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Testing assumptions about sex change and spatial management in the protogynous gag grouper, *Mycteroperca microlepis*

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ABSTRACT: Gag grouper *Mycteroperca microlepis* are protogynous hermaphrodites, for which the assumption of female-driven reproductive potential may be inaccurate. In protogynous species, male abundance, fertilization success, and stock productivity are affected by where and when sex change occurs and how fishing pressure affects male recruitment and survivorship. In this study, we integrated large spatial-scale data with high-resolution data from a 3 yr study sampling gag at deep-water sites with varying spatial management (a marine protected area [MPA], a seasonally closed area, and an 'Open area'). Gag exhibited complex spatial ecology; females formed pre-spawning aggregations before migrating to deep-water spawning sites, which overlapped with locations where males were sampled year-round. The observed male sex ratio in the MPA was 5% compared to the expected 15%. It was 0% in less protected areas. Sex change occurred occasionally in small fish and before, during, and after the spawning season. In addition, sex change was observed in pre-spawning female-only aggregations as well on the spawning grounds, indicating that male social cues are not requisite. We propose that shallow-water, pre-spawning aggregations are a key spatio-temporal bottleneck to gag productivity. They appear to be an important source of transitionals and are heavily fished, which may negatively impact male recruitment to the spawning grounds. Our results indicate that overall gag abundance is low, MPAs do not protect all recruiting males (as previously assumed), and current regulations are not sufficient for the male population to recover to historic levels (~17% male).

KEY WORDS: Movement ecology · Mating strategy · Sex change · Gag grouper · Marine protected areas · MPAs

1. INTRODUCTION

Important fisheries are supported by species with complex spawner-recruit ecology. Although stock productivity is typically modeled based on mature female biomass or total egg production, it is becoming increasingly clear that other factors also affect reproductive success. These include sequential hermaphroditism, sex-specific movement ecology, the formation of aggregations (pre-spawning or spawning), and species-specific life cycle distribution, all of which result in species-specific vulnerability to fishing pressure and stressors (Lowerre-Barbieri et al. 2017a, 2019). In sequential protogynous hermaphrodites, males recruit from females, and this can present unique challenges to both traditional stock assessments (Alonzo & Man-

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gel 2005, Brooks et al. 2008, Ellis & Powers 2012, Shepherd et al. 2013) and spatial management (Chan et al. 2012, Easter & White 2016). Stock assessment models typically aggregate outputs across the spatial domain of the species (Berger et al. 2017) and assume that 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). Sex allocation theory predicts that adult sex ratios should result in the highest reproductive success for the population, with maturation selected to occur when the benefits of increased fitness outweigh the costs of maturation. In protogynous fishes, males typically exhibit delayed maturation (i.e. males are larger and older than females) and this is predicted to evolve when male competition plays a role in the mating system. Thus, to predict productivity and provide effective management for these species requires an understanding of where and when sex change cues occur as well as knowledge of a species' mating strategy (Alonzo et al. 2008, Easter & White 2016). It is also important to assess the spatial distribution and size of the reproductive unit, i.e. group of fish which come together at a spawning site for courtship and reproductive behavior (pairs, harems, group spawners, spawning aggregations, or leks). Similarly, for spatial management to increase productivity, there is a need to understand the spatial ecology of a species, and in protogynous species, to understand how this spatial ecology affects the sex-change process and mating strategy/optimal sex ratio.

Gag *Mycteroperca microlepis* support extensive commercial and recreational fisheries (McErlean 1963, Schirripa & Goodyear 1994), and all males recruit from the mature female population (Koenig et al. 1996). Their movement ecology is complex, including a series of sequential ontogenetic habitat shifts from estuarine nursery grounds to shelf-edge spawning sites (Carruthers et al. 2015), where males are believed to be resident. In addition, they are reported to form both pre-spawning and spawning aggregations (Koenig et al. 1996). They are highly regulated in the Gulf of Mexico (GOM) (http://sedarweb.org/s33rd06-gulf-mexico-gag-management-history) through size and bag limits, closed seasons, and spatial management (2 marine protected areas [MPAs] at spawning sites developed in 2000, and a seasonally closed spawning reserve established in 2009). Spatial management was predicted to increase male abundance (Heppell et al. 2006, Ellis & Powers 2012). However, Ellis & Powers (2012) pointed out that a male density-dependent feedback loop and relatively small, isolated MPAs would result in lower male abundance than predicted if sex change is endogenously driven (Heppell et al. 2006). Current stock status for GOM gag is uncertain, as the most recent stock assessment (SEDAR 33; SEDAR 2014) produced conflicting results: 'not overfished' when using the female-only spawning stock biomass (SSB) model and 'overfished' when using the combined-sexes SSB model. The final decision was to use the female-only SSB model due to implausible SSB-combined biomass trajectories, high uncertainty in reproductive parameter estimates, and low predicted male sex ratios in its terminal year (~2% male; SEDAR 33). These predicted male sex ratios were similar to the 2–3% male in the 1990s when gag were overfished (Hood & Schlieder 1992, Coleman et al. 1996, Koenig et al. 1996, Collins et al. 1998) and significantly lower than the predicted estimate of ~15% in spawning reserve MPAs (Heppell et al. 2006).

To improve our ability to manage protogynous species, we need field testing of model assumptions and results as well as increased strategic modeling (Easter & White 2016). Gag in the GOM make an excellent case study for this process given the current uncertain stock status, previously published modeling efforts, and the implementation of 2 spawning reserves almost 20 yr ago. In this study, we assess assumptions and model predictions about sex change cues and spatial management for gag. Data from a 3 yr study in the GOM, sampling the spawning grounds within the Madison Swanson MPA, the seasonally closed Edges, and a nearby 'Open area', are integrated with data collected from the following sources: a cross-shelf fisheries-independent monitoring (FIM) survey, fishery-dependent monitoring (FDM), and samples from a collaborating commercial fisherman targeting relatively near-shore areas (Fig. 1). We used these data to test the following hypotheses: (1) females form pre-spawning aggregations in December, January, and February and then undergo spawning migrations to deep-water spawning aggregation sites, where males remain year-round and females move only to spawn (Hood & Schlieder 1992, Koenig et al. 1996); (2) percent male and male age at 50% male (A50) have increased since the 1990s, and percent male within the MPA is ~15%, as predicted by the model of Heppell et al. (2006); (3) sex change cues occur only on the spawning grounds, and sex change is either endogenously driven or constrained by a size threshold of 800 mm total length; and (4) male sex ratios on the spawning grounds are a requisite social cue for sex change, producing a density-dependent feedback loop for the rate of sex change, associated with the sex ratio on the spawning grounds the previous year (Ellis & Powers 2012).

