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Gag grouper reproduction in the Gulf of Mexico

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Introduction:

Gag (*Mycteroperca microlepis*) support extensive commercial and recreational fisheries, especially along the Gulf coast of Florida, where most Gag are landed (McErlean, 1963; Schirripa and Goodyear, 1994). Because Gag have a sequential protogynous hermaphroditic gender system, all males must recruit from the mature female population (Koenig et al. 1996). A wide range of previous studies on Gag have documented their high susceptibility to overexploitation due to this gender system and spatial ecology (Bannerot et al. 1987; Coleman et al. 1996, 2000; Armsworth 2001; Alonzo and Mangel 2005; Heppell et al. 2006).

Gag spawn at the shelf edge and produce pelagic eggs. Pelagic larval duration is from 35 to 45 d, after which Gag settle in estuaries (Fitzhugh et al. 2005). The arrival time and duration of juveniles within estuaries varies with latitude, and estuarine juvenile abundance varies temporally, with peaks in juvenile recruitment every 2 to 4 years (Switzer et al. 2012). Previous research suggested that Gag form spawning aggregations (Coleman et al. 1996; Domeier and Colin, 1997), but recent research did not find evidence of this (Lowerre-Barbieri et al., 2020). Males are believed to remain at the shelf edge year-round, whereas females return to nearshore reefs after spawning (Coleman et al., 1996). Females also form pre-spawning aggregations in late fall/early winter in nearshore habitats and these appear to be heavily fished (Lowerre-Barbieri et al., 2020).

Age truncation in protogynous species is expected to result in low male abundance and possible sperm limitation, decreased egg production and/or decreased genetic diversity and resilience (Collins et al. 1998; Chapman et al. 1999; Alonzo and Mangel 2004, 2005; Brooks et al. 2008; Shepherd et al. 2013). This presents unique challenges for stock assessment and fisheries management (Brooks et al. 2008; Ellis and Powers 2012; Shepherd et al. 2013). Traditionally, reproductive success is integrated into stock assessments through the stock–recruitment relationship. These relationships typically are based on female-only spawning stock biomass (SSB). Data used to estimate SSB includes estimated abundance of mature females at age, mean weight at age, the proportion of females that are mature at a given age, and estimates of natural mortality and fishing mortality to predict survivorship in any given year (Murawski et al. 2001; Lowerre-Barbieri et al. 2011a). However, in protogynous species, reproductive potential cannot be assumed to correlate only with female biomass. For example, the spawner-recruit curve may be dependent on relative abundance of both males and females. Natural mortality and catchability estimates may be sex-specific, and although sex change removes females from the population, they continue to contribute to total biomass (Shepherd et al. 2013). Thus, it has been recommended that SSB of both sexes be used to measure biomass status (Brooks et al. 2008).

In the last stock assessment, steepness was considered highly uncertain and set at 0.85 and male sex ratio was low (2-3%; SEDAR 33). However, assessment results from combined SSB seemed unrealistically pessimistic (Figure 1). In that assessment, all histological data was used for maturity estimation, although a potential decrease in size at maturity was noted, resulting in an A50 of 3.5 years and L50 of 543 mm FL. A similar small but decreasing trend was noted in size and age at transition. Data from all years was used resulting in an L50 of 1022 mm FL and an A50 of 10.7 years.

Survey Design, Sampling Methods, and Analyses:

Most samples came from NMFS (n=2,749), collected from 1991 to 2019 from fishery-dependent (FD) and fishery-independent (FI) sources. Additional samples came from FWC from 2009 to 2019 (n=1,835). The FWC samples came from fishery-independent monitoring surveys (FIM), fishery-dependent monitoring surveys (FDM) and from a study targeting Gag along the western coast of Florida (Lowerre-Barbieri et al., 2020).

FWC samples of gonad tissue were collected 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). Samples from NMFS were collected and preserved in 10% buffered formalin on board of scientific vessels, in ports where the fish were intercepted, or at the laboratory soon after the gonad tissue was removed from the fish. The gonad tissue remained in storage in 10% buffered formalin until time of processing. Trimmed subsamples were sent in histology cassettes to specialized laboratories for histological processing. The histological samples were then embedded in paraffin, sectioned to 4-6 *μm* in thickness, and stained using hematoxylin and eosin. Gag samples from 2014 to 2019 collected by NMFS (n=257) were processed at FWC/FWRI using their protocol.

Gonadal analysis

Gonadal tissue was histologically assessed for all samples and sex and reproductive phases assigned. Different histological methods were used by the different labs, and between years within lab for NMFS Panama City. Samples assessed by FWC followed Lowerre-Barbieri et al. (2009) and Brown-Peterson et al. (2011); criteria are outlined in Table 1. Histological indicators for female Gag reproductive phases and histological criterion used by FWC/FWRI are outlined in Table 2 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 are considered to have received the physiological cue to develop oocytes for the coming spawning season (Lowerre-Barbieri et al., 2011). However, gonadal development does not always correspond to functional maturity.

Oocyte maturation was broken down into sub-stages: 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 1 and 2). The duration of both OM and POFs in Gag was considered to be 48 h.

There is no definitive histological indicator to distinguish immature from mature regenerating females, which both have only PG oocytes. 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. Parasites occurred in both ovaries and testes and immature and mature females and had previously been mis-identified as brown bodies (melanomacrophages) and thought to be an indicator of previous spawning and maturity in the NMFS developmental classification. Consequently, only 5 immature status assignments can be made from historical variables distinguishing reproductive phases (spawning state[1] used by NMFS for samples collected until 2002 and gonad class[2] used by NMFS in samples collected between 2002 and 2013) out of 2362 females sampled between 1991 and 2013. Maturity status in SEDAR 10 was therefore not based on reproductive phase assignment but on combinations of other histological classification variables: leading gamete stage, presence of atresia, and brown bodies. Females "with PG oocytes as the leading stage, with no atresia of yolked oocytes and minimal to zero melanomacrophages were deemed immature" (SEDAR 10), resulting in 66 females assigned as definitely immature. Females exhibiting atresia of unyolked oocytes and some melanomacrophages were assigned an uncertain maturity status (SEDAR 10).

We were not able to reproduce the SEDAR 10 maturity analysis, and we note that criteria used for maturity assignment are a major source of uncertainty for estimating maturity at age. Our recommendation is to use gonadosomatic index (GSI) rather than brown bodies to help distinguish between resting and immature females. GSI is calculated as:

$$GSI \; = \; 100 \; x \; \frac{gonad \; weight}{total \; weight}$$

Drawbacks to using GSI are that fish total weights were not always available due to sampling limitations, and measured weights are subject to measurement error. GSI for spawning capable females, with confirmed gonadal weights assessed by FWC (n = 164) ranged from 0.43 to 8.48 and GSI for females assigned as immature by FWC (n = 6) ranged from 0.03 to 0.08. Of the females assigned as resting by FWC (n = 527), the 5th percentile of GSI values corresponded to 0.05. Based on these values, we recommend using 0.05 as the threshold for distinguishing immature from regenerating gag. Our definition for immature females thus was 1) leading gamete stage is PG oocytes, 2) no atresia of yolked oocytes is present, 3) no POFs are present, and 4) GSI is less than 0.05. We assigned females as definitely mature if any of the following were observed: 1) leading gamete stage were vitellogenic or hydrated oocytes, 2) atresia of yolked oocytes, or 3) POFs were observed. In addition, any females assigned by FWC as immature were included as immature and any assigned as reproductive phase 3 through 5 were included as mature for the purpose of this analysis.

