Scamp grouper reproduction on the West Florida Shelf

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Scamp grouper reproduction on the West Florida Shelf

Susan Lowerre-Barbieri, Hayden Menendez, Ted Switzer, and Claudia Friess

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Introduction:

Scamp grouper, *Mycteroperca phenax*, are distributed along the western Atlantic and throughout the Gulf of Mexico (Hoese and Moore 1977) and support important recreational and commercial fisheries (Lombardi-Carlson et al., 2012). However, stock status in the Gulf of Mexico (GOM) is currently unknown. Scamp grouper are sequential protogynous hermaphrodites, in which males recruit from females and this can present unique challenges to both traditional stock assessments (Alonzo & Mangel 2005, Brooks et al. 2008, Shepherd et al. 2013) and spatial management (Easter & White 2016) because males recruit from females. Stock assessment models typically aggregate outputs across the spatial domain of the species (Berger et al. 2017) and assume reproductive success is female-driven (Easter & White 2016). Both of these assumptions can be erroneous in protogynous species (Brooks et al. 2008, Shepherd et al. 2013). Because of this, in addition to estimating female reproductive potential it is important to estimate sex ratios and the potential for sperm limitation

Scamp grouper are a moderately long-lived species with a maximum age in the Gulf of Mexico of 31 years (Lombardi-Carlson et al., 2012). They are reported to have an extended spawning season from January through June, with a peak in April. Females mature by age 2 and overlap in female and male sizes has been previously reported (Lombardi-Carlson et al., 2012). The objectives of this work are to: (1) develop histological indicators to assess maturity, reproductive timing, transition, and reproductive phases (Lowerre-Barbieri et al., 2011a, Brown-Peterson et al., 2011); and (2) based on these indicators evaluate maturity, size at transition, and sex-specific spatial ecology.

Survey Design, Sampling Methods, and Analyses. Reproductive samples of Scamp were collected from three different sources: fishery-independent monitoring surveys (FIM), fishery-dependent monitoring surveys (FDM) and two targeted grouper studies that were conducted by FWC along the western coast of Florida (Figure 1).

Fishery independent monitoring on the west Florida shelf provided samples from 2009 through 2018 using primarily standardized hook-and-line methods, including a short bottom longline, a vertical longline (Christiansen et al. 2018a), and a repetitive timed drop survey (Christiansen et al. 2018b). The short bottom longline was an experimental gear consisting of 12 equally-spaced gangions (1.83-m spacing) along a length of monofilament (181.4 kg test) backbone. Each 1.52-m gangion terminated with a single Mustad circle hook (either 8/0, 11/0, or 15/0; Ref 39960D) baited with Atlantic Mackerel (*Scomber* spp.). The spatial extent, seasonal coverage, sampling intensity, and specific sampling gear used varied somewhat over time with the greatest number of samples collected in May (n=78) and August (n=73).

Fishery dependent samples were collected opportunistically by dockside samplers who frequented commercial fishing docks and local fishing tournaments in the central Florida region. From 2015 to 2019 gonadal tissue along with date landed, fork length (FL), and occasionally a general fished location was recorded.

Scamp samples were also collected as by-catch in two targeted studies on Gag reproduction. The first targeted study sampled in a marine protected area (Madison Swanson MPA), a seasonally-

closed area known as the Edges, and a nearby open area. Hook-and-line sampling alongside video surveys were completed from December to May over a three-year span (2015-2018). To better spatially distribute effort, each sampling location was broken up into roughly equal-sized sampling zones, with each zone fished for a total of 4 hours per zone (Lowerre-Barbieri et al., *in press*). Fishing rods with electric reels as well as electric bandit reels were used with 80 to 130 lb test monofilament. Hooks (12/0) were primarily baited with Atlantic Mackerel (*Scomber* spp.), however upon availability, live bait and cut bait were also used (20-40% of the time). Video data were collected with an un-baited, remote camera array made up of three compact action camera (Veho MUVI K-series). Cameras were mounted around a circular freestanding, weighted base to maximize the horizontal field-of-view to ~300°. The camera array was deployed in each zone prior to fishing, once per trip for 20 minutes to capture undisturbed fish communities and social behavior. Once the camera system was retrieved, hook-and-line fishing was conducted for a minimum of 20 minutes at the camera site.

Samples from the second study, came from another MPA, Steamboat Lumps and an open area to the south of Steamboat Lumps called the Sticky grounds and the Pinnacles. Sampling at these locations started in December 2018 and is on-going. Fish are again sampled with hook-and-line and video for a total of four days per month from December through May. Conventional, spinning, and electric reels were used to capture fish although monofilament and hook size differed depending on sampling depth and habitat. The camera array was deployed twice in each zone and followed the same procedure of lowering the camera prior to fishing for 20 minutes, followed by a minimum fishing time of 20 minutes at the camera site.