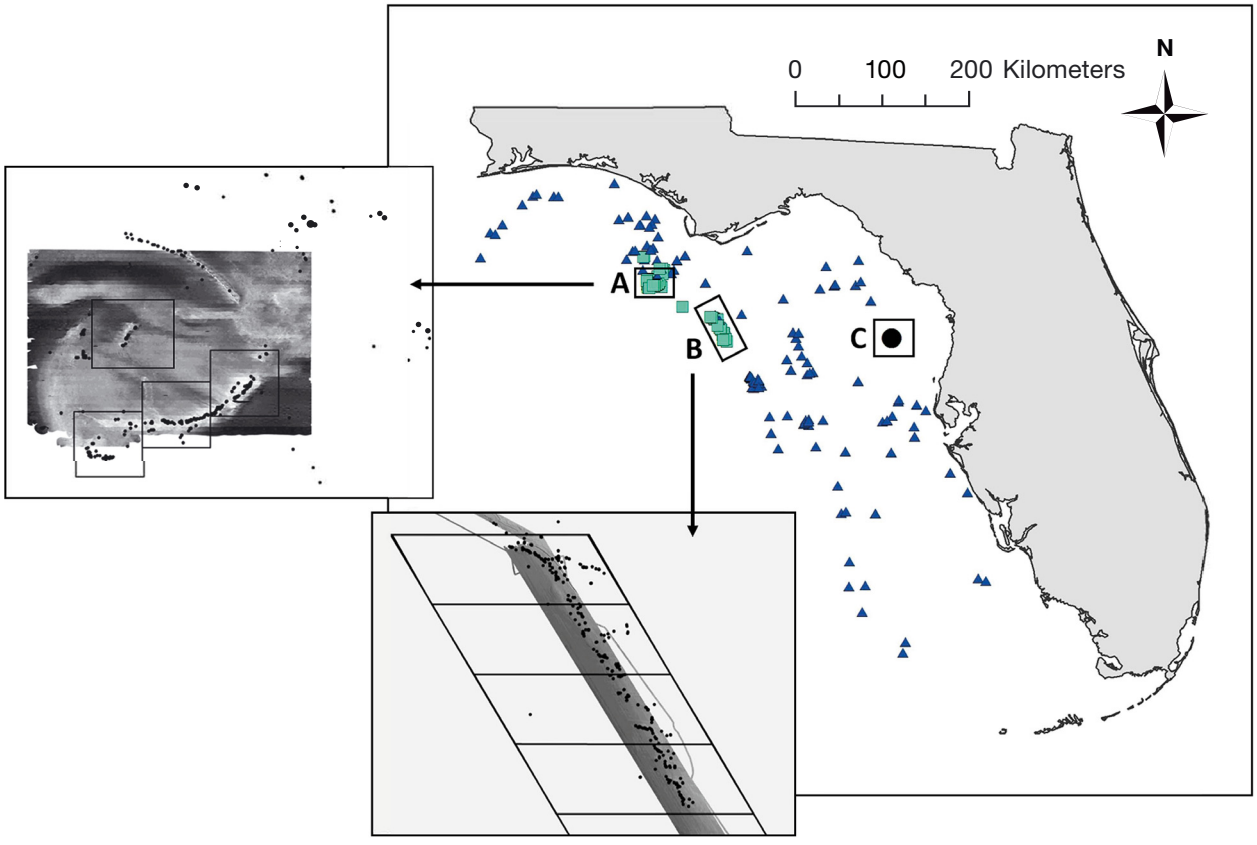

Fig. 1. Distributon of gag *Mycteroperca microlepis* samples with location data by data source: (1) targeted study sites (■, A ,B) sampled from December through March; (2) samples from the fisheries-independent reef survey (▲) and (3) samples from a collaborative fisherman (●, C), Insets include sampling zones in (A) Madison Swanson and (B) the Edges. Note that comparable spatial information was not available for fisheries-dependent samples collected

2. MATERIALS AND METHODS

2.1. Data collection

This study was conducted along the west coast of Florida, USA, with data collected at varying spatio-temporal scales depending on the source: FIM, collaborative hook-and-line fisher, a targeted study, and FDM. All data sources, except FDM, also provided data on sample location and depth. The targeted study sampled with video and hook-and-line from December to May, which encompasses the spawning season, over 3 yr (2015–2018). Monthly video and biological data were collected in 3 deep-water areas (mean depth range: 59.7–85.6 m; Fig. 1): (1) Madison Swanson, an MPA closed to bottom fishing year-round and all fishing from 1 November to 30 April (Fig. 1A); (2) an artificial reef in an unprotected area just to the northeast of Madison Swanson (not shown); and (3) the Edges (Fig. 1B), an area closed to all fishing from 1 January to 30 April. To assess sex ratios outside of the predicted spawning season, 4 opportunistic sampling trips were made to Madison Swanson in the months of June, July, October, and November.

To improve the spatial distribution of samples within the targeted MPA and seasonally closed study areas, these areas were further subdivided into sampling zones. In Madison Swanson, we targeted known gag habitat, with 3 zones along the ridge, and 1 within the center of the MPA (Fig. 1A). The Edges was broken into 4 zones from north to south (Fig. 1B), and locations with hard bottom and ledges were fished within each zone. The Open area had 1 zone that included the 'Zinnia' wreck and has been previously identified as a potential pre-spawning aggregation site (C. Koenig unpubl. data). Target sampling was monthly, with ~4 h of fishing per zone. Sampling was both video- and capture-based. Video data were collected with an unbaited, remote camera array made up of 3 compact action cameras (Veho MUVI K-series). Cameras were mounted around a circular, freestanding, weighted base to maximize the hori-

zontal field-of-view to ~300°. The camera array was deployed in each zone prior to fishing, once per trip for 20 min 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 min at the camera site, whereas other fishing sites had a minimum fishing time of 5 min. Fishing rods with electric reels as well as electric bandit reels were used with 80 to 130 lb (36 to 59 kg) 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). At each site, start and end times for the fishing event were recorded. However, fished time is only a rough estimate of fishing effort, as the number of hooks in the water and fishing skill of the crew varied. In addition, fishing was interrupted for at least one of the crews to release by-catch on a SeaQualizer pressure-activated descending device, as well as to photograph gag pigmentation at landing.

FIM on the west Florida shelf provided samples from 2009 through 2018 primarily with 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 181.4 kg monofilament backbone. Each 1.52 m gangion terminated with a single Mustad circle hook (either 8/0, 11/0, or 15/0; Mustad 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 = 106) and August (n = 101).

Fishery-dependent samples came from 2 sources: a collaboration with a hook-and-line commercial day boat fisherman who provided samples, depths, and location data and the Florida Fish and Wildlife Research Institute (FWRI) FDM program, which collected biological data from gag at select tournaments and docks in the central Florida region. The day boat captain and crew used standard hand gear (manual rod and reel) with 50 lb (23 kg) test monofilament, light sinkers, and 9/0 circle hooks baited with live pigfish *Orthopristis chrysoptera*. They fished an area with a network of ledges 18–21 m high (C in Fig. 1). On each fishing day, they started at known ledges, and 4 people would continuously fish until gag catch significantly decreased (typically within ~15 min), at which time they would move to the next ledge. The collaborating fisherman provided sampling depth and location and invited biologists to collect otoliths, size measurements, and gonadal tissue from landed fish in 2016 through 2018. Samples were collected primarily in January and February (n = 59). FDM samples came from 2015 through 2019 and included date landed, fork length (FL), and gonadal tissue. All FL measurements were converted to total length (TL) using the following equation (SEDAR 33):

$$TL = 5.85 + 1.03 \times FL \quad (1)$$

Although sampling occurred year-round, the greatest number of samples were collected in December (n = 108) and February (n = 120).

2.2. Sample processing

Fish were kept on ice until they could be processed, generally within 24 h of capture. Fish were measured for TL (±1 mm), sagittal otoliths were removed and stored dry, and ovarian tissue samples were taken for histological analysis. Gonad weight and total weight were measured to the nearest g for samples taken in the targeted study. Gonadosomatic indices were calculated as [GW/(TW–GW)] × 100. Ovarian tissue was fixed in 10 % neutral buffered formalin for a minimum of 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). Fish were aged using standard protocols and both whole and sectioned otoliths (Fitzhugh et al. 2003). Age was assigned based on annulus counts of opaque zones, marginal edge growth, and a January 1 birth date.

Reproductive state, phase, and histological indicators of spawning were based on accepted classification methods (Brown-Peterson et al. 2011, Lowerre-Barbieri et al. 2015), with a few slight modifications. Five oocyte development stages were identified: primary growth (PG), cortical alveoli (CA), partially yolked (PY/ Vtg1), fully yolked (FY/Vtg2 and Vtg3), and early and late oocyte maturation (OM) (Lowerre-Barbieri et al. 2009). Criteria used to distinguish immature from mature regenerating females included physical attributes of the cross section: size, organization, ovarian wall thickness, and standing stock of PG oocytes. Fish with secondary-growth oocytes (CA or more developed) were considered mature (Lowerre-Barbieri et al. 2011). Post ovulatory follicles (POFs) were identified but were difficult to age and thus categorized as within 24 h of spawning or older (Hunter

& Macewicz 1985a). Spawning indicators were: OM, POFs, and hydrated oocytes undergoing ovulation. The spawning season was based on the first and last day on which these indicators were observed in the pooled data. Actively spawning females had oocytes in late OM (i.e. had completed germinal vesicle migration) and were assumed to spawn the day they were captured. Skip spawners were defined as mature females sampled on the spawning grounds and in the spawning season with no indicators of secondary oocyte growth (i.e. regenerating phase). Male gonadal development was categorized as developing, spawning capable (early and late), or regenerating based on the presence of spermatocytes, spermatids, or spermatozoa and a continuous or discontinuous germinal epithelium. Transitional fish, i.e. those individuals changing sex from female to male, were identified using criteria similar to those applied to honeycomb grouper *Epinephelus merra* (Bhandari et al. 2003) and orange-spotted grouper *E. coioides* (Wu et al. 2015). These included presence of oocytes (typically PG) in conjunction with spermiogenesis, as indicated by a continuous germinal epithelium, spermatagonial nests, spermatocytes, and sometimes spermatids or even small pockets of sperm (see Section 3 and Fig. 7).