Because gag transition from female to male, testes continue to have ovarian walls and often large numbers of primary growth oocytes. Because of this, sex cannot be assigned based on macroscopic examination of the gonads and histological analysis is needed. Fish were considered male if only spermatogenic cells were present (i.e., no PG) or they had spermatozoa present (Trip et al., 2011).

[1] 2004 AGR Manual Histology Chapter

[2] 2008 AGR Manual Histology Chapter

Similarly, sex was determined as female if there was nothing but female tissue and/or pockets of spermatogonia but no later stages of spermatogenesis.

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 3). Estimates of size and age at transition were based on all females and male but did not include transitionals.

Maturity and transition

Binomial generalized linear models (GLMs) were used to model maturity and transition at age and length. Different link functions (logit, probit, cloglog and cauchit) were specified, and the best model was chosen via Akaike Information Criterion (AIC). Models were fitted in R and model comparison was performed using the R package 'MuMIn'. Estimated parameters were the intercept and slope. The inflection point (age or length at 50% maturity or transition) was calculated by dividing the absolute value of model intercept by slope. We present model results separately for all years combined (1991 to 2019) as well as the early (1991-1999) and later (2000-2019) periods. Additionally, sex transition models were run separately with and without fish collected in the Madison-Swanson protected area, as males appear to be resident within the MPA and thus not representative of male size/age distributions in the fished stock (Lowerre-Barbieri et al., 2020). Maturity models were run for: 1) all females assigned as definitely mature and definitely immature (based on histological criteria above); and 2) a more conservative measure of maturity based on reproductive phase 3 or 4 (i.e., spawning capable) and definitely immature females collected within the spawning season. The spawning season was based on the first and last date actively spawning females were observed over all years and sample locations (December 18 - May 14). However, only one spawning female was collected in December (1992) and one in May (1991) and the core spawning season is expected to be more restricted, with active spawners collected in Madison Swanson from 1 February to 18 April (Lowerre-Barbieri et al., 2020)

Because there is no definitive histological identifier to distinguish between immature and mature females and increasingly there is the recognition that gonadal development is not always the best indicator of functional maturity, we were interested in developing estimates based on fish on the spawning grounds under the assumption that they are functionally mature. This works especially well for gag, given their spatial ecology (with younger females occurring closer to shore) and the lack of immature females sampled at depths of 50 m or greater, where this stock spawns. Thus we derived functional maturity estimates from logistic model fits to the ascending limb of the length and age composition for individuals sampled from habitats where all occurring gag would be expected to be mature: 1) within the Madison-Swanson closed area, a known gag spawning aggregation site and 2) fish collected through FI sampling from depths greater than 50 meters. These alternative estimates of length and age at maturity represent a proxy to the 50% recruitment to the spawning population and were estimated as the inflection point of the logistic fitted to the ascending limb of the composition data.

Best measure of reproductive potential:

Reproductive success is accomplished through trade-offs between the rate of reproductive output and the survivorship rate associated with that output. To integrate the concept of reproductive success into stock assessment processes, Trippel (1999) introduced the term "stock reproductive potential," defined as the "annual variation in a stock's ability to produce viable eggs and larvae that may eventually recruit to the adult population or fishery." Traditionally, it has been assumed that reproductive success in marine fishes is primarily driven by fecundity. Although reproductive success is tightly coupled with adult abundance and fecundity in many terrestrial animals, it is less so in marine fishes which have extreme adult to offspring size ratios, offspring mobility and mortality. Spawner-recruit systems in marine fishes are species-specific with traits occurring over multiple spatial, temporal and biological scales. There is no one measure of reproductive potential which is accurate for all species. The best species-specific measure will depend on data availability, spatio-temporal reproductive behavior, demographic drivers of reproductive value, and gender system (i.e., sequential hermaphrodite or gonochoristic; (Lowerre-Barbieri et al. 2017). Given that Gag are protogynous, it is important to consider the contribution of males, as well as females to reproductive potential (Brooks et al., 2008; SEDAR 2015).

Results / Discussion:

A total of 4,600 Gag were sampled from 1991 to 2019 in the Eastern Gulf of Mexico and had histological analysis of gonadal development. Of all fish sampled, there were: 384 males, 4,198 females and 18 transitionals. A shift in the length and age distribution of males toward smaller and younger individuals is observable in the 2010 to 2019 period compared to the 90s (Figure 2). Of the females, 325 were assigned as immature, 2422 as maybe mature, and 1451 as mature. A greater proportion of females had to be assigned uncertain maturity status due to missing GSI in the 2010s (Figure 3).

Age and size at maturity

Immature females sampled during the presumptive spawning season (n = 889) ranged in size from 238 to 762 mm FL (Table 6) with a mean of 509 mm and were 1 to 6 years old (Table 7) with a mean of 3.4. Estimated parameters with uncertainty estimates for length and age at maturity and transition are shown in tables 4 and 5, respectively. The logit link function provided either the best fit or was within 1 AIC point of the model with the best fit for all but the early period model where all mature and immature samples were used. Estimated lengths and ages were fairly consistent between models, ranging from 598 to 611 mm FL and 3.6 to 4.1 years, respectively (Tables 4 and 5). Due to the above-described difficulties assigning maturity, we recommend using the model for all years with only those samples collected during the presumptive spawning season included. Under this model, estimated size at 50% maturity was 603 mm (Table 4, Figure 4), and estimated age at 50% maturity was 3.9 years (Table 5, Figure 4). We included sensitivity runs where we excluded fish collected in the Madison Swanson protected area to see what the impact of those samples on the results are, and it was minimal (excluding MS, L50 was 605 mm FL and A50 was 4.0 years).

Estimates of 50% recruitment to the spawning population were similar to results from traditional estimates for maturity for age (A50 = 3.9, SE = 0.016; Figure 5) but were about 70 mm higher for fork length (L50 = 668, SE = 0.005; Figure 6).

Age and size at transition

Length and age at transition were estimated for the entire time period (1991-2019) as well as the historical (1991-1999) and recent (2000-2019) periods separately. Additionally, we compared runs with samples from all areas to those for Madison-Swanson fish only and those excluding Madison-Swanson, for the years 2015-2018 (the years for which MS samples were available).

The smallest observed male was 600 mm FL (Table 10; there was no age for this individual), and the youngest observed males were four years old (Table 11). Transitionals (n=18) ranged in size from 661 to 1075 mm FL, with a mean size of 896 mm FL. Both the logit and probit link provided best fits to the length data, while the probit fit was always the preferred model for fits to the age data. Estimates of size at 50% male ranged from 974 (non-MS, 2015-2018) to 1139 (all areas, 1991-1999)(Table 8) and estimates of age at 50% male ranged from 10.5 (non-MS, 2015-2018) to 12.9 (all areas, 1991-1999)(Table 9). Estimates for male size at transition were similar within and outside Madison Swanson from 2015 to 2018 (987 versus 974) but estimates of age at 50% male were ~ two years higher in Madison Swanson compared to other areas (12.8 versus 10.5). Because age at transition increased in Madison Swanson but sex ratios did not recover to historic levels, the MPA appears to have protected resident males without providing similar full protection to recruiting males, resulting in an aging male population which is not representative of the fished stock. Thus, we recommend the non-MPA estimates for all years (L50 = 1,050, A50 = 11.6) as the best measure of age at transition for gag in the Gulf of Mexico. It is also important to note that age at transition is not static. We are observing smaller and younger males in recent years (2015-2018 non-Madison Swanson L50=973, A50=10.5) compared to what was observed in the historical period (L50=1,103, A50=12.9), suggesting adaptation to age truncation.

