Expanded Annual Stock Assessment Survey 2011: Red Snapper Reproduction

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Summary

In 2011, an Expanded Annual Stock Assessment (EASA) survey provided Gulf-wide synoptic sampling of red snapper throughout the reproductive period (April – October). A total of 2487 red snapper were caught; the sex ratio was 1F:1M. Females were found in spawning condition throughout the US Gulf, primarily in outer shelf waters. Adding to previous findings, maturity was found to be delayed in the western Gulf (Louisiana) and females also exhibited higher spawning fraction signaling regional differences. Spawning fraction results indicated that both the frequency of spawns and the duration of spawning increased with age indicating older females spawn more batches of eggs within a year. Gear related differences were evident in that females caught by long-line exhibited higher spawning fraction than catches from vertical-line gear, consistent with differences in size/age selection. When age-dependent numbers of batches were included in the model, annual fecundity was greater at ages > 6 in comparison to previous estimates. A quality control study regarding the use of frozen ovaries indicated that freezing degrades the quality of reproductive information that can be obtained. However histological detail from frozen tissue was sufficient to determine sex and identify spawning markers (postovulatory follicles and hydrated oocytes).

Introduction

In 2011, an Expanded Annual Stock Assessment (EASA) survey was funded to contract commercial vessels, outfit them with contracted biologists, and use bottom long-line and vertical-line gear to sample species in the reef fish complex. The sampling design targeted red snapper, *Lutjanus campechanus*, among other species, across the US Gulf of Mexico. The project was unique in that it allowed Gulf-wide synoptic sampling to be conducted throughout the red snapper reproductive period (April through October 2011). Thus this project enabled a more spatially and temporally extensive survey than conducted via fishery-independent approaches in the past.

The aspect of the EASA project reported herein summarizes the red snapper reproductive traits that could be measured during this more extensive survey. One objective was to improve the measure of stock reproductive potential via estimates of sex ratio and age specific spawning fraction and batch fecundity. An early decision in the survey design was to freeze gonad samples in order to minimize the hazards from use of fixatives (formalin) in the field and to simplify procedures at sea with a desire to maximize the number of samples and ancillary data that could be taken. Thus, a field gonad sampling experiment was added to evaluate freezing the reproductive tissues as opposed to the more typical treatment of fresh fixation in preservative. In this fresh-preserved vs. frozen tissue comparison the objectives were to 1) develop any necessary calibrations for ovary weight based upon fresh, frozen and preserved ovaries, 2) determine if viable fecundity counts could be obtained from frozen subsamples of red snapper ovaries and develop corrections as needed, and 3) test the utility of frozen ovaries and subsequent histological preparations as a basis to assess key reproductive traits including development stage, and spawning markers.

Methods

More details on the sampling design, station selection, gear, and conduct of the survey can be found in Campbell et al. 2012. Briefly, red snapper were sampled via vertical-line and long-line gear from the west Florida shelf through Texas from the inner shelf to a depth of about 180 m. Fishing was conducted 12 hours each day. As each cruise was randomly assigned to time of fishing operations (midnight-to-noon or noon-to-midnight) any red snapper had an equal chance of capture at any hour of the day by design. The time-of-day of all catches and thus all samples were recorded as the gear was retrieved. All harvested red snapper were kept and processed which included measures of length (TL, FL, mm) and weight (kg). In addition, otoliths were extracted for aging, and gonads were dissected, sexed, macroscopically staged, and ovaries were frozen for further processing in the laboratory. In the laboratory, frozen ovaries were weighed (nearest 0.1 g) and sub-samples were taken from the right posterior lobe and placed into 10% neutral buffered formalin (NBF) for histological preparation (Mass Histology Services,Worcester, Mass., hematoxilyn and eosin-y stain). For any ovaries that were macroscopically observed to be hydrated (actively spawning) 2 sub-samples (ovarian core and periphery, approximately a cubic cm) were placed into 10% NBF to complete thawing and undergo fixation. Using batch fecundity methodology (Porch et al. 2007 and references within) fecundity subsamples were weighed (nearest 0.1 mg) at the lab and counts were made of hydrated oocytes. Red snapper were aged from sectioned otoliths as described in Allman and Fitzhugh (2007) and Allman et al (2012).

For the quality control investigation of the reproductive study, sampling was conducted by an experienced biologist aboard one vessel. As in the broader survey, all visibly hydrated

females were sampled for fecundity however sub-samples were taken and directly placed into 10% NBF as well as frozen (later thawed and preserved) for fecundity comparison. In addition, random females were chosen for reproductive histology comparisons (fresh-preserved and frozen) and ovary weight comparisons (fresh, frozen and thawed). Ovaries were removed from each female and weighed fresh at sea with the appropriate hanging scale (10, 100, or 1000 g range scales) and then again back in the laboratory after treatment as earlier described.

To analyze the quality control results, frozen and thawed ovary weights were regressed against fresh ovary weight. Relative fecundity (hydrated eggs/g) was compared using linear regression of frozen and fresh-preserved tissues. Slides of ovarian tissue chosen for histological comparison of frozen versus fresh were randomly selected from 50 females. Histological quality was compared using a non-parametric rating system of 1-5 with 1 being poor and 5 being outstanding for a number of different categories (nuclear detail, follicle detail, lipid vacuole detail, yolk globual detail, overall morphology, and staining). The ratings for each ovary were averaged across categories and compared between fresh and frozen ovary treatments using a paired samples Wilcoxon test performed on the software package R (ver. 2.13.1). Histological classification categories were compared using a percent agreement measure between fresh and frozen treatments.

For the broader reproductive study, batch fecundity results were graphically examined against a larger data set and model fits for red snapper (Porch et al. 2007). Maturity results were also compared with prior studies. Results of development stage and relative fecundity were examined as a function of time; two-week periods assigned sequentially beginning January 1, 2011. The fraction of females exhibiting spawning markers (hydrated oocytes and postovulatory follicles) were logistically regressed (XLSTAT) by time period and age.

Results & Discussion

Survey summary and sex ratio

The 2011 EASA survey was conducted over 846 sea days (April to October) and included 1171 long-line (4 vessels) and 1939 vertical-line (2 vessels) stations (Campbell et al 2012). A total of 2487 red snapper were caught and were the most abundant species in the vertical-line catch and the 3rd most abundant species in the long-line catch. Of the total red snapper, 2424 individuals were sexed with an exact ratio of 1:1 males and females. Further, the finding of an approximate 1:1 sex ratio applied to both gear types (835F:863M vertical-line: 377F:349M long-line). Of the females, n=1002 were further analyzed for ovarian histology and n= 992 for both age and histology (Table 1).

Females with spawning markers (hydrated oocytes, postovulatory follicles) were distributed throughout the U.S. Gulf survey area (Figure 1). Spawning females overlapped the depth range of non-spawning females and were largely caught on the outer shelf (15 to 158 m; ave. depth 60 m non-spawning females; ave. depth spawning females 62 m). In viewing the spatial results (Figure 1) by time period females in spawning condition were found throughout the U.S Gulf early in the survey (May-June) as well as late (Sept-October). On a Gulf-wide scale, these trends suggest a lack of temporal-spatial segregation in spawning. However, more red snapper were captured in the western Gulf and it was evident that females from waters off Louisiana –particularly outer shelf, exhibited a higher spawning fraction (Figure 1, Table 2). While catches and sample sizes were much lower, females from the west Florida shelf exhibited lowest spawning fraction (Table 2). These findings may correspond to the respective differences in regional stock structure and age/size composition.

Females also exhibited a notable temporal pattern in development and spawning. Females with spawning markers reached peak fraction by late June – mid July (Table 1, Figure 2A) indicating the period of highest seasonal spawning frequency. This also corresponded to the time of increased allocation to the ovary by weight via the gonadosomatic index (Figure 2B), and is generally consistent with previous findings of reproductive seasonality showing June - August peaks (SEDAR7-DW-35 and references within).

Maturity

Maturity was estimated in manner consistent with previous approaches (Jackson et al. 2007, SEDAR7-DW-35, Cook et al. 2009). Only female data from June, July and August were retained and females exhibiting vitellogenic or more advanced stage oocytes were considered mature. In 2004, data were compiled over a decade (1991 to 2002 n= 2261; 78% of samples from 1998-2002; SEDAR7-DW-35). In 2009, data were updated (n=110 records, Cook et al. 2009) primarily to fill in gaps in youngest ages and thus the fitted maturity functions overlapped (Figure 3). Graphically comparing the past results with the 2011 EASA data (Figure 3) suggests fraction of females mature-at-age have decreased over time (notably ages 2-6). If these are indications that females are delaying maturation it could signal changes in system productivity or stock recovery (Walters and Martell 2004, Jackson et al. 2007).