Videos were read by 2 independent readers at standardized intervals (n = 60 s) synced across the cameras to provide synoptic abundance estimates. Abundance was summed across videos at each corresponding interval, and the maximum number of individuals ($MaxN_V$) per video was used to estimate relative abundance at a given site.

2.3. Data analysis

The spatio-temporal window of spawning was based on the seasonal period over which fish with spawning indicators were collected and the spatial distribution of active spawners. Depth was used as an indicator of habitat selection. Variables were tested for normality using the Shapiro-Wilk test. Differences in depth by capture were not distributed normally for either sex ($p < 0.0001$) and thus the non-parametric Wilcoxon Mann-Whitney test was used to assess if differences in depth at capture by sex were significant. To evaluate sex-specific movement ecology associated with spawning, we used the non-parametric Kruskal-Wallis test to assess if depth-at-capture varied significantly with reproductive phase for each of the sexes separately, and for females we used the Dwass, Steel, Critchlow-Fligner (DSCF) post hoc method to identify pairwise differences in depth-at-capture with developmental phase. Because sizes were not distributed normally, all statistical analyses of sizes were based on non-parametric Wilcoxon Mann-Whitney tests. Size and age at 50% male (A50) within the MPA were estimated using logistic regression. To evaluate if female age (integers only) differed significantly by sampling area (i.e. the seasonally closed Edges versus the Madison Swanson MPA), we used a general linear model (GLM) with a Poisson distribution.

We used catch rates (standardized to 1 h of fishing), maximum number caught ($MaxN_C$), and maximum number observed on video ($MaxN_V$) to evaluate potential aggregating behavior. To evaluate changes in density associated with spawning aggregations, the above indicators were compared between sampling events within the spawning season (spawn events) or outside the spawning season (non-spawn events). Spawning aggregations have been defined as fish repeatedly concentrating for the purpose of spawning at a predictable space and time, with at least a 4-fold increase in density (Domeier 2012). Thus, we used a 4-fold increase in catch rates as a threshold for identifying aggregating behavior (both pre-spawning and spawning). Unique fishing events were defined as those occurring at a specific time and place in the targeted study. Because our interest was only in temporal changes in density, and the same methods were used in all targeted sampling, we estimated nominal catch per unit effort (CPUE) rather than standardizing it with a statistical standardization model. CPUE was calculated as the number of fish caught per minutes fished for each unique fishing event and standardized to expected catch per hour. Although our measure of effort was imprecise, the number of hooks in the water per minute fished was assumed to be comparable across fishing events. CPUE within the spawning season (spawn-time events) was compared to CPUE outside the spawning season (non-spawn time events) to test the hypothesis that fish moved to deep-water sites to spawn. Logistic regression was used to estimate size and age at 50% male and to predict the probability of being male at a given size or age so that we could compare observed sizes and ages of the smallest observed males to the model-predicted probability of that occurring. The underlying assumption was that if fish transition at size/ages much lower than expected, these fish are not surviving the migration to the MPA. Means are presented ±SD.

3. RESULTS

In total, 1657 gag were captured with hook and line and had a sex assigned, based on histological analysis. Samples with associated depth and location data (n = 1020) came from the targeted study on the spawning grounds (December–May, n = 572, and opportunistically in other months, n = 43), the fishery-independent survey (n = 345), and the commercial hook-and-line fisherman (n = 58; Fig. 1). Gag were sampled at depths ranging from 5 to 122 m. However, sampling was not equally distributed over these depths and differed with data source. Gag from FIM samples had the widest range of depths, from 6.3 to 128 m, with a mean depth of 64.1 ± 26.5 m. Gag sampled in the targeted study came from deeper waters (39–122 m; mean = 83.4 ± 11.0 m). The commercial hook-and-line fisher targeted relatively shallow water (5–15 m; mean = 13.9 ± 3.5 m). An additional 621 samples with FL and gonadal tissue were sampled by FDM but did not have location or depth data.

3.1. Spatial ecology

The spawning season, based on the first and last day females with spawning indicators occurred (all sources), was from 1 February through 18 April. Pre-spawning aggregating behavior consistently occurred at the shallow sites that our collaborative fisher targeted and was observed in 1 year (out of 3) at an artificial reef in the deep-water Open area. The commercial fisher captured fish as early as November and as late as mid-February. Maximum catch per day at his shallow sites peaked at ~100 fish on 22 January 2016. Of these 100 fish, 21 were sampled for biological data. Among the sampled fish, 100% were female and 50% had developing or spawning-capable ovaries. In contrast, at the deep-water Open area, catches were always low (Table 1), and density changes indicative of aggregating behavior were only observed in 1 of the 3 years based on video sampling. $MaxN_V$ was 1 for the first 2 years, but it increased to 12 fish in December 2017. In subsequent months $MaxN_V$ ranged from 1 to 3 fish. Given the low catches, it was not possible to determine if these fish were female.

Males and actively spawning females were sampled at deep-water sites, but we found no strong evidence for spawning aggregations. Actively spawning females were sampled at depths from 65 to 99 m and males from 49.1 to 128 m, and the spatial distribution of actively spawning females and males overlapped (Fig. 2). Most fishing events in the targeted study were at depths greater than 49 m (1102 out of 1132 events). Only 269 of these events landed gag for which a sex could be assigned, and 20% of these events captured males: either males only (n = 21 events, 1–2 fish per event) or both sexes (n = 33 events, n = 2–10 fish per event). When both sexes were caught in a fishing event, males were captured first 36% of the time (n = 12 events). Actively spawning females were sampled in Madison Swanson and The Edges, but not in the Open area. At these 2 spawning grounds, density increased with the spawning season, while a concomitant decrease in density occurred in the Open area (Table 1). Even in the MPA, catch rates were patchy and relatively low, with 57% of fishing events resulting in no gag captured and a mean CPUE of 1.7 ± 2.8 fish h^{-1}. In the MPA, the only indicator meeting the threshold for aggregating behavior was Max CPUE which was 4.8 times greater in spawn events (i.e. sampled during the spawning season) than non-spawn events. Other indicators had density increases ranging from 1.2 for mean CPUE to 3.3 for $MaxN_V$. All indicators were lower in the Edges, and $MaxN_C$ was the only indicator meeting the threshold of a 4-fold increase.