Parameter comparisons with the past stock assessment

Our best estimate of maturity based on traditional reproductive indicators resulted in size at 50% maturity of 603 mm, and estimated age at 50% maturity was 3.9 years. Prior estimates of maturity based on histology included a maturity indicator which has since been ruled out. Thus our estimates are somewhat higher than in previous SEDARs—SEDAR 33: L50=543 mm FL and A50=3.5 years and SEDAR10: L50=585 TL, A50=3.7. Estimates of 50% recruitment to the spawning grounds, which is considered to better mirror functional maturity resulted in an L50 of 668 and an A50 = 3.9 years.

For size at transition for all years and excluding Madison Swanson fish, we estimate an L50 of 1,050 mm FL and an A50 of 11.6 years. The length estimate is similar to previous SEDAR parameters (SEDAR33 L50=1022 FL, SEDAR10 L50 = 1085 TL) but the age estimate is slightly higher (SEDAR33A50=10.7, SEDAR10=10.8 years). In prior years sex assignment was based on pigmentation and potentially included some miss-specified females. We did not have that data for comparison.

Measure of reproductive potential

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 (Alonzo & Mangel 2005, Heppell et al. 2006, Ellis & Powers 2012, Easter & White 2016). In the last stock assessment, male sex ratio was estimated at ~2% based on age composition and an A50 of 10.7 years at transition. This was believed to be incorrect due to several MPAs developed to help protect male gag, but recent research estimated ~1% male sex ratio in the fished stock and ~5% in Madison Swanson (an MPA). It also indicated that gag transition in areas other than the spawning grounds and thus are not fully protected by current MPAs (Lowerre-Barbieri et al., 2020). 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.

Historically gag have demonstrated a male sex ratio of 17%, a sex ratio of 2% in the 1990's when they were severely over-fished, and currently exhibit a male sex ratio of ~2-3%. The expected increase in male abundance due to spawning reserve MPAs is not being realized. Even amongst protogynous species, gag are unique in having such low male sex ratios. For example, scamp, which are also protogynous and spawn in similar habitat to gag currently are estimated to have a male sex ratio of 41% (Lowerre-Barbieri et al., 2020 SEDAR68). They also demonstrate greater size and age overlap between the sexes and higher rates of transition (Figure 8). Thus, we recommend integrating a measure of male reproductive potential into this assessment, either through using combined spawning biomass or setting a sex ratio target (similar to SPR) to maintain a minimum of 20 to 30% of virgin sex ratio.

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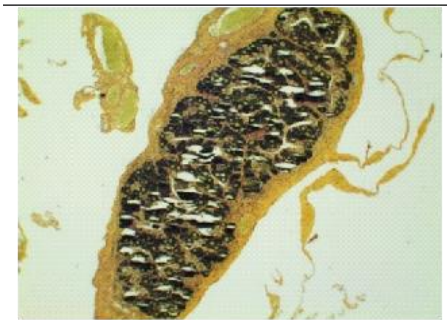
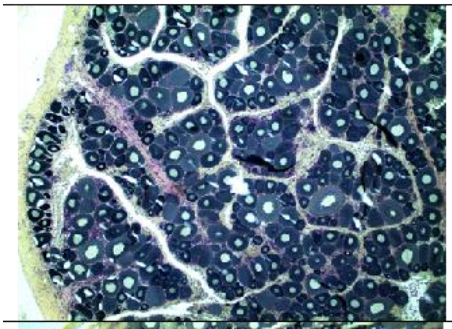
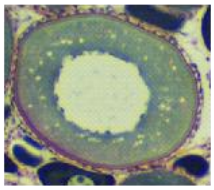
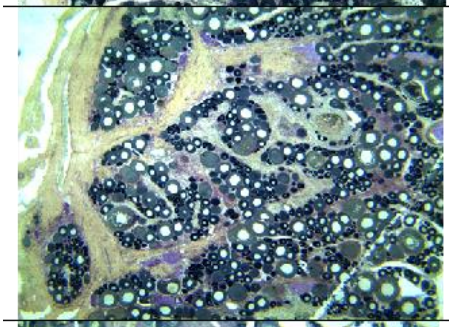
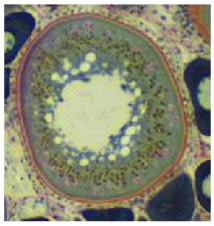
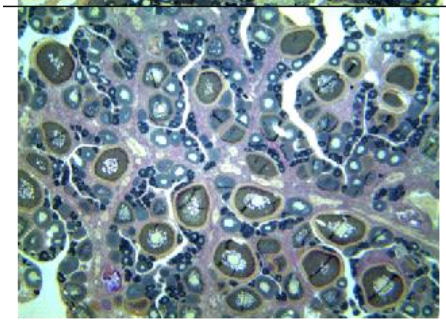
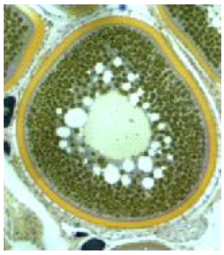
Tables and Figures

Table 1. Ovarian classification and terms based on histological analysis (modified from Lowerre-Barbieri et al., 2009).

Reproductive state	Phase	Histological indicators	Significance
Non-spawning	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.
Non-spawning	Developing	Cortical alveolar and sometimes early yolked oocytes. No evidence of POFs. Some atresia may be present.	Mature or maturing. Environmental signals have triggered the maturation process, but fish are not yet developed enough to spawn.
Spawning	Spawning- capable	Yolked oocytes. May have some early OM and/or some atresia; fish which have spawned within the past 48 h may have remnant POFs	Part of the spawning population. Fish developed enough to spawn.
Spawning	Sub-phase: Actively Spawning	Late OM (completed GVM or GVBD with yolk coalescence and partial to full hydration); ovulation; or newly-collapsed POFs	Part of the spawning population. Fish sampled in close proximity to the time of spawning and thus useful for assessing spawning sites.
Non-spawning	Regressing	A high percentage of yolked oocytes undergoing atresia (alpha and beta).	Mature fish at the end of the spawning season, resorbing left over developed oocytes.
Non-spawning	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 Gag, *Mycteroperca phenax*, used by FWC/FWRI

