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Growth, Reproductive Life-History Traits and Energy Allocation in Epinephelus guttatus (red hind),E. striatus (Nassau Grouper), and Mycteroperca venenosa (yellowfin grouper) (Family Serranidae, Subfamily Epinephelinae)

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UNIVERSITY OF MIAMI

GROWTH, REPRODUCTIVE LIFE-HISTORY TRAITS AND ENERGY ALLOCATION IN *EPINEPHELUS GUTTATUS* (RED HIND), *E. STRIATUS* (NASSAU GROUPER), AND *MYCTEROPERCA VENENOSA* (YELLOWFIN GROUPER) (FAMILY SERRANIDAE, SUBFAMILY EPINEPHELINAE)

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Growth, Reproductive Life-History Traits and Energy Allocation in *Epinephelus guttatus* (red hind), *E. striatus* (Nassau grouper), and *Mycteroperca venenosa* (yellowfin grouper) (Family Serranidae, Subfamily Epinephelinae) (June 2010)

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Fish populations are regulated by both external environmental factors, e.g., water quality parameters and habitat, and internal reproductive biology and physiology processes. For many species and populations there is often ample external information, while critical internal, i.e., life-history trait (LHT), information is not available. For this study, I determined LHTs and energy allocation patterns for *Epinephelus guttatus* (red hind), *E. striatus* (Nassau grouper), and *Mycteroperca venenosa* (yellowfin grouper) harvested from The Bahamas. I determined age ranges, and how growth patterns and rates differed among the study species. The maximum ages were: 17 (years old, yo), *E. guttatus*; 22 yo, *E. striatus*; and 13 yo, *M. venenosa*. Between the study species, *E. striatus* was estimated to have the slowest, while *M. venenosa* had the fastest growth rate. I described a gonad reproductive histology classification system which was used to quantify reproductive LHTs and to identify the reproductive maturity stage of Epinephelinae species. The classification system and the ageing data were used to determine the spawning seasons, sex ratios, size and age of sexual maturation and sex change and gonadosomatic indices (GSIs) for the study species. The peak spawning months were January-February for *E.*

guttatus, December-January for *E. striatus* and March-April for *M. venenosa*. The fifty-percent sexual maturity estimates were 235 total length mm (Tlmm), 2.05 yo; 435 Tlmm, 4.00 yo; and 561 Tlmm, 4.66 yo for *E. guttatus*, *E. striatus* and *M. venenosa*, respectively. The size and age range of sex change for *E. guttatus* was between 257-401 Tlmm, ~4-5 yo and between 716-871 Tlmm, ~8-9 yo for *M. venenosa*. Females had significantly larger GSIs than males, while some male *M. venenosa* had atypically large GSIs as compared to other Epinephelinae species. I determined protein and lipid concentrations in muscle and gonad tissues to ascertain energy allocation patterns. The GSI wet weight values (g) were converted to GSI energy values (kJ) and were found not to be equivalent for a given species and sex, emphasizing that caution should be used when interpreting GSI (g) in terms of energy investment. For all species and sexes except for female *E. guttatus*, the proportion of energy delegated to somatic growth declines as a fish grows longer, while reproduction energy allocation increases. The results of each study were compared to previous studies conducted throughout the tropical western Atlantic Ocean, and were related to species-specific ecological and spawning behaviors. The findings of each study highlight that the LHTs of the study species greatly differ and these differences will impact population dynamics and need to be considered for management initiatives. In the final chapter, the effects of fishing on LHTs are reviewed and fishery management options are discussed.