Table 1. Indicators used to evaluate density changes of gag *Mycteroperca microlepis* between the spawning season (S) and non-spawning seasons (NS), indicative of aggregating behavior by area sampled. These include: mean catch per unit effort (CPUE), maximum CPUE, maximum number of fish captured in a fishing event ($MaxN_C$), and the maximum number of fish observed on video ($MaxN_V$)

Area	CPUE (fish h^{-1})				$MaxN_C$ (n fish)		$MaxN_V$ (n fish)	
	Mean S	Mean NS	Max S	Max NS	S	NS	S	NS
Madison Swanson	1.9	1.5	38	8	17	10	8	4
Edges	0.3	0.2	7.4	4	8	2	7	9[a]
Open	0.2	0.2	3.5	5	1	1	2	12

[a]These data are from 30 January 2016, i.e. 1 d before the spawning season. The maximum number observed in the remainder of the non-spawning season was 6 fish

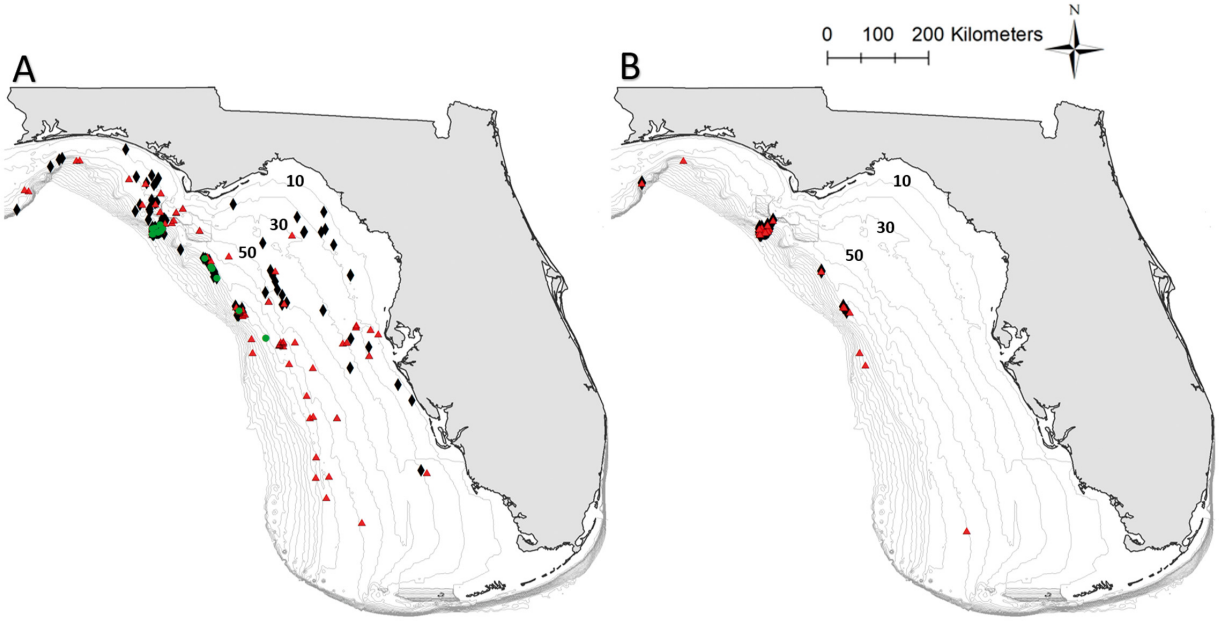

Fig. 2. Spatial distribution of gag samples used in this study by (A) females and (B) males. Red triangles denote fish sampled in the spawning season (1 February through 18 April), and black diamonds are fish sampled outside that time period. Green circles denote actively spawning females and the 10, 30, and 50 m depth contours are noted

Sex-specific movement ecology was evident based on the depths at which fish were captured. Mean depth at capture differed significantly with sex (Wilcoxon Mann-Whitney, n = 1015, $p < 0.0001$), with a minimum female depth of 4.6 m versus 49.1 m for males. Female depth at capture also differed significantly with reproductive phase (Kruskal-Wallis, $\chi^2 = 88.322$, $p < 0.0001$), whereas male depth at capture did not (Kruskal-Wallas, $\chi^2 = 2.4151$, $p = 0.4908$). Immature females (n = 13) ranged in age from 1 to 4 yr old and occurred in shallower water than other phases (mean = 21.8 m), and these differences were significant (Fig. 3). Developing females (n = 47) were as young as 2 yr old and sampled over a wide range of depths. Although depth range overlapped with that of immature and spawning capable females, mean depth differed significantly for females in these developmental phases. Females with fully-yolked oocytes ranged in age from 3 (n = 1) to 15 yr (n = 2) and were sampled over a depth range similar to developing females (15.2–12 m) but primarily at deeper sites. Their capture depths were not significantly different from those of actively spawning females (age range: 4–15 yr). Regressing females, those resorbing leftover secondary-growth oocytes (CA, PY, and FY) had a similar age range (4–11 yr) and sampling depths to active spawners, whereas regenerating females did not. Regenerating females had the widest range of ages (2–17 yr) and depths (4.6–128 m). Females in this reproductive phase represent both fish that had spawned and completed the reproductive cycle, as well as skip spawners sampled on the spawning grounds during the spawning season. The ages of skip spawners did not differ significantly from spawning-capable females (Wilcoxon Mann-Whitney test, n = 284, p = 0.0656), and the age range for both was 3–15 yr.

The assumption that females migrate to deep-water spawning sites was supported by the presence of only mature females at these sites, as well as increased female abundance during the spawning season, but not all females left deep-water sites after spawning. Percent of females increased at deep-water sites with the spawning season (Fig. 4). In the Madison Swanson MPA, catches from spawn-time events were 95% female, significantly greater than the 82% female observed in the non-spawn time ($\chi^2 = 18.1201$, $p < 0.0001$, n = 481). However, some females appear to be year-round residents at these deep-water sites. In opportunistic sampling in Madison Swanson in June, July, October, and November (n = 44), 85% of the catch was female, and these females were mostly young, ranging in age from 2 to 10 yr, with a mean age of 5 yr (n = 35).

3.2. Sex ratio

Gag exhibit low milt reserves and lower than expected male sex ratios. Spawning-capable males during the spawning season had low gonadosomatic

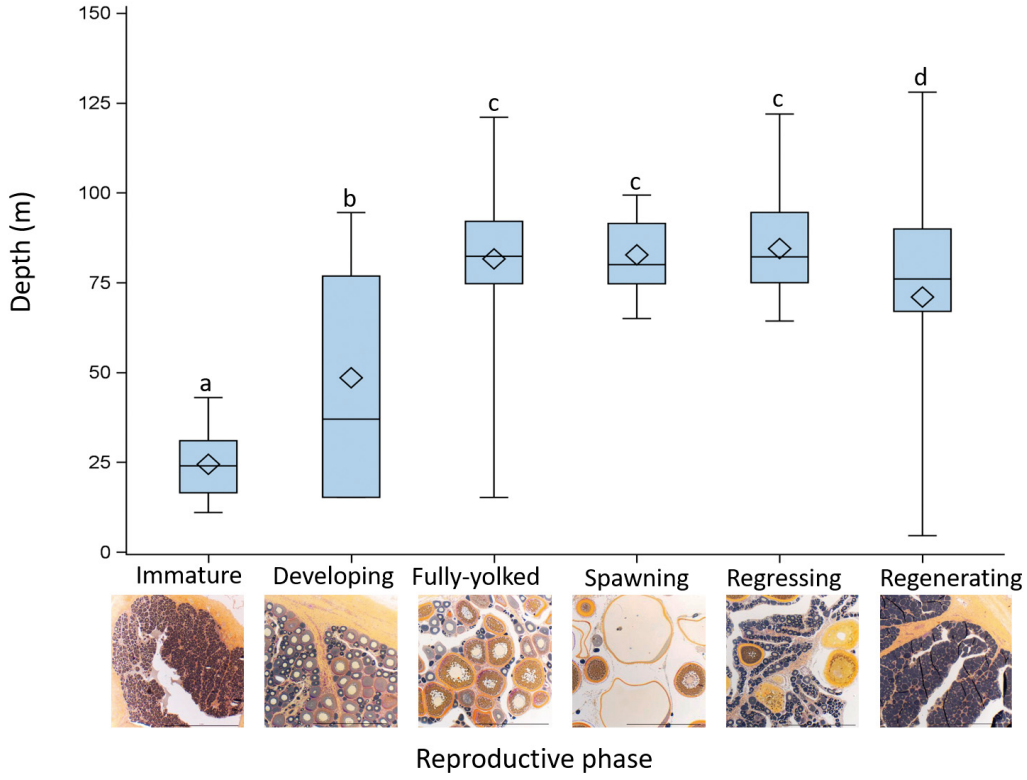

Fig. 3. Depth of female gag at capture by reproductive phase from fishery-independent sampling (i.e. 3 yr study and survey). 'Fully-yolked' corresponds to spawning capable and 'spawning' designates active spawners. Boxes represent the 25th to 75th quantiles, and whiskers are the range. Diamonds represent the means, and horizontal lines are the medians. Those groups which significantly differed from each other, based on the Dwass, Steel, Critchlow-Fligner method, are indicated by different letters

