



Egg quality traits and predictors of embryo and fry viability in red snapper *Lutjanus campechanus*



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ABSTRACT

The quality of red snapper eggs is highly variable and unpredictable in aquaculture, leading to high mortality during early larval rearing. In this work, the viability of red snapper eggs was monitored from fertilization until unfed larvae expired because of exhaustion of vitelline reserves to determine egg quality traits in this species. The spawns were obtained via strip spawning wild-caught females following hormonal induction with chorionic gonadotropin. Females were induced immediately after capture (wild group, $n = 17$) or held captive for the entire maturation period prior to induction (captive group, $n = 7$). Candidate predictors of egg quality measured on the female parent at the time of induction or on the spawn at ovulation were evaluated using correlation and multiple regression analysis. The fertilization rate, the hatching rate, and the duration of survival of unfed larvae post hatch were weakly correlated to each other ($-0.23 < r < -0.08$), revealing occurrence of distinct and independent components of egg quality. Spawns from captive females were characterized by a longer latency interval between hormonal induction and ovulation, lower fecundity, and lower hatching rates, as compared to those from wild females. Among the wild brood fish, a positive correlation was observed between the age of the female and the hatching rate. The best model optimized during stepwise multiple regression analysis of hatching rate data only explained 34% of the variance for this trait and no model could be optimized for the prediction of fertilization rate or the duration of survival post hatch. These results highlight the need to develop alternative egg quality measures to predict the viability of fry with confidence.

1. Introduction

The red snapper is one of the most economically important marine fish species in the southeastern United States, especially in the Gulf of Mexico (GOM), where it is targeted by recreational and commercial fisheries. The red snapper is a primary candidate for the developing marine aquaculture industry in the United States because of its high value as a food and game fish, and its overfished status. Achieving a reliable supply of high quality eggs is a primary challenge that needs to be overcome when developing the aquaculture of new marine species (Mylonas et al., 2010). Thus, initial research efforts on red snapper have focused mainly on controlling reproduction (Phelps et al., 2009). While spermiation is inhibited in red snapper males reared in captivity, most red snapper female broodstock initiate oogenesis (Bardon-Albaret et al., 2015); however, oocyte maturation is infrequent and spontaneous spawns in tanks are scarce and typically unfertilized (Phelps et al., 2009). Volitional tank spawning is expected to lead to a better fry quality (Mylonas and Zohar, 2001; Papanikos et al., 2003). In red snapper, however, the rarity and unpredictability of volitional spawns,

and their low fertility lead to the use of hormonal induction and in vitro fertilization to produce embryos for aquaculture. Maturation and release of gametes are routinely induced in wild-caught females with a single injection of human chorionic gonadotropin (hCG) (Minton et al., 1983) and eggs are collected by strip spawning and fertilized *in-vitro*. Fertile spawns are achieved with high rate of success ($> 50\%$), although the viability of the obtained eggs is highly variable and unpredictable. The uncontrolled variability of survival to first feeding impairs aquaculture production because of the waste of time and resources spent on egg batches with low survival potential. It also impacts interpretations of experiments aiming to improve protocols for early feeding. Developing reliable criteria to characterize egg quality is therefore critical to select spawns that will produce fry with high viability potential for culture trials, and to evaluate effectively candidate factors influencing egg quality.

Research on the determinants of egg quality has been extensive during the past few decades (Migaud et al. 2013; Bobe and Labbé, 2010; Brooks et al., 1997; Kjörsvik et al., 1990 for reviews). Yet the success of these efforts is still moderate in marine fishes (Thorsen et al., 2003) and

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comparisons among studies is difficult because of the lack of standardization of methodologies and criteria used to define egg quality. Most studies include the fertilization and the hatching rates in the evaluation of egg quality as recommended by Brooks et al. (1997). The impacts of egg quality on the fitness of fry until first feeding and at later stages of development was recognized by some authors (e.g. Kjørsvik et al., 1990; Bromage et al., 1994), but was rarely studied. As pointed out by Bobe and Labbé (2010), assessments of egg quality must include the evaluation of survival rates at multiple time points during embryonic and early larval life. Because developmental failures at fertilization, hatch or post hatch may be due to different causes, the viability at these stages need to be treated as separate egg quality traits. In red snapper, peaks of mortality were reported during embryonic development before gastrulation (Papanikos et al., 2003; Woodard, 2003) and at different times post hatch mainly when the larvae transition from endogenous to exogenous feeding (Williams et al., 2004). Fry mortality was particularly elevated during the first 36 h post hatch (hph), where it was estimated to average 60% (Phelps et al., 2009). However, the timing of these mortality episodes, in particular the severity and variability of late mortality events (after 36 hph), has not been clearly documented because survival was only recorded at one arbitrary pre-set time point, usually 36 hph. It is unclear if developmental failures at different stages (fertilization, embryonic development, and after hatch) are independent and therefore reflect different causes and distinct aspects of egg quality, as discussed above.

The first objective of this work was to study the timing of mortality events of red snapper eggs at various stages post fertilization to determine a suite of egg quality traits characterizing comprehensively the developmental potential of a spawn. The correlation of these traits with spawning female characteristics or parameters observed on the spawn at the time of fertilization was also studied aiming to predict egg quality. Because of the reported disruptions of reproduction in aquaculture, spawns from mature females caught in the wild and induced immediately for ovulation were compared to those from females held captive during maturation to evaluate possible effects of captive rearing conditions on egg quality traits.

2. Materials and methods

A group of females were caught in the wild and held in captivity for at least one year prior to spawning experiments (captive females). Hence, the captive holding period encompassed the entire gametogenesis. A second group of females were caught in the wild on the day of hormonal induction (wild females). The handling of females from the two groups for hormonal induction, spawning, and evaluation of spawns was identical to allow comparison of the results. The spawns were obtained by strip spawning and *in-vitro* fertilization of ovulated eggs following induction with chorionic gonadotropin. All spawning trials were performed between 2010 and 2013, during the natural spawning season reported for wild red snapper in the northeastern GOM (May to September, Collins et al., 1996).