Samples of gonad tissue were collected from culled Scamp in years 2009-2020 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 1-2 h, and stored in 70% ethanol. Samples were embedded in glycol methacrylate, sectioned to 3–5- μm thickness, stained with periodic acid–Schiff's hematoxylin, and then counterstained with metanil yellow (Quintero-Hunter et al. 1991). Gonadal tissue was histologically assessed for all samples and thus sex and reproductive phases would be assigned. However, because FIM and FDM sampling was field-based, gonad weights were rarely taken. Thus, the gonadosomatic index (GSI) could be calculated for only 310 fish (161 males, 140 females, and 9 transitionals), collected primarily in the targeted sampling. GSI was calculated as:

GSI= 100 * (gonad weight/total gutted weight)

Gonadal analysis. Reproductive state, phase and histological indicators of Scamp were assigned following Lowerre-Barbieri et al. (2009) and Brown-Peterson et al. (2011); criteria are outlined in Table 2. Histological indicators for female reproductive state 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. Secondary growth oocytes (SG) included CA, Vtg, and OM and fish with this level of development were considered mature (Lowerre-Barbieri et al., 2011b). To help distinguish immature from mature regenerating females, which both have only PG oocytes, the density of the PG population, thickness of ovarian wall and presence of muscle bundles extending from the ovarian wall into the ovarian lamellae were used as indicators (Lowerre-Barbieri et al., 2011b).

Because oocyte maturation can take up to 16 h under the temperature regimes in which Scamp 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 and 3). Only developing, spawning capable (early and late) and regenerating reproductive phases were assigned to males, as active spawning can only be identified based on macroscopic analysis and there is no clear histological indicator to separate males late in the spawning season but still capable of spawning from those which are undergoing regression.

Scamp are not dimorphic. Because Scamp transition from female to male, testes continue to have ovarian walls and often large numbers of primary growth oocytes (Figure 2). Because of this, histological analysis is needed to assign sex. Fish were considered male if only spermatogenic cells were present (i.e., no PG) or they had spermatozoa present (Trip et al., 2011). Similarly, sex was determined as female if there was nothing but female tissue or healthy SG oocytes were present. Parasitic nematodes were frequently observed in histological slides. Small cross sections of parasites looked similar to yolked oocytes undergoing atresia, with the exception of an external epithelial layer (Figure 3). Parasites occurred in both ovaries and testes and immature and mature females.

We defined fish undergoing sex change as transitional (no sex assigned) and broke this down into early and late transition. Early transition is defined as those fish with spermatagonia, spermatocytes, and some spermatids. Late transition includes proliferating amounts of male tissue with spermatids or later stages of spermatogenesis present (Table 4).

Results / Discussion:

A total of 913 Scamp were sampled from 2009 through 2019 in the eastern Gulf of Mexico. Although Scamp were sampled in all months, the months sampled varied with year. Samples from December through May were taken primarily in the years of 2017-2019 (Figure 4). A total of 894 fish had gonadal tissue which was histologically analyzed to assign sex and reproductive phase and all consequent analyses are based on these data We sampled: 459 females, 414 males and 21 fish undergoing transition from female to male.

Although male, female, and transitional sizes overlapped (Figure 5) mean size by sex differed. The mean size of females was 454.1 +/- 82.0 mm FL compared to 558.9 +/- 64.1 mm FL for males and 506.7 +/-52.3 for transitionals. These differences were significant (Kruskal-Wallis, $\chi 2$=222.5292, $P < 0.0001$). These fish are currently being aged and age data will be added in May. The observed male sex ratio, 47%, was close to parity and higher than previously reported. In the 1970's the male sex ratio was estimated at 37.9%, with a decrease to 18-24% in the 1990's (Coleman et al., 1996).

Spawning capable males occurred in every month, whereas spawning capable/actively spawning females were collected only from January through June, with a peak in April (Figure 4). This is similar to previous reports (Lombardi-Carlson et al., 2012). Spawning seasonality based on female spawning indicators was 11 March to 3 June, a duration of 85 days. However, given that 88% of actively spawning females were sampled in March and April and that GSIs begin to decrease in May (Figure 3), the core spawning season appears to be roughly 60 days, from mid-March through mid-May.

Within the spawning season of 11 March to 3 June, 152 adult females were sampled. Six fully hydrated females were collected in targeted sampling and were captured between 7:10 to 10:15 AM, suggesting morning spawning. Seven of the hydrated females also had day one POFs, indicating Scamp can spawn two days consecutively. The % hydrated method resulted in a spawning fraction of 17.8% (27/152) and a spawning interval of 5.6 d. Post ovulatory follicles (without co-occurring hydrated oocytes) were present in 14 out of the 152 adult females, resulting in a spawning fraction of 9% and a spawning interval of 11.1 d, almost twice that of the % hydrated method. Using both spawning indicators and an estimated indicator duration of 48 h resulted in a spawning fraction of 13.5% and a spawning interval of 7.4 d. Given that hydrated females will be contagiously distributed if Scamp aggregate to spawn, we do not recommend using the % hydrated method.