In parsing data by region, sample sizes are reduced (given the June-August constraint) but there is some evidence that maturity-at-age is lower in offshore Louisiana waters, particularly ages 4-6 (Table 3). Regional differences in maturity have been previously suggested with findings of lower fraction mature-at- age off Louisiana compared to Alabama (Jackson et al.

2007) and lower fractions mature-at-size and age were evident for western samples from multi-year aggregated data (SEDAR7-DW-35 and addendum).

When data were aggregated such that ages have equal weight, a potentially interesting observation is that one age cohort, age 4 females, appears to express higher maturity at age than expected by viewing adjacent year classes (Figure 3). This was also graphically evident when examining maturity by size; females in the 300-450 size range indicated a greater fraction mature. It may be informative to continue survey efforts to further investigate whether cohort effects can be discerned.

Diel pattern of catch and spawning markers

Previous studies provide estimates of the durations that spawning markers are evident. Final oocyte maturation and hydration have been estimated to begin during morning hours (ca 8:30) and remain evident through-out the day (to about 18:00 or 10 hours; Jackson et al. 2006). Field observations and spontaneous spawning in tanks indicate that ovulation occurs in mid to late afternoon peaking about 1600 hours with spawning commonly occurring before sunset around 2000 hours in summer months (Jackson et al. 2006, Papanikos et al. 2003, Papanikos et al. 2008). While postovulatory follicles have been noted to appear as early as 1100 hours, new postovulatory follicles are not thought to be common until after 1600 hours as ovulation peaks and postovulatory follicle duration is not thought to exceed about 24 hours in the Gulf of Mexico during summer (Jackson et al. 2006, Nieland et al. 2002). Thus combined, the prevalence of hydrated oocytes and postovulatory follicles (histological spawning markers) can be detected over a period of about 34 hours.

In estimating spawning fraction, it may be important to consider the time-of-day that fish are caught, samples preserved, and the diel timing that histological spawning markers can be detected. By design, red snapper could be randomly caught during the EASA study at any time of day (see methods) but the results indicate that the diel pattern of vertical-line catches were quite different from long-line catches (Figure 4 A). Vertical-line catches were notably higher in the day time hours while long-line catches were overall lower for red snapper and more evenly distributed throughout a 24 hour period. Corresponding to this catch pattern, there were gear-based differences in the distribution of spawning markers by hour. Most notable, females undergoing hydration were detected during daylight hours (06:00 to 20:00 hours) in the vertical-line samples (Figure 4 B) whereas hydration stage females were detected throughout a 24 hour period in long-line samples (Figure 4 C). Previous studies such as Jackson et al 2006 also sampled red snapper with vertical-line gear and noted hydration during daylight hours. The long-line result which shows red snapper may be captured bearing hydrated oocytes throughout a 24 hour period may indicate a broader range in time that spawning occurs and a more even distribution of diel spawning intensity than suggested in previous studies (Jackson et al 2006, Papanikos et al. 2008). In both gears, females bearing postovulatory follicles (POF) were detected in higher fraction than those with hydrated oocytes consistent with the idea that POF stage is detectable over a longer period (e.g., about 24 hours; Jackson et al. 2006). The difference in results between the two gears regarding hydration stage (H) may occur due to several reasons. One possible explanation could relate to behavioral differences in the way the fish interacts with gear. For instance there may be a tendency for red snapper to be more susceptible to vertical-line gear during the day as schooling fish may have a greater tendency to rise up in the water column and become susceptible to hooks suspended vertically. Another explanation may be a tendency

of larger/older females, which are selected in long-line gear due to hook size selection (e.g. Allman and Fitzhugh 2007, Campbell et al. 2012) to expend more energy in reproduction and be more active in spawning throughout a diel cycle. Whatever the explanation, it was clear that females from long-line catches showed a higher fraction of spawning markers overall (H and POF stages, Table 4) apparently related to size/age (see age-based results below).

The fractions of females with spawning markers (M) can be converted to daily spawning fractions (S) following Priede and Watson (1993):

$$S=24*M/T_M$$

Where T_M is the duration in hours that spawning markers can be detected. On average, the mean fraction of females with spawning markers was 0.39 (Table 4) and when adjusted for the duration that spawning markers should be detected (about 34 hours) yields an estimate of S = 0.28. Corrected to a 24 h day, the inverse of S gives the average inter-spawning interval of 3.6 days. This is similar to previous estimates; 3.97 -5.95 d (Collins et al. 1996), 3.4 – 4.2 d (time calibrated method, Woods et al. 2003, and 4.3 d (Brown-Peterson et al. 2009).

Spawning fraction and number of batches at age

Spawning fraction specific to age, is difficult information to obtain, but a review of reproductive studies generally predicts that older fish will spawn more often among species that exhibit indeterminate fecundity (Fitzhugh et al. in press). Based on the 2011 expanded survey, it was apparent that fractions of red snapper with spawning markers also increased with age indicating older females produced more batches of oocytes than did younger females (Figure 5). As shown earlier, there is a seasonal component (Figure 2 A,B) as well as a size/age component to spawning fraction. A logistic regression of fraction bearing spawning markers by age and

time period revealed that older females, particularly older than age-4, showed higher spawning fraction throughout the reproductive season (Figure 6). In addition there was evidence that older females may have a more extended reproductive season than younger females (Figure 6, Table 5). Based upon expected diel timing that spawning markers are evident, conversions were made for daily fraction S, and the inter-spawning interval in days as earlier indicated. Further dividing the reproductive duration by the inter-spawning interval would yield the expected number of batches per year.

At older ages (age-8+), data had to be aggregated due to the rarity of old fish in the population and decreasing numbers of older fish sampled. Increased sampling in future surveys may allow greater resolution among these older ages. Porch et al. 2007 found some evidence that batch fecundity may be lower on average among older ages (> age 15) and speculated that senescence may become a factor in females surviving to older age.

Batch fecundity

We learned that because of the effects of freezing and desiccation of mature oocytes (see below), batch fecundity counts were feasible on females that were clearly in late stages of hydration. By additionally censoring females with evidence of recent postovulatory follicles, these represent a smaller subset (n = 50) of females histologically observed to be undergoing final maturation and hydration (n = 237). While there was a tendency to overestimate batch fecundity from frozen samples (see quality control below), the 2011 results generally indicated lower fecundity at age than in previous results presented in Porch et al. 2007 (Figure 7).

Annual fecundity

Annual fecundity was calculated two ways. The first approach was to multiply the batch fecundity at age (asymptotic relationship; Porch et al. 2007) by the number of estimated batches irrespective of age. This implicitly assumes that the number of spawned batches is constant with age. The second approach accounts for an increase in daily spawning fraction with age (older fish spawn more frequently − interspawning interval decreases, Table 5) and factors in an increase in season duration with age (older fish spawn longer, Figure 8). Both traits indicate that older fish spawn more batches. Thus calculated, the age effect of increased batches reflects increased fecundity-at-age. This is apparent at ages 6 and above when the constant model is compared with the age-dependent model (Figure 9). As a demonstration, the age-dependent model was based upon a non-linear function for spawning duration (Figure 8) and point estimates of spawning fraction (Table 5). We recognize there may be alternative approaches to model annual fecundity.

Quality control investigation

The linear relationship between frozen and thawed whole ovary weight and fresh whole ovary weight was 1-to-1 (Figure 10), which indicated no need for further weight conversion. However, the linear relationship between frozen and fresh fecundity samples yielded a slope less than one and a positive y-intercept, which indicated there were more frozen hydrated follicles counted per gram of sample than in fresh preserved tissue from the same female (Figure 11). When the subsamples were extracted to meet a standard 75 mg weight, the frozen-fixed sample contained more hydrated oocytes. The frozen subsamples varied in appearance compared to fresh preserved tissue with some HOs being flat or dehydrated (Figure 12). We concluded there was a

tendency to over-count HOs in frozen samples primarily due to changes in subsample weight. Because some of the fecundity subsamples were not obtained from a whole frozen ovary but rather a small amount of tissue frozen in a dry vile, the freezing likely caused a reduction in weight via dessication in those samples. Ramon and Bartoo (1997) noted the same phenomena in that freezing desiccated smaller pieces of ovarian tissue in contrast to larger intact ovaries. Klibansky and Juanes (2007) actually saw an increase in ovarian weight but attribute that result to freezing the tissue immersed in fresh water, and they suggest freezing the tissue immersed in an isotonic solution to solve the problem.