2.1. Broodstock acquisition

Broodstock were collected by hook and line on habitats located 20–25 miles south of the Alabama coast. Capture depth ranged between 15 and 25 m. Fish weight ranged between 1.43 and 4.32 kg, averaged 2.53 ± 0.70 kg (mean \pm SD). Red snapper in this size range were likely to have reached sexual maturity and tolerate better handling than larger fish (Phelps et al., 2009). The sex of each fish was determined at capture by visual examination of the aspect of the genital papilla and by

the observation of sperm release following gentle abdominal pressure in males. Fish were transported to facilities at the Thad Cochran Marine Aquaculture Center of the University of Southern Mississippi Gulf Coast Research Laboratory (TCMAC).

2.2. Prophylaxis procedures and acclimation of captive fish

Fish assigned to the captive group received prophylactic treatments upon arrival at the hatchery. Treatments include a 5 min freshwater bath, followed by a 24 h static bath of Praziquantel (5 mg L^{-1}); then fish were subjected to a copper treatment (0.2 mg L^{-1} copper-sulfate) for a period of at least 3 weeks to remove all stages of *Amyloodinium ocellatum* parasites. After that, fish free of parasites in gills were allocated to brood tanks.

A passive integrated transponder (PIT) tag (Oregon RFID, Portland OR) was implanted in each brood fish to allow individual identification. Then, fish were allocated to one of four 12-m³ broodtanks, which were stocked at $3.85 \pm 1.91 \text{ kg m}^{-3}$ and sex ratio 1:1. Each tank was connected to individual recirculating seawater filtration system. Artificial photothermal cycling simulated natural seasonal variations previously recorded in offshore Mississippi waters (NOAA National Data Buoy Center, Station 42 067). The diet included fish, shrimp, and squid at a 2:1:1 ratio respectively. Fish were fed to satiation, three times per week. During the gametogenesis and spawning periods, the diet was partially substituted with a supplement supplied twice a week at 1% body weight. The supplement consisted of lecithin (0.2%), a vitamin premix (2%) (prepared according to Moon and Gatlin, 1991), fish-meal (3%), and fish-oil (3%), which were combined in a gelatin based preparation.

2.3. Fish selection

Captive fish selection was performed during three trials when temperature in the tanks was greater than 26 °C, matching spawning conditions for red snapper in the natural environment. Fish were individually weighed and measured under anesthesia (100 mg L^{-1} of Tricaine Methane Sulfonate, MS-222). The males were selected for spawning experiments when they released sperm following application of a gentle pressure on both sides of the abdomen toward the papilla. The oocyte maturity stage was determined via observation of a sample of oocytes obtained by an intra-ovarian biopsy, using a Frydman[®] memory form polyethylene catheter (CDD laboratory). Oocytes were scattered in a petri dish and covered with Serra's solution (ethanol:formalin:acetic acid, 6:3:1 by volume) to clear the cytoplasm and determine the oocyte maturation stage. Females with fully-grown oocytes were selected, and pictures of the biopsy were taken for subsequent staging as described in Section 2.7. Fully-grown oocytes were characterized by diameter greater than 300 μm , with cytoplasm entirely clear in Serra's solution.

Mature wild fish were captured during five collection trips performed during the natural spawning season. Fish were directly transported to the hatchery facility and selected for spawning experiments as described above for the captive group. Selected fish were weighed, measured, and hormonally induced. Hormonal induction occurred within 8 h of fish collection.

2.4. Hormonal induction

Gamete maturation was induced with a single intramuscular injection of human Chorionic Gonadotropin (hCG, VWR Scientific Products Inc., Suwanee, GA) at a dose of 1100 IU kg^{-1} of body weight for females and 550 IU kg^{-1} for males (Minton et al., 1983). Following

injection, fish were transferred into 400-L aquaria (4–5 fish per aquarium) where they could be easily observed during the induction period. Aquaria were connected to a recirculating seawater filtration system maintained at $27 \pm 1^\circ\text{C}$ and $30 \pm 2\text{ g L}^{-1}$. Fish were individually marked by a small specific cut on their caudal fins to ease visual identification of individual females.

2.5. Monitoring of ovulation and strip spawning

Females were checked for ovulation beginning 24 h post induction. Ovulation was detected by the release of ova following a gentle pressure on the flanks of the female. Ovulating females were anesthetized by adding 100 mg L^{-1} MS-222 to the water in the holding aquarium. Two males were simultaneously sedated in a separate bath with the same dose of MS-222. Ova were stripped from the female into a graduated bowl containing 500-mL of seawater. Sperm was simultaneously stripped from the males into the bowl and immediately mixed with the eggs. Sperm was added in large excess (obtained from multiple strokes applied to each male), to ensure that milt was not a limiting factor for fertilization. Two to five minutes were allowed for fertilization following addition of the sperm. After fertilization, a 1–3-mL sample was taken from the homogenized mixture of eggs in seawater, and the eggs were counted under a stereo microscope. The total number of eggs of the spawn (fecundity) was estimated by extrapolation of the count to the volume containing the spawn.

The floating eggs were separated by decantation, stocked in an incubator at 1 egg mL^{-1} , and homogenized via gentle aeration until the determination of the fertilization rate at 1 h post fertilization (hpf). The fertilization rate was determined by counting the proportion of eggs having completed the second embryonic division in a subsample of at least 100 eggs. Hatching rates were estimated at 36 hpf by counting the numbers of hatched larvae and unhatched eggs in two replicate samples of approximately 100 eggs each, kept in separate 1-L glass beakers in static conditions ($26 \pm 1^\circ\text{C}$, $30 \pm 1\text{ g L}^{-1}$). Spawns with fertilization rates greater than 70% were used in the present study. Normally, spawns showing low fertilization rate are not used for hatchery production.

