses were performed by Eurofins Scientific, Inc. Nutritional Analysis Center (Des Moines, IA, USA). For each egg (\(n = 4\)) and gonad sample (\(n = 2\)) analysed, a complete fatty acid profile, phospholipid profile, vitamin E, A and C and amino acid profile were performed.

For the nutritional analyses, a complete fatty acid analysis was performed on each sample using gas liquid chromatography described in Metcalf and Schmitz (1961). A phospholipid profile for phosphatic acid (PA), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), 3-sn-lysocephatidylcholine (LPC) and phosphatidylserine (PS) was performed. These phospholipids were analysed using high-performance liquid chromatography (HPLC) further described in Patton, Fasulo and Robins (1982).

A total vitamin E analysis was performed using HPLC and fluorometric detection described in Desai (1984). Vitamin A and provitamin A carotenoids analyses were performed on each sample by using saponification of the samples followed by HPLC with fluorometric and UV detection described in Furr (2004). Vitamin C (ascorbic acid) was analysed for each sample by oxidizing ascorbic acid with activated charcoal and reacting the oxidative form with o-phenylenediamine and measuring fluorescence intensity as described in detail in Wu et al. (2003).

Lastly, for each sample analysed, a complete amino acid profile was performed. The amino acid tryptophan was analysed using alkaline hydrolysis and reverse phase liquid chromatography. Methods are further described in detail in Spies (1967). Cystine and methionine were analysed 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 analysed using cation-exchange liquid chromatography described in Kaiser, Gehrke, Zumwalt and Kuo (1974).
Statistical analysis was performed using IBM® SPSS® Statistics Version 22. Empirical data for the egg morphometrics were analysed using one-way Welch ANOVA followed by a Games-Howell post hoc test with 95% confidence. Empirical data for survival activity indices were analysed using one-way ANOVA followed by Tukey’s post hoc test with 95% confidence. Multiple linear regression was performed on all empirical data. Any nutritional changes in the egg composition were correlated using Pearson’s moment correlations with empirical data (p < .05). All results were presented as means ± SEM.

3 | RESULTS

3.1 | Water parameters

The environmental parameters remained consistent throughout the study (Table 1) with increase in salinity from an initial PELEC reading of 32.93 ± 0.28 ppt to a final PELEC reading of 35.37 ± 0.29. The final PELEC total ammonia (NH₄⁺) was 0.03 ± 0.02 mg/L.

3.2 | Broodstock growth in captivity

In this study, all broodstock were sexually mature at the time of capture at an initial total length (TL) of 55.0 ± 0.98 cm and a mass of 1.01 ± 0.05 kg. At the end of the 10-week trial, all broodstock were euthanized and had a final TL of 97.42 ± 3.74 cm and a mass of 5.57 ± 0.76 kg.

3.3 | Empirical results

3.3.1 | Egg morphometrics

Egg diameter and egg oil globule diameter from spawns collected over time are presented in Figure 1. Egg diameter and egg oil globule diameter were significantly different (p < .05) over time respectively. Both egg diameter and egg oil globule diameter had the lowest values on 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). The egg diameter and egg oil globule had the highest values on day 39 (1594.88 ± 5.95 μm) and day 47 (307.05 ± 1.66 μm) respectively. In examining the relationship between salinity and egg diameter, there was no significant inverse relationship between the two variables generating a regression coefficient, R² = 0.384 (p < .05; Figure 2).

3.3.2 | 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 < .05) but no later time periods in the time course of this study were significantly different from each other (Figure 3). A multiple linear regression was performed on the empirical data gathered in this study to investigate a linkage between time in captivity of the broodstock, egg morphometrics and larval survival. In this multiple linear regression model, the independent variables of elapsed time in captivity, egg diameter and larval SAI at 1 dph significantly predicted larval SAI at 3 dph (R² = 0.996, p < .05). Egg oil globule diameter as an independent variable over the time course of this study did not significantly add 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.