Immature females ranged in size from 178 to 342 mm FL, with a mean of 273.0 mm FL. The size of females with secondary growth oocytes (cortical alveoli or more developed; 265 to 695 mm FL) overlapped somewhat with that of immature females (Figure 6). Estimated size at 50% maturity used all months, given the relatively large number of regenerating females throughout the year (n=432). Estimated size at 50% mature was 265.1 mm FL (SE=3.6038) and 95% confidence limits of 258 mm FL to 272.2 mm FL (Figure 7A). Filtering the female data to assign maturity only to spawning capable females (which includes the actively spawning females) greatly decreased the sample size (n=81). Using this criterion the estimated size at 50% maturity was 350 mm FL (SE=82.8332) and 95% confidence limits of 185.1 to 514.9 mm FL (Figure 7B). Although the precision of this estimate decreased due to decreased sample size the accuracy seems improved given that the smallest female with spawning indicators was 375 mm FL (Figure 6). Although estimates of size and age at maturity are critical to stock assessments and typically determined based on a logistic curve fitted to sex-specific maturity data, the accuracy of these estimates is difficult to determine given: (1) the complex process of maturation; (2) reproductive phases are not randomly distributed in space; and (3) the inability to conclusively distinguish between immature and regenerating based on the most developed oocyte stage (Lowerre-Barbieri et al., 2011).

Estimated size at 50% male was 508.8 mm FL (SE=2.9325), with 95% confidence limits of 503 mm FL to 514.5 mm FL (Figure 8). Transitionals (n=21) ranged in size from 409 to 610 mm FL, with a mean size of 506.7 mm FL, very similar to the estimated size at 50% mature. The temporal distribution of transitionals was extensive, with samples collected in every month of the year. However, spatially they were limited to deeper water sites with a minimum depth of 46 m, similar to depths where actively spawning females were sampled (41 to 235 m; Figure 9), suggesting that transitionals may remain on the spawning grounds. This has important implications for the efficacy of spawning reserve MPAs to protect male abundance in this species in contrast to Gag grouper, which exhibit female pre-spawning aggregations where

transition can occur prior to spawning migrations to deepwater spawning sites (Lowerre-Barbieri et al., in press). This may be a key factor in the higher sex ratios of Scamp in MPAs, compared to Gag which only exhibited 5% male inside the Madison Swanson spawning reserve MPA.

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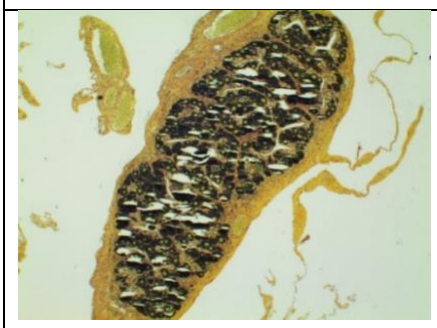
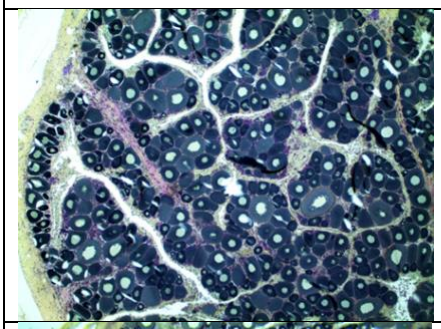
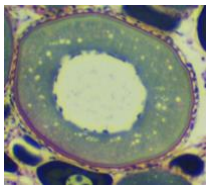
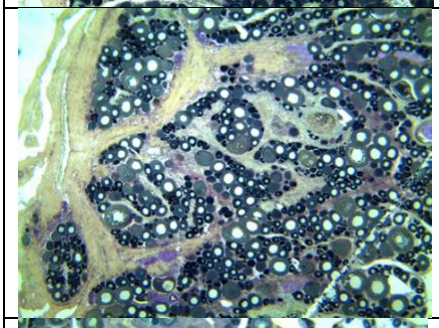
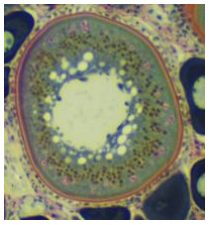
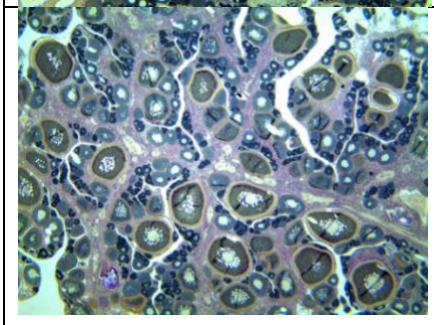
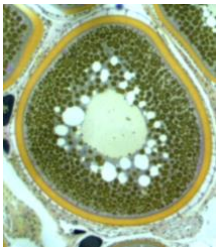
Table 1. Ovarian classification and terms based on histological analysis.