2.6. Kinetics of mortality post fertilization

The survival of eggs and starved larvae from spawns of captive and wild broodstock was monitored over time. Egg quality may still impact viability and fitness during the early larval phases of exogenous feeding. Nonetheless, the effects of egg quality on survival and growth become confounded with environmental and husbandry factors once live food is added to culture tanks. Thus, in this study, egg quality was monitored as the fertilization rate, the hatching rate and survival post hatch until the end of the endogenous feeding phase. Random samples of ~100 eggs from each spawn were stocked in 14 1-L beakers filled with sea water ($30 \pm 1\text{ g L}^{-1}$ salinity), maintained in static conditions at $26 \pm 1^\circ\text{C}$. Two of the beakers were randomly selected every 24 h, beginning at 36 hpf, and the unhatched eggs, live and dead larvae were counted. Because of the potential stress inflicted on the larvae by the process of counting, replicate beakers were counted once and then discarded. Subsequent survival counts were performed on other duplicate beakers from the 14 beakers stocked for each spawn. Daily monitoring continued until all larvae died (maximum 7 dpf in all cases).

2.7. Spawn and parental parameters

The parameters recorded for each spawn were the date of spawn

(Date), female weight (Wt), total length (TL), Fulton's condition coefficient (K), oocyte stage (Stage), latency time (Latency), ova pH, relative fecundity, age, and hepatosomatic index (HSI).

The parameter 'Date' was the number of days elapsed between the beginning of the spawning season and the date of the spawn. May 1st was considered to be the start of the spawning season in the northern GOM (Collins et al., 1996).

Female weight (Wt, in g) and total length (TL, in cm) were measured before hormonal induction and the Fulton's condition coefficient (K) calculated according to the formula $K = 100\text{ Wt TL}^{-3}$.

The maturation stage reached by selected females (parameter 'Stage') was determined during examination of the ovarian biopsies fixed at the time of induction. Pre-ovulatory oocytes were classified into five stages reflecting consecutive changes of the aspect of the vitellus in maturing oocytes described by Żarski et al. (2011): Stage I: uniform yolk, no oil droplet visible; Stage II: small and poorly visible oil droplets filling the entire cytoplasm of the oocytes; Stage III: oil droplets well defined, peripheral hyalinization; Stage IV: ring of large forming droplets centered around the germinal vesicle; and Stage V: less than five large oil droplets coalesced with diameter about $\frac{1}{4}$ of the germinal vesicle diameter. The entire biopsy-sample was examined (> 30 oocytes) and the stage reached by the most advanced cohort of oocytes was recorded.

The latency period ('Latency') was defined as the time interval between hormonal stimulation and detection of ovulation.

The pH of the unfertilized ova was measured immediately when ovulation was detected. A subsample of the spawn was obtained in a dry 10-ml beaker during stripping and the pH was measured using a pH pen-meter (YSI®, pH-100 with piercing electrode).

Fecundity was measured as the total number of eggs released by the female and was estimated volumetrically by counting the number of fertilized eggs in a 1–3-mL subsample of the spawn. Fecundity was standardized to the female weight to obtain the relative fecundity (Rfecundity).

After spawning, contributing females were euthanized using a lethal dose of MS-222 (> 400 mg L^{-1}). Otoliths were removed to determine the age of each female by otolith increment analysis according to protocols described in VanderKooij and Guindon-Tisdell (2003). The liver was weighed and standardized to body weight to calculate the hepatosomatic index ($\text{HSI} = 100\text{ liver Wt} \times \text{total body Wt}^{-1}$).

The spawn quality traits measured were the fertilization rate, the hatching rate, and the 'survival duration'. The latter parameter was derived statistically as described below and characterized the viability of larvae post hatch.

2.8. Statistical analyses

The embryonic mortality rate was calculated as fertilization rate minus hatching rate. The hatching rate was standardized to the fertilization rate (i.e. standardized hatching rate = hatching rate/fertilization rate) to distinguish the rate of success during embryonic development from fertilization rate. Similarly, survival post hatch was standardized to the hatching rate to distinguish mortality post hatch from embryonic mortality.

Statistical tests were performed using the Statistical Analysis Software (SAS®) version 9.3 (SAS Institute, NC), SAS procedures are cited preceded by the term PROC below. The assumptions of normality and homoscedasticity were tested using the Shapiro-Wilk test in PROC UNIVARIATE and the Levene test in PROC GLM, respectively. To improve the normality of distributions and reduce heterogeneity of variances, variables with poor normality ($P < 0.01$) were transformed.

Table 1

Variables (mean \pm SD; range in brackets) obtained from wild and captive females /spawns. These two groups were compared by Student's *t*-test. Significant ($P < 0.05$) *P*-values are in bold. In all tests $df = 22$, except for Age and HSI ($df = 21$).

	Wild (n = 17)	Captive (n = 7)	<i>P</i> -values
Date (days)	67.0 \pm 37.0 (23–135)	85.6 \pm 12.0 (73–109)	0.212
Age	5.9 \pm 0.93 (4–7)	4.1 \pm 1.2 (3–6)	< 0.001
Wt (kg)	2.50 \pm 0.67 (1.55–4.32)	2.58 \pm 0.83 (1.43–3.70)	0.802
TL (cm)	56.5 \pm 4.4 (49.5–65)	53.9 \pm 5.1 (47.5–60.5)	0.213
K	1.36 \pm 0.12 (1.12–1.57)	1.60 \pm 0.21 (1.34–1.94)	0.001
Oocyte stage	3.5 \pm 1.5 (1–5)	2.6 \pm 1.4 (1–5)	0.179
Latency Time (h)	28.89 \pm 1.06 (27.0–31.0)	32.86 \pm 1.18 (32.0–34.5)	< 0.001
Ova pH	7.44 \pm 0.51 (6.56–8.35)	7.17 \pm 0.54 (6.15–7.82)	0.251
HSI	7.86 \pm 1.81 (5.66–11.53)	9.48 \pm 4.75 (5.04–17.70)	0.411
Rfecundity (egg.kg ⁻¹)	100,891 \pm 48,878 (23,502–202,024)	30,653 \pm 21,028 (4729–65,840)	< 0.001
Fert. rate (%)	92.7 \pm 5.3 (80–100)	83.7 \pm 10.0 (71–97)	0.016
Hatch. rate (%)	81.3 \pm 17.0 (27–95)	54.6 \pm 19.7 (31–82)	0.005
Survival duration (dpf)	3.9 \pm 1.6 (1–6)	3.4 \pm 1.8 (1–6)	0.598
Quality (0/1)	0.71 \pm 0.47	0.71 \pm 0.49	0.970