![Figure 1](image)

**FIGURE 1** Egg morphometrics of captive mahi-mahi spawns over time (n = 90). (a) Egg diameter (μm) of captive mahi-mahi spawns over time. (b) Egg oil globule diameters of captive mahi-mahi spawns over time. Lettering indicates significant difference (p < .05)
3.4 | Gonad analysis

There were differences between the two pooled gonads of wild-caught mahi-mahi females (WG; \( n = 1 \)) and gonads of captive broodstock (CG; \( n = 1 \)). Results of the vitamin composition of pooled gonads are represented in Table 3. In assessing the compositional differences between several vitamins, there was a higher level of retinol in the CG (397 IU per 100 g) in comparison to the WG (174 IU per 100 g). There were higher levels in \( \alpha \)-tocopherol in the CG with an \( \alpha \)-tocopherol level of 2.91 mg per 100 g while the WG had 1.63 mg per 100 g. Conversely, there was 6.27 mg per 100 g of ascorbic acid in the CG while gonads of the WG had 14.5 mg per 100 g. The composition of fatty acids in the gonads of wild and captive broodstock are both represented in Table 4. In comparing the composition of the two pooled gonads, there were lower levels of DHA (0.80 g per 100 g of sample) in the CG compared to the WG (0.94 g per 100 g of sample). In addition, there were lower levels of AA in the CG (0.07 g per 100 g of sample) compared to the WG (0.13 g per 100 g of sample) while there were higher levels of EPA in the CG (0.32 g per 100 g of sample) compared to the WG (0.14 g per 100 g 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 fatty acids (SFAs) in the WG (1.25 g per 100 g of sample) while the levels of saturated fats in the CG were 0.84 g per 100 g of sample. There were also lower levels of PUFA in the CG compared to the WG but higher levels of monounsaturated fatty acids (MUFA) in the CG. There were no differences between the phospholipid composition of the WG and CG for phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylinositol and lysophosphatidylcholine. There were higher levels of phosphatic acid in the WG (<8,000 ppm) than the CG (<5,000 ppm). The composition of the amino acids of the WG and CG is represented in Table 5. There were no suggestive differences for any amino acids between the two gonad compositions.

3.5 | 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

<table>
<thead>
<tr>
<th>Variable</th>
<th>( B )</th>
<th>( SE_{B} )</th>
<th>( \beta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>17.3</td>
<td>0.997</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>0.42</td>
<td>0.003</td>
<td>0.101*</td>
</tr>
<tr>
<td>Egg diameter</td>
<td>-0.01</td>
<td>0.001</td>
<td>0.167*</td>
</tr>
<tr>
<td>SAI (1 dph)</td>
<td>1.66</td>
<td>0.014</td>
<td>1.038*</td>
</tr>
</tbody>
</table>

\( B \), unstandardized regression coefficient; \( SE_{B} \), standardized regression coefficient; \( \beta \), standard coefficient.

\(* \ p < .05.\)

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>Wild gonad</th>
<th>Captive gonad</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol</td>
<td>174</td>
<td>397</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>14.5</td>
<td>6.27</td>
</tr>
<tr>
<td>Alpha-tocopherol</td>
<td>1.63</td>
<td>2.91</td>
</tr>
<tr>
<td>Beta-tocopherol</td>
<td>&lt;0.100</td>
<td>&lt;0.100</td>
</tr>
<tr>
<td>Gamma-tocopherol</td>
<td>&lt;0.100</td>
<td>&lt;0.100</td>
</tr>
<tr>
<td>Delta-tocopherol</td>
<td>&lt;0.100</td>
<td>&lt;0.100</td>
</tr>
<tr>
<td>Total vitamin E (tocopherols)</td>
<td>1.63</td>
<td>2.91</td>
</tr>
</tbody>
</table>

Retinol in IU per 100 g of sample and ascorbic acid and tocopherols in mg per 100 g of sample. <0.100 mg per 100 g is below the sensitivity of the analysis for tocopherols.
for fatty acid correlations, vitamin E and amino acid correlations to SAIs over time at 1 and 3 dph represented in Figures 4–7 and Table 6. 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, $r_{14} = .964$ and $r_{14} = .919$ ($p < .05$) respectively (Figure 4). 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 $r_{14} = .898$ and $r_{14} = .872$ ($p < .05$) for SAIs at 1 and 3 dph respectively (Figure 5).

There was a strong negative correlation between SAIs over time and DHA/EPA concentration in the eggs $r_{14} = -.996$ and $r_{14} = -.980$ ($p < .05$) for larval SAI at 1 and 3 dph respectively (Figure 6). There was a strong positive correlation with vitamin E concentrations (mg per 100 g) in the eggs and larval SAIs at 1 and 3 dph during the time course of this study, generating coefficients of $r_{14} = .904$ and $r_{14} = .940$ ($p < .05$) for SAIs at 1 and 3 dph respectively (Figure 7). 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 the larval SAI at 3 dph with correlation coefficient of $r_{14} = -.561$.