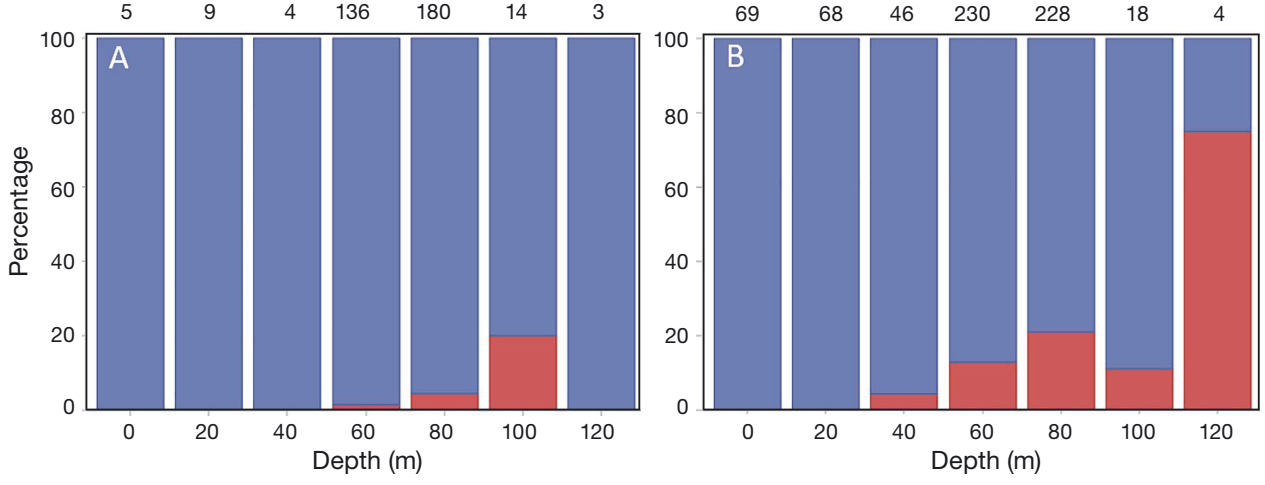

Fig. 4. Sex-specific percentages of gag by depth bin and spawning season (red: males, blue: females) for all sampling areas. (A) Sampled during the spawning season from 1 February through 18 April; (B) sampled outside this time period. Sample sizes for each 20 m depth bin and season are noted above each bar

indices (mean = 0.35 ± 0.16, n = 12) and released little to no milt when strip spawned — a pattern associated with pair spawners. In the targeted study, catches in Madison Swanson from May to December were 11% male. However, the spawning season is the only time most mature males and females are in the same location. During this time period, catches were only 5% male, significantly less than the predicted 15% male

(χ^2 = 1310.7452, p < 0.0001, n = 261). Sex ratios were effectively 0% male in the Edges (seasonally closed) and Open area. Outside of the spawning season, 1 male was captured at each of these locations.

3.3. Sex change mechanism

Sex change cues were not limited to the spawning grounds. Although transitionals were rare (n = 8), they were sampled at deep-water targeted spawning sites (n = 2), in FDM samples (n = 5), and from the shallow-water pre-spawning aggregation sites that our collaborating fisher targeted (n = 1). They were also sampled prior to the spawning season (3 fish in December and January), during the spawning season (n = 2), and just after the spawning season (n = 3 in late April and May). All transitionals had remnant populations of primary growth oocytes, spermatogonia, and spermatocytes (Fig. 5). Only one fish sampled on 29 March had histological indicators of yolked oocytes, and these were in late beta atresia

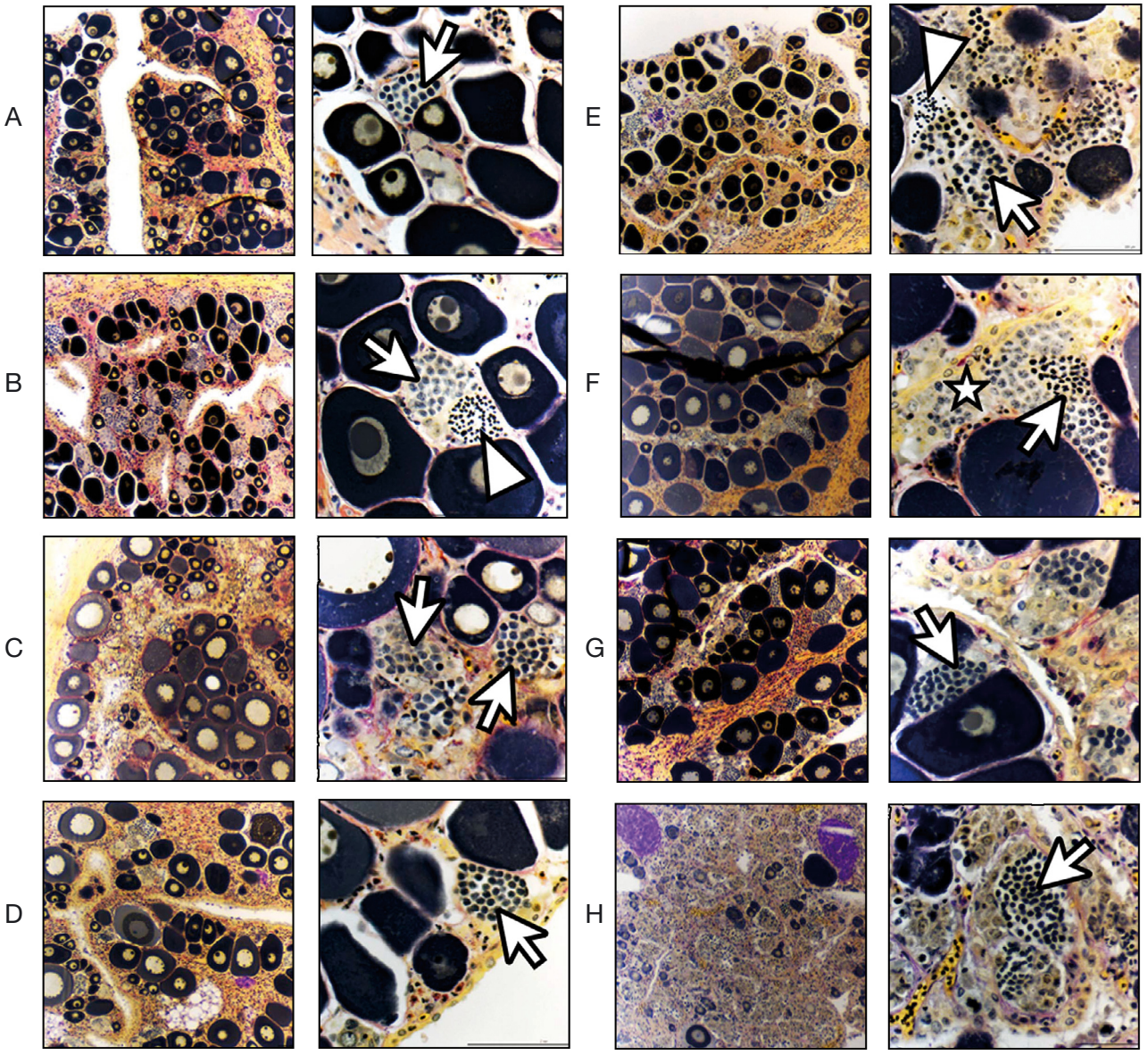

Fig. 5. Histological micrographs of gag undergoing transition. Date sampled (given as mo/d/yr) and total length at capture were (A) 5/25/2016, 845 mm; (B) 5/9/2017, 1007 mm; (C) 1/11/2018, 675 mm; (D) 3/29/2017, 895 mm; (E) 4/30/2018, 1015 mm; (F) 12/31/2018, 901 mm; (G) 2/8/2019, 841 mm; and (H) 1/9/2019, 1056 mm. Micrographs for each fish are at 10× (first photo) and 40× (second photo). Star: spermatogonia; arrows: spermatocytes; triangles: spermatids

(Fig. 5D). The 2 transitionals sampled in the targeted study on the spawning grounds both occurred in May, one in the Edges and the other in the MPA.