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

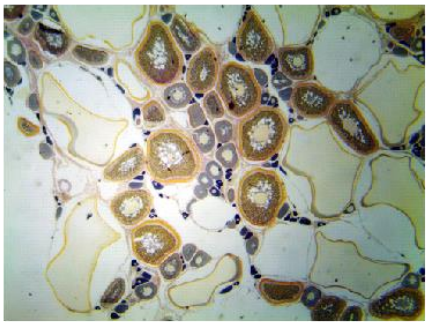
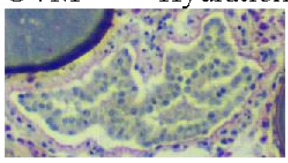
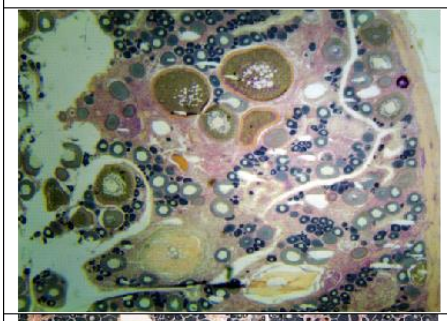
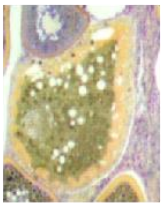
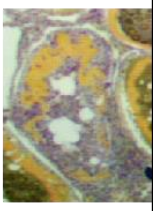
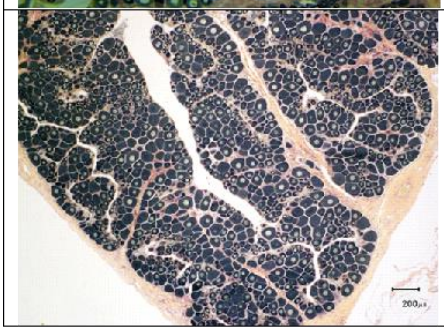
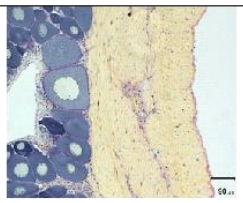
	***Actively Spawning subphase*** • Late germinal vesicle migration (GVM) • Germinal vesicle breakdown (GVBD) • Hydration and can have fresh POFs	 GVM Hydration  Fresh POF
	**Regressing** • Most Vtg oocytes undergoing atresia	  Alpha atresia Beta atresia
	**Regenerating** • Oogonia and PG oocytes present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

Table 3. Histological indicators of fish undergoing transition in male Gag, *Mycteroperca microlepis.*

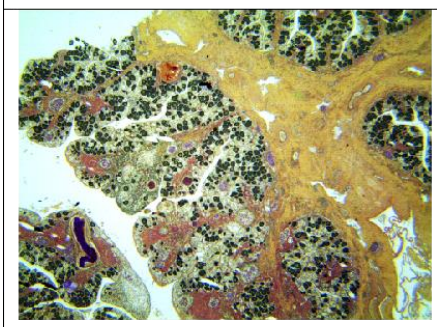
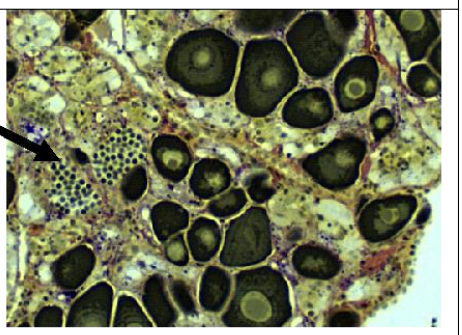
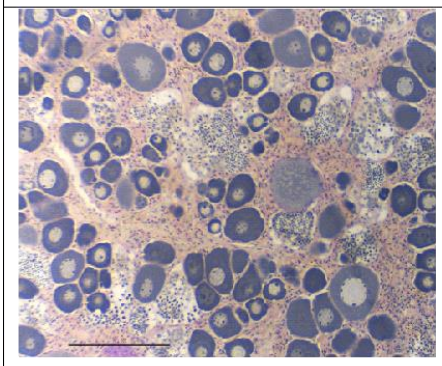
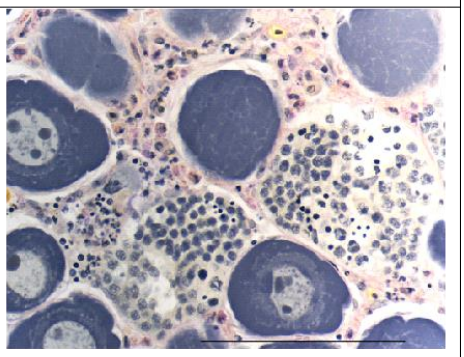
Teste Cross Section	Phase Characteristics	Key histological indicator
	**Early transition** • Spermatagonia (Sg) & spermatocytes (Sc) present • Continuous germinal epithelium • PG abundance decreasing	
	**Mid-late transition** • Sg, Sc & spermatids (St) present, occasional sperm crypts • Male tissue proliferation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

Table 4. Parameter estimates for gag maturity-at-length binomial generalized linear models for the entire time period and the early (1991-1999) and late (2000-2019) time periods separately. Models were run for all assigned mature and immature fish collected throughout the year (all year) and only those collected during the period when actively spawning individuals were observed (SS). Four link functions (logit, probit, cloglog and cauchit) were specified, and the best model was chosen via Akaike Information Criterion (AIC). For all models, the logit was either the best-fitting model or was within 1 AIC point of the best-fitting model, so we show only logit link parameter values.

Model	Link	N	Parameter	Estimate	St. Error	Value at 50% Probability
All year 1991-2019	logit	1776	Intercept	-15.046	0.909	600
			Slope	0.025	0.001	
All year 1991 -1999	logit	809	Intercept	-13.551	1.207	601
			Slope	0.023	0.002	
All year 2000-2019	logit	967	Intercept	-16.661	1.401	598
			Slope	0.028	0.002	
SS 1991-2019	logit	1015	Intercept	-18.525	1.887	603
			Slope	0.031	0.003	
SS 1991 -1999	logit	612	Intercept	-16.175	1.924	601
			Slope	0.027	0.003	
SS 2000-2019	logit	403	Intercept	-28.787	6.636	611
			Slope	0.047	0.010	

Table 5. Parameter estimates for gag maturity-at-age binomial generalized linear models for the entire time period and the early (1991-1999) and late (2000-2019) time periods separately. Models were run for all assigned mature and immature fish collected throughout the year (all year) and only those collected during the period when actively spawning individuals were observed (SS). Four link functions (logit, probit, cloglog and cauchit) were specified, and the best model was chosen via Akaike Information Criterion (AIC). When the logit model was within 1 AIC point of the best-fitting model, we show estimates for the logit model, otherwise best-fitting model values are shown.

Model	Link	N	Parameter	Estimate	St. Error	Value at 50% Probability
All year 1991-2019	logit	1573	Intercept	-7.391	0.475	3.8
			Slope	1.959	0.116	
All year 1991 -1999	cauchit	637	Intercept	-19.765	4.517	3.9
			Slope	5.006	1.127	
All year 2000-2019	logit	936	Intercept	-7.147	0.614	3.6
			Slope	1.972	0.155	
SS 1991-2019	logit	881	Intercept	-9.778	0.978	3.9
			Slope	2.513	0.234	
SS 1991 -1999	logit	485	Intercept	-10.009	1.188	4.1
			Slope	2.47	0.274	
SS 2000-2019	logit	395	Intercept	-10.162	1.98	3.7
			Slope	2.764	0.492	

Table 6. Observed and predicted proportion mature at age for the logit model for all years, with sampling restricted to the period during which actively spawning individuals were observed (1991-2019, SS).