Data expressed in percentages (fertilization rate, standardized hatch rates, and survival duration standardized to its maximum value, i.e. 6 dpf) were subjected to angular (arcsine square root) transformation, and the relative fecundity was log transformed. An allometric transformation was used for HSI. The weights of the liver and the female body were log transformed before HSI calculation, and residuals of the resulting linear regression between the two variables (resHSI) were used to meet the assumption of the lack of correlation between response variables for multiple linear regressions (Anderson, 2001).

The percentage of surviving larvae was compared among days post fertilization in a one way ANOVA in PROC GLM (time was treated as a fixed factor), followed by a post-hoc Fisher's Least Significant Difference (LSD) test. For each spawn, the day immediately preceding the first significant decrease in larval survival is referred hereafter as the survival duration. In all studied spawns, the percentage of surviving larvae decreased rapidly after that day.

An unpaired Student's *t*-test implemented in PROC TTEST was used to compare mean values of all parameters between spawns from captive and wild females (Captive parameter).

Pearson's correlation coefficients (*r*) were calculated in PROC CORR and were determined between variables along with the probability *P* that $r = 0$. Correlations were considered significant when the associated *P*-value was less than 0.05. Stepwise multiple linear regressions were conducted in PROC REG to build models predicting the spawn quality traits (relative fecundity, fertilization rate, standardized hatching rate, and standardized survival duration) with an optimal subset of the spawn and maternal parameters. Because significant differences were observed between spawns from wild and captive females for several parameters, models were built using the data from wild females (n = 17). Models were computed using a stepwise algorithm allowing variable entry and retention at the default significance level $P = 0.15$.

3. Results

Fifty-seven wild and 19 captive females were selected during eight trials. Ovulation was detected in 44 females (33 wild and 11 captive) between 24 and 35 h post induction. Twenty-four females (17 wild and 7 captive) were monitored.

3.1. Kinetics of mortality during early larval development

The results of monitoring of the 24 spawns until complete mortality was observed (up to 7 dpf) are available in Appendix A. The fertilization rate averaged $90.0 \pm 8.0\%$ and ranged from 71.3 to 100.0%. The fertilization rates of spawns from captive fish were slightly lower than those of spawns from wild fish (83.7% and 92.7% on average, respectively, Table 1). The mean hatching rate for the 24 studied spawns was $73.5 \pm 21.4\%$. The distribution of hatching rates had greater variance (27–95%, SD = 21.4%) than that of fertilization rates (71–100%, SD = 8.0%).

The hatching rates of spawns from captive females were significantly lower than those from wild females (54.6% versus 81.3%, Table 1). The embryonic mortality rate averaged 29% in spawns from captive females versus 16% in spawns from wild females ($P = 0.023$). Three out of seven spawns from captive females had hatching rates lower than 40%. Hatching rates for the four other spawns from captive females ranged between 56.5% and 82.3% (Appendix A). The correlation between the fertilization rate and the standardized hatching rate was moderate ($r = 0.42$, $P = 0.041$).

The survival duration for the 24 spawns averaged 3.8 ± 1.7 dpf, ranged from 1 to 6 dpf, and did not differ significantly between wild and captive spawns ($P = 0.618$). Larvae still alive at 4 dpf would have the opportunity to attempt exogenous feeding for at least 24 h in aquaculture tanks where live feeds are offered beginning at 3 dpf. Therefore, spawns with survival durations of 4 dpf or more (12 wild spawns and 5 captive spawns) would be expected to yield high proportions of larvae successfully initiating exogenous feeding. Seven spawns had a survival duration between 1 and 3 dpf, meaning that a significant decrease in survival rate happened before larvae could attempt exogenous feeding. The survival duration was not significantly correlated to the fertilization rate ($r = -0.167$, $P = 0.435$) or the hatch rate ($r = -0.239$, $P = 0.261$). The weak correlation between the survival duration and the fertilization rate or the hatching rate indicates that some spawns can have a good fertilization, or a good hatching rate but poor survival duration.

Table 2

Pearson's correlation coefficients between parameters measured on wild (below diagonal) and captive (above diagonal) females. Significant values are in bold font. Symbols denote the level of significance of correlations: * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$.