Reproductive state		Phase	Histological indicators	Significance
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	Oocyte maturation, hydration or POFs.	Fish with indicators of spawning activity.
		Subphases:		
		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 2. Histological basis for reproductive phases in female Scamp, *Mycteroperca phenax*

Ovarian Cross Section	**Phase Characteristics**	**Most advanced oocyte or key histological indicator**
	**Immature Phase** • Only oogonia & PG • No muscle bundles • Thin ovarian wall • Small ovaries • Organized lamellae	 Perinucleolar primary growth (PG)
	***Early Developing – Sub phase*** • PG & CA • Can be some atresia	 Cortical alveolar (CA)
	**Developing** • PG & CA, Vtg1 (partially yolked) • No Vtg3 or POFs • Can be some atresia	 Vtg1 partially yolked
	**Spawning Capable** • Vtg3 (fully yolked oocytes FY) present • Can have early oocyte maturation (OM) • Can be some atresia	 Vitellogenic 3

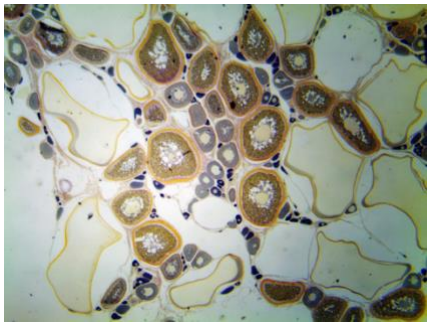
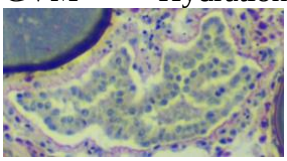
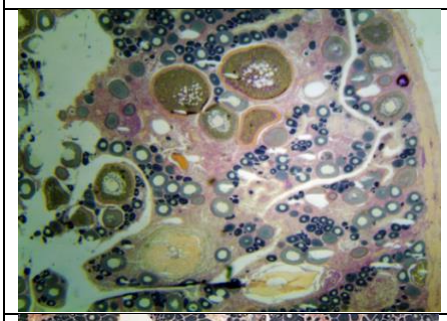
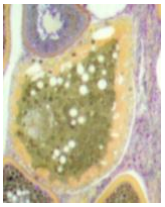
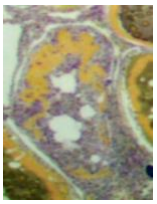
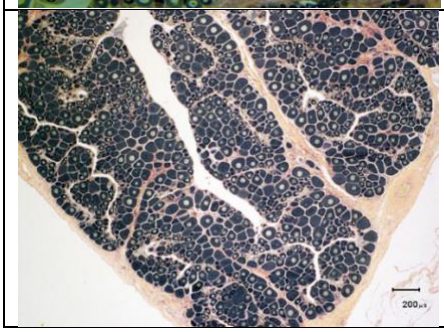
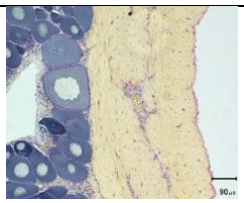
	***Actively Spawning subphase*** • Late germinal vesicle migration (GVM) • Germinal vesicle breakdown (GVBD) • Hydration and can have fresh POFs	 GVM Hydration  Fresh POF
	**Regressing** • Most Vtg oocytes undergoing atresia	  Alpha atresia Beta atresia
	**Regenerating** • Oogonia and PG oocytes present • Muscle bundles • Thick ovarian wall	 Thick ovarian wall

Table 3. Histological indicators of fish undergoing transition in male Scamp, *Mycteroperca phenax morio.*

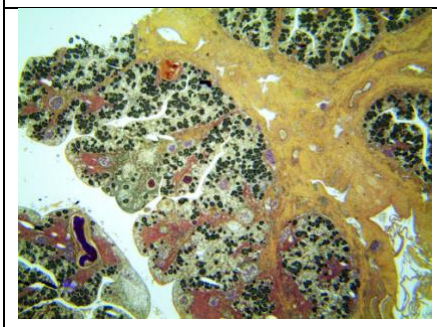
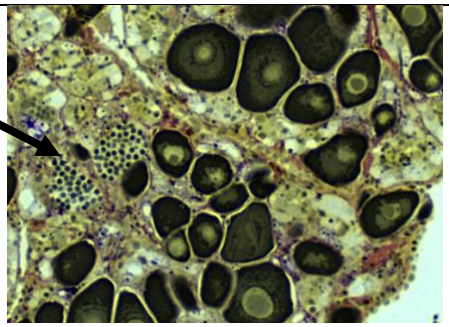
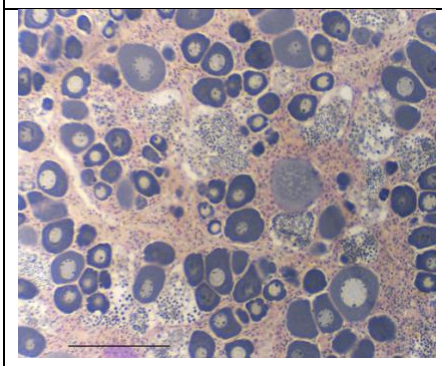
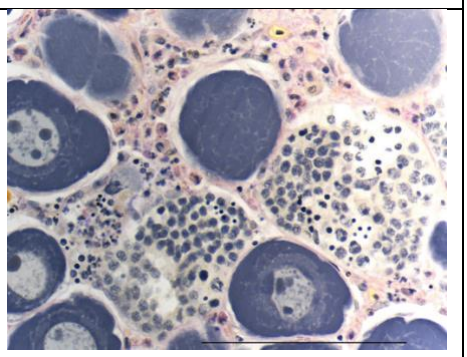
Teste Cross Section	Phase Characteristics	Key histological indicator
	**Early transition** • Spermatagonia (Sg) & spermatocytes (Sc) present • Continuous germinal epithelium • PG abundance decreasing	
	**Mid-late transition** • Sg, Sc & spermatids (St) present, occasional sperm crypts • Male tissue proliferation is dominant	

Figure 1. Locations where Scamp grouper samples were collected from 2009 to 2019. Data sources include the FWRI fisheries independent monitoring reef fish survey, FWRI Fishery dependent monitoring (FDM), and samples collected as by-catch from studies on Gag spawning grounds. For the FDM samples, it is important to note that fishermen assign only a general location.