Frozen samples were ranked significantly lower in histological quality than the fresh histological samples according to the paried-samples Wilcoxon test (Table 6; $V=741$, $p<0.0001$). That histological quality was superior in the fresh-fixed compared to the frozen-fixed samples was visually apparent. However, the scores of the histological quality indicated that specific traits of germinal vesicle resolution, follicle-, lipid vacuole-, and yolk globual detail were all in question, which directly effects identification of sexual classification characteristics such as postovulatory follicles, leading gamete stage, and atresia. To address those specific concerns these classification characters were specified in a percent agreement measure between fresh and frozen histology. There was 82% agreement in leading gamete stage between fresh and frozen treatments, but presence of atresia was only agreed upon in 64% of the red snapper examined. Identifications of postovulatory follicle stage along with prior indicators of spawning agreed 76% of the time between the two treatments. There was 82% agreement for reproductive histology spawning class; a key variable which is determined from all the other histological traits (Figure 13). The relatively high agreement in the histology spawning class gives some confidence in the ability to derive reproductive estimates especially spawning fraction from

frozen ovaries. However, postovulatory follicles appeared older and early stages of oocyte maturation was more difficult to identify because the germinal vesicle was not always visible (but see Young et al 2003) thus more exact timing of the these spawning markers (e.g., into day-0 versus day-1 cohorts) were not attempted. Fully hydrated oocytes from frozen and fresh-preserved preparations appeared similar in histology slides (Figure 14) in contrast to plated whole oocytes (for fecundity counts, Figure 12). As onset of final oocyte maturation and late POF degeneration may be more difficult to discern, the effect may be to lessen the overall duration that spawning markers can be detected in frozen compared to fresh-preserved tissue. But because we know of no information suggesting duration of spawning markers is related to age, any adjustments of marker duration should not affect the interpretation of number of batches relative to age which is a chief objective of the study.

Histology comparisons between frozen and fresh-fixed tissue are rare. Titford and Horenstein (2004) compared a number of different fixatives for histological purposes using a rating system of one to five. Results indicated formalin to be the best fixation agent among all the chemicals, but it was noted that histologists may have chosen formalin unknowingly because it is what they are accustomed to rather than yielding the better result (Titford and Horenstein, 2004). Young et al. (2003) compared fresh-fixed and frozen-fixed ovarian histology of broadbill swordfish (*Xiphias gladius*) to justify using frozen gonads in characterizing reproductive dynamics. Neither oocyte stage nor post-ovulatory follicle (POF) presence/absence was misidentified in the frozen histology sections when compared with the fresh-fixed sections. However, while Young et al. determined spawning frequency from both the POF and hydrated follicle method, Arocha and Barrios (2009) used frozen ovaries to determine spawning frequency

only using the hydrated follicle method because of the uncertainty in the identification of frozen POFs (Hunter and Macewicz, 1985).

We believe our finding of high percent agreement of spawning class allows us to estimate maturity and fractions of females bearing spawning markers. However, we would not recommend freezing ovarian tissue if there is an alternative. If formalin cannot be used, one alternative may include the less toxic Streck tissue fixative, which behaves similar to formalin in preserving ovarian tissue (McCarthy et al. 2008). Another improvement would entail freezing small amounts of gonadal tissue in an isotonic solution instead of a dry vial or freshwater. Because of possible species specific reactions to freezing, the best option may be to conduct a preliminary comparison between fresh and frozen gonads before large scale sampling is undertaken.

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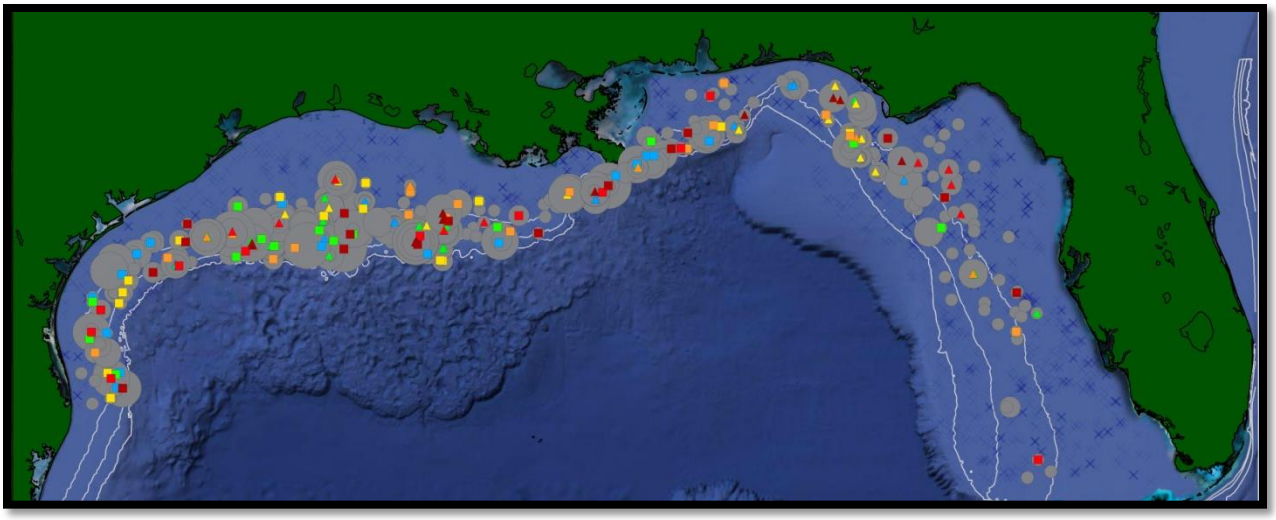
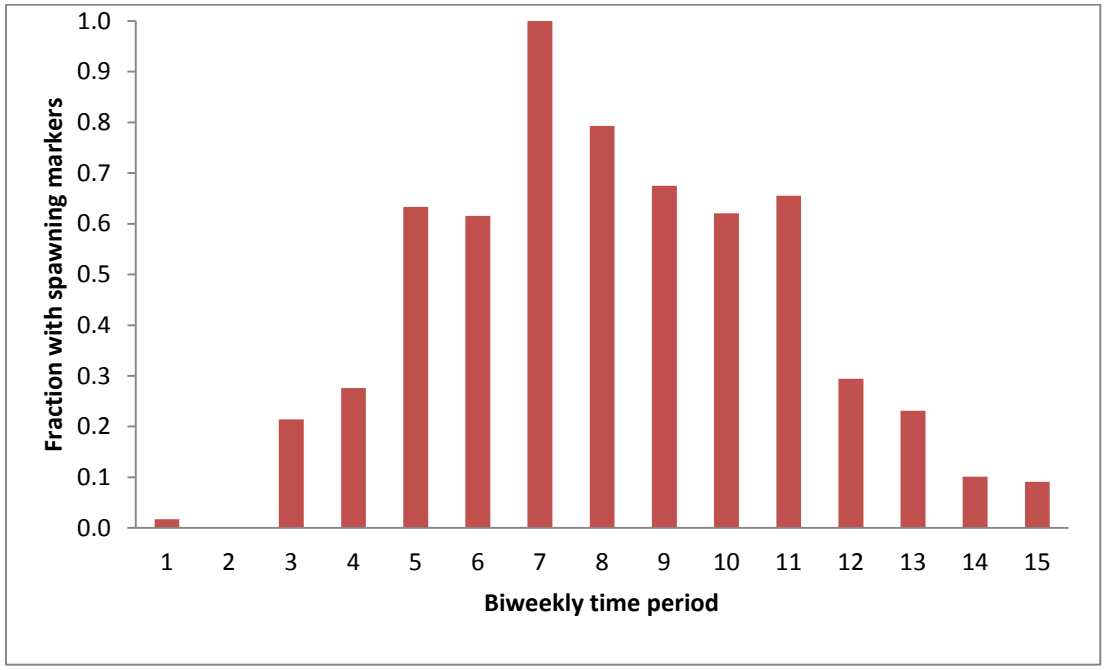


Figure 1. Distribution of sampling stations (cross symbol-no red snapper caught) and positive catches of red snapper (grey circle; size indicates number landed per set: 1-4, 5-10, 10-13, 14-17, 18+). Colored symbols indicate females with spawning markers (square symbols - bottom longline gear, triangles – bandit gear). The colors (cool to warm) indicate increasing age; blue <= age 3, dark red >= age 8. The 50, 100 and 200 m isobaths are indicated. Note that symbols are for graphical illustration and do not reflect scale of area fished per station.

A.