	Date	Age	Wt	TL	K	Stage	Latency	Ova pH	HSI	R fecundity	Fert. rate	Hatch. rate	Survival duration
Date	1	0.42	0.02	0.36	-0.48	0.6	-0.46	-0.28	-0.54	0.79*	0.26	0.16	-0.48
Age	0.13	1	-0.22	-0.22	0.1	-0.15	-0.27	0.15	0.09	0.08	0.75	0.64	-0.38
Wt	-0.59*	0.15	1	0.91**	0.58	0.27	-0.01	-0.81*	0.21	-0.15	-0.03	0.17	-0.68
TL	-0.50*	0.20	0.92***	1	0.21	0.57	-0.09	-0.87*	-0.14	0.2	-0.14	0.02	-0.69
K	-0.49*	-0.01	0.46	0.12	1	-0.48	-0.04	-0.2	0.78*	-0.67	0.38	0.5	-0.4
Stage	0.34	0.02	-0.24	-0.26	-0.08	1	-0.4	-0.73	-0.73	0.84*	-0.37	-0.32	-0.08
Latency	0.73***	0.03	-0.27	-0.08	-0.61**	0.26	1	0.23	0.34	-0.63	-0.11	0.01	0.02
Ova pH	0.55*	0.11	0.05	0.14	-0.31	-0.08	0.64**	1	0.16	-0.32	0.11	-0.07	0.49
HSI	-0.51*	-0.26	-0.08	-0.12	0.14	-0.16	-0.62*	-0.32	1	-0.83*	0.6	0.69	-0.28
R fecundity	-0.42	0.06	0.33	0.44	-0.16	0.27	-0.11	-0.38	0.06	1	-0.19	-0.29	0.06
Fert. rate	0.05	0.10	0.1	0.16	-0.05	0.15	0.2	-0.06	-0.05	0.13	1	0.96***	-0.56
Hatch. rate	0.06	0.58**	-0.09	-0.10	-0.21	-0.01	-0.26	-0.27	0.11	0.21	-0.23	1	0.49
Survival duration	-0.34	0.04	-0.19	0.64	-0.09	-0.08	0.08	-0.03	0.21	0.39	-0.08	-0.13	1

3.2. Comparison of the wild and captive female groups

The female body weight and total length ranged from 1.43 to 4.32 kg and 47.5–65.0 cm, respectively (Table 1). The female mean length and weight did not differ significantly between the wild and captive groups. However, Fulton's condition coefficient K was significantly larger for captive fish (1.60 versus 1.36, Table 1).

Ovulation was detected on average 30 h after administration of the hormonal stimulation, but wild females responded significantly earlier than captive ones (Table 1). Mean response time for wild females was 28.9 h (range 27–31 h) versus on average at 32.9 h (range 32–34.5 h) for captive females. Wild females produced up to 200,000 eggs Kg^{-1} (mean 100,891 eggs Kg^{-1}), but captive females only produced 30,000 eggs Kg^{-1} on average with a maximum of 65,000 eggs Kg^{-1} (Rfecundity parameter, Table 1).

Otolith increment analysis revealed that female age ranged between three and seven years old (average 5 years old). Wild females were significantly older (5.9 years old on average) than captive ones (4.1 years old on average).

3.3. Correlation between pre-spawning parameters and egg quality traits

Because captive females differed from wild females for several pre-spawning parameters, correlation analysis was conducted separately for the 17 spawns from wild fish and the 7 from captive fish. Pearson's correlations between variables for the two groups are presented in Table 2.

For both captive and wild fish, significant positive correlations were observed between weight and length ($r > 0.9$, $0.001 < P < 0.005$). There was a positive correlation between the age of wild females and the hatching rate of their spawn ($r = 0.58$, $P = 0.018$), indicating that a better hatching rate was generally obtained with older fish in this group. None of the other parameters recorded prior to spawning were significantly correlated to the egg quality traits (fertilization rate, standardized hatching rate, and survival duration).

Captive and wild females showed different patterns of correlations. In the wild group, the date of spawn was significantly negatively correlated to the weight, the length, the HSI, and Fulton's condition coefficient ($r = -0.49$ to -0.59 , $0.01 < P < 0.05$), indicating a decrease of these parameters as the spawning season progressed.

Spawning date was positively correlated with the ova pH ($r = 0.55$, $P = 0.023$) and the latency time ($r = 0.73$, $P = 0.001$), indicating an increase of the ova pH and the time to respond to hormonal induction over the course of the spawning season. The latency time was negatively correlated with Fulton's condition coefficient ($r = -0.61$, $P = 0.009$) and HSI ($r = -0.62$, $P = 0.011$), indicating a longer response time for fish with lower condition. Finally, a significant positive correlation was found between latency time and ova pH ($r = 0.64$, $P = 0.006$). None of these correlations were significant in the captive group ($n = 7$).

For the captive females group, fertilization and hatching rates were strongly correlated ($r = 0.96$, $P = 0.001$). The relative fecundity was positively correlated to the spawning date ($r = 0.79$, $P = 0.034$). Significant positive correlations were also detected between relative fecundity and oocyte stage ($r = 0.84$, $P = 0.019$) and between HSI and Fulton's condition factor ($r = 0.78$, $P = 0.038$). Negative correlations were found between relative fecundity and HSI ($r = -0.83$, $P = 0.022$), and between the ova pH and female weight ($r = -0.81$, $P = 0.026$) and length ($r = -0.87$, $P = 0.011$) respectively. The latter correlation indicates that the pH of the ova from larger captive females was lower than that of smaller fish. Stepwise multiple linear regression analysis was used to predict egg quality traits (fertilization rate, hatching rate, and survival duration) as well as relative fecundity of wild fish ($N = 17$) based on the parameters recorded on females and their ova at the time of spawning. The optimal regression model for the prediction of the fecundity employed one single parameter (the oocyte stage assessed prior to hormonal induction). The model explained only a small part of the variance ($r^2 = 0.18$) that was not significant ($P = 0.099$). The stepwise model retained for standardized hatching rate only included one predictor parameter (the age of the female parent), and explained 34% of the variance. These two models are described below.

$$\text{Fecundity} = 0.05 \text{ oocyte stage} + 4.80 \quad P = 0.099 \quad r^2 = 0.18$$

$$\text{Hatching rate} = 0.17 \text{ female age} + 0.29 \quad P = 0.018 \quad r^2 = 0.34$$

No model could be optimized for the fertilization rate and survival duration, reflecting the lack of correlation of these two egg quality traits to any of the parameters measured in the study.

