Spatio-temporal dynamics in red snapper reproduction on the West Florida Shelf, 2008-2011.

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Spatio-temporal dynamics in red snapper reproduction on the West Florida Shelf, 2008-2011.

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Introduction:

Red snapper, *Lutjanus campechanus*, support important commercial and recreational fisheries from the southeastern United States Atlantic Ocean, the Gulf of Mexico, and the Caribbean. The stock currently is considered 'overfished' and a rebuilding plan is in effect leading to increased regulation of the fishery (SEDAR 2005). Although the Gulf of Mexico population has traditionally been considered one stock, a number of important parameters vary between the Eastern and Western Gulf of Mexico. Recent genetic studies report an effective population size approximately four orders of magnitude smaller than the census population and genetic heterogeneity of age-0 fishes over small spatial scales (Saillant et al. 2010). This work highlights the need to assess red snapper reproduction over the appropriate spatial scales and the potential for reproductive success to vary with local populations.

Biological reference points associated with maximum sustainable yield (MSY) used to determine red snapper overfishing and overfished criteria and rebuilding plans (SEDAR 7) have been derived either from spawner-recruitment (S-R) functions or by assuming that MSY is associated with a specific level of spawning per recruit (expressed as a percentage of the unfished level and designated "SPR"). Both methods are based on 2 underlying assumptions: (1) that egg production can be predicted based on SSB and (2) that egg production drives reproductive success (Lowerre-Barbieri et al. 2009). However, there is increasing awareness that spawning stock biomass is a poor predictor of reproductive potential and increasing evidence of other factors affecting reproductive success, such as: the effect of spawning site location on recruitment success (deYoung and Rose 1993, Begg and Marteinsdottir 2002), spawning site fidelity (Thorrold et al. 2001; Robichaud and Rose 2003; Svedang et al. 2007, Adams et al. 2011), reproductive timing (Wright and Trippel 2009; Lowerre-Barbieri et al. 2011) and demographic trends in these behaviors (Scott et al. 2006; Anderson et al. 2008; Cooper et al. *in press*).

The objectives of this work were to: (1) develop histological indicators to assess reproductive timing and assign reproductive phases (Brown-Peterson et al. 2011); and (2) based on these indicators evaluate where and when actively spawning females occurred.

Survey Design, Sampling Methods, and Analyses:

Reproductive samples of red snapper were collected in the FWC reef fish survey, in SEAMAP trawls, and in sampling associated with focused research studies in the Florida Middle Grounds and the Florida panhandle. The reef fish survey includes a portion of the WFS bounded by 26^{o} and 28^{o} N latitude and depths from 10 – 110 m. To assure adequate spatial coverage of sampling effort, the WFS survey area is subdivided into four sampling zones comprised of two NMFS statistical zones (Tampa Bay: NMFS statistical zone 5; Charlotte Harbor: NMFS statistical zone 4) and two depth zones (Nearshore: 10 – 37 m; Offshore: 37 – 110 m).

Samples of gonad tissue were collected from culled red snapper and immediately fixed in 10% phosphate-buffered formalin. For histological analysis, ovarian tissue was fixed in 10% neutrally

buffered formalin for 24 h, soaked in water for 24 h, and stored in 70% ethanol. Samples were embedded in glycol methacrylate, sectioned to 3–5-mm thickness, stained with periodic acid–Schiff's hematoxylin, and then counterstained with metanil yellow (Quintero-Hunter et al. 1991).

Ovarian analysis. Reproductive state, phase and histological indicators of red snapper were assigned following Lowerre-Barbieri et al. (2009) and Brown-Peterson et al. (2011) and criterion are outlined in Table 2. Histological indicators are outlined in Table 3 and included: (1) oocyte developmental stages: primary growth (PG), cortical alveoli (CA), vitellogenic (Vtg1-3), and oocyte maturation (OM); (2) post ovulatory follicles (POFs); and (3) atresia. In addition, thickness of ovarian wall and presence of muscle bundles extending from the ovarian wall into the ovarian lamellae were used to help distinguish between immature and regenerating females (Lowerre-Barbieri et al. 2011). Because oocyte maturation can take up to 16 h under the temperature regimes in which red snapper are spawning, the process of oocyte maturation was further broken down into: germinal vesicle migration (GVM), germinal vesicle breakdown (GVBD), yolk coalescence or clarification, and oocyte hydration (Jalabert 2005). Postovulatory follicles (POFs) were classified as either newly collapsed (recognizable by the size and appearance of the granulosa cells' nuclei) or 12 h or older based on POF size, organization, and elapsed time from peak spawning (Hunter & Macewicz 1985). Actively spawning females were considered to be those undergoing late OM, ovulation, or with fresh POFs (Tables 2 & 3).