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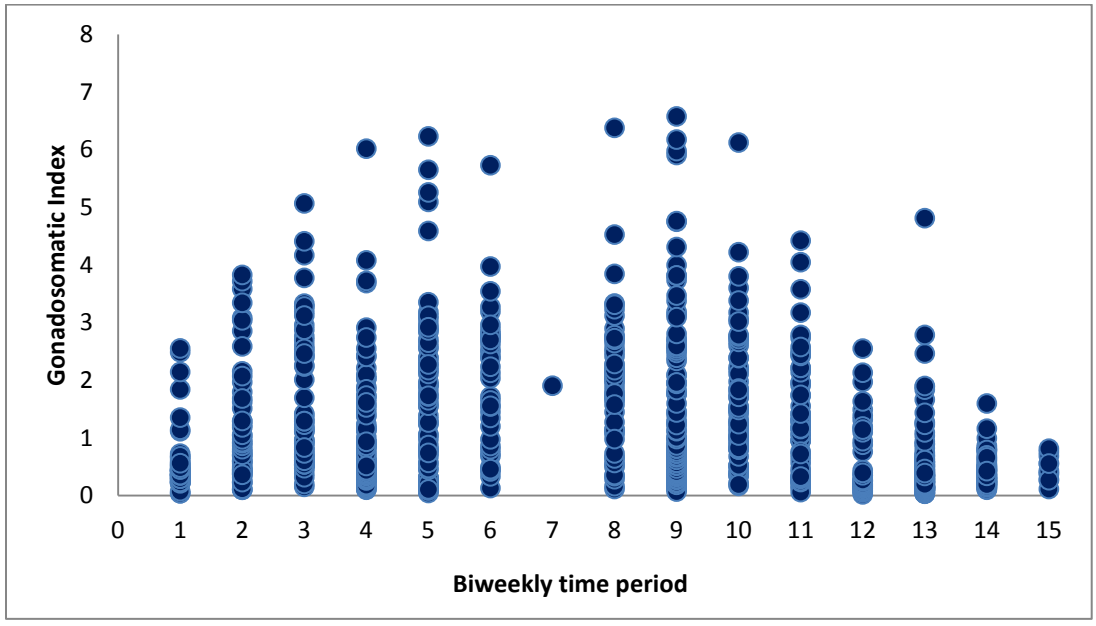


Figure 2. Seasonal reproductive development of female red snapper summarized by biweekly time period during the 2011 survey (see Table 1 for dates). A. Fraction of females with histological spawning markers (H, POF) and B. Gonadosomatic index.

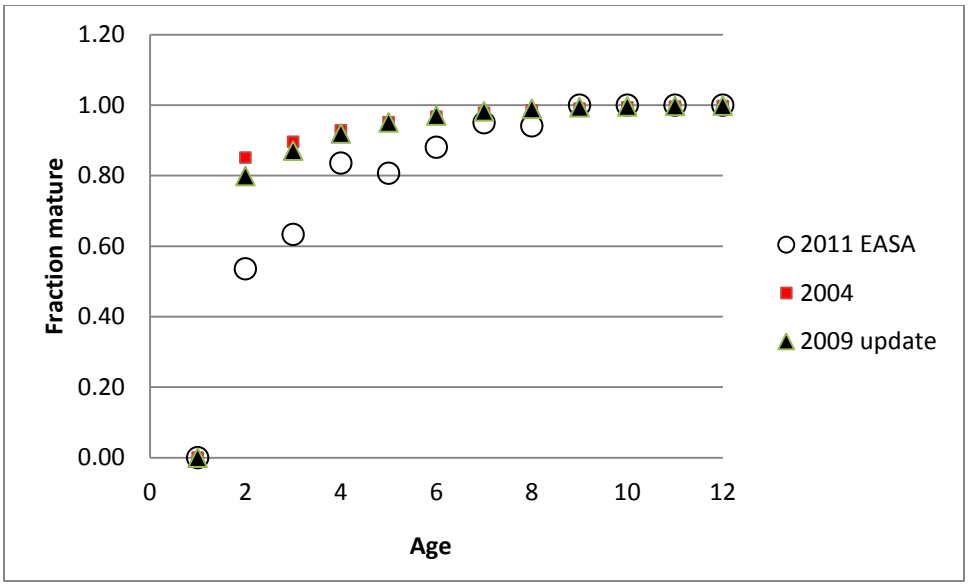
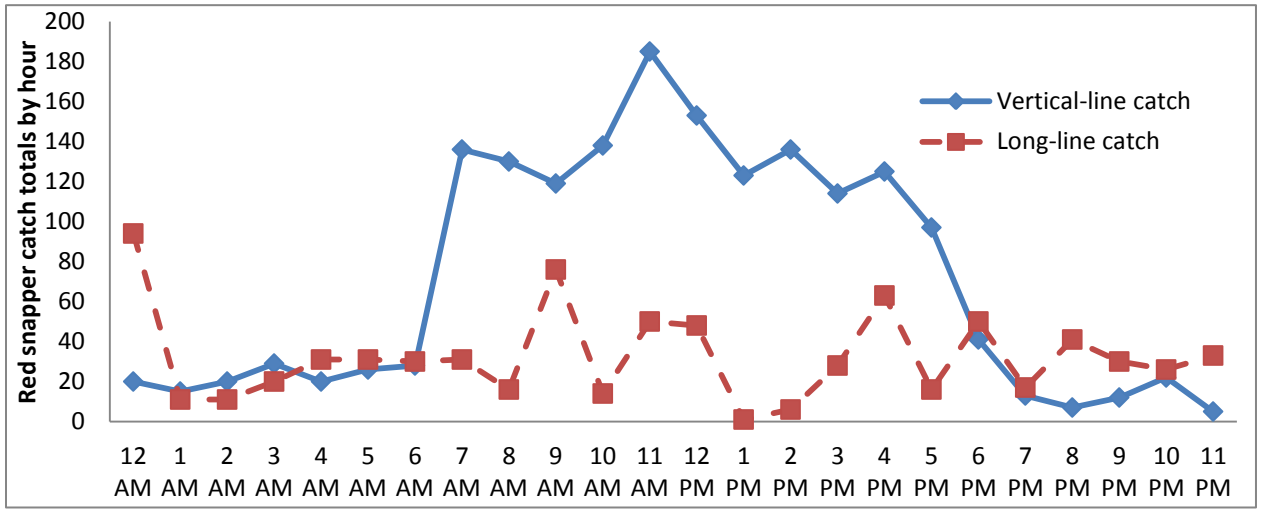


Figure 3. Fitted points (solid symbols) for female age-at-maturity are shown for previous assessments; 2004 and update in 2009 (n=2371 females). Estimated fraction mature aggregated at age are shown (open circles) for the 2011 EASA project (n=433 females). Age 12 is a plus group for EASA data.

A.



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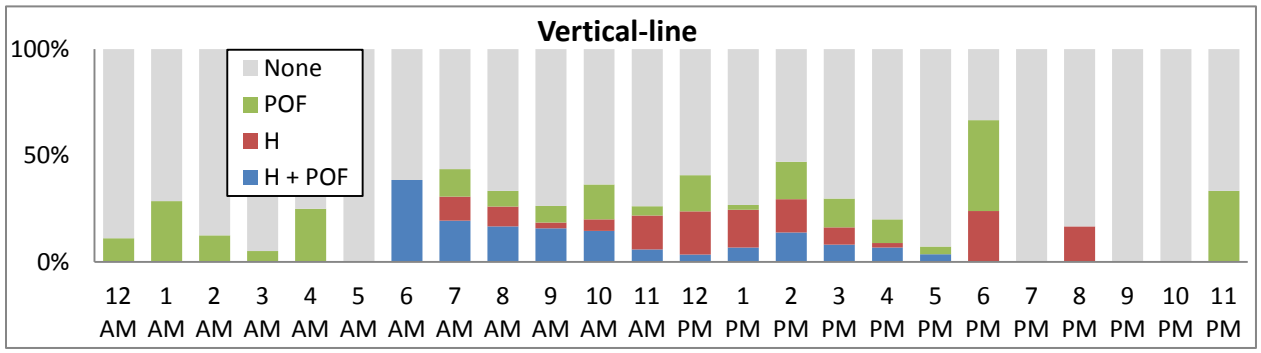


C.

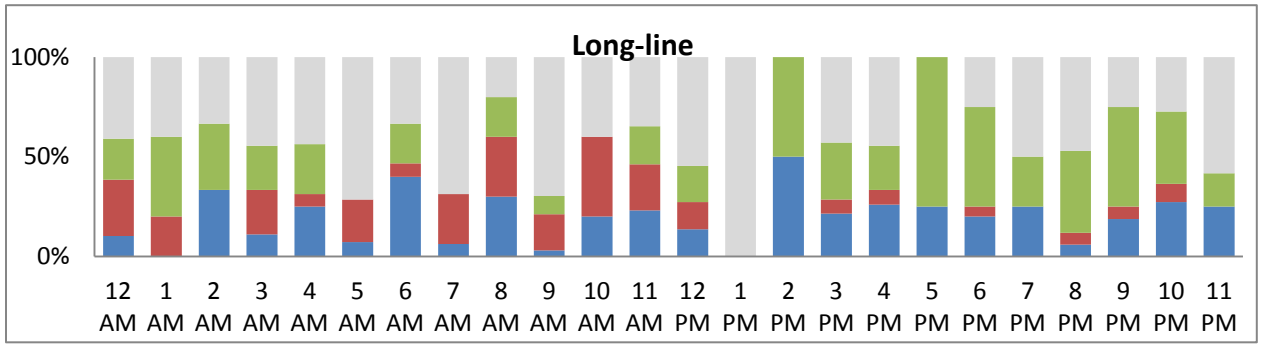


Figure 4. Catch totals by gear and hour retrieved are indicated for males and females combined (A). Of the females captured each hour, the percent with spawning markers (H, hydrated oocyte; POF, postovulatory follicle) are also shown for vertical-line catches (B) and long-line catches (C).

