

Reproductive classification and spawning seasonality of *Epinephelus striatus* (Nassau grouper), *E. guttatus* (red hind) and *Mycteroperca venenosa* (yellowfin grouper) from The Bahamas

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Abstract. Fish of the family Serranidae: subfamily Epinephelinae are some of the most heavily harvested reef fish in the Caribbean. However, large knowledge gaps exist about their reproductive biology. Understanding a species' reproductive biology is critical for species management and provides evolutionary insight into species-specific reproductive strategies. Epinephelinae species possess a diversity of reproductive strategies, comprised of life-history traits which are highly variable between and amongst populations (e.g. size at sexual maturity, spawning duration, sex ratio). For this study, a reproductive histological classification system was refined for application to Bahamian fish populations of: *Epinephelus striatus* (Nassau grouper), *E. guttatus* (red hind) and *Mycteroperca venenosa* (yellowfin grouper). The use of a single classification system on multiple species provided a reliable framework to assess the status of reproductive life-history traits. Spawning seasonality in The Bahamas was described for populations of *E. striatus*, *E. guttatus* and *M. venenosa*. Spawning for *E. striatus* peaked November through January, while *E. guttatus* peaked in January and *M. venenosa* peak in March and April. The classification system will be used to establish consistent monitoring techniques and the results provide information for future management efforts in The Bahamas and allow for comparison to populations throughout the Caribbean.

Key words: Nassau grouper, red hind, yellowfin grouper, reproductive biology, gonad histology classification

Introduction

The reproductive biology of a fish is defined both by the combination of the species-specific reproductive mode and reproductive traits (Winemiller and Rose 1992, Murua and Saborido-Rey 2003, Morgan 2008). The reproductive mode does not vary between populations and is defined by the combination of the sexual development pattern (e.g. gonochoristic or hermaphroditic) and the gamete production system (e.g. determinate or indeterminate). Reproductive life-history traits (e.g. spawning seasonality and duration, age or size of sexual maturity, and sex ratio) vary between and amongst populations (Winemiller and Rose 1992, Murua and Saborido-Rey 2003, Morgan 2008). All are critical to understand a given population because they provide insight into how different strategies influence gamete production (Winemiller and Rose 1992) and how life-history trait plasticity can greatly alter a population's productivity or reproductive potential over time (Winemiller and Rose 1992, Morgan 2008).

Histological analysis of gonads provides more accurate and specific information to quantify life-

history traits than traditional, macroscopic gonad examinations. Balon (1975) and Winemiller and Rose (1992) established that a classification system should also allow for intra-species comparisons for ecosystem based management and evolutionary life history comparison purposes. The use of a single histological classification system on multiple species provides a means to assess the reproductive biology of species that possess different reproductive strategies with variable life-history traits, and allows for comparisons between species.

Species of the family Serranidae: subfamily Epinephelinae (commonly called groupers and hinds) are heavily fished in the Caribbean. Despite their importance, large knowledge gaps exist about their reproductive biology. As with most marine fish, species-specific data is required for Epinephelinae because reproductive life-history traits are variable both within and between species (Sadovy 1996). In The Bahamas, fishery management and monitoring initiatives are focusing on securing consistent reproductive biology and landing data for commercially valuable Epinephelinae species.

Epinephelinae landings, especially *Epinephelus striatus* (Nassau grouper), and to a lesser, but considerable extent *E. guttatus* (red hind), and *Mycteroperca venenosa* (yellowfin grouper) constitute a major portion of fin-fish catches in The Bahamas (Cushion and Sealey 2007). To date, some reproductive life-history studies have been completed on *E. striatus* in The Bahamas (see Sadovy and Eklund 1999 for a review); while no formal studies on *E. guttatus* and *M. venenosa* have been conducted in The Bahamas.