Results / Discussion:

A total of 237 female red snapper (size range 160-750 mm FL) had ovarian tissue sampled for this study, most collected in 2009 and sampled with hook and line (Table 1). Eight immature females were collected in July, ranging in size from 160 to 219 mm FL with an average size of 198.7 mm FL +/- 7.3 mm SE. The majority were age 1, however there were two age 2 immature females. The smallest spawning capable female was 247 mm FL, as was the smallest female with hydrating oocytes. All mature females were age 2 or older. These results are similar to previous studies, reporting spawning females with a minimum size of 296 mm FL (Fitzhugh et al. 2004) and 285 mm FL (Woods et al. 2007) and aged two years old. Although Cook et al. 2009 collected the first evidence of some red snapper being capable of maturing by age 1, with their smallest spawning female being 196 mm FL

Ovarian samples were collected from March through December, with a total of 58 actively spawning females collected during the months of June through September. Their ages ranged from 2-7 years old with a mean age of 3.7 SE=0.16 years. Spawning capable females (n=66) were collected over a wider time period from March through November. However, spawning capable 2-year olds which would be first time spawners were only collected in the months of June through August, suggesting these fish may have a shorter spawning season than older females. Many species demonstrate demographic differences in spawning periods, with older, larger fish spawning sooner and often for longer durations than younger fish (Kjesbu et al. 1996; Wright and Trippel 2009), presumably increasing the reproductive success of these individuals and the population as a whole.

A wide range of locations were sampled, as can be seen based on the chevron trap and hook and line samples in 2009 and 2010 (Fig. 1A) and reproductive samples were taken from a subset of these collections. Actively spawning females were collected off the panhandle, as well as off

of Tampa Bay (Fig. 1B). Of the 58 actively spawning females 43% were collected west of Tampa Bay and the average size of these spawners was slightly smaller (mean=427.4 +/- SE 12.8 mm FL) than those collected off the panhandle (mean=457.3 +/- SE 21.3 mm FL). Similarly the maximum age was younger of the spawners collected off of Tampa Bay (5 years) than those collected off the panhandle (7 years). Active spawners were collected in a wide range of depths from 11 to 77 meters, with an average depth of 45.3 m +/- 2.6 m SE.

Currently red snapper in the Gulf of Mexico are managed as one stock, with the recognition that there are two sub-units, separated roughly by the Mississippi River (SEDAR 2009). However, there is a need to better understand reproductive dynamics at smaller spatial scales to assess potential source sites and how they may impact the larger stock's production (Saillant et al. 2010).

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Table 1. Summary of ovarian samples by sampling gear and year.

Year	Chevron Trap	Hook & Line	Trawl	Vertical longline	Unknown	Total
2008	2					2
2009	53	76	23			152
2010	2	66		1		69
2011				8	6	14
Total	**57**	**142**	**23**	**9**	**6**	**237**

Table 2. Ovarian classification and terms based on histological analysis.

Reproductive state		Phase	Histological indicators	Signficance
Immature	Nonspawn-ing	Immature	Only oogonia and primary growth oocytes, including chromatin nucleolar and perinucleolar oocytes. Usually no atresia.	Virgin that has not yet recruited to the spawning population.
Mature	Nonspawn-ing	Developing	Cortical alveolar and sometimes early yolked oocytes. No evidence of POFs. Some atresia may be present.	Environmental signals have triggered development, but fish are not yet developed enough to spawn.
Mature Spawning population	Spawning-capable	Spawning-capable	Yolked oocytes. May be some atresia.	Fish developed enough to spawn.
	Spawning	Spawning *Subphases*:	Oocyte maturation, hydration or POFs.	Fish with indicators of spawning activity.
		Imminent	Early OM (GVM with little yolk coalescence)	Will spawn in 14 h.
		Active	1. Late OM (completed GVM or GVBD with yolk coalescence and partial to full hydration), 2. Ovulation 3. Newly-collapsed POFs	Spawning +/- 2 h.
		Recent	POFs (12-36 h old)	Spawned within 2 d.
Mature	Nonspawn-ing	Regressing	A high percentage of yolked oocytes undergoing atresia (alpha and beta).	Cessation of spawning.
	Nonspawn-ing	Regenerating	Only primary growth oocytes present, including chromatin nucleolar and perinucleolar. Muscle bundles, enlarged blood vessels, thick and/ or convoluted ovarian wall, and gamma or delta atresia may be present.	Sexually mature, reproductively inactive. Most common outside of the spawning season.

Table 3. Histological micrographs of red snapper, *Lutjanus campechanus* histological indicators used to assign reproductive phases.