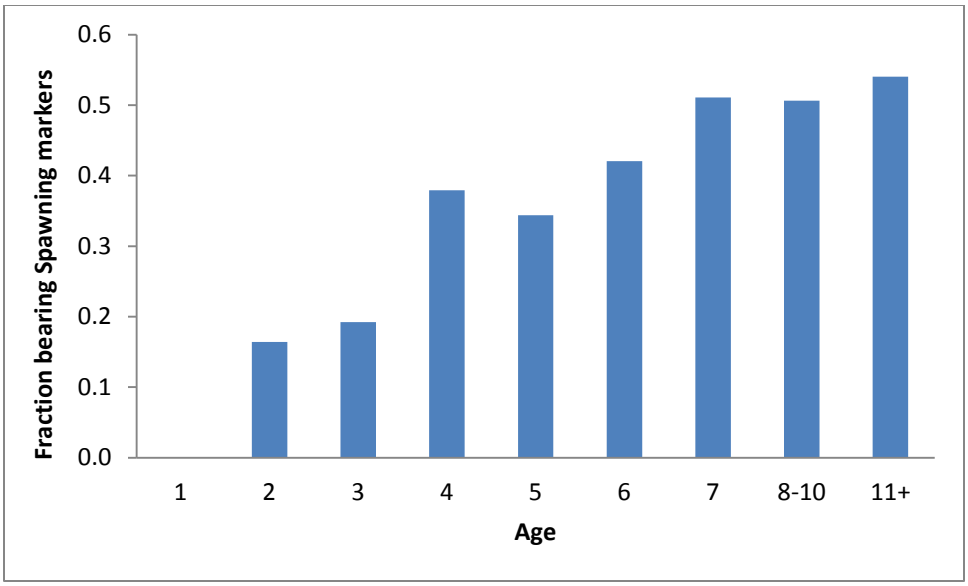
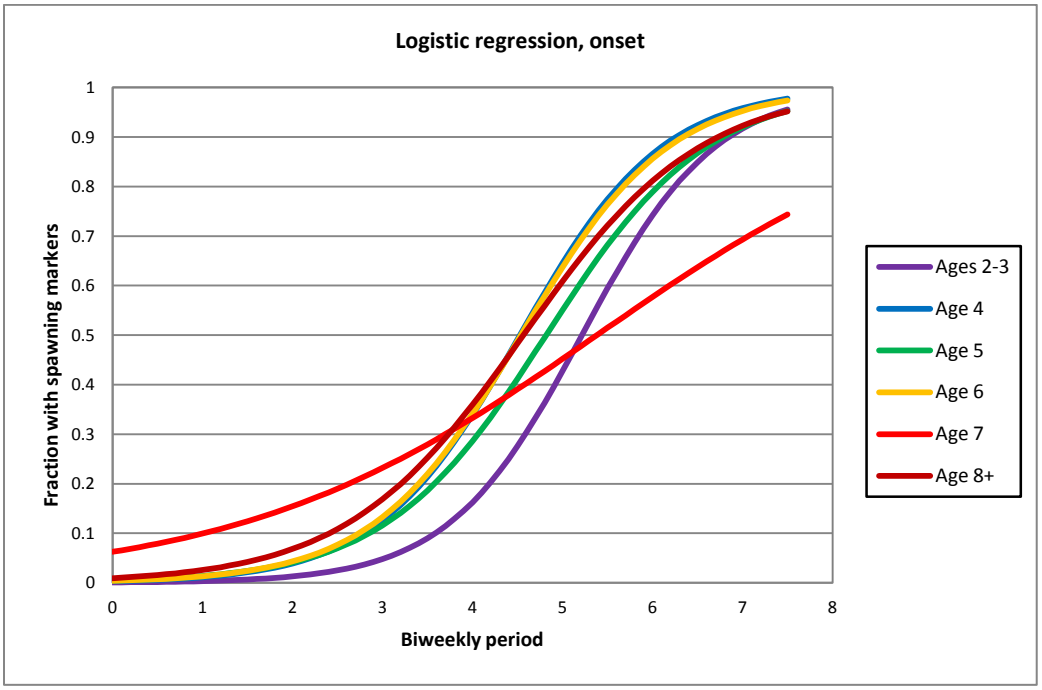


Figure 5. Fraction of females histologically assessed bearing spawning markers (H, POF stage). See Table 5 for sample sizes.

A.



B.

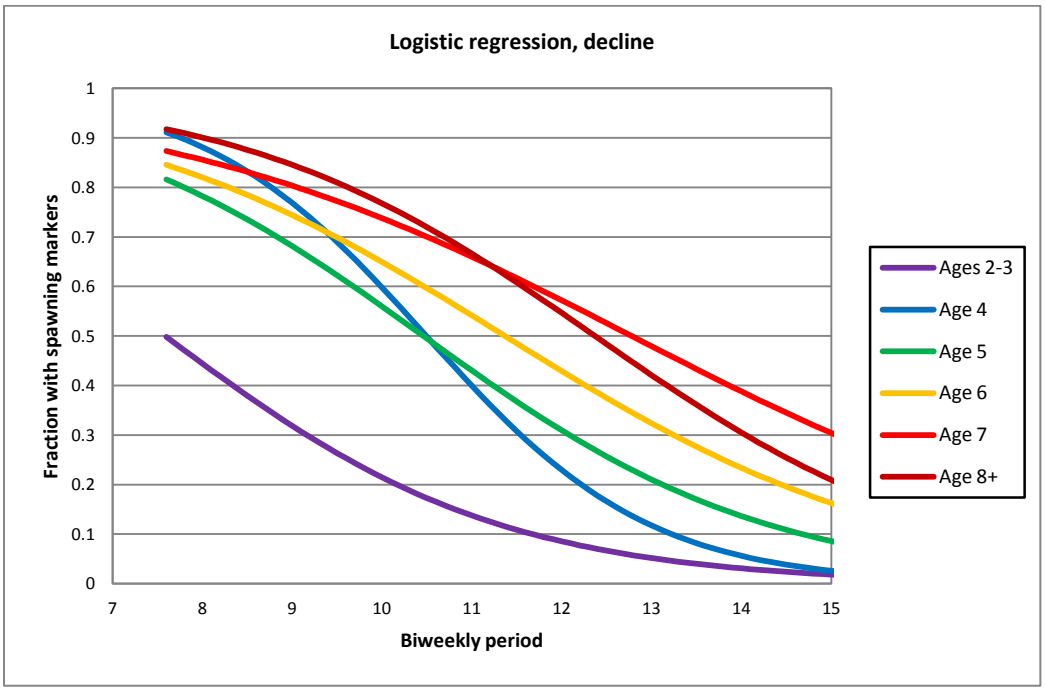


Figure 6. Logistic regression of female fraction bearing spawning markers by age. See Table 1 for sample period dates.

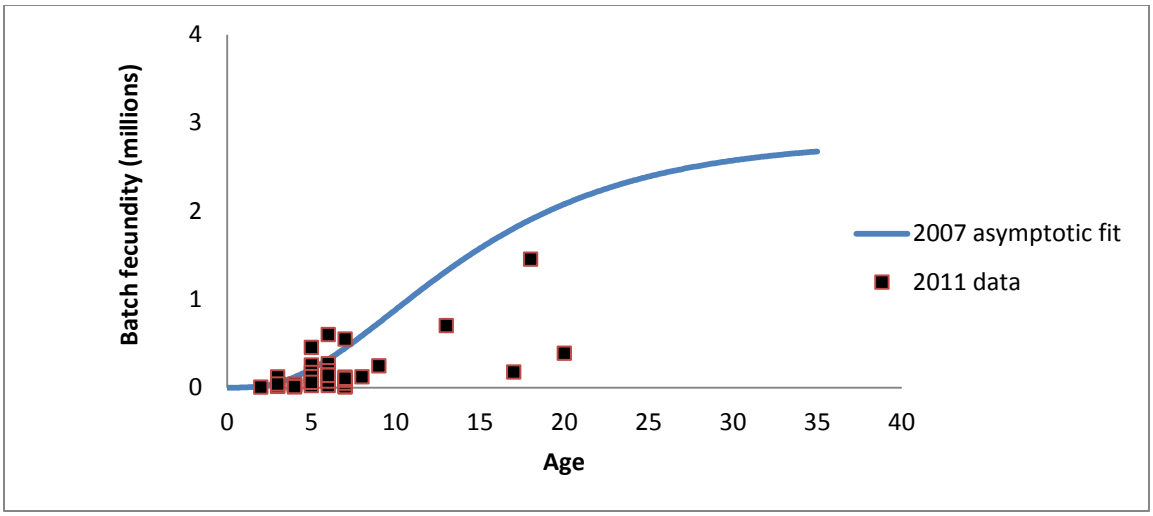


Figure 7. Batch fecundity by age from females in late hydration from the 2011 EASA survey. For comparison, the asymptotic age-batch fecundity relationship is shown (Porch et al. 2007) based upon a larger batch fecundity data set.