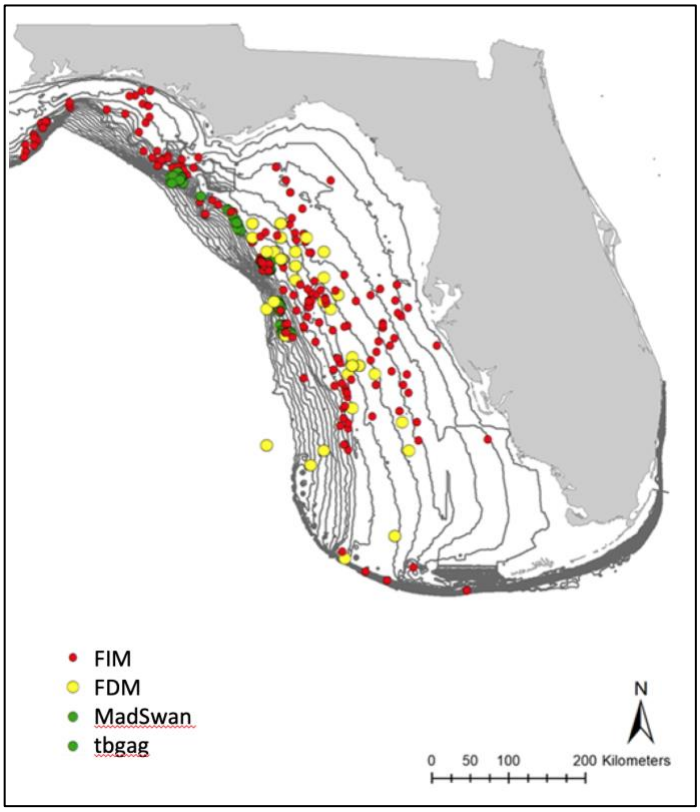


Figure 2. Sex cannot be determine based on macroscopic examination of Scamp gonads, as they all look similar: All of the below examples were sampled on 12/28/2019. A. transitional, 483 mm FL B. regenerating female, 546 mm FL and C. male, 569 mm FL.

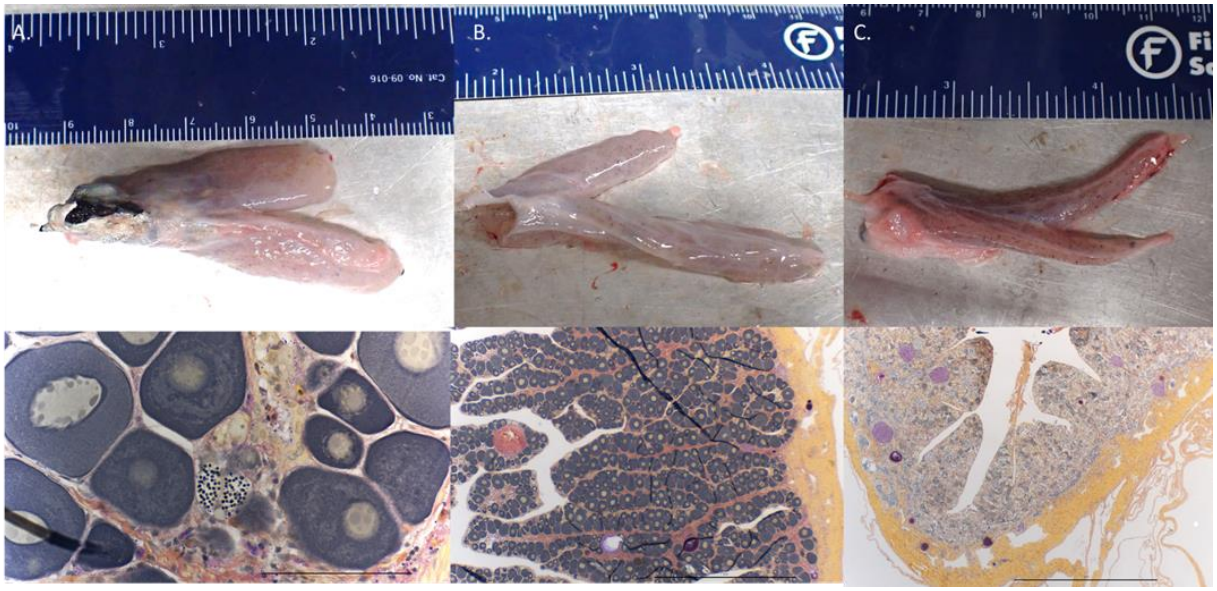


Figure 3. Similar to Red Grouper, Scamp commonly had parasites in both female (top) and male (bottom) gonads.. These looked much like vitellogenic oocytes.