This paper describes the effectiveness of a histology classification system for quantifying reproductive life-history traits and identifying the reproductive maturity stage of Epinephelinae species. The goal was to affirm that the proper criteria and diagnostics were incorporated into the system, so it could be applied to multiple Epinephelinae species that possess different reproductive strategies. The system was then used to determine the spawning seasonality for *E. guttatus*, *E. striatus*, and *M. venenosa* harvested in the central Bahamas. The classification system will form the basis for consistent long-term monitoring initiatives in The Bahamas and provide a means to evaluate temporal and spatial differences in Epinephelinae reproductive life-history traits that influence reproductive potential.

Material and Methods

A fishery-dependent monitoring project commenced in January 2007 at a major commercial fish market in New Providence (the most populated island), Nassau, Bahamas to acquire Epinephelinae landings, population, and reproductive biology data (Cushion and Sealey 2007). Data was obtained via monthly monitoring corresponding with the full moon phase (the spawning period of many Epinephelinae). A standard histological classification system was incorporated into the project to evaluate, compare and monitor reproductive traits among Epinephelinae species.

Monthly sampling was conducted at the market from January 2007-April 2008. Length, weight and gonad weight were measured and recorded for each fish. A section of each gonad was collected and preserved in 10% neutral buffered formalin. Gonad sections were later imbedded in paraffin, sectioned and stained using hemotoxylin and eosin following standard histological procedures (Fitzhugh et al. 1993). Gonad homogeneity tests to confirm that a subsample was representative of the entire gonad were previously performed for each species by Sadovy and Colin (1995) (*E. striatus*), Sadovy et al. (1994) (*E. guttatus*), and García-Cagide and García (1996) (*M. venenosa*).

The reproductive biology classification system was adopted (with minor changes) from Lyon et al. (2008). Lyon et al. (2008) outlined a classification system based on previous studies including Moe (1969) and (Brown-Peterson et al. 2006). This system was adopted to classify Epinephelinae species for this study. Minor revisions were made to account for many Epinephelinae being protogynous species (thus having transitional gonads) and the common occurrence of “bisexual” gonads that contain both oogenic and spermatogenic tissue, but for which primary function as either male or female cannot be determined (Sadovy and Shapiro 1987).

Female and male fish were classified using diagnostic features to determine sexual maturity, the leading gamete stage (the most advanced oocyte or spermatogenic stage present), and whether oocytes were recently released (Table 1 a and b). The presence of vitellogenic oocytes indicates spawning will occur within days or weeks. Female spawning indicators are advanced vitellogenic oocytes (lipid and yolk coalescence) that represent the initiation of spawning and fully hydrated oocytes that are indicative of actively spawning fish. Recently spawned females were detected by the presence of post-ovulatory follicles. The end of the spawning season was determined by massive cell atresia (indeterminant spawners) or the lack of vitellogenic oocytes (determinate spawners). Male sexual maturity was indicated by initiation of spermatogenesis and formation of spermatocysts. Males are classified as spawning capable when spermatozoa were evident and filling sperm ducts and lobules.

Fish were classified as transitional if degenerating oogenic and proliferating spermatogenic tissue were present (Sadovy and Shapiro 1987). Fish were classified as bisexual if fairly equal amounts of oogenic and spermatogenic tissues were present, but no sexual function was determined (Sadovy and Colin 1995).

All histological slides for each species were analyzed and classified by two readers. Results were used to determine reproductive class. Also, the percentage of samples in each class was determined monthly and used to estimate the spawning seasonality for each focal species. Months were designated as spawning months if over 50% of the female samples for the month were classified as active or spawning and over 50% of the male samples were classified as spawning capable.

Results

The histological classification scheme modified and utilized for this study provided the appropriate criteria (Table 1) for designation of 96% of gonad samples

Table 1. A histological reproductive classification system and diagnostics for female, transitional, bisexual and male Epinephelinae.