Dedicated to my parents

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

CHAPTER ONE

PROJECT OVERVIEW AND THEORETICAL BACKGROUND: GROWTH, REPRODUCTIVE LIFE-HISTORY TRAITS AND ENERGETICS OF FISHES

Project Overview and Rationale

Understanding how fishes have evolved to maximize evolutionary fitness via variation in reproductive strategies and life-history traits (LHTs) is a significant research area in evolutionary science, as well as in reproductive biology studies (Beverton 1963, Stearns 1982, Roff 1992, Winemiller and Rose 1992, Charnov 1993, Morgan 2008). Intra- and inter-species comparison studies provide a better understanding of both the diversity of reproductive strategies and the influence of natural selection on LHTs (Winemiller and Rose 1992, Hairston and Bohonak 1998). Intra- and inter-species comparisons of LHTs can therefore provide crucial understanding into how variation influences reproductive output and population dynamics (Jones and McCormick 2002) (e.g., baboons: Popp 1983, snakes: Brown and Shine 2005, fish: Huntsman and Schaaf 1994, fish: Myers and Barrowman 1996). The overall objective of the present study was to thus determine growth parameters, LHTs and growth and reproductive energy allocation for three closely related species of the family Serranidae, subfamily Epinephelinae (*Epinephelus guttatus*, red hind; *E. striatus*, Nassau grouper, and *Mycteroperca venenosa*, yellowfin grouper). The findings were examined in the context of their species-specific reproductive strategies. The premise of the present study was "not all Epinephelinae species are created equal", and there are species-specific reproductive strategy and LHT differences which are important to understand.

For fish, the reproductive strategy of a species is defined by a combination of variable, 'plastic', reproductive LHTs and 'fixed' aspects, collectively termed the reproductive mode (Figure 1.1) (Winemiller and Rose 1992, Murua and Saborido-Rey 2003, Morgan 2008). For the present study, growth parameters, reproductive LHTs (growth, sex ratios, age and size of sexual maturation, age and size of sex change, and spawning seasonality) were determined and growth and reproduction energy allocation were investigated in *E. guttatus*, *E. striatus* and *M. venenosa* harvested from The Bahamas. These study species were chosen because they are closely related (Craig and Hastings 2007), yet possess unique reproductive strategies which have evolved since the closing of the Isthmus of Panana ~3.5 mya (Coites et al. 1992). Also, most commercially harvested Epinephelinae are presently considered "over-fished" (Morris et al. 2000) and species-specific information is needed for management. The findings for the three study species were contrasted to: a) postulate how reproductive LHT variation between species may differentially influence reproductive output, b) compare LHTs to other populations within the tropical western Atlantic Ocean (TWA) and c) evaluate how aspects of a species' reproductive mode (e.g., sexuality and the spawning mode) may relate to LHT variation between the study species.

Research Objectives and Hypotheses

(See Figure and Table 1.1 for an outline of reproductive strategies and the reproductive biology of the study species)

Objective One: To ascertain age and growth data for Bahamian populations of *E. guttatus, E. striatus* and *M. venenosa* to elucidate species-specific growth patterns. (Chapter 2)

Q1) What are the body mass allocation parameters (as measured by the allometric equation: $M = aL^b$) and growth rates (as measured by the von Bertalanffy growth function (1938): $L_t = L_\infty (1-e^{-k(t-t0)})$) for the study species?

Q2) How do the parameters and growth rates compare to other studies performed in the TWA?

Q3) What are the physiological and reproductive biology implications of differing body mass allocations and growth rates between the study species?

H1) The growth forms of *E. guttatus*, *E. striatus* and *M venenosa* will differ.

H2) The von Bertalanffy growth curves will differ between *E. guttatus*, *E. striatus* and *M. venenosa*.

Rationale: Epinephelinae species of the genera *Epinephelus* and *Mycteroperca* are commonly subdivided into hinds and groupers, based on morphologically (Heemstra and Randall 1993) and physiologically different growth patterns (Sullivan and DeGarine 1990). Hinds are mainly of the genus *Epinephelus* and generally reach a smaller maximum size than groupers. Groupers, which include all *Mycteroperca* species and the majority of *Epinephelus* species, also differ in their growth patterns. *Mycteroperca* species have relatively faster growth rates than *Epinephelus* species, are more cylindrical and tapering in body form, and appear to be more agile swimmers. *Epinephelus* species generally live longer than *Mycteroperca* species (Heemstra and Randall 1993).

Objective Two: To establish a reproductive classification system for Epinephelinae species harvested in The Bahamas and to use the system to determine the spawning seasonality of *E. guttatus, E. striatus* and *M. venenosa*. (Chapters 3 and 4)

Q1) What is the spawning seasonality of Bahamian populations of *E. guttatus*,

E. striatus and *M. venenosa*?

H1) Spawning seasonality for Bahamian populations of *E. guttatus*, *E. striatus* and *M. venenosa* will differ from other populations in the TWA.

Rationale: Spawning seasonality is a variable LHT and may differ latitudinally for a given species because spawning seasonality is related to environmental parameters.