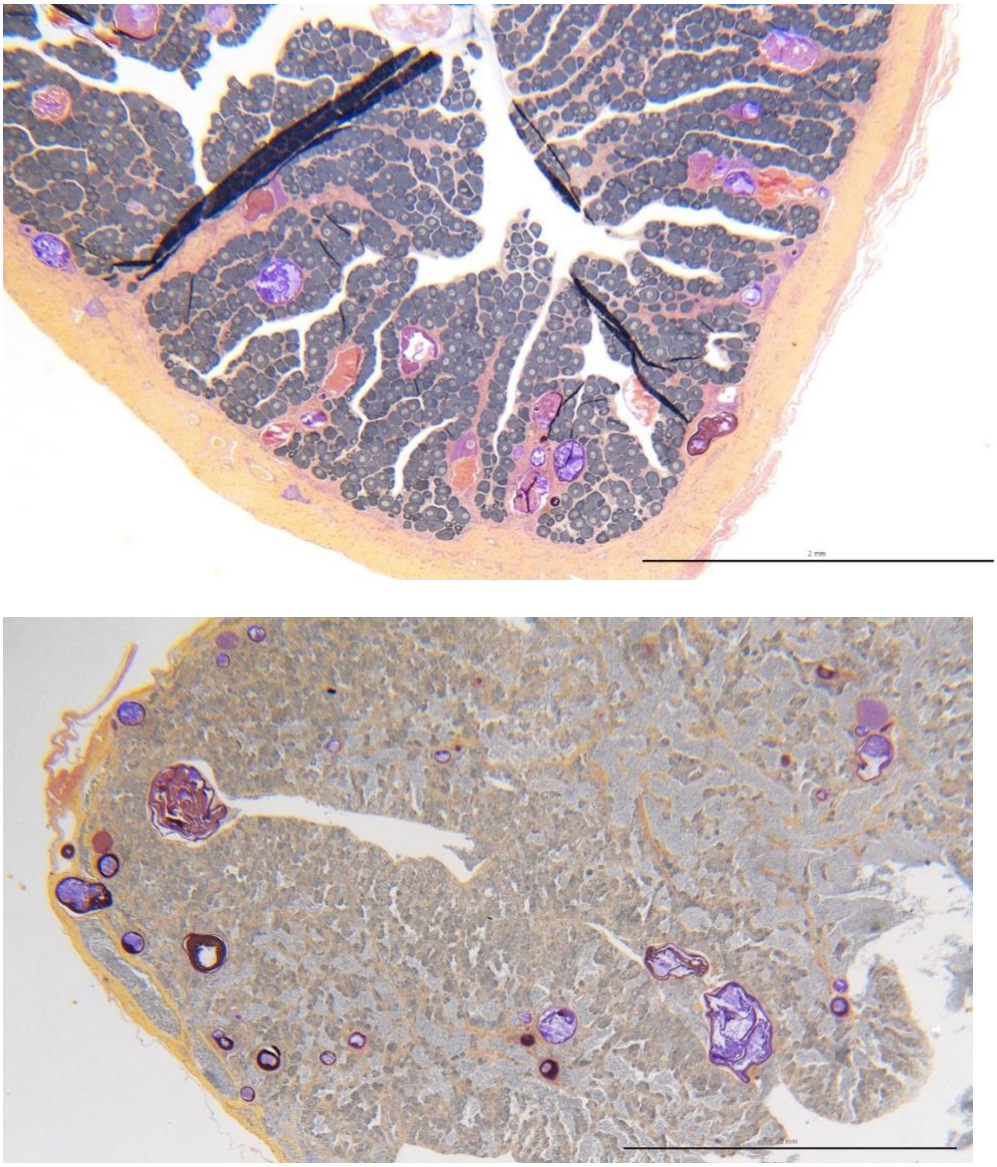


Figure 4. Temporal distribution of female and male reproductive phases and monthly patterns in sex-specific gonadosomatic index (GSI). Spawning capable and actively spawning females occurred from December through May, with a peak in April. GSIs were calculated for the months of January through August, as well as in December (not shown). Mean December GSI was 0.31 +/-0.12 for females and 0.09 +/- 0.05 for males. Monthly ample sizes for reproductive phases are indicated at the top of the bars. Sample sizes for GSI were 161 males and 140 females.