4. Discussion

4.1. Timing of mortality events and egg quality traits

Survival at the time of first feeding varied greatly among spawns (from 0 to 93%) and there was no correlations between the fertilization rate, the hatching rate, and the survival duration. This indicates that, even when the fertilization rate is relatively high (> 70% for all studied spawns), mortality during the incubation period can occur resulting in poor hatching rates. Similarly, in seven spawns, hatching rates were high (> 70%), but survival decreased significantly before mouth opening (3 dpf), rapidly followed by complete mortality with less than 30% larvae alive a day later (i.e. larvae from these spawns would not have had the opportunity to initiate exogenous feeding). Some studies in other species showed correlations between the fertilization rate and the hatching rate (e.g. *Hippoglossus hippoglossus*, Babiak et al., 2008), whereas others, as our study, revealed low and non-significant correlations between spawn quality parameters (Kjørsvik et al., 1990; Yousefian et al., 2010). The lack of correlation between the spawn parameters recorded routinely in the hatchery (fertilization and hatching rates) and the potential of larvae to transition from the maternally controlled endogenous feeding phase to exogenous feeding indicates that different developmental failures are likely involved in the three types of mortality events. In consequence, the simple measurement of fertilization and/or hatching rates is insufficient to predict the viability of larvae at first feeding. All three egg quality traits defined above (fertilization rate, hatching rate, and survival duration) need to be monitored for a comprehensive assessment of the viability of a spawn.

4.2. Egg quality and reproductive traits in 'wild' and 'captive' females

Spawns obtained from captive red snapper females had significantly higher rates of embryonic mortality than those of wild females. In addition, the results obtained for several parameters measured in the study indicated that the reproductive process was disrupted in captive females. The majority of wild females displayed fully grown oocytes. This observation is consistent with previous reports of red snapper maturation in the wild that indicate a high frequency of spawning (up to every 4 days, Brown-Peterson et al., 2008). In contrast, partial or complete lack of vitellogenesis was observed in more than 80% of the 106 captive females. No signs of ovulation, prior to induction, was detected in the captive group and oocyte staging revealed that only 28.6% of the captive females displayed Stage IV oocytes (58% in wild females) versus 57% found with Stage II oocytes (23.5% for wild females). Relative fecundity was three times lower for spawns from captive fish, indicating that only a small number of oocytes were responsive to the hormonal treatment and could complete the final steps of maturation and ovulation. The latency time was about 4 h longer in captive females, implying that even responsive oocytes were less competent than those in wild females (Gohin et al., 2010; Zuccotti et al., 2011). These results suggest that the lower fecundity and viability of embryos in captive females may be due to the lower competency of oocytes at induction, probably resulting from disruptions of the reproductive process in captive conditions.

The females in the captive group were significantly younger than those of the wild group. These differences could not be prevented, as age could only be determined after spawning experiments via otolith analysis. The age at first maturity for red snapper females in the Northern Gulf of Mexico was reported to be 2–4 years old (Collins et al., 1996; Woods et al., 2003). All the females from the wild group were 4

years old or older and therefore had likely engaged in spawning activity prior to our spawning trials. On the other hand, 57% of captive females were 3–4 years old at spawning and had been caught in the wild when they were 2–3 years old. Some of these females may not have reached sexual maturity prior to being brought to the laboratory and would not have completed a full cycle of oocyte maturation prior to hormonal induction. First-spawning fish often show low fecundity and low-quality spawns (Berkeley et al., 2004; Jeuthe et al., 2013; Targońska et al., 2012; White and Palmer, 2004). Thus, the occurrence of first-spawning females in the captive group could explain in part the reduced performance of this group.

Captive fish had a higher Fulton's condition factor. This parameter may indicate the general fitness and nutritional status (Bolger and Connolly, 1989). In the present study, higher K in captive females probably reflects greater food availability, and also the lack of investment in spawning prior to the trials. The better condition of captive females may have influenced the availability of nutrients to constitute embryos endogenous reserves during vitellogenesis. However, the spawns from captive females did not show higher viability of fry, suggesting that the captive diet was deficient for some specific essential nutrients. Unsuitable broodstock diets have led to poor fry quality in several marine fishes (Izquierdo et al., 2001; Zambonino-Infante and Cahu, 2010). Suitable diets for red snapper broodstock have not been developed yet.

4.3. Correlation of egg quality traits and female or spawn parameters

The only significant correlation between egg quality and female phenotype was between the hatching rate and the female age, suggesting that older females produced eggs with a higher viability to hatch. This finding is consistent with the results of several other studies in fish (Brooks et al., 1997; Jerez et al., 2012; Jeuthe et al., 2013; Kjørsvik, 1994). The optimal regression model accounting for female age only explained 34% of the total variance, suggesting that additional parameters, not measured in this study, accounted for the observed variation in hatching rate. The morphology of blastomeres at the early embryonic cleavage stage has been found to be correlated with hatching rates in other fishes such as the Atlantic cod (Avery et al., 2009; Kjørsvik, 1994), Atlantic halibut (Shields et al., 1997), and Pacific hapuku (Kohn and Symonds, 2012). This parameter was not assessed in this work and would warrant evaluation in future studies of red snapper egg quality.

Wild fish tended to be smaller at the end of the spawning season. This result may reflect in part the reduced availability of larger fish in late summer on collecting sites following size-selective removal by fisheries. This result could also be due to a higher success obtaining spawns from smaller fish at later stages of the spawning season. Smaller fish were likely experiencing their first active reproductive cycle. Therefore, these females may have been still unresponsive if caught and induced at the beginning of the season (May) but could have responded to the hormonal treatment if captured and induced in August or September. The condition of wild fish, measured by K and the HSI, decreased during the course of the spawning season. This trend was significant and possibly reflected the reproductive investment of fish leading to the exhaustion of reserves over the course of spawning (Galloway and Munkittrick, 2006; Llanos-Rivera and Castro, 2004). The latency time following hormonal induction increased as the reproductive season progressed. This trend may reflect the lower condition and exhaustion of fish at the end of the spawning season discussed above. It could also be caused by the slightly lower temperature in the hatchery induction system (26–27 °C) as compared to the temperature

on offshore capture sites during the second part of the season (typically close to 30 °C). The viability of red snapper larvae obtained by spawning wild-caught females decreased over the course of the spawning season in the study of Bourque and Phelps (2007). Such a correlation was not detected in the present work.