Objective Three: To determine reproductive LHTs (sex ratios, size-frequencies, size and age of sexual maturation and sex change, and the gonadosomatic indices (GSI)) for Bahamian populations of *E. guttatus*, *E. striatus*, and *M. venenosa*. (Chapter 4)

Q1) What are the reproductive LHTs for the study species?

Q2) How do the reproductive LHTs compare to findings from other studies performed in the TWA?

Q3) Do aspects of the spawning mode (e.g. sexuality and mating system) correspond to differing LHTs between the study species?

H1) The sex by size-frequency distributions will differ (*E. guttatus*, *E striatus* and *M. venenosa,* tested independently).

H2) The sex ratios will differ (*E. guttatus*, *E. striatus* and *M. venenosa,* tested independently).

H3) The GSIs will differ between sexes and species (inter-and intra-species analyses).

Rationale: H1 and H2) Sex by size-frequency distributions and sex ratios may differ between the study species as a related to their sexualities. H3) Sexuality and spawning behaviors may relate to different gamete investment. Because *E. striatus* migrates and reproduces in large aggregations with many males, sperm competition may lead to an

increased reproductive energetic investment, as compared to *E. guttatus* and *M. venenosa*.

Objective Four: To determine how much energy *E. guttatus*, *E. striatus* and *M. venenosa* invest into growth and reproduction. (Chapter 5)

Q1) Approximately how much energy (kj of protein and lipids) do the study species invest into growth annually and when do the study species begin to delegate more energy to reproduction versus growth?

Q2) Approximately how much energy (kj of protein and lipids) do the study species invest into sexual maturation of their gonads during a given reproductive season?

H1) Reproductive energy investment will differ between male and female *E. striatus* and *E. guttatus* and *M. venenosa* (each species tested independently).

Rationale: Energy allocation will differ between the study species in relation to their species-specific reproductive modes.

Conceptual Framework and Background Information

Life-History Theory

Evolutionary persistence requires a range of mechanisms to cope with the challenges of survival, and successful, adaptive reproductive strategies are critical for species to persist (Leaman 1991). Life-history theory asserts that natural selection to maximize the number of surviving offspring (Stearns 1982 and 1992, Roff 2000). LHTs will vary accordingly in consistent patterns to meet this constraint and thus, species that inhabit different environments show different patterns of life-history characteristics. Epinephelinae species occupy a diversity of habitats globally and throughout TWA (Heemstra and Randall 1993). Many TWA Epinephelinae studies have found inter- and intra-species

spatial and temporal LHT variation (e.g., Claro et al. 1990, Sadovy et al. 1993, Shapiro et al. 1993, Potts and Manooch 1995, Sadovy and Ecklund 1999). Accordingly, reproductive LHT investigations into different populations and closely related species are warranted to ensure that the most accurate information is used to assess population dynamics, elucidate fundamental differences in reproductive strategies and understand how these differences influence the reproductive potential of a population (Winemiller and Rose 1992, Myers and Barrowman 1996, Morgan 2008).

Reef Fish Population Dynamics

Reef fish populations (defined as an isolated group that does not exchange post-settlement stages) are maintained by a balance of external gamete supply and post-settlement mortality. Reproductive output and subsequent recruitment is affected by external factors including currents, predation, habitat and natural and fishing mortality, as well as internal biological and physiological factors, such as growth, sexual maturation, fecundity and energy allocation (Figure 1.2) (Jones and McCormick 2002). Exterior processes affect the present quantity of fish, while the internal reproductive biology determines the quality and quantity of gametes, and thus affects long-term population changes (Jones 1984).

Evidence maintains that coral reef fish populations typically experience variable replenishment and recruitment and that the variability has lasting impacts on patterns of demography and abundance (Doherty 1991, Myers 1997). One tactic used by many reef fish, including the study species, is to produce prolific numbers of gametes and to reproduce in large groups to maximize reproductive output and fertilization potential (Sadovy 1996). This tactic has been theoretically explained by the lottery hypothesis,

which states that the arrival and number of recruits to a reef, rather than subtle differences in ecological requirements or competitive abilities as adults appear to primarily determine which species become established at a site (Sale 1976). Thus, ample gametic output is a bet-hedging strategy that provides a buffer for high losses during early life stages (Sale 1976; Shapiro et al. 1994). The internal traits and reproductive strategies for a given species are thus adapted to ensure some offspring survive to become part of the breeding population (Sale 1976, Roff 1992).