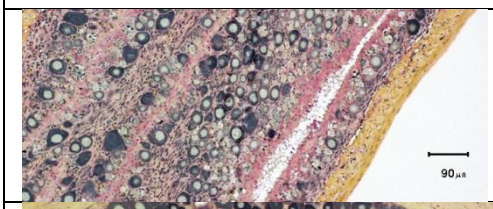
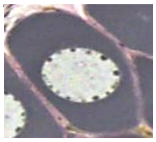
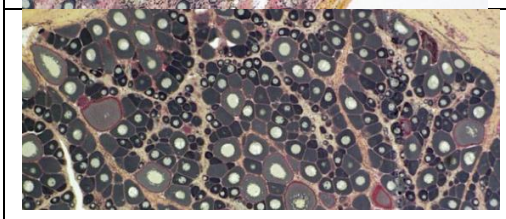
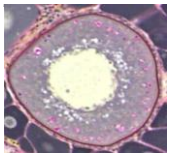
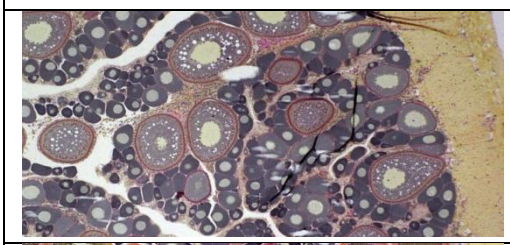
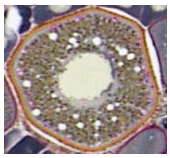
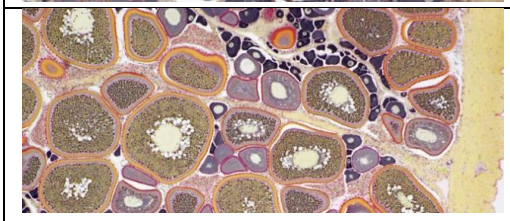
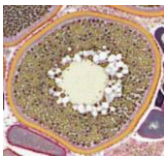
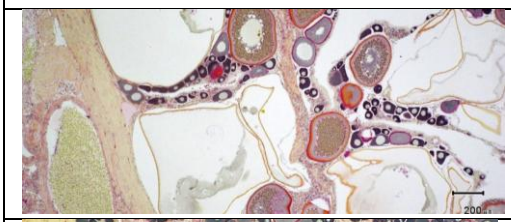
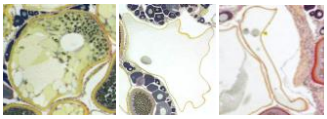
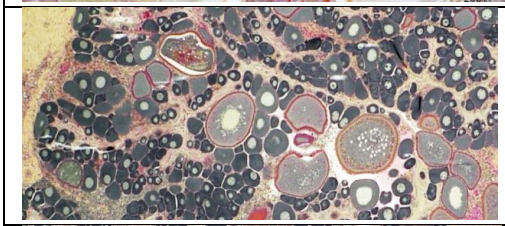
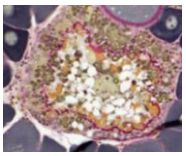
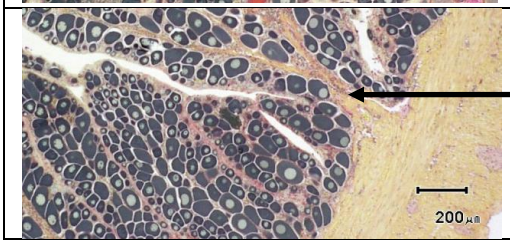
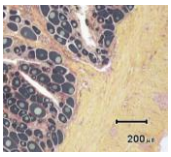
Ovarian Cross Section	Phase Characteristics	Most advanced oocyte or key histological indicator
	**Immature Phase** Only oogonia & PG No muscle bundles. Thin ovarian wall. Well-organized lamellae.	 Perinucleolar
	**Early developing subphase** Primary growth & cortical alveolar oocytes only Zona pellucid formed Can be some atresia	 Cortical avleolar
	**Developing** Primary growth, cortical alveolar, vitellogenic oocytes stages 1 & 2 Atresia can be present	 Vitellogenic 2
	**Spawning Capable** Completed vitellogenesis (Vtg3) Can have post ovulatory follicles present	 Vitellogenic 3
	**Actively Spawning subphase** Late oocyte maturation (GVM, GVBD, & hydration) Or ovulation Fresh POFs	 GVM Hydration
	**Regressing** Most vtg oocytes atretic; 1st indicator alpha atresia discontinuous zona pellucida PG and CA oocytes present	 Alpha atresia
	**Regenerating** Oogonia and PG oocytes o Muscle bundles often Thick ovarian wall May have late stage atresia	 Thick ovarian wall

Figure 1. A. Spatial distribution of sampling sites (chevron traps and hook and line) and red snapper collections in 2009 and 2010. B. Spatial distribution of female reproductive samples. Stage 1=immature, Stage 2=developing, Stage 3=spawning capable, Stage 4 (red) = actively spawning subphase, Stage 5=regressing and Stage 6=regenerating.