Sex change was not associated with a minimum size. Although males were significantly larger (mean TL = 1034 mm) than females (mean TL = 793 mm; Wilcoxon Mann-Whitney test, n = 1633, $p < 0.0001$), they exhibited a wide size range: 623–1336 mm TL. In addition, although the male interquartile range (89 mm TL) was ca. half that of females (151 mm TL), the male size distribution was not positively skewed, the expected pattern if there is a threshold size associated with transition (Fig. 6). The estimated size at 50% male was 1010 mm TL, but 75% of transitionals were smaller than this, ranging in size from 675 to 1056 mm TL. The logistic model used to estimate size at 50% male predicted that males smaller than 700 mm TL had less than 1% probability of occurring. Males were larger in the MPA (mean = 1053 mm TL, n = 81) than in other areas (mean = 1020 mm TL, n = 117), and the size and age of the transitional sampled in the MPA (age 14 yr and 1107 mm TL) were considerably larger and older than observed in the seasonally closed area (age 6 yr, 845 mm TL).

The A50 for the MPA was 13 yr, older than previously reported for the GOM. However, sex-specific ages overlapped (Fig. 7), with the youngest male aged 7 yr and the oldest female aged 17 yr. Because only 2 males were captured outside the MPA in the targeted study, it was not possible to estimate 50% male size or age in the Edges or the Open area. However, the males from these areas were the oldest fish captured in the area (9 yr and 950 mm TL at the Edges and 11 yr and 1082 mm TL in the Open area). In the Open area, the male was also the largest fish sampled. However, in the Edges, the largest fish sampled was a female (1080 mm TL). Females in the Madison Swanson MPA were significantly older than those sampled in the Edges (GLM, $\chi^2 = 6.16$, $p = 0.0130$), with a female mean age of 6.71 ± 2.54 yr in Madison Swanson compared to 5.79 ± 1.99 yr in the Edges.

4. DISCUSSION

Spawner-recruit ecology in marine fishes is considerably more complex than the spawner-recruit relationships typically used in stock assessments. These ecological systems are species-specific and made up of fixed (selected for over evolutionary time periods), behavioral, and variable traits (Lowerre-Barbieri et al. 2017a). Gender system is a fixed trait and most fish are gonochoristic (i.e. separate sexes). However, sequential hermaphroditism is not uncommon, with protogyny the most common form. Many protogynous species, like gag, that begin life as females and transition to males, support important commercial and recreational fisheries such as: California sheephead *Semicossyphus pulcher*, black sea bass *Centropristis striata*, scamp *Mycteroperca*

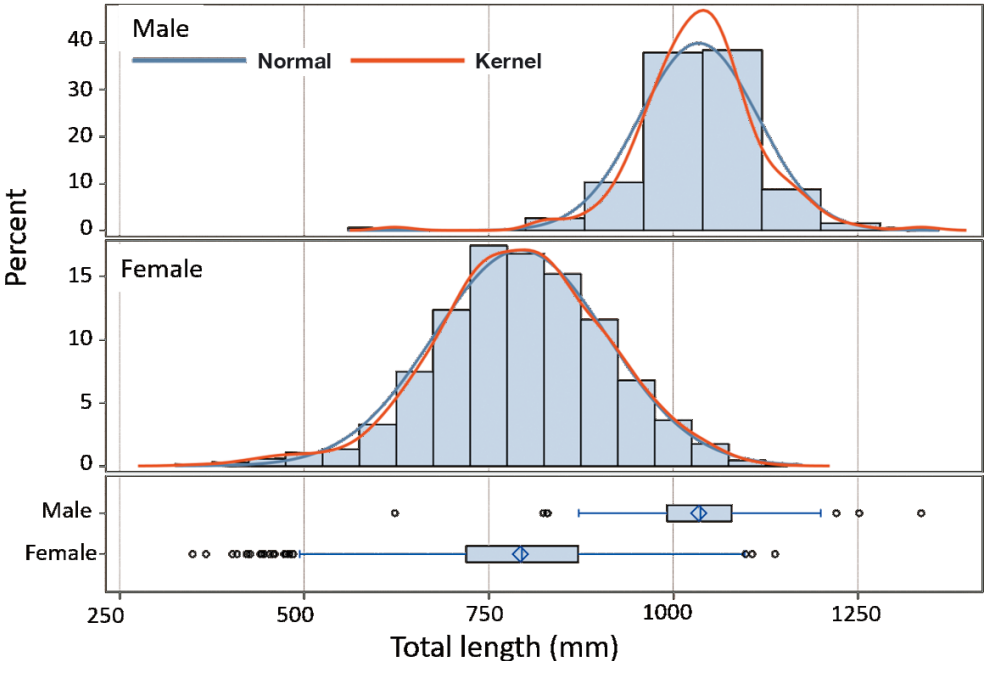

Fig. 6. Size distribution of gag by sex compared to expected normal (blue) and kernel (red) distributions. Although size approximated the normal distribution, it did not pass the Wilcoxon Mann-Whitney test, and differences were tested with non-parametric statistics. In the boxplots, mean is denoted by a diamond and median with a vertical line. The ends of the boxes represent the 25th and 75th percentiles, whiskers represent expected range, and dots show data points outside of this range

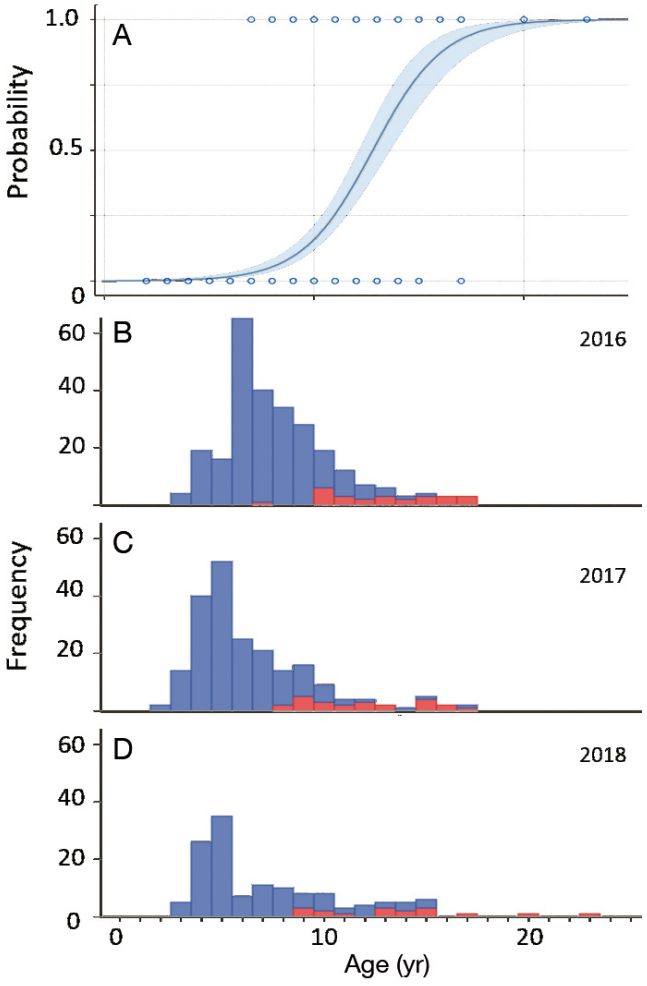

Fig. 7. (A) Predicted age at 50% male for gag in the Madison Swanson marine protected area and (B–D) age composition by year (including the previous December) sampled for all 3 areas (Madison Swanson, Edges, and Open) by sex (blue: females and red: males). In (A), observed ages by sex are represented by circles, and the predicted sex change relationship is indicated by a line with 95% confidence intervals indicated by the shaded area

phenax, red grouper *Epinephelus morio*, red porgy *Pagrus pagrus*, and coral trout *Plectropomus leopardus* (Sadovy de Mitcheson & Liu 2008). However, gender system is not the only trait impacting reproductive success. Protogynous species differ in terms of the spatial distribution of their life cycles, mating behavior, reproductive unit, and developmental/sex change cues, all of which will be impacted by ecological context. It is increasingly recognized that these traits must be considered to predict how a protogynous stock will respond to fishing pressure or spatial management (Huntsman & Schaaf 1994, Alonzo & Mangel 2005, Heppell et al. 2006, Ellis & Powers 2012, Easter & White 2016).