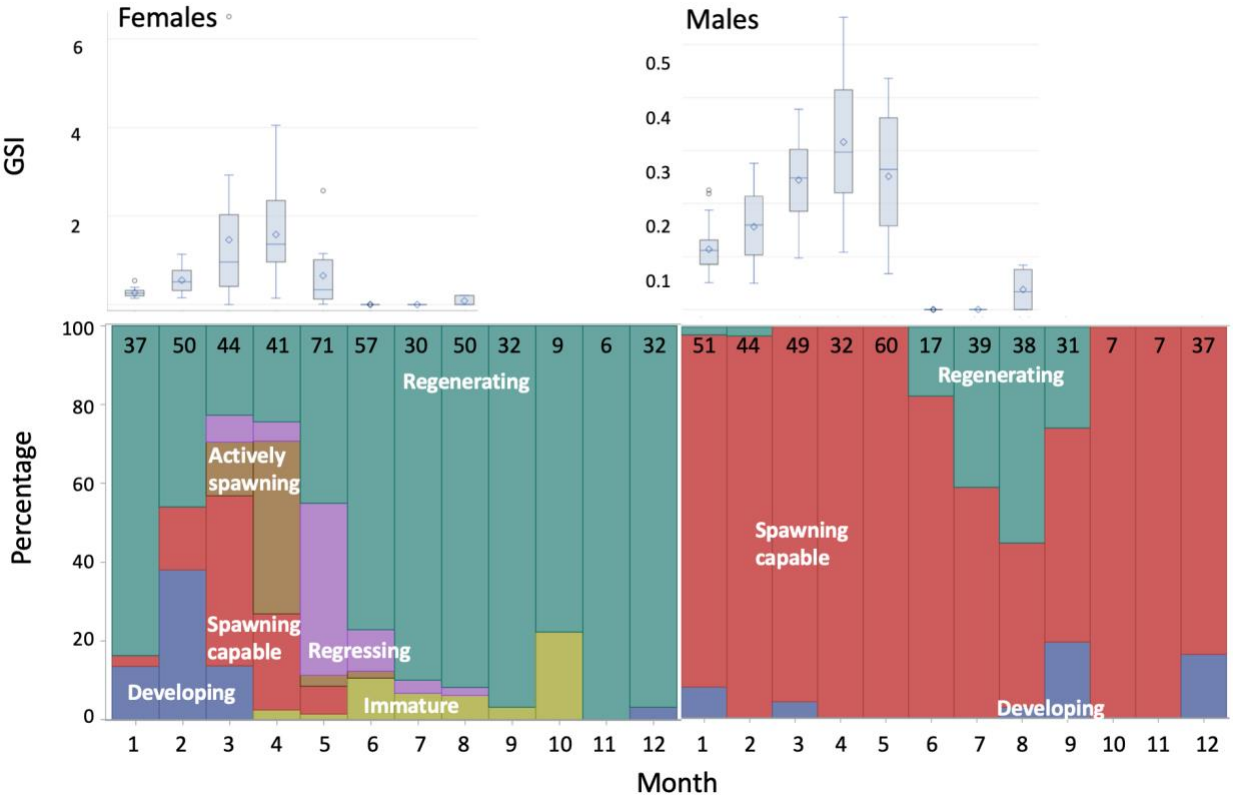


Figure 5. Sex-specific length frequency distribution (A) and mean length (B).

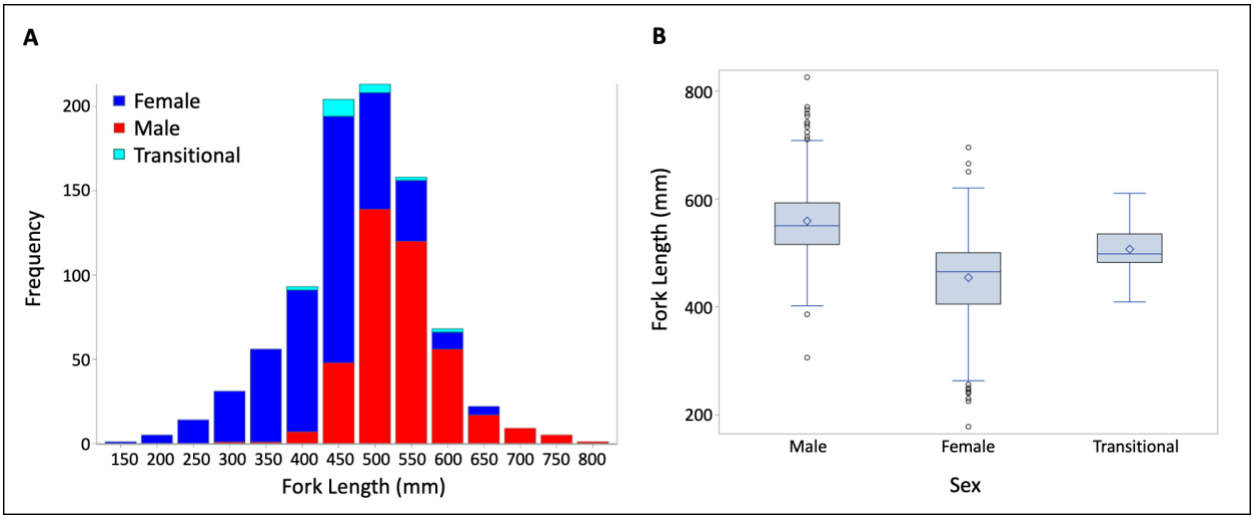


Figure 6. Female length frequency distribution by maturity status.