Length Bin Midpoint	N	N_{mat}	Observed Proportion Mature	Predicted Proportion Mature
0	0	0	NA	0.002
1	3	0	0.000	0.012
2	55	7	0.127	0.076
3	178	21	0.118	0.369
4	196	123	0.628	0.806
5	253	233	0.921	0.967
6	371	369	0.995	0.995
7	200	199	0.995	0.999
8	123	123	1.000	1.000
9	73	72	0.986	1.000
10-18	121	121	1.000	1.000

Table 7. Observed and predicted proportion mature at length for all years, with sampling restricted to the period during which actively spawning individuals were observed (1991-2019, SS).

Length Bin Midpoint	N	N_{mat}	Observed Proportion Mature	Predicted Proportion Mature
235-345	2	0	0.000	0.000
355	0	0	*NA*	0.001
365	0	0	*NA*	0.001
375	2	0	0.000	0.001
385	1	0	0.000	0.001
395	2	0	0.000	0.002
405	4	0	0.000	0.003
415	4	0	0.000	0.004
425	7	0	0.000	0.005
435	9	0	0.000	0.007
445	12	0	0.000	0.009
455	5	0	0.000	0.012
465	4	0	0.000	0.016
475	2	0	0.000	0.022
485	6	0	0.000	0.030
495	5	1	0.200	0.040
505	8	1	0.125	0.054
515	5	0	0.000	0.072
525	7	1	0.143	0.095
535	2	0	0.000	0.125
545	4	0	0.000	0.163
555	6	1	0.167	0.210

565	6	1	0.167	0.265
575	4	2	0.500	0.329
585	4	1	0.250	0.400
595	4	2	0.500	0.475
605	8	5	0.625	0.552
615	5	1	0.200	0.626
625	3	0	0.000	0.695
635	7	7	1.000	0.756
645	10	9	0.900	0.808
655	11	10	0.909	0.851
665	13	12	0.923	0.886
675	11	9	0.818	0.913
685	16	15	0.938	0.935
695	13	13	1.000	0.951
705	21	20	0.952	0.964
715	18	16	0.889	0.973
725	20	19	0.950	0.980
735	29	28	0.966	0.985
745	23	23	1.000	0.989
755	27	27	1.000	0.992
765	31	30	0.968	0.994
775	32	32	1.000	0.996
785	29	29	1.000	0.997
795	33	33	1.000	0.998
805	24	24	1.000	0.998
815	35	35	1.000	0.999
825	34	34	1.000	0.999
835	29	29	1.000	0.999
845	41	41	1.000	0.999
855-1185	378	378	1.000	1.000

Table 8. Parameter estimates for gag sex transition-at-length binomial generalized linear models. Four link functions (logit, probit, cloglog and cauchit) were specified, and the best model was chosen via Akaike Information Criterion (AIC). Parameter values for the best fitting model and the logit link are provided. MS = Madison-Swanson

Model	**Link**	**N**	**Parameter**	**Estimate**	**St. Error**	**Value at 50% Probability**
All areas, 1991-2019	logit	4582	Intercept	-18.521	0.738	1039
			slope	0.018	0.001	
All areas, 1991-1999	logit	1671	Intercept	-19.783	1.496	1103
			slope	0.018	0.001	
All areas, 2000-2019	logit	2911	Intercept	-24.763	1.303	988
			slope	0.025	0.001	
Non-MS, 1991-2019	logit	4038	Intercept	-17.383	0.735	1050
			slope	0.017	0.001	
MS, 2015-2018	probit	544	Intercept	-23.181	2.907	987
			slope	0.023	0.003	

	logit		Intercept	-41.639	5.591	987
			slope	0.042	0.006	
Non-MS, 2015-2018	probit	1021	Intercept	-12.098	0.901	974
			slope	0.012	0.001	
	logit		Intercept	-22.141	1.778	973
			slope	0.023	0.002	

Table 9. Parameter estimates for gag sex transition-at-age binomial generalized linear models. Four link functions (logit, probit, cloglog and cauchit) were specified, and the best model was chosen via Akaike Information Criterion (AIC). Parameter values for the best fitting model and the logit link are provided. MS = Madison-Swanson

Model	**Link**	**N**	**Parameter**	**Estimate**	**St. Error**	**Value at 50% Probability**
All areas, 1991-2019	probit	4179	Intercept	-4.062	0.122	12.0
			slope	0.340	0.014	
	logit		Intercept	-7.513	0.258	11.8
			slope	0.635	0.027	
All areas, 1991-1999	probit	1369	Intercept	-4.537	0.2675	12.9
			slope	0.352	0.0266	
	logit		Intercept	-8.653	0.587	12.8
			slope	0.676	0.055	
All areas, 2000-2019	probit	2810	Intercept	-3.963	0.143	11.6
			slope	0.342	0.016	
	logit		Intercept	-7.273	0.299	11.5
			slope	0.635	0.032	
Non-MS, 1991-2019	probit	3638	Intercept	-4.105	0.136	11.6
			slope	0.353	0.016	
	logit		Intercept	-7.666	0.289	11.5
			slope	0.667	0.032	
MS, 2015-2018	probit	541	Intercept	-4.419	0.3588	12.8
			slope	0.345	0.0336	
Non-MS, 2015-2018	probit	1002	Intercept	-3.738	0.219	10.5
			slope	0.357	0.027	

Table 10. Observed and predicted proportion male at length for the logit model, all years, excluding Madison-Swanson.

Length Bin Midpoint	**N**	**N_{male}**	**Observed Proportion Male**	**Predicted Proportion Male**
325-585	490	0	0.000	0.000
595	55	1	0.018	0.001
605	63	0	0.000	0.001
615	72	0	0.000	0.001
625	66	1	0.015	0.001
635	76	0	0.000	0.001

645	72	0	0.000	0.001
655	89	0	0.000	0.002
665	101	0	0.000	0.002
675	95	0	0.000	0.002
685	118	0	0.000	0.003
695	102	0	0.000	0.003
705	94	0	0.000	0.004
715	99	2	0.020	0.004
725	125	0	0.000	0.005
735	104	0	0.000	0.006
745	104	0	0.000	0.007
755	104	0	0.000	0.008
765	132	1	0.008	0.010
775	111	0	0.000	0.011
785	101	0	0.000	0.013
795	100	2	0.020	0.016
805	102	1	0.010	0.019
815	99	2	0.020	0.022
825	96	0	0.000	0.026
835	84	1	0.012	0.030
845	86	2	0.023	0.035
855	73	1	0.014	0.042
865	60	2	0.033	0.049
875	76	2	0.026	0.057
885	51	5	0.098	0.066
895	58	1	0.017	0.077
905	55	7	0.127	0.090
915	58	5	0.086	0.105
925	51	8	0.157	0.121
935	52	9	0.173	0.140
945	44	8	0.182	0.161
955	39	10	0.256	0.185
965	29	7	0.241	0.211
975	39	15	0.385	0.240
985	31	10	0.323	0.272
995	28	9	0.321	0.306
1005	33	13	0.394	0.342
1015	29	13	0.448	0.380
1025	30	13	0.433	0.420
1035	26	8	0.308	0.460
1045	25	14	0.560	0.502

1055	20	14	0.700	0.543
1065	14	8	0.571	0.584
1075	13	4	0.308	0.623
1085	18	12	0.667	0.661
1095	14	7	0.500	0.697
1105	20	15	0.750	0.731
1115	26	19	0.731	0.763
1125	9	5	0.556	0.791
1135	10	9	0.900	0.817
1145	14	9	0.643	0.841
1155	15	13	0.867	0.862
1165	3	2	0.667	0.880
1175	7	6	0.857	0.897
1185	4	2	0.500	0.911
1195	6	6	1.000	0.924
1205	5	5	1.000	0.934
1215	2	2	1.000	0.944
1225	2	2	1.000	0.952
1235	2	2	1.000	0.959
1245	1	1	1.000	0.965
1255	1	1	1.000	0.970
1265	1	1	1.000	0.975
1275	1	1	1.000	0.978
1295	2	1	0.500	0.984
1325	1	1	1.000	0.990

Table 11. Observed and predicted proportion male at age for the probit model, all areas, 2000-2019.