Reef Fish Reproduction and Strategies

Winemiller and Rose (1992) described a trilateral spectrum of strategies for teleost fish based upon both reproductive LHTs and gamete production systems. These included the age or size of sexual maturity, maximum size, growth rate, maximum age in years, fecundity (number of eggs per individual), mean diameter (mm) of mature oocytes, and parental investment. Murua and Saborido-Rey (2003) classified female reproductive strategies of fish from the north Atlantic Ocean based on reproduction traits including, the type of gamete production and release, mating system and the sexuality of a species. While Sadovy et al. (1994) outlined three specific mating systems employed by Epinephelinae species (see below). The Winemiller and Rose (1992), Sadovy (1994) and Murua and Saborido-Rey (2003) classification systems were adapted for the present study to define the '*reproductive strategy*' of each study species (Figure 1.1).

The overall reproductive strategy of a species is defined by a combination of reproductive LHTs and the species-specific reproductive mode (Winemiller and Rose 1992, Murua and Saborido-Rey 2003, Morgan 2008). Reproductive LHTs may vary temporally within a population and spatially between populations, because they are

environmentally and anthropogenically influenced (e.g. water temperature, population density and fishing). A species' reproductive mode is fixed and specified by three factors exhibited by the majority of populations of a species, including the sexuality of the species, gamete production mechanism and the mating system (Winemiller and Rose 1992, Sadovy et al 1994, Murua and Saborido-Rey 2003, Morgan 2008). Aspects of mating systems may include the migration distance, timing and size of spawning aggregations and courtship behaviors.

The sexuality a species is defined as "the typical expression of sexuality exhibited by (the majority) individuals of a population or species" and relays the sexual function of a fish (Sadovy and Domeier 2005). Sexuality types include gonochorism, in which individuals develop as two distinct sexes and sexual function does not change, and three forms of hermaphroditism. Hermaphroditism forms include: a) simultaneous hermaphrodites, in which an individual possesses both male and female gonads, b) protogynous species that mature as females and later change to males, and 3) protandrous species that mature as males and later change to females (Shapiro 1987, Sadovy 2005) (Figure 1.3). Sex change has obvious evolutionary implications, and according to sex allocation theory, hermaphrodites should change sex whenever net future reproductive success would be higher for the opposite than for the existing sex (Charnov 1982). Thus, key factors regulating gametic output and reproductive potential in hermaphroditic fish are average size or age of sexual reproduction, and sex change (Shapiro 1987).

Epinephelinae species may be gonochoristic, simultaneous hermaphrodites or various forms of protogynous hermaphrodites (female to male sex change), including monandry and diandry (Shapiro 1987, Sadovy and Domeier 2005). In monandry, all

males develop from functional females by sex changes. In diandry, two male patterns exist: one being direct male development (no sex change, i.e. primary males), and a second involving female to a male sex change (i.e. secondary males). Protandry (adult male to female sex change) has not been described for wild Epinephelinae, although it has been reported in captivity (Sadovy 2005).

Gamete production types are defined by a combination of the duration of the annual spawning season, and the frequency of gamete production and release during a spawning bout (spawning frequency). For Epinephelinae species, gamete production may be determinate or indeterminate. Determinate gamete production means that spawning fish produce and release all eggs for a spawning bout (there may be multiple bouts during a single spawning season) at once. Indeterminate production means that multiple egg clutches are produced during a spawning bout and they are released intermittently as the gametes mature (Sadovy 1996, Murua and Saborido-Rey 2003). Spermatogenesis happens early in maturation within the same spawning timeframe as females, but contrary to females, males are often capable of spawning early on through late into a reproductive season (Brown-Peterson and Wyanski 2006).