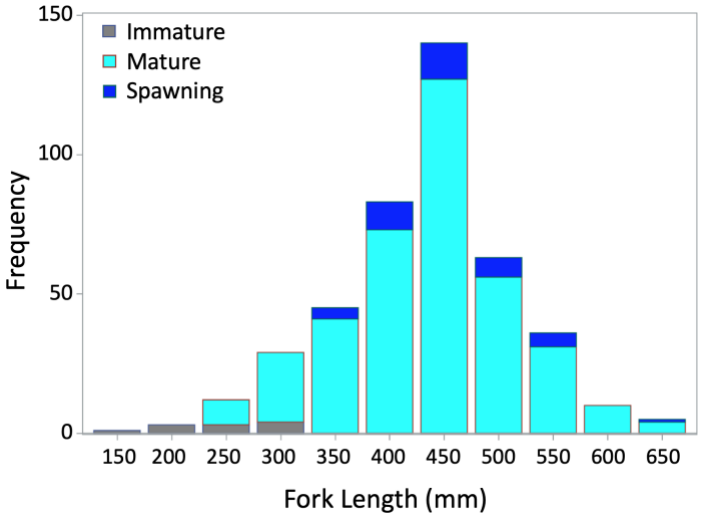


Figure 7. Observed and predicted values of size at maturity with 95% confidence limits (light blue shaded area) using (A) all female samples (n = 432) and (B) only immature and spawning capable and actively spawning females (n = 81).

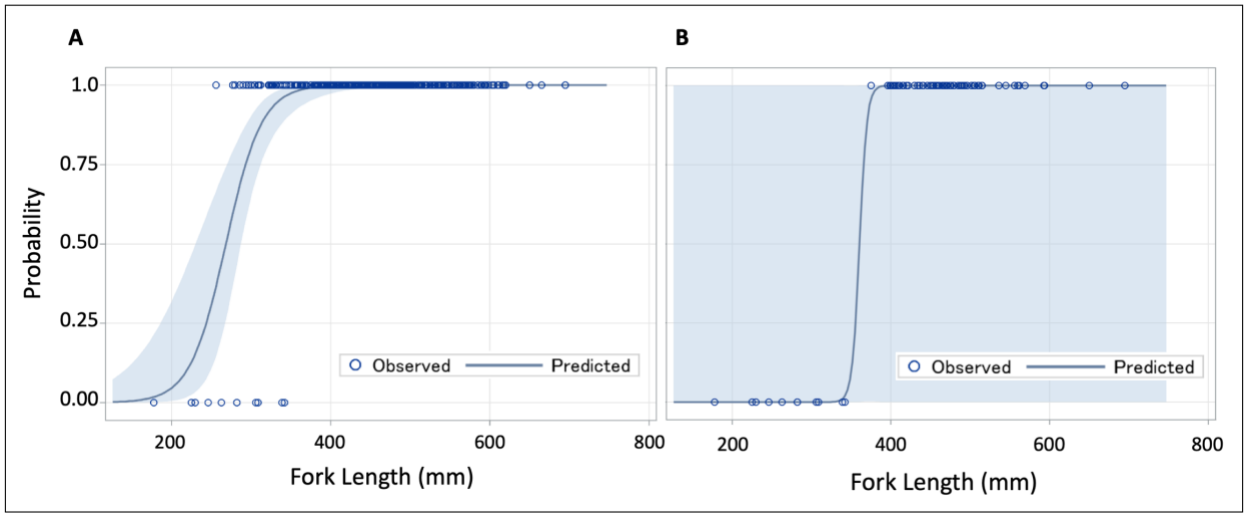


Figure 8. Observed and predicted values of size at transition with 95% confidence limits (light blue shaded area). Estimated size at 50% male was 508.8 mm FL.

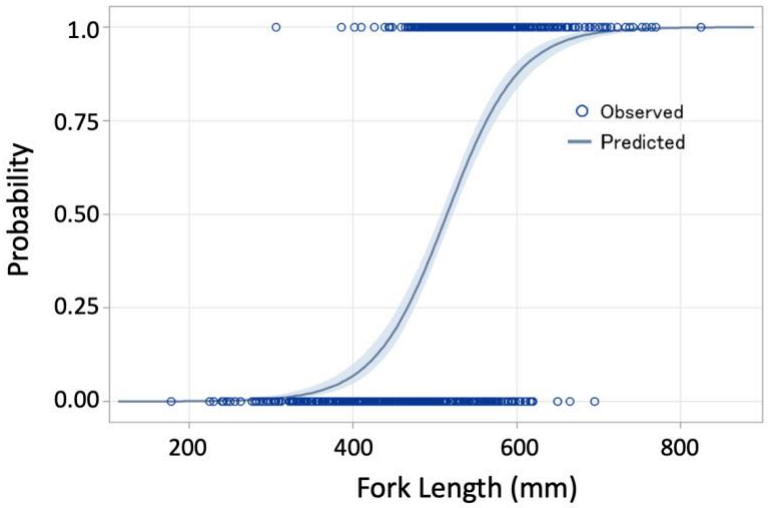


Figure 9. The spatial distribution of females, males, and transitional Scamp used in this study. Only 2 out of the 3 sources were plotted (FIM and targeted grouper studies) whereas FDM samples were not plotted due to the inaccuracy of location given to scientists by fishermen. Red circles represent males, blue triangles are females, and green diamonds mark transitional fish.