There was also no correlation with larval SAIs 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 (Table 6).

**4 | DISCUSSION**

Results from this study illustrated a strong relationship between the egg morphometrics and larval survival with time in captivity. In addition, there were 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 ($R^2 = 0.384$, $p < .05$) during this study that has been reported in Baltic cod *Gadus morhua* L. (Vallin & Nissling, 2000) and European flounder *Pleuronectes flesus* (Linnæus, 1758; Solemdal, 1967). There was a significant relationship over the time course of this study among elapsed time in captivity of the broodstock, egg diameter and larval SAI at 1 dph with larval SAI at 3 dph. This indicated that there was a linkage among egg diameter size, hatch rate and larval survival in mahi-mahi which has been reported in Atlantic cod (Knutsen & Tilseth, 1985;
effects on the developing embryos and larvae. Corticosteroid binding levels of maternal cortisol in the oocytes can have deleterious through the process of vitellogenesis (Brooks et al., 1997). Elevated Sumpter, 1992). Cortisol can be maternally transferred to the eggs have been shown to produce eggs smaller in diameter in rainbow trout Oncorhynchus mykiss (Stieglitz et al., 2017). In addition, elevated maternal cortisol levels stimulate ovulation and spawning as seen on day 1 of this study handling stress can act on the hypothalamic-pituitary-gonadal axis to on the induction of spawning and limit the process of vitellogenesis broodstock possibly causing elevated levels of corticosteroids to act that the smaller egg diameter and low larval survival at day 1 of this study was attributed to the stress of capture and handling of the larval SAIs at 1 and 3 dph both represented by a solid black line with filled circles while EPA concentrations are represented by a dashed grey line with filled triangles 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, Contreras-Sanchez & Fitzpatrick, 2001). Cortisol can also severely downregulate the process of vitellogenesis. Vitellogenin synthesis, which occurs at liver, is initiated by an estrogen-dependent mechanism. Cortisol can strongly inhibit the estrogen receptors in the liver at transcription and therefore decrease plasma vitellogenin levels (Lethimonier, Flouriot, Valotaire, Kah & Ducouret, 2000). For an income spawner like mahi-mahi where the 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 the smaller egg diameter and low hatch rates due to the 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 in survival over the 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

**FIGURE 4** Correlations of larval survival activity indices based on larval survival at (a) 1 dph and (b) 3 dph with EPA (g per 100 g of sample) from spawns over time in captivity. Statistical significance indicated by lettering (†) (p < .05). Larval SAIs at 1 and 3 dph are both represented by a solid black line with filled circles while EPA concentrations are represented by a dashed grey line with filled triangles.

**FIGURE 5** 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 < .05). Larval SAIs at 1 and 3 dph are both represented by a solid black line with filled circles while EPA/AA relative ratios are represented by a dashed grey line with filled triangles.
spawning seasons has also been shown in cobia Rachycentron canadum (Linnaeus, 1766; Nguyen, Reinertsen, Rustad, Tran & Kjørsvik, 2012).

Evidence from this study suggests a strong correlation between several key nutrients with the replicated larval SAIs over time. 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 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. This could be attributed to high levels of EPA in comparison to very low traceable levels of AA found in Sardinella aurita which was supplied in the captive diet (Njinkoué, Barnathan, Miralles, Gaydou & Samb, 2002). DHA was also observed to be lower over time, although not statistically significantly correlated with larval survival. The relative ratio of DHA/EPA was negatively correlated with larval SAIs at 1 and 3 dph. DHA is vitally important to larval ontology and correct development. DHA is especially important as a precursor for many different types of phospholipids used in the development of neural and retinal tissue; this is crucially relevant in the case of rapid growing larvae like mahi-mahi that have a higher percentage of neural tissue relative to the larval body mass (Bell et al., 1995). Deficiencies in DHA have been linked to vision impairment in herring Clupea harengus (Linnaeus, 1758; Bell et al., 1995) and the inability for juveniles to school properly shown in yellowtail Seriola quinqueradiata (Temminck and Schlegel, 1845; Ishizaki et al., 2001). DHA and EPA compete for the formation of structural phospholipids with DHA being more biologically favored and is selectively incorporated at higher rates than EPA into polar lipids of the yolk.
One tocopherol molecule has the ability to protect larval survival in this study. Vitamin E acts as antioxidant in its primary effect on larval survival in mahi-mahi. Possible reduced DHA levels seen in captivity did not illicit a negative effect on larval survival in mahi-mahi. It could be argued that for mahi-mahi which has one of the fastest embryonic development as a selective advantage.