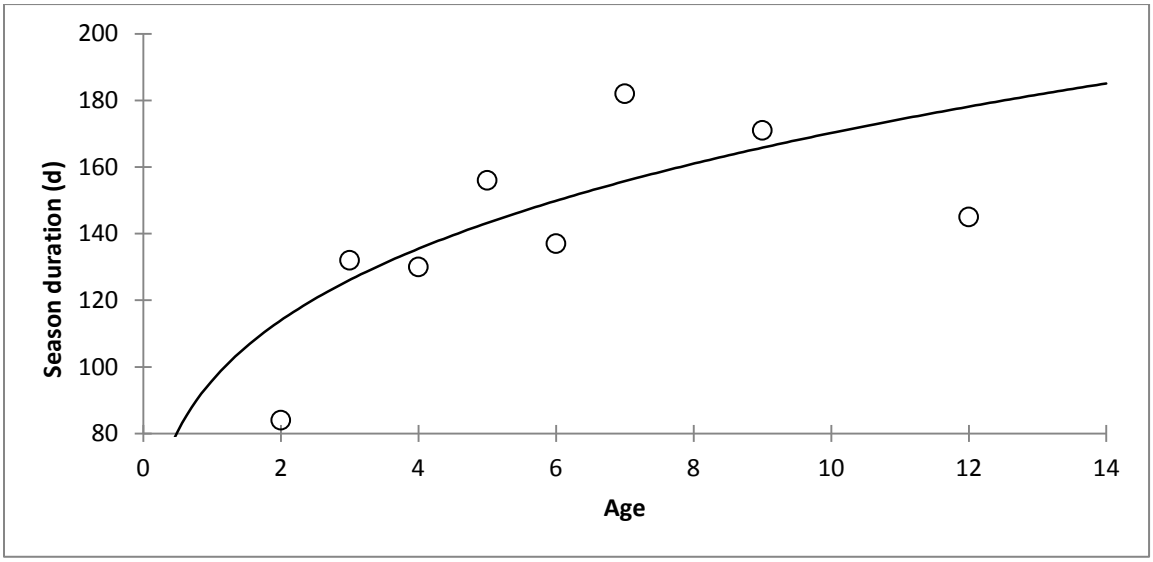


Figure 8. Non-linear weighted regression of the spawning season duration in days by age. Duration determined based upon the earliest and latest appearance of spawning markers by age. Due to sample size, ages 8-10 were aggregated and oldest ages aggregated as plus group, age 11+. Duration = 95.895 $(Age)^{0.249}$. R^2=0.47.

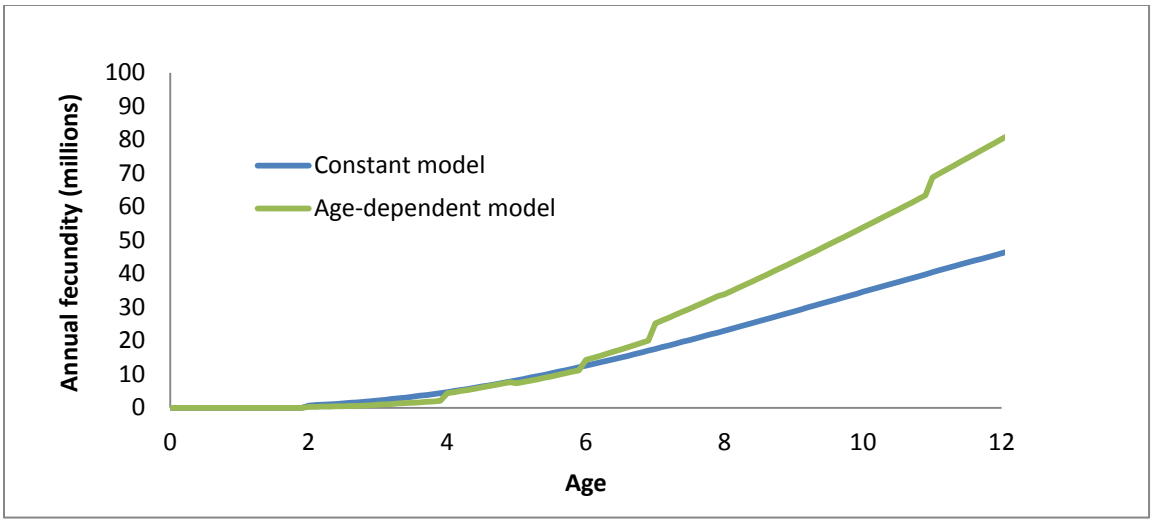


Figure 9. Annual fecundity based upon number of spawns assumed constant by age as opposed to increasing by age. Constant annual fecundity model based upon asymptotic batch fecundity relationship (see Fig 7), spawning season duration of 142 d (averaged across ages, this study) and mean interspawning interval of 3.6 d (this study). Age-dependent annual fecundity model based upon non-linear function for duration by age (see Fig 8) and the age-based estimates of interspawning interval (Table 5).

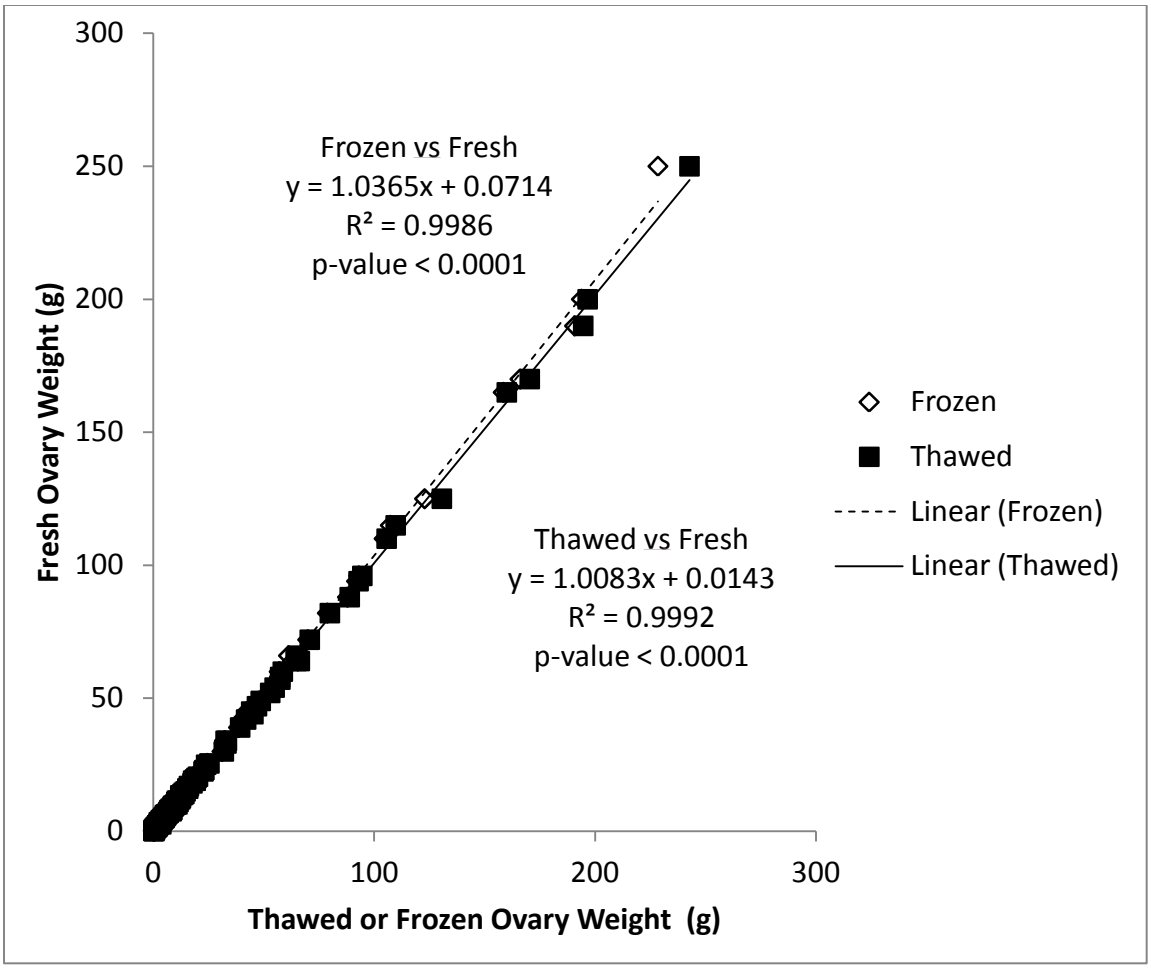


Figure 10. Thawed and frozen whole ovary weight as predictors of fresh whole ovary weight with equations, coefficient of determination, and p-value displayed for each linear relationship. Both relationships are close to a 1:1 relationship, which indicates little need to convert frozen or thawed whole ovary weights to obtain fresh ovary weights.