Sex	Class	Diagnostics
Female	Immature, inactive	Primary growth oocytes only, no evidence of prior spawning. Chromatin nucleolus stage (small cells with large nucleus), and initial perinucleolar stage (larger oocytes). Well-organized gonad.
	Inactive, uncertain	Not capable of spawning in distant future & prior spawning unclear.
	Developing virgin, Developing	Cortical alveolar oocytes present. Prior spawning indicators confirm maturity (D). No spawning indicators (Dv).
	Active, mature	Vitellogenic oocytes present, will spawn within days or weeks.
	Spawning, hydrated	Early or late hydrated oocytes or post-ovulatory follicles present.
	Post-ovulatory, spent	End of spawning cycle, majority of oocytes (>50%) experiencing atresia. Post-ovulatory follicles may be present.
	Regressed, inactive, mature.	PG oocytes only, evidence of sexual maturity & recent spawn.
Regressed, mature.	Sexually mature but will not spawn in current season, development ended prematurely.	
Transitional	Sperm crypts proliferating throughout gonad. Gamete stages from primary spermatocyte through spermatid should be present. Remnant oocytes possibly undergoing atresia. Must possess evidence of degenerating oogenic and proliferating spermatogenic tissue. (Protogynous species only).	
Bisexual	Oogenic and spermatogenic tissues present, but neither is dominant or proliferating. No sexual function can be determined.	
Male	Immature, inactive	Includes males with spermatogonia (SGG) and no evidence of spermatogenesis (SG).
	Developing virgin (only gonochoristic species)	Spermatogenesis begins; spermatocytes present & no prior indicators of maturity (Dv).
	Developing	Initiation of spermatogenesis and formation of spermatocysts (D).
	Spawning capable	Fish is reproductively active and capable of spawning. All stages of spermatogenesis may be present.
	Spent	Spermatogenesis is ceasing. Some residual spermatozoa present. Spermatogonia proliferation and regeneration of germinal epithelium common in periphery of testis.
	Regressed, inactive, mature	Spermatogonia dominate; no active spermatogenesis. Continuous germinal epithelium throughout.

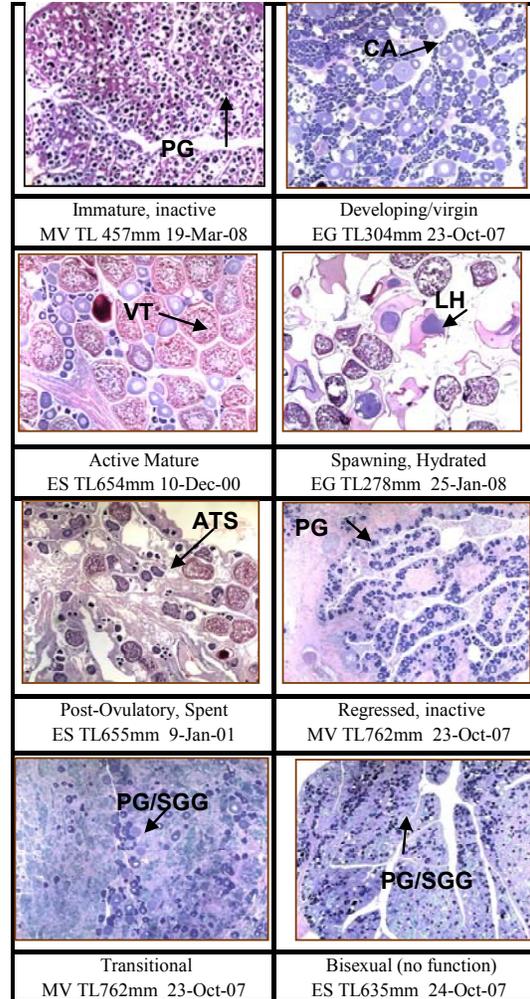


Figure 1a: Epinephelinae histological reproductive classification system for females, bisexual and transitional fish. Reproductive classes, diagnostic features, size and sample collection date are highlighted for female *E. striatus* (ES), *E. guttatus* (EG) and *M. venenosa* (MV). Primary growth (PG), cortical alveolar (CA), vitellogenic oocytes (VT), late hydrated (LH) and atresia (ATS) are highlighted.