4.1. Spatial ecology and management

We hypothesized that females would form pre-spawning aggregations prior to migrating to deep-water spawning sites, where males remain year-round, similar to what other studies have shown. Our results supported these hypotheses and showed that gag exhibit clear depth preferences with life stage, sex, and spawning (Fig. 8). However, we did not find strong evidence of spawning aggregations nor that all females leave deep-water spawning sites after the spawning season. Based on this and prior studies, we developed a conceptual model of gag spatial ecology. Ontogenetic shifts from nursery to adult habitat are common in fishes, and this pattern has been previously reported in gag (Koenig et al. 1996, Carruthers et al. 2015, Gruss et al. 2017). Juveniles remain within estuaries for 5–7 mo (Koenig & Coleman 1998, Switzer et al. 2012, Jue et al. 2015), and immature females (ages 1 to 4 yr) occurred in fairly shallow water (mean 22 m). However, the depth and age distributions of immature females overlapped with those of mature females (ages 4 to 17 yr). Male ages (7 to 23 yr) also overlapped with mature females, but their depths-at-capture differed significantly. Males were sampled only in deeper water (~50+ m), indicating a change in behavior that is hormonally controlled. Although prior research reported gag spawning aggregations (Collins et al. 1987, Coleman et al. 1996, Domeier & Colin 1997), they have been small: fewer than 100 fish (Coleman et al. 1999) or 5–50 individuals observed schooling during submersible dives in 1977–1982 (Gilmore & Jones 1992). In both the latter and the present study, scamp were always observed in larger numbers than gag. The maximum number of gag captured per event in this study was 17, in the MPA. The maximum number observed on video was 12 fish, in the Open area which has been previously noted as a pre-spawning aggregation site. Pre-spawning aggregations have the potential to be large, but further research is needed to understand this behavior.

4.2. Sex ratio

We hypothesized that male abundance would recover in the MPA, resulting in a male sex ratio of ~15% (Heppell et al. 2006) and an A50 older than that reported in the 1990s (10.9 yr). In our targeted study, we observed only a 5% male sex ratio in the MPA and 0% males in the Edges and the Open area. However, accurate estimates of gag sex ratio are dif-

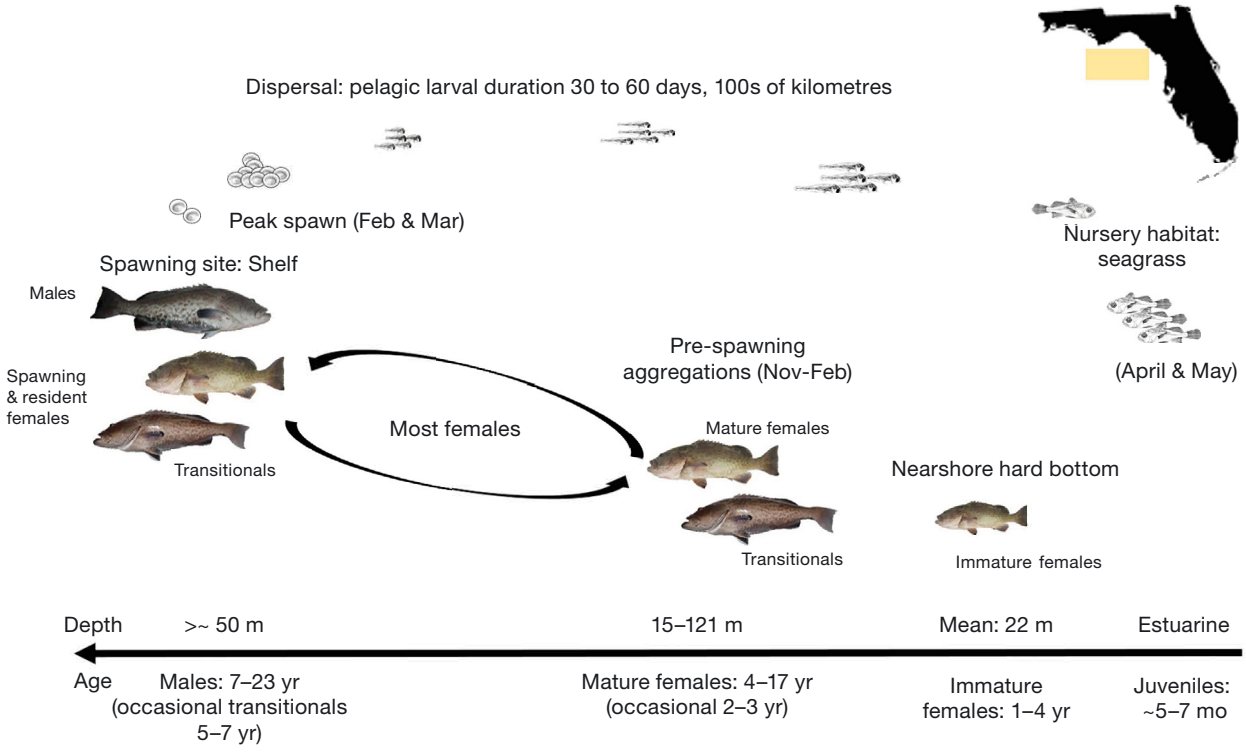

Fig. 8. Conceptual model of the spatial ecology of gag. Seasonal information is in parentheses, yr = years, mo = months

ficult due to sexual segregation, with the sexes living predominantly in different locations and coming together only to spawn. This behavior is common in many vertebrates and especially those with strong sexual size dimorphism and polygynous mating systems (Ruckstuhl & Neuhaus 2002, Wearmouth & Sims 2008). For gag, the result of this behavior is that representative samples of the population sex ratio can only be made during times and locations associated with spawning (1 February to 18 April and at deep-water sites). We did not find evidence of males feeding more aggressively than females. However, further research to confirm that male catchability does not decrease during the spawning season due to 'swamping' by females migrating to the spawning grounds or decreased male foraging during peak spawning would be helpful (Lowerre-Barbieri et al. 2017b) but difficult to conduct given deep-water spawning sites and high discard mortality. Nevertheless, the low numbers of males sampled in our targeted study outside the MPA (n = 2) and indicators of low overall gag abundance, even in the MPA, strengthen the conclusion of low male gag abundance.

In contrast to male sex ratios, male A50 increased as hypothesized, reflecting the protection provided by the MPA, even with continued poaching (Koenig & Coleman 2011). However, rather than indicating a recovering stock, the increase to 13 from 10.9 yr, combined with low observed sex ratios, suggests the male population in the MPA may be aging with limited recent recruitment of younger males. This is similar to what has been predicted based on simulation models for other protogynous species (Chan et al. 2012, Easter & White 2016) and highlights the need to better assess male recruitment and the processes driving it. Transitionals were very rare in our samples. The probability of sampling them, however, is not proportional to annual recruitment of males, as this process is both seasonal and relatively ephemeral. Transitionals were observed from December through May, and the duration of transition is assumed to be at least 23 d based on the observed yolk resorption rate for northern anchovies (Hunter & Macewicz 1985b) and lack of alpha atresia in gag transitionals. However, further research is needed to assess if transition is evenly distributed over the observed months and habitats, or if there is a spatio-temporal trend.