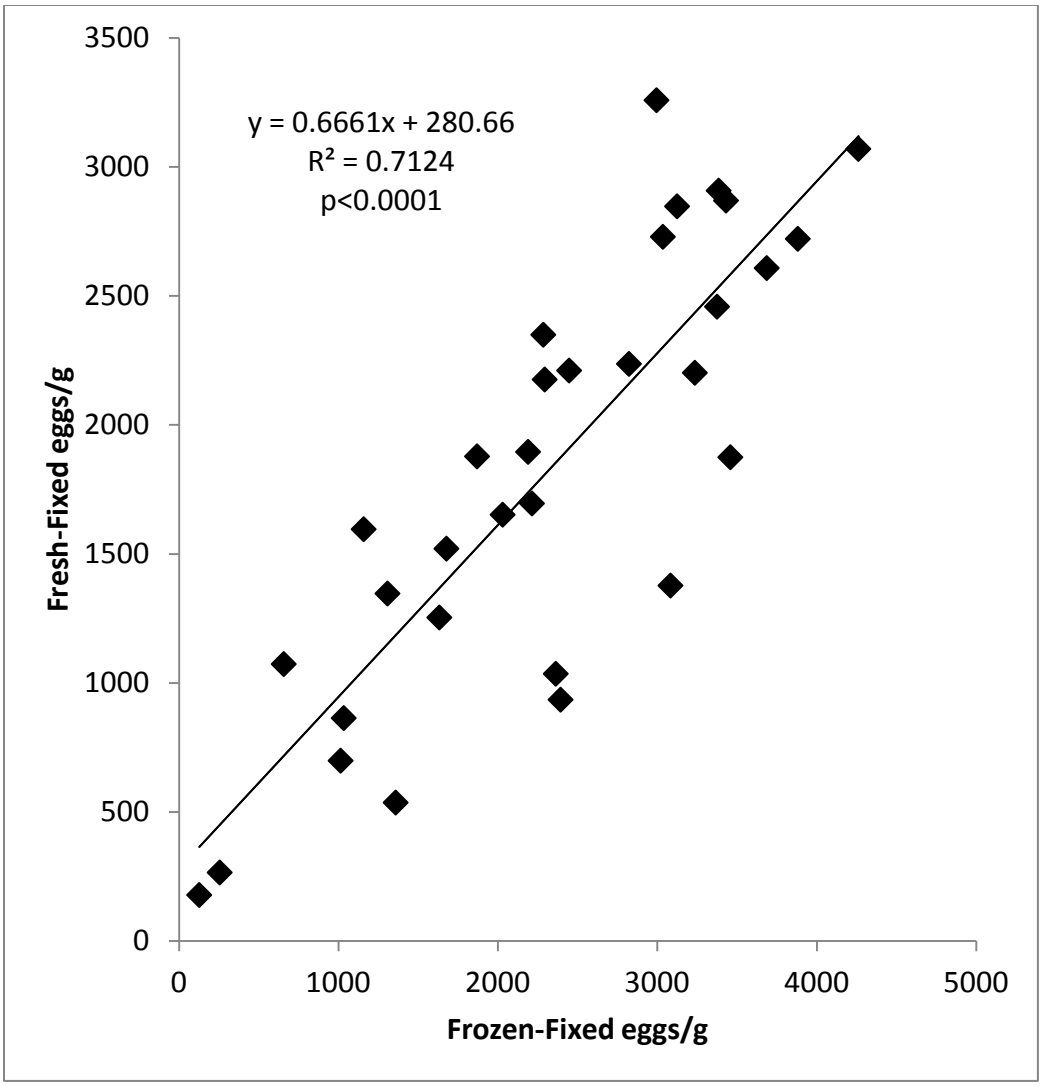


Figure 11. Fecundity counts of frozen-fixed egg/g as a predictor of fresh-fixed eggs/g with linear equation, coefficient of determination, and p-vaule displayed. The high y-intercept and small slope value indicates more eggs were counted per gram of sample in the frozen-fixed treatment, but this may be due to the weight of the subsample more than the number of recognized hydrated oocytes.

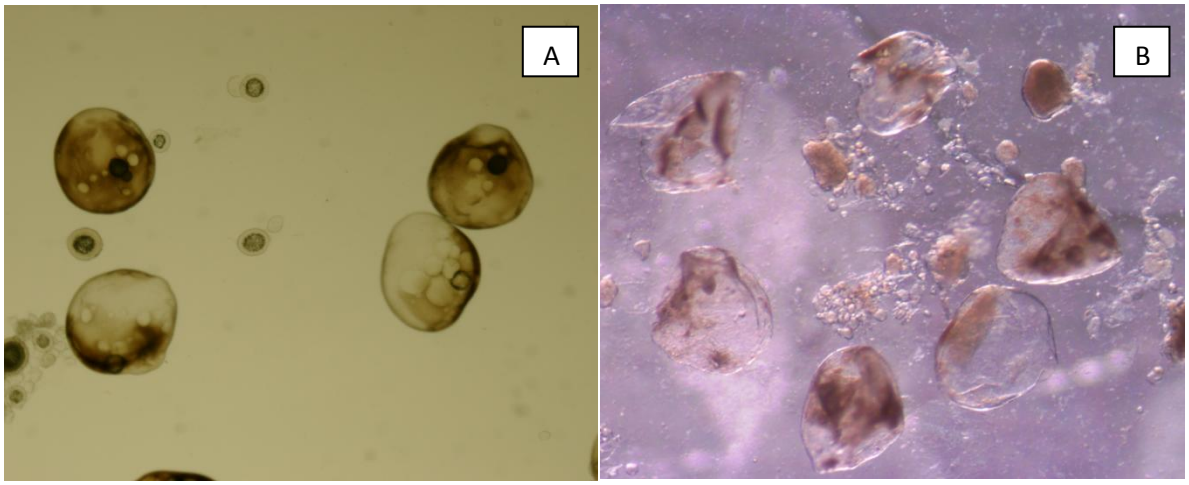


Figure 12. Whole mount of late hydration stage oocytes as viewed for batch fecundity counts, A) from fresh preserved tissue and B) from tissue preserved after initial freezing.

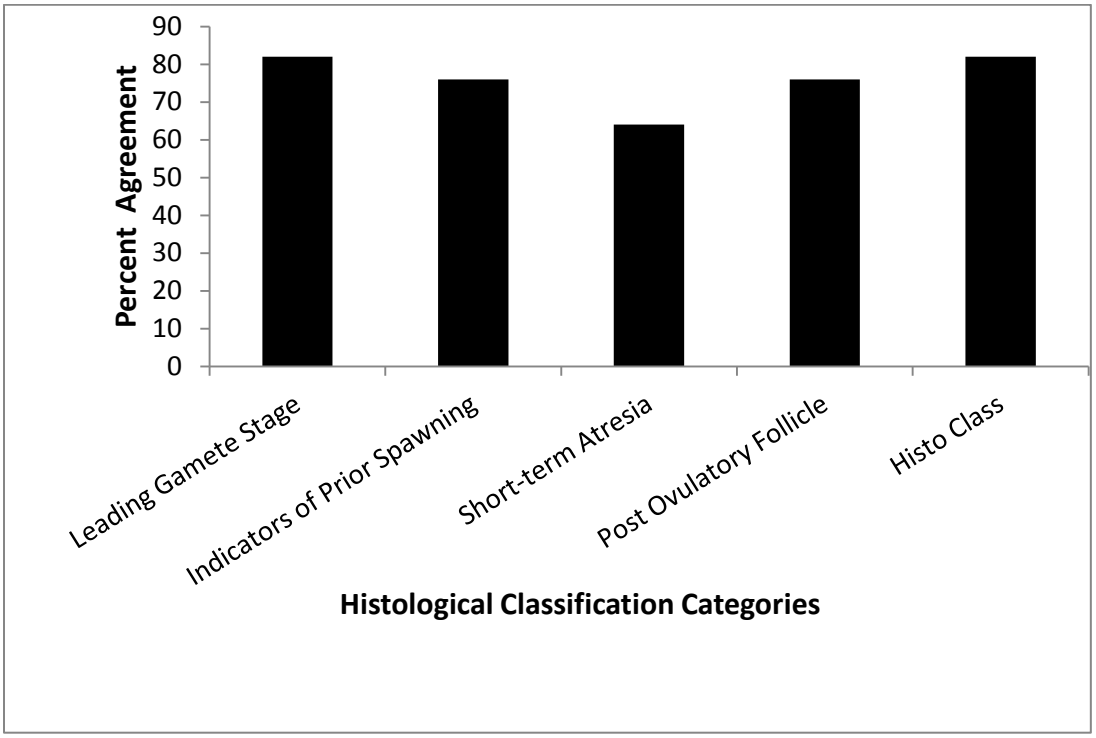


Figure 13. The percent agreement between frozen and fresh histology slides for each histological classification category (displayed on the x-axis). Although there was varying agreement of specific characters of the ovary, histo class, which is determined by the rest of the categories, has relatively high agreement at 82%.