The relative fecundity was best predicted by oocyte stage at hormonal induction, although the model only explained 18% of the variance. In this study, the oocyte stage was recorded as the maximum stage reached by oocytes present in a biopsy sample but the proportion of oocytes having reached this stage was not determined. A simple way to improve this assessment would be to estimate the proportion of oocytes at advanced maturational stages in the sample and use this variable as a predictor of relative fecundity. The size of the ovaries could also be assessed using ultrasound (Novelo and Tiersch, 2012) and combined with the proportion of potentially responsive oocytes discussed above to improve the prediction of relative fecundity.

No prediction model could be optimized for the other egg quality measures (fertilization rate and survival duration). This result is consistent with the lack of significance of correlations between these egg quality traits and most of the parameters evaluated in this study. The fertilization rate had a relatively narrow range because spawns with a minimum fertilization rate of 70% were intentionally selected. The fertilization potential of ova decreases rapidly (within one hour) post ovulation in red snapper (Phelps et al., 2009), sometimes before females are checked for ovulation. In consequence, the variability of fertilization rate and other egg quality traits could be due to over-ripening (post-ovulatory aging, Mohagheghi Samarin et al., 2015). The selection of spawns with high fertilization rates in this study was expected to reduce the risk of sampling overripe spawns. This risk could not be eliminated completely because there is no reliable method available to measure post-ovulatory aging or to determine the exact timing of ovulation. However, the lack of correlation between the latency duration and egg quality traits suggests that the impacts of post-ovulatory aging was moderate.

The survival duration parameter aimed to characterize the potential of hatched larvae to successfully transition to exogenous feeding. During the endogenous feeding phase that precedes this transition, larvae rely on their vitelline reserves for energy and growth. Both the quantity and quality of vitellus are important factors influencing early development (Finn and Fyhn, 2010) and, thus, future studies should evaluate the quantity and composition of vitelline reserves. In particular the content and balance of essential fatty and amino acids (Finn and Fyhn, 2010; Mommens et al., 2013), but also other nutrients such as those involved in carbohydrate metabolism (e.g. Moguel-Hernández et al., 2015) may be important predictors of larval viability post-hatch. Early results in red snapper revealed changes in the free amino acid content of eggs during the course of development (Hastey et al., 2010), although experimental alteration of this content did not improve egg viability (Hastey et al., 2015).

The low power of candidate parameters for the purpose of

predicting egg quality traits is consistent with studies in other species that concluded that egg quality is very difficult to predict with confidence (Bobe and Labbé, 2010; Brooks et al., 1997; Kjorsvik et al., 1990). Other parameters may improve our understanding of egg quality. In particular, endogenous variables such as nutrient content (Cahu et al., 2003; Zambonino-Infante and Cahu, 2010) and maternal RNA content (Bonnet et al., 2007; Chapman et al., 2014; Lubzens et al., 2017) have been shown to strongly influence fry viability in other fish species and would warrant examination in red snapper. Finally, the stress level of females and its effects on egg quality would also need to be studied, in particular in the context of strip spawns that involve manipulations of the fish.

4.4. Conclusions

This study showed that egg quality in red snapper is a complex trait. The yield of viable larvae capable of initiating exogenous feeding is determined by the cumulative outcomes of fertilization, development to hatch, and the survival duration post hatch. The lack of correlation between these three traits observed in this study indicates that they reflect distinct and independent components of egg quality that will require separate evaluation in order to predict effectively the potential of a spawn. Significant differences were found between captive and wild females for several reproductive traits including egg quality, reflecting disruptions occurring in culture conditions. Accordingly, further study of egg quality predictors will need to be conducted separately for captive and wild females. Finally, egg quality traits could not be predicted reliably by simple phenotypic variables measured on females and their gonads prior to hormonal induction. Further studies of endogenous characteristics of the ova using genomic approaches and analytical chemistry may prove useful in order to better predict and improve the quality of red snapper spawns.

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Appendix A

Date, female identification number, mean fertilization rates with 95% confidence interval, mean hatching rates \pm SD, mean survival percentage at various dates post hatch \pm SD, and survival duration (in dpf), of 24 spawns from captive and wild red snapper. Groups labeled with the same letter are not significantly different as inferred from a posteriori classification. dpf = days post fertilization.