Age	N	N_{male}	Observed Proportion Male	Predicted Proportion Male
1	3	0	0.000	0.000
2	70	0	0.000	0.001
3	428	0	0.000	0.002
4	669	1	0.001	0.006
5	757	6	0.008	0.015
6	672	10	0.015	0.035
7	350	17	0.049	0.072
8	222	28	0.126	0.134
9	128	31	0.242	0.225
10	114	34	0.298	0.344

11	62	31	0.500	0.480
12	40	24	0.600	0.619
13	35	23	0.657	0.744
14	20	13	0.650	0.843
15	14	11	0.786	0.913
16	18	15	0.833	0.957
17	12	11	0.917	0.981
18	5	4	0.800	0.992
19	4	4	1.000	0.997
20	6	6	1.000	0.999
21-28	9	9	1.000	1.000

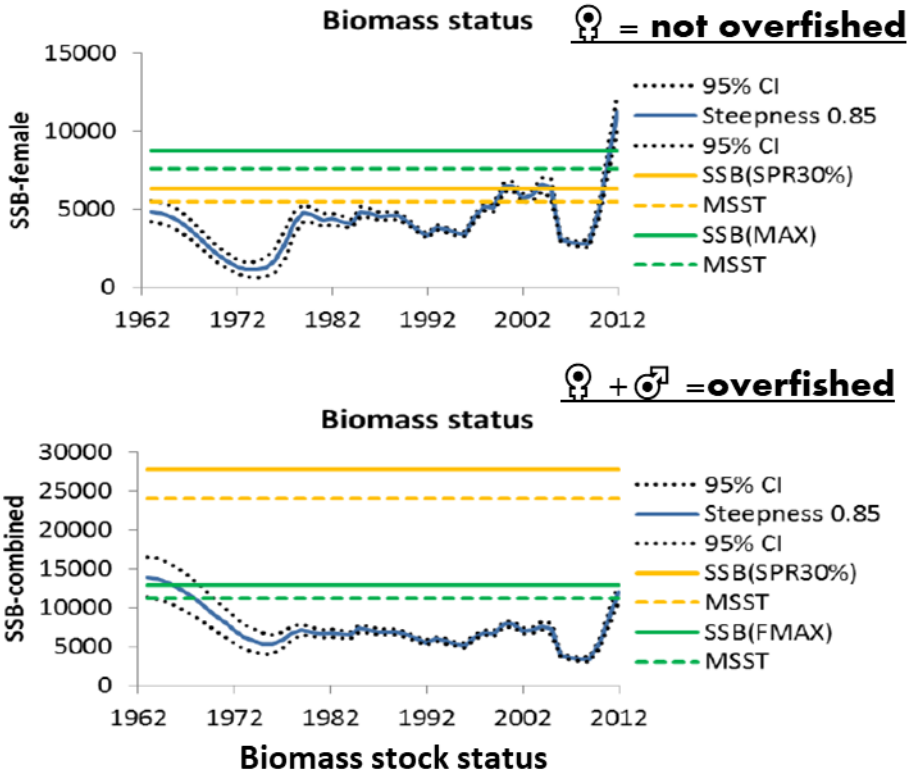


Figure 1. Biomass status for female only spawning stock biomass (top) versus for combines sexed (bottom).