Reef fish employ a variety of mating systems. The systems of many Epinephelinae species are correlated to environmental and biological factors, lunar cycles and elaborate courtship behaviors, such as color changes and male territorial defense behaviors (Sadovy 1996). Three main Epinephelinae mating systems have been observed in tropical western Atlantic Ocean species (Sadovy et al. 1994). These are: a) non-migratory, haremic, small-group spawning (one male, a few females), found in a smaller hinds such as *Epinephelus fulvus* and *E. cruentatus*; b) migratory, haremic, small-group

spawning found within smaller *Epinephelus* species and some larger *Mycteroperca* species; and c) migratory aggregating group spawning found in many larger *Epinephelus* and *Mycteroperca* species. The exact cues leading to a synchronous state of maturation and spawning aggregations are unknown. Spawning aggregations typically happen for a short duration during winter full-moon periods, when water temperatures are at their lowest. Some species may migrate distances in excess of 100 km to reach spawning aggregations at distinct sites (e.g., *E. striatus*) (Bolden 2000).

Fish Growth and Energy Allocation

Life-history theory predicts that there is optimal size or age-specific allocation of resources to growth, maintenance and reproduction to maximize fitness (Wooton 1985, Jonsson and Jonsson 2003). Sattar et al. (2006) stressed that "energy allocation, how available energy is diverted towards alternative uses, is the mechanism that integrates the trade-offs through shaping the individual's growth trajectory." A very clear trade-off exists between growth and reproduction in fish. Predation rates may be greater at a smaller size (Sogard 1997), so theoretically a fish should channel more energy in to growing larger versus sexual maturation. But, future breeding opportunities are never guaranteed, so there is also an alternate inherent risk in delegating resources to growth over reproduction (Sadovy 1996).

At the population level, growth rates and reproductive investment are adapted to local environmental conditions to balance these tradeoffs (Roff 2000). Allocation to reproduction earlier or later in life is likely to influence the entire demographic structure and population dynamics. Both mature biomass and overall egg production will be affected by population changes in maturity, size, and fecundity-at-age (Winemiller and

Rose 1992, Sattar et al. 2006). Thus, LHTs must be known for a given population to realistically estimate reproductive potential (Sadovy 1996, Morgan 2008).

Growth in fishes is indeterminate (continuous). Growth rates are determined by measuring body length and mass, and the age associated with these measurements, and can be defined as an increase in body length, condition and tissue energy concentration (Jobling 1994). Growth is strongly associated with both biotic and abiotic environmental factors such as temperature, photoperiod and salinity levels. Thus, growth rates are highly variable within and between populations (Manooch 1987, Conover 1990, Sadovy et al. 1992, Claro and Garcia-Arteaga 2001). At the population level, growth rates impact reproductive output, because they are directly related to the size or age of sexual maturity (Beverton 1992, He and Stuart 2001 and 2002, Charnov 2008).

The estimated energy value of a given fish tissue can be calculated as: Energy value (kJ g-1) (estimated) **=** (kJ g-1 protein) + (kJ g-1 lipids). Energy can be allocated to somatic (white muscle growth) and/or reproduction, which includes behavioral and gonadal investment. Reproductive effort is the proportion of the energy income that is devoted to reproduction, and the partitioning of energy into reproduction changes (typically increases for females) as a fish matures (Wooton 1985; Rijnsdorp and Ibelings 1989; Jonsson and Jonsson 2003).

Study Species

The Subfamily Epinephelinae comprises about 159 species of marine fish globally, and 24 species inhabit the TWA (Heemstra and Randall 1993). Ecologically, most species tend to live solitarily during non-reproductive periods and they are mid- to top-level predators in coral reef systems. *Epinephelus guttatus*, *E. striatus*, and *M. venenosa* from

the subfamily Epinephelinae were selected for this study because they are confamilial species with a strong monophyletic lineage (Craig and Hastings 2007), yet they possess varying LHTs that compose their overall reproductive strategies (Table 1.1). The variability in reproductive strategies between the species provided a foundation to compare the species and to investigate the influences of reproductive modes on life-history traits.

Epinephelus guttatus is the most common *Epinephelus* species in the West Indies (Heemstra and Randall 1993). The life-history of *E. guttatus* is characterized by a maximum size of approximately 700 mm, and a maximum reported age of 17 years (Froese and Pauly 2010). *Epinephelus guttatus* is a protogynous, monandric hermaphrodite (Sadovy et al. 1994) and reaches sexual maturity at between approximately 220-240 mm (approximately three years). Sex change has been estimated to occur at an average size of 280 Tlmm, and most fish larger than 400 mm are males (Shapiro et al. 1994). Spawning seasonality coincides with full and new moons, approximately three months a year (Shapiro et al. 1994). Gametogenesis is classified as determinate, batch spawning and the spawning system is defined by short migrations, haremic, male dominated small-groups found at several sites within loosely-defined areas (Sadovy et al. 1994) (Table 1.1).