(n=675) into a class (Fig. 1 a and b), all species combined. The results of this study corroborate previous reproductive biology studies on the focal species. Gamete production in *E. striatus* is indeterminate and the species is functionally gonochoristic (Sadovy and Colin 1995). *E. guttatus* is a protogynous hermaphrodite with determinate gamete production (Shapiro et al. 1993); while *M. venenosa* is a protogynous hermaphrodite with indeterminate gamete production (García-Cagide and García 1996).

E. striatus samples were typically the most challenging to classify due to ~12% (26 out of 220) of all samples containing both inactive oogenic and spermatogenic tissue. The domination of oogenic or spermatogenic tissue was used to classify these fish, but 4% were classified as “bisexual” because no

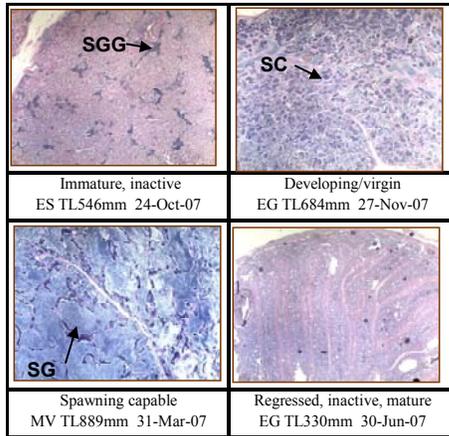


Figure 1b: Epinephelinae histological reproductive classification system for male fish. Reproductive classes, diagnostic features, size and sample collection date are highlighted for transitional, bisexual and male *E. striatus* (ES), *E. guttatus* (EG) and *M. venenosa* (MV). Spermatogonia (SGG), spermatogenesis (SG), and spermatocytes (SC) are highlighted. (Spent male not pictured.)

Table 2. Spawning seasonality for Bahamian Nassau grouper (*E. striatus*), Red hind (*E. guttatus*) and Yellowfin grouper (*M. venenosa*). Samples collected from January 2007- April 2008 in New Providence, corresponding to the full moon cycle. Spawning months were designated as so if over 50% of the female samples were classified as “Active” or “Spawning hydrated” and over 50% of the male samples were classified as “Spawning capable” (Table 1).

Species	Spawning Years/ Months (Sample number in parentheses).
<i>E. striatus</i>	November 2007 (21) January and February 2008 (27 and 23)
<i>E. guttatus</i>	February 2007 (17) January and February 2008 (22 and 30)
<i>M. venenosa</i>	March 2007* (12 and 28) March and April 2008 (28 and 34)
*Two sampling periods: one at the beginning and one at the end of the month, following the full moon schedule.	

sexual function could be determined. Also, eleven *E. striatus* samples (5%) were classified as “inactive, uncertain”. For *E. guttatus* 12 out of the 200 hundred samples (6%) were classified as “inactive, uncertain”. For *M. venenosa*, 15 out of the 175 samples (9%) were classified as “inactive, uncertain”. For all species, the majority of samples classified “inactive, uncertain” were from the summer, non-spawning months.

Spawning seasonality for the three focal species was analyzed (Table 2). Over 50% of the male and female *E. striatus* samples in November 2007, January and February 2008 were in spawning condition (n=21, 27 and 23, respectively) (no samples were obtained in December 2007). For *E. guttatus*, over 50% of the male and female samples collected in February 2007, January and February 2008 were in spawning condition (n=17, 22 and 30, respectively). *M. venenosa* samples revealed their spawning season

to be slightly later. Over 50% of the male and female samples collected during two sampling periods in March 2007 (one at the beginning and one at the end of the month, following the full moon schedule), and March and April 2008 (n=12, 28, 28 and 34, respectively) were in spawning condition. Additionally, 45% of the February samples were in spawning condition.