Evidence on the nutritional composition of gonads and spawns collected over time in captivity corroborated that key nutrients such as vitamin E and amino acids were strongly correlated with larval survival over time. It has been demonstrated that 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 into the developing oocytes to give additional energy reserves needed for hatching and larval development as a selective advantage.

**5 | CONCLUSION**

Overall, this study elucidated changes in the egg morphometrics and larval survival over time. It has been demonstrated 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. Evidence on the nutritional composition of gonads and spawns collected over time in captivity corroborated that key nutrients such as vitamin E and amino acids were strongly correlated with larval survival 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 with larval survival in this study. Vitamin E acts as antioxidant in its primarily role to prevent lipid peroxidation from ROS during high periods of metabolic activity such as embryonic development (Palace & Werner, 2006). One tocopherol molecule has the ability to protect up to 1,000 lipid molecules from peroxidation with α-tocopherol being the most biopotent and therefore it 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 (Linnaeus, 1758) that elevated levels of PUFAs with insufficient levels of vitamin E in the broodstock diet caused larval yolk sac hypertrophy, oxidative type lesions and mortality (Fernández-Palacios, Izquierdo, Gonzalez, Robaina, & Valencia 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 its incorporation into developing oocytes to protect against lipid peroxidation. Vitamin E levels were conserved in the CG (2.91 mg per 100 g) in comparison to the WG (1.63 mg per 100 g) alluding to a diet in captivity that is high in this particular antioxidant. Vitamin C increases the biopotency of vitamin E and is also a potent antioxidant (Palace & Werner, 2006). This vitamin was not correlated with larval survival and also 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 per 100 g) than in the WG (197 IU per 100 g) 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 levels of vitamin A esters found in the liver (Furuita, Tanaka, Yamamoto, Shiraishi & Takeuchi, 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 the earlier work of Ostrowski and Divakaran (1991) with utilization of amino acids as energy substrates in mahi-mahi eggs and larvae. Amino acids act as an important building block for structural proteins and as a secondary energy source. Almost all amino acids showed strong correlations with larval survival except for tryptophan and valine that 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 during hatching minimizes oxygen consumption which may be limiting during this critical period. In addition, marine finfish larval tail buds at the time of hatching consist of undifferentiated white muscle that do not utilize lipids very well (Crabtree & Newsholme, 1972). Interestingly, there were no differences between 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 into the developing oocytes to give additional energy reserves needed for hatching and larval development as a selective advantage.

**TABLE 6** Pearson correlations of amino acids with egg morphometrics and larval survival indices over time in captivity

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>SAI (1 dph)</th>
<th>SAI (3 dph)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>.996*</td>
<td>.973*</td>
</tr>
<tr>
<td>Histidine</td>
<td>.992*</td>
<td>.962*</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>.996*</td>
<td>.978*</td>
</tr>
<tr>
<td>Leucine</td>
<td>.998*</td>
<td>.982*</td>
</tr>
<tr>
<td>Total lysine</td>
<td>.991*</td>
<td>.980*</td>
</tr>
<tr>
<td>Methionine</td>
<td>.812*</td>
<td>.874*</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>.998*</td>
<td>.979*</td>
</tr>
<tr>
<td>Threonine</td>
<td>.991*</td>
<td>.980*</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Valine</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

Asterisks denote statistical significance (p < .05).

*Denotes no change in level of amino acid over time.

KLOEBLEN ET AL.
survival. It was also found that EPA and its related ratios did not have a negative effect on larval survival, and 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 groundwork for future broodstock nutrition studies for high-performance pelagic species.

ACKNOWLEDGMENTS

This research was made possible by a grant from the Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER). Data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org (https://doi.org/10.7266/n7wd3xm0). It should also be acknowledged that the author, Steven Kloeblen, was funded by a research agreement between the University of Miami and Open Blue Sea Farms. In addition, the authors would like to thank the staff and students at the University of Miami Experimental Hatchery for all their support and hard work to make this study possible. The authors would also like to thank Kevin Schauer for his assistance in performing the ammonia analysis for the PELEC systems. Lastly, we are thankful for the assistance of Captain Ray Rosher and the crew of the Miss Britt for their help in capturing the mahi-mahi broodstock for this study. All procedures and animals used in this study were done so in accordance with the University of Miami Institutional Animal Care and Use Committee (IACUC) protocol numbers 15-019 and 12-064.

REFERENCES