The mating function (the relationship between sex ratio and fertilization success) plays an important role in the productivity of protogynous species (Easter & White 2016) but is poorly understood for all species, including gag. Thus, we do not have the data to estimate the optimal gag male sex ratio, but several lines

of evidence suggest that the current low male sex ratio is of concern. The low male gonadosomatic indices and milt reserves are indicative of pair spawners. For comparison, the mean gonadosomatic index for spawning-capable male gag was 0.35, compared to 2–3% for weakfish *Cynoscion regalis*, a group spawner (Lowerre-Barbieri et al. 1996). Because gag are pair spawners and females are multiple batch spawners (Collins et al. 1998), males would have to spawn with multiple females per day, every day of the ~78 d spawning season. Although male spawning frequencies are virtually unknown, given the low milt reserves during the spawning season this seems unlikely. In addition, the current male sex ratio appears to be close to what it was in the early 1990s (2%) when gag were overfished and undergoing overfishing, and sperm limitation was first considered (Coleman et al. 1996). This male sex ratio is considerably lower than seen in other protogynous reef fish. For example, male sex ratios of scamp declined from ~38 to 18% from the 1970s to the 1990s and those of red grouper increased from ~14% in the 1960s to 22% in the 1990s (Coleman et al. 1996). The male sex ratio in the protogynous hogfish *Lachnolaimus maximus* off the west Florida coast is estimated as ~12–17% (Collins & McBride 2011). Thus, although we cannot prove that sperm limitation is occurring, the above evidence suggests that male abundance is well below what would be expected in a healthy stock.

4.3. Sex change mechanism

We tested the following hypotheses about sex change: (1) it occurs only on the spawning grounds, (2) it is endogenously driven (Heppell et al. 2006); (3) it is associated with a minimum size threshold (800 mm TL); and (4) male sex ratios on the spawning grounds are a requisite social cue for sex change that results in a density-dependent feedback loop based on male abundance (Ellis & Powers 2012). Our results of males and transitionals smaller than 800 mm TL agrees with previous studies concluding that gag sex change is not endogenously driven (Coleman et al. 1996, 2000, Koenig et al. 1996), nor confined by a minimum size threshold. It also indicates potential for physiological adaptability to age truncation as gag can produce younger, smaller males, as seen in other species. This raises the question of why male gag sex ratios remain so low. We believe this is due to the incorrect assumption that sex change occurs only on the spawning grounds and that spawning reserve MPAs will thus protect male recruitment. Although Coleman et al. (1996) reported observing 2 transitionals collected in shallow water during the spawning season, the management implications of this have not yet been addressed. Our findings that transitionals occur before, during, and after the spawning season and at both all-female pre-spawning aggregation sites and on the spawning grounds indicates that male sex ratios are not a requisite cue for sex change. This highlights the importance of pre-spawning aggregations and the potential for transitionals to be captured before migrating to the spawning grounds.

Sex allocation theory suggests sex change is favored when individual reproductive value changes with size or age, and this relationship differs by sex (Charnov 1982, Warner 1988, Allsop & West 2004). However, individual reproductive value also depends on the make-up, size, and isolation of the reproductive unit, which in protogynous species can be harems, spawning aggregations, or leks. Our results suggest that gag may not form spawning aggregations, and their sex-specific movement ecology rules out harems, a common pattern in protogynous species. A harem is defined by a dominant male that mates with a group of females in a defended territory. Removal of the dominant male results in either a female transitioning to a male or another male taking over the territory. If harems are well-dispersed, sex change must occur rapidly or the reproductive unit will be unable to reproduce for the rest of that spawning season. This pattern has been observed in the bluehead wrasse *Thallassoma bifasciatum*, which can complete sex change in 1 to 2 wk (Warner & Swearer 1991). However, in haremic species where reproductive units are in close proximity and can exchange individuals, there would not be the same need for this rapid response, and presumably this is the case for the rock hind *Epinephelus adscensionis*, as it takes 54 d to complete sex change (Kline et al. 2011). Similarly, if species form spawning aggregations, there is no need for rapid sex change, as the removal of 1–2 males will not have the same impact on the reproductive unit. An example of this appears to be black sea bass, which form spawning aggregations (Farmer et al. 2017) and take 42 d to complete sex change (Benton & Berlinsky 2006).

Gag exhibit dimorphic size and sexual segregation, and they appear to form leks as part of their mating strategy. Leks in fishes have been defined as, 'when non-resource-based aggregations of males are visited by females for the purpose of mating' (Casaretto et al. 2015, p. 13) and have been reported in haddock *Melanogrammus aeglefinus* and cod *Gadus morhua*

and more recently in the Gulf grouper *Mycteroperca jordani* (Rowell et al. 2019). Leks are a fairly common bird mating strategy, with males 'displaying' for females and trying to out-compete each other for mating success (Stearns 1992). Male gag displaying for females has been reported (Gilmore & Jones 1992). In this mating strategy, male size plays a role in male-to-male competition and reproductive success, and life history theory predicts a low probability of early male maturation (Stearns 1992). If this is the case with gag, then smaller, younger males recruiting to Madison Swanson may have lower reproductive success than those recruiting to areas such as the Edges which continue to be fished and have few males and smaller females. Like spawning aggregations, in a lek there is not the same need for rapid sex change, as the removal of one or more males is not expected to dramatically affect the reproductive success of the reproductive unit. If the duration of gag sex change (i.e. the time it takes to become a functional male after receiving the cue to change sex) is similar to that of rock hind or black sea bass, i.e. approximately 2 mo, then female gag would need to receive the sex change cue by December or January to contribute as males in the upcoming spawning season. This is the time at which females form pre-spawning aggregations.

Given our results, we propose a new conceptual model for gag sex change, which may have important implications for other protogynous species. Female-to-female interactions within pre-spawning aggregations clearly play an important role in gag sex change, as reported for other protogynous species (Lamm et al. 2015). Presumably the largest, most aggressive females in a pre-spawning aggregation will transition. On the spawning grounds, these female-to-female interactions will be moderated by male abundance and size dominance, but when male abundance is low, female-to-female interactions will be less disrupted by males and/or spawning and result in higher numbers of transitionals both during and after the spawning season on the spawning grounds.

4.4. Conclusions

In gag grouper, the spatial distribution of their life cycle, their gender system, and their mating strategy all impact sex change, male recruitment, and the spatio-temporal level of fishing mortality they can sustain. The inability of gag sex ratios to adapt to fishing pressure has been hypothesized to be driven by sex change cues associated with an inflexible size or age threshold. In contrast, our results indicate that sex change can occur over a wider range of sizes, months, and habitats than previously believed, including in pre-spawning female-only aggregations. Given that both gag biomass and fishing pressure is greatest in shallow waters (Carruthers et al. 2015), we hypothesize that shallow-water, pre-spawning aggregations are a key spatio-temporal bottleneck to gag productivity. This is because of the potential to remove both fish undergoing transition and females cued to change (but without identifiable gonad restructuring) before they can contribute to gag productivity as males on the spawning grounds.

It is increasingly recognized that adult sex ratios affect a range of sex-specific behaviors, mating strategies, and extinction risk — although understudied in most animals (Schacht et al. 2017). Gag have especially complex spawner-recruit ecology, with dimorphic size and sexual segregation, a common pattern in ungulates (Ruckstuhl et al. 2006), which in captivity may be kept at unnaturally low male sex ratios. For example, sheep in captivity can be kept at male sex ratios as low as 1%, but in wild flocks their sex ratios are close to parity (Clutton-Brock & Iason 1986), presumably providing the genetic diversity and population resilience needed to ensure population stability in the wild. We do not suggest that parity is the natural sex ratio for gag. However, historically gag have demonstrated a male sex ratio of 17%, and the expected increase in male abundance due to spawning reserve MPAs is not being realized. Even amongst protogynous species, gag are unique in having had male sex ratios as low as 2%. They have also experienced overfishing during much of the time series they have been managed (1974–2010; SEDAR 33 update). Thus, although the GOM gag population has been able to sustain itself in the past at very low male abundance, the concern is for how long it can continue to do so in the current marine environment of multiple stressors and few spatial refugia.

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