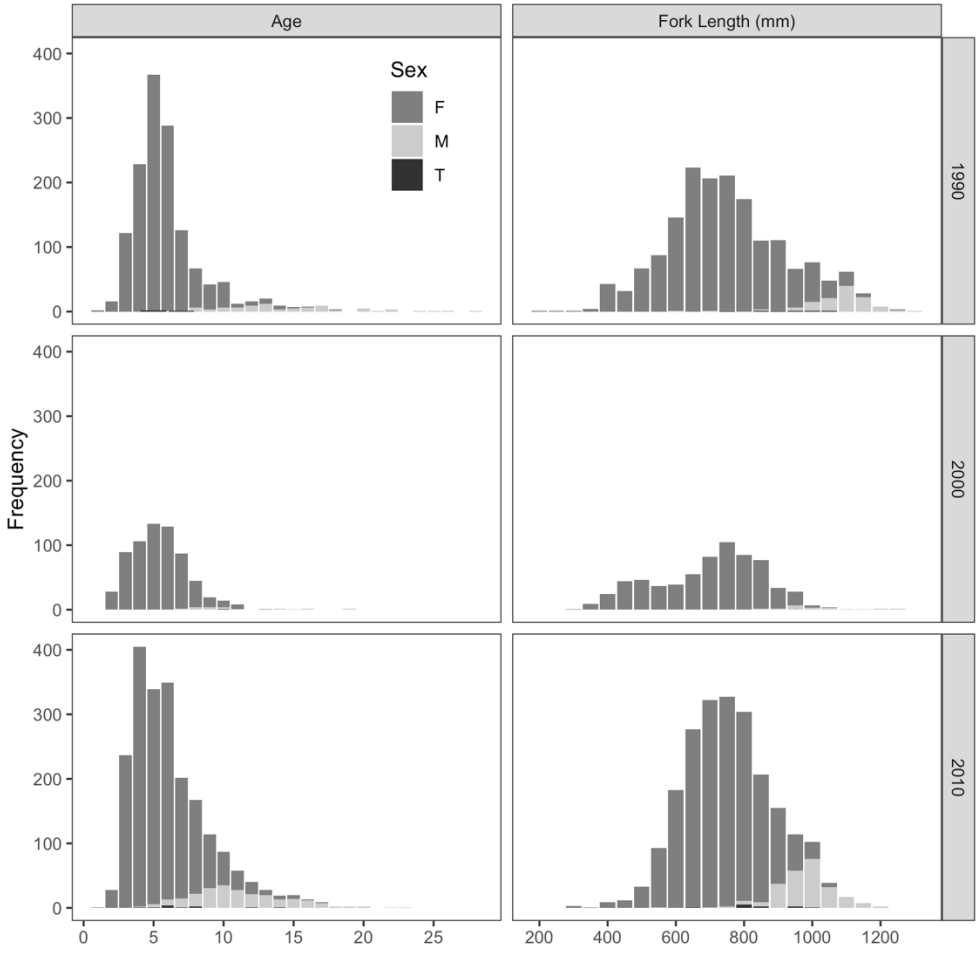


Figure 2. Age (left) and length (right) distribution by sex and decade

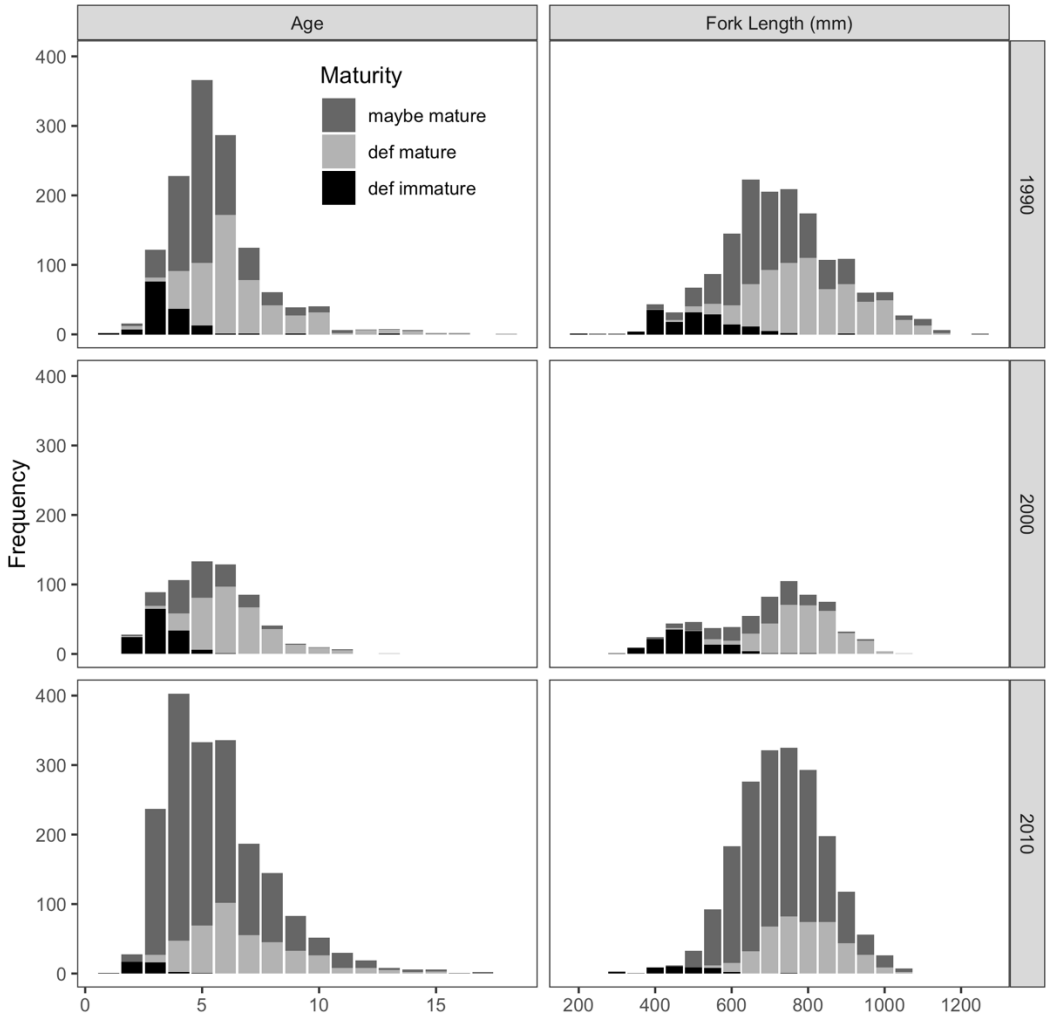


Figure 3. Age (left) and length (right) distribution by assigned maturity status and decade

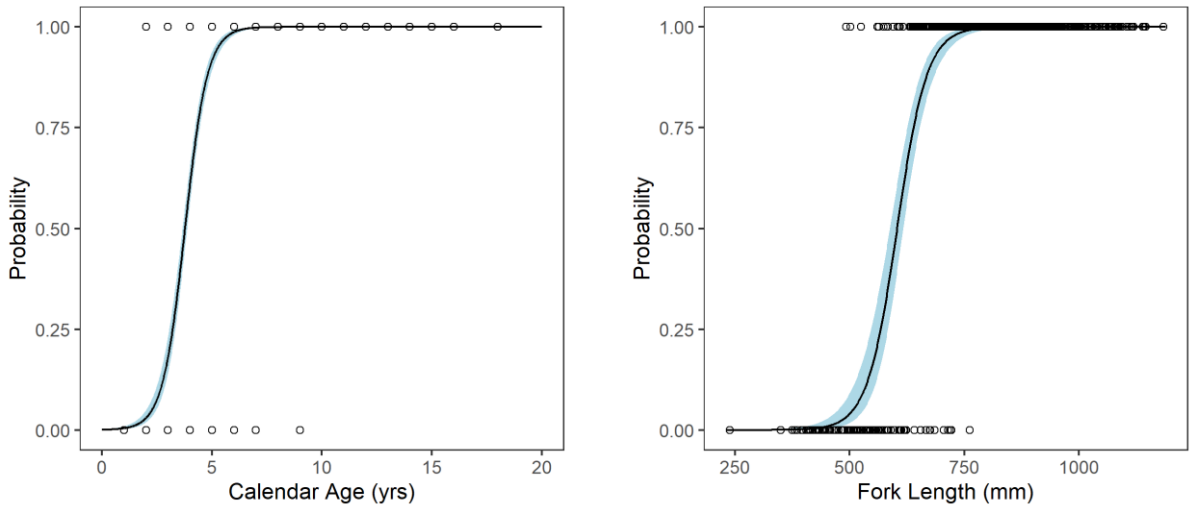


Figure 4. Observed and predicated age (left) and fork length (right) at maturity with 95% confidence intervals, for the models in which sampling was restricted to the period during which actively spawning individuals were observed (1991-2019, SS). The estimated size and age at 50% maturity under the logit models were 603 mm FL and 3.9 years, respectively.

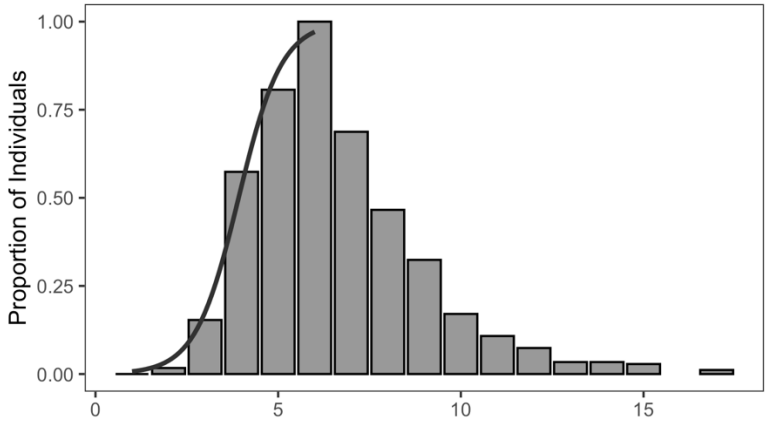


Figure 5. Logistic model fit to age for gag collected at Madison-Swanson and in waters deeper than 50 meters by fisheries-independent sampling ($N_{a<7}$ = 443). The estimate of the inflection point (~ 50% recruitment to the spawning population) is 3.9 years (SE = 0.017).