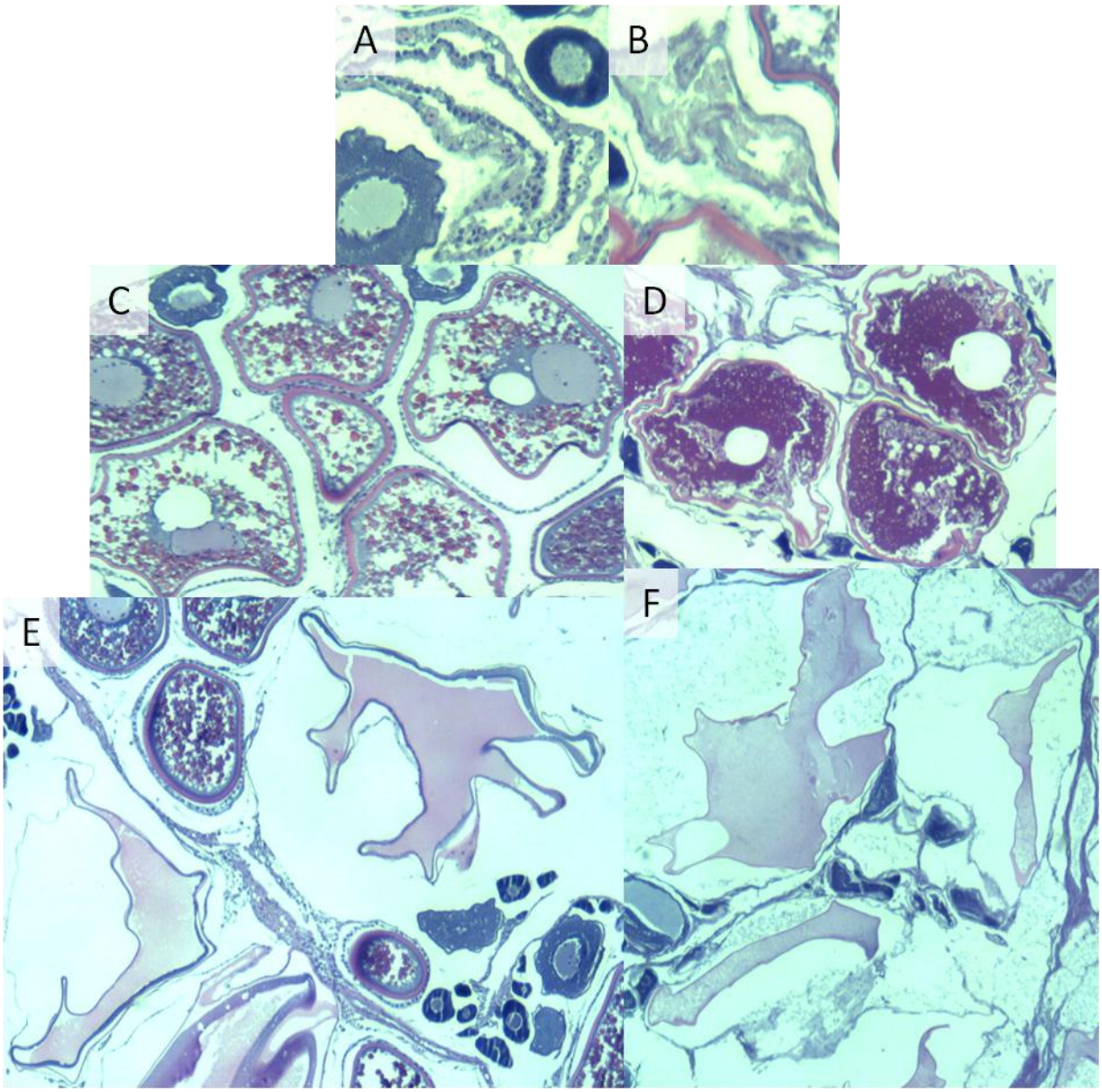


Figure 14. Fresh ovarian histology slides with the corresponding frozen ovarian histology slide. Fresh compared to frozen post-ovulatory follicles (A&B), Oocyte maturation (C&D), and Late hydration (E&F).

Table 1. Summary of red snapper females from the 2011 expanded survey. Within each approximate two week period, the initial date of sampling is listed. The numbers of sampling dates and females, and fraction and of females bearing histological spawning markers are indicated, along with the mean and range of age. The survey covered a total period of 198 days.

Period	Start date	N dates	N females	Fraction with spawning markers	Mean Age	Range
1	4/9	9	58	0.02	6	2-14
2	4/19	9	52	0.00	8	2-31
3	5/3	8	70	0.21	7	3-14
4	5/17	9	87	0.28	5	2-14
5	5/31	12	90	0.63	6	2-29
6	6/15	6	39	0.62	9	2-34
7	6/29	1	2	1.00	21	8-34
8	7/13	8	58	0.79	8	3-28
9	7/26	10	123	0.67	6	2-22
10	8/9	10	58	0.62	7	3-21
11	8/23	7	58	0.66	5	1-15
12	9/10	7	34	0.29	6	2-24
13	9/20	9	173	0.23	5	2-25
14	10/4	12	79	0.10	6	2-22
15	10/19	4	11	0.09	7	4-9

Table 2. Fraction of females bearing histological spawning markers (H, POF stage) by state/region (assigned by longitude and shrimp statistical grid).

	Statistical Grids	N females	Fraction with spawning markers
W FL shelf	1-7	60	0.25
FL panhandle	8-9	125	0.35
AL	10-12	80	0.39
LA	13-17	428	0.43
TX	18-21	309	0.38

Table 3. Fraction mature-by-age based upon state (assigned by longitude). Maturity based upon presence of vitellogenic or more advanced oocytes for females collected in June, July, and August.

	FL + AL		LA		TX	
Age	N females	Fraction Mature	N females	Fraction Mature	N females	Fraction Mature
1			2	0		
2	4	0.25	19	0.63	5	0.40
3	1	0	25	0.72	4	0.25
4	26	0.96	40	0.75	7	0.86
5	25	0.88	51	0.75	12	0.92
6	8	0.88	33	0.82	26	0.96
7	12	1	31	0.90	17	1
8			7	0.86	10	1
9	1	1	4	1	6	1
10			1	1	5	1
11			2	1	7	1
12+	2	1	12	1	27	1

Table 4. Fraction of females bearing histological spawning markers by gear.

	Fraction H	Fraction POF	Fraction combined	Total N
Vertical-line	0.19	0.21	0.31	660
Long-line	0.32	0.41	0.55	342
Gear combined	0.24	0.28	0.39	1002

Table 5. Age-based fractions of females bearing histological spawning markers. Conversions are shown for daily spawning fraction (S) and inter-spawning interval in days. The duration refers to the number of days between first and last observation of a female with spawning markers by age-class.

Age	Duration (d)	N females	Fraction with spawning markers	S	Inter-spawning interval (d)
2	84	67	0.16	0.12	8.63
3	132	78	0.19	0.14	7.37
4	130	174	0.38	0.27	3.73
5	156	224	0.34	0.24	4.12
6	137	157	0.42	0.30	3.37
7	182	137	0.51	0.36	2.77
8-10	171	79	0.51	0.36	2.80
11+	145	74	0.54	0.38	2.62

Table 6. Average scores of histological quality between fresh and frozen treatments for six different categories in ovarian histology. The scores were comprised of a one to five nonparametric rating system.

Treatment	Nuclear Detail	Follicle Detail	Lipid Vacuole Detail	Yolk Globual Detail	Morphology	Staining
Fresh	4.60	4.00	4.34	4.41	4.02	5.00
Frozen	1.64	2.02	2.23	1.38	3.34	4.04