Discussion

The high percentage of classification for each focal species highlights the cross-utility of the classification system. The system allows for the requisite reproductive biology information to be quantified for Epinephelinae species in the Bahamas. The confirmation *E. striatus* as functionally gonochoristic was supported by the overlap of males and females in all size classes. This is unlike protogynous *E. guttatus* and *M. venenosa*, in that no males were found in the relatively smaller size classes and no females were found after a certain size (unpublished data). The percentage of *E. striatus* that were classified as bisexual, with no sexual function being determined was not unusual. Sadovy and Colin (1995) investigated the sexual development pattern of *E. striatus* and found four mature bisexual individuals and 23% of all samples were immature bisexuals. The classification of 4% of *E. striatus*, 6% of *E. guttatus* and 9% of *M. venenosa* as inactive, uncertain was also not uncommon. These samples were primarily from summer months when fish are not spawning. Inactive and regressed fish are the main classes during this time period and both are typified by compact gonads with primary growth oocytes. Thus, without sufficient evidence of prior spawning (e.g. old hydrated oocytes) it is not possible to confirm regression. Shapiro et al. (1993) investigated sex change and reproduction in *E. guttatus* and could not distinguish between inactive and late, regressed females.

Spawning seasonality for many Epinephelinae and other reef fish is a variable reproductive trait (within and between populations), especially for populations at different latitudes (Sadovy 1996). Spawning seasonality has previously been determined for *E. striatus* in the Bahamas. Colin (1992) found that the *E. striatus* populations off Long Island spawned during the full moon periods of December and January, possibly not during November and likely not during February. This study highlights that *E. striatus* spawning seasonality is slightly variable within the Bahamas. Spawning began in November 2007 and continued through February 2008. However, for *E. striatus*, spawning seasonality is strongly correlated with the lunar full moon as well as temperature, not the month *per se* (Sadovy and Eklund 1999). Colin

(1992) found *E. striatus* spawning occurred at water temperatures between 25.0-25.5°C. Thus, water temperature is likely a strong contributing factor for latitudinal and annual fluctuations in spawning seasonality.

This is the first documentation of spawning seasonality for *E. guttatus* and *M. venenosa* in The Bahamas. Shapiro et al. (1993) found a similar spawning seasonality for Puerto Rican *E. guttatus* populations. Using a gonadal size index and histology, spawning peaks were found in January and February. Meanwhile, *E. guttatus* spawning peaks much later in Bermuda occurring during the full moon periods from May to July (Luckhurst et al. 2004). It is noteworthy that *E. guttatus* spawning seasonality is not as tightly correlated to the full moon (Sadovy et al. 1994), as with *E. striatus* and *M. venenosa*. Thus the monthly, full-moon sampling regime did not likely capture all *E. guttatus* spawning activity in the Bahamas.

Small groups of *M. venenosa* are often associated with aggregations of *E. striatus* (e.g. Whaylen et al. 2004 (Cayman Islands), Nemeth et al. 2004 (USVI)). However, January and February are not the dominant spawning times for *M. venenosa*. Personal communications with fishermen in this study, in conjunction with gonad sampling, confirmed full moon periods during March and April as peak spawning months of *M. venenosa* in The Bahamas. A large proportion of the specimens were spawning capable in February, thus indicating the spawning period may commence in February. In Cuba, García-Cagide and Garcia (1996) found April and May to be the strongest spawning months for *M. venenosa* which is consistent with later spawning at more southerly latitudes.

Because reef fisheries in The Bahamas are multi-species, it is important to implement a system that can be applied to multiple species to ensure that consistent and reliable information is obtained. The fishery-dependent sampling protocol with a standard reproductive classification allowed for the collection and analysis of samples year round. This combination system will provide a means for long-term monitoring of Epinephelinae species to consistently assess reproductive life-history traits and the reproductive potential of populations.

Acknowledgements

Funding for this project came from the University of Miami Department of Biology, The College of The Bahamas Marine and Environmental Studies Programme, Disney Wildlife Conservation Fund and Earthwatch Institute. Thanks to the fishermen and people at Montagu ramp and to Anastasia Gibson and the Department of Marine Resources staff for field assistance.

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