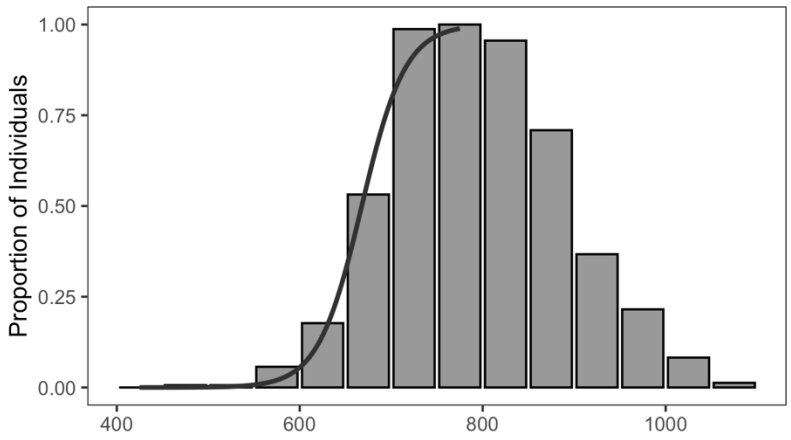


Figure 6. Logistic model fit to length bin for gag collected at Madison-Swanson and in waters deeper than 50 meters by fisheries-independent sampling ($N_{fl<800}$ = 432). The estimate of the inflection point (~ 50% recruitment to the spawning population) based on 50mm length bins is 668 mm FL (SE = 0.005).

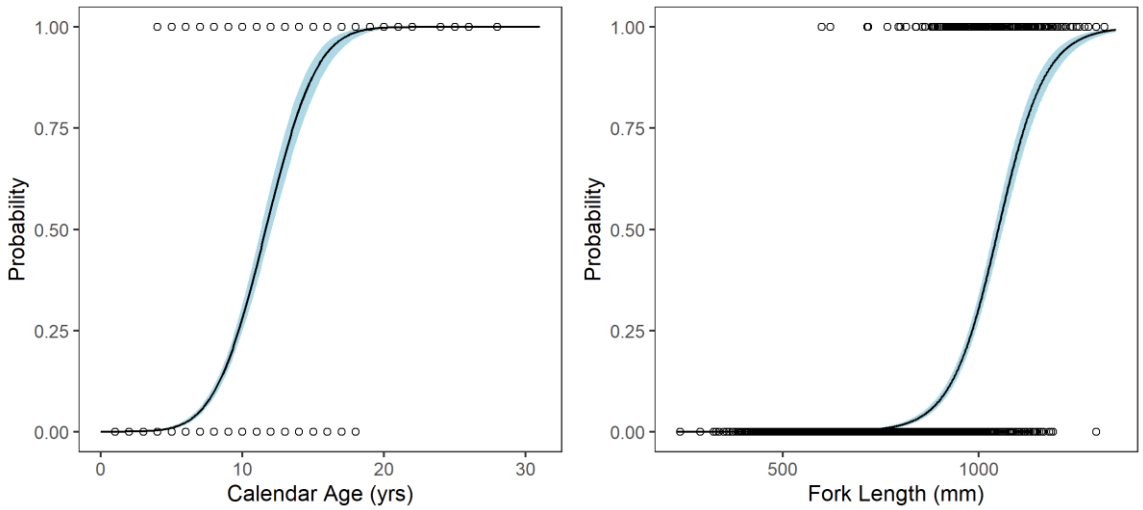


Figure 7. Observed and predicted age (left) and fork length (right) at sex transition with 95% confidence intervals for all years, excluding Madison-Swanson. The estimated size at 50% male under the best-fitting model (logit) was 1050 mm FL, and the estimated age at 50% transition under the best-fitting model (probit) was 11.6 years.

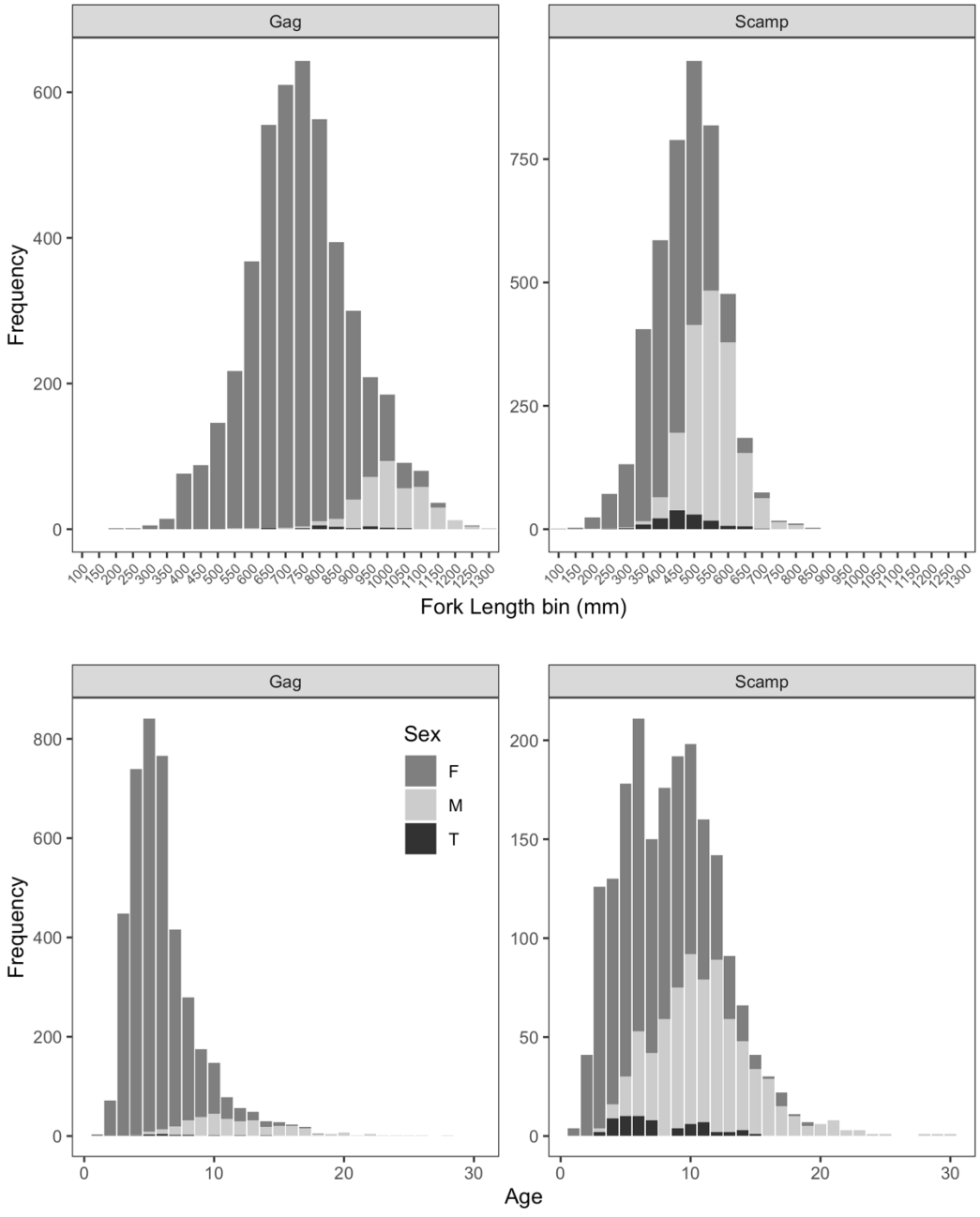


Figure 8. Age and length frequency distribution by sex for gag and scamp *Mycteroperca phenax*