Date (2011–2012)	Female	Fertilization ± CI	Hatch ± SD	Survival at 2 dpf ± SD	Survival at 3 dpf ± SD	Survival at 4 dpf ± SD	Survival at 5 dpf ± SD	Survival at 6 dpf ± SD	Survival duration
Captive fish									
17-Aug 2011	c1	89.72 ± 1.87	63.88 ± 8.33 ^a	39.17 ± 2.37 ^b	20.68 ± 5.49 ^{bc}	15.13 ± 21.39 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	1
12-Jul 2012	c2	90.74 ± 2.10	73.10 ± 5.35 ^a	42.13 ± 5.35 ^b	32.10 ± 2.41 ^{cd}	36.04 ± 4.10 ^{bc}	26.33 ± 0.46 ^{de}	22.68 ± 6.1 ^e	1
12-Jul 2012	c3	71.29 ± 4.03	31.39 ± 7.35 ^a	29.77 ± 7.35 ^a	37.46 ± 5.03 ^a	38.53 ± 6.74 ^a	35.08 ± 1.82 ^a	26.04 ± 8.28 ^a	6
25-Jul 2012	c4	71.34 ± 5.13	36.03 ± 2.30 ^{ab}	42.25 ± 4.70 ^a	27.97 ± 4.00 ^c	32.01 ± 5.77 ^{bc}	0.00 ± 0.00 ^d	0.00 ± 0.00 ^d	4
25-Jul 2012	c5	96.67 ± 0.49	82.31 ± 4.18 ^a	77.92 ± 4.95 ^a	82.27 ± 2.66 ^a	87.62 ± 3.71 ^a	48.02 ± 27.51 ^b	0.00 ± 0.00 ^c	4
25-Jul 2012	c6	87.50 ± 2.35	56.54 ± 3.07 ^{ab}	49.84 ± 0.22 ^b	59.29 ± 0.07 ^a	61.70 ± 5.14 ^a	8.71 ± 6.88 ^c	0.00 ± 0.00 ^d	4
25-Jul 2012	c7	78.33 ± 3.64	39.06 ± 5.20 ^a	37.41 ± 1.84 ^a	37.74 ± 3.38 ^a	41.72 ± 3.47 ^a	13.66 ± 0.88 ^b	0.00 ± 0.00 ^c	4
Captive fish	mean	83.66	54.62	45.50	42.50	44.68	18.83	6.96	3.4
n = 7	SD	10.03	19.69	15.52	21.24	23.41	18.31	11.93	1.8
Wild fish									
17-Aug 2011	w1	89.84 ± 2.02	88.56 ± 0.46 ^a	75.65 ± 3.50 ^b	88.91 ± 0.95 ^a	76.67 ± 7.07 ^b	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	4
23-May 2012	w2	87.18 ± 2.37	93.58 ± 0.87 ^a	91.65 ± 0.42 ^a	77.14 ± 9.38 ^b	89.53 ± 4.87 ^a	93.73 ± 0.44 ^a	2.70 ± 1.23 ^c	5
23-May 2012	w3	92.68 ± 1.70	85.05 ± 7.13 ^a	84.39 ± 9.85 ^a	85.28 ± 0.82 ^a	87.42 ± 3.65 ^a	91.62 ± 3.08 ^a	4.18 ± 4.43 ^b	5
23-May 2012	w4	87.59 ± 2.49	64.40 ± 0.94 ^a	58.45 ± 7.61 ^a	56.43 ± 20.55 ^a	11.98 ± 0.35 ^{bc}	22.84 ± 11.80 ^b	1.24 ± 1.76 ^{bc}	3
23-May 2012	w5	94.57 ± 1.37	74.88 ± 8.79 ^a	48.73 ± 14.43 ^b	4.29 ± 1.29 ^c	5.79 ± 8.19 ^c	1.60 ± 2.26 ^c	0.00 ± 0.00 ^c	1
23-May 2012	w6	91.30 ± 1.83	77.75 ± 1.43 ^a	85.59 ± 4.18 ^a	79.12 ± 11.86 ^a	77.96 ± 6.05 ^a	80.87 ± 4.51 ^a	58.52 ± 11.05 ^b	5
3-Jul 2012	w7	99.67 ± 0.11	95.09 ± 2.81 ^a	87.60 ± 7.23 ^a	89.50 ± 3.04 ^a	88.91 ± 6.43 ^a	89.03 ± 6.12 ^a	31.05 ± 13.77 ^b	5
3-Jul 2012	w8	99.34 ± 0.22	92.07 ± 2.13 ^a	77.54 ± 12.90 ^b	88.32 ± 1.72 ^{ab}	91.19 ± 0.68 ^a	93.05 ± 2.72 ^a	44.01 ± 1.63 ^c	5
3-Jul 2012	w9	96.99 ± 1.09	92.45 ± 1.56 ^a	88.37 ± 5.95 ^a	93.14 ± 0.86 ^a	88.64 ± 1.50 ^a	93.78 ± 2.59 ^a	33.04 ± 2.10 ^b	5
3-Jul 2012	w10	96.62 ± 0.78	92.56 ± 0.08 ^a	91.83 ± 2.42 ^a	91.04 ± 1.05 ^a	93.82 ± 0.65 ^a	14.23 ± 10.94 ^b	0.00 ± 0.00 ^c	4
3-Jul 2012	w11	100.00 ± 0.00	86.53 ± 0.39 ^a	86.53 ± 0.39 ^a	28.72 ± 1.34 ^b	30.07 ± 1.20 ^b	28.67 ± 5.00 ^b	9.43 ± 0.31 ^c	2
3-Jul 2012	w12	79.72 ± 5.56	85.74 ± 7.77 ^a	85.74 ± 7.77 ^a	85.13 ± 7.77 ^a	83.91 ± 1.70 ^a	74.70 ± 11.83 ^a	13.92 ± 7.06 ^b	5
26-Jul 2012	w13	89.06 ± 2.16	91.65 ± 0.49 ^a	92.49 ± 0.07 ^a	92.85 ± 0.32 ^a	92.34 ± 0.05 ^a	91.22 ± 0.45 ^a	33.94 ± 22.71 ^b	5
26-Jul 2012	w14	89.23 ± 2.15	68.08 ± 3.24 ^a	68.08 ± 3.24 ^a	58.46 ± 4.35 ^a	58.37 ± 3.75 ^a	45.88 ± 9.75 ^b	45.37 ± 6.55 ^b	4
26-Jul 2012	w15	95.28 ± 0.99	26.95 ± 4.74 ^a	26.95 ± 4.74 ^a	28.03 ± 1.76 ^a	31.46 ± 2.29 ^a	35.67 ± 2.78 ^a	28.39 ± 11.56 ^a	6
12-Sep 2012	w16	94.62 ± 1.77	72.74 ± 0.61 ^a	33.26 ± 5.34 ^b	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	1
12-Sep 2012	w17	91.79 ± 2.06	94.81 ± 0.72 ^a	88.27 ± 2.05 ^b	83.39 ± 3.89 ^c	25.15 ± 0.54 ^d	0.00 ± 0.0 ^e	0.00 ± 0.0 ^e	1
Wild fish	mean	92.68	81.35	74.77	66.46	60.78	50.41	12.88	3.9
n = 17	SD	5.33	17.05	20.76	31.76	34.80	39.32	21.28	1.7
Overall	mean	90.04	73.55	66.23	59.47	56.08	41.20	10.58	3.8
n = 24	SD	7.97	21.39	23.40	30.71	32.27	37.12	18.04	1.7

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