Epinephelus guttatus are typically found in shallow reefs and rocky bottoms and are carnivores which feed mainly on crabs (*Calapa* and *Mithrax*) and other crustaceans (alpheid shrimps and scyllarid lobsters), octopus and fishes (labrids, wrasses and haemulids, grunts) (Dominici-Arosemena and Wolff 2005). Some of the largest populations of *E. guttatus* may be in the US Virgin Islands, based on sighting frequency

(%) fishery independent data from the Reef Environmental Education Foundation (reef.org) (2010) (Table 1.2).

The life-history of *E. striatus* is characterized by rapid growth until sexual maturity and a lifespan that can exceed 29 years. *Epinenphelus striatus* is thought to be functionally gonochoristic (and not hermaphroditic, as are most groupers), the average age of sexual maturity for males and females is approximately 400-450 mm (between four and eight years), and the sex ratio of many populations has been reported as 1M:1F (Sadovy and Eklund 1999). The spawning season occurs approximately over one week for three months a year, and is timed around the full moon and varies by latitude (Colin 1992, Carter et al. 1994). The spawning system employed by *E. striatus* is migratory, aggregating group spawning, with sperm competition behaviors demonstrated by the males (Colin 1992) (Table 1.1).

Epinephelus striatus transition habitats as they grow to adulthood. Juvenilles have been observed hiding among the leaves of the turtle grass *Thalassia testudinium* (Grover and Shenker 1999), while adults occur from the shoreline to at least 90 m depth and are usually close to caves (Sadovy and Eklund 1999, Chiappone et al. 2000). *Epinephelus striatus* are carnivores which feed primarily on fish and crustaceans (Randall 1965). Some of the largest populations of *Epinephelus striatus* may be found in the Turks and Caicos Island, based on sighting frequency (%) data from the Reef Environmental Education Foundation (reef.org) (2010) (Table 1.2).

Mycteroperca venenosa is a protogynous hermaphrodite. *M. venenosa* reaches a maximum length of 900 mm and the average size of sexual maturation for females is 550-600 mm and 710 mm for males, with sex change thought to happen at approximately

650 mm (Garcia-Cagida et al. 2001). Gametogenesis is indeterminate, asynchronous, with batch spawning (Garcia-Cagida and Garcia 1996). The spawning system is thought to be similar to the system adopted by *E. striatus*, with the majority of reproduction corresponding with the full moon, three months a year (Sadovy et al 1994b, Nemeth et al. 2004) (Table 1.1). Similar to *E. striatus*, *M. venenosa* juveniles occur in shallow turtle grass beds, and adults are found on rocky and coral reefs as deep as 137 m, and feed mainly on fishes and squids (Randall 1967). Some of the largest populations of *M. venenosa* may be found in the Cayman Islands, based on sighting frequency (%) data from the Reef Environmental Education Foundation (reef.org) (2010) (Table 1.2).

Table1.1 . Summary of reproductive strategies for *Epinephelus guttatus* (red hind), *E. striatus* (Nassau grouper), and *Mycteroperca venenosa* (yellowfin grouper).

Species	Sexual Development	Growth	Length at 50% Mature	Gametogenesis	Sperm competition	Spawning mode
Red Hind (*Epinephelus guttatus)*	Protogynous hermaphrodite (monandric)	Small Max size- ~760 mm, common to 400 mm Max age- 22 years[7]	F~250: M:400 mm[4]	Iteroparous, Determinate[5]	No	Small aggregations: single-male[8]
Nassau grouper (*Epinephelus striatus*)	Functionally gonochoristic[2]	Large Max size- 12000 mm[7], Max age- 29 years[7]	F and M 400-450mm[6]	Iteroparous, Indeterminate[5]	Yes[5]	Group synchronous: multi-male
Yellowfin grouper (*Mycteroperca venenosa*)	Protogynous hermaphrodite (monandric)[2]	Large May reach 900 mm[3] Max age- 15 years[10]	F 550-600: M 710 mm[2]	Iteroparous, Indeterminate[2]	Likely[2]	Large aggregations with sub groups[9]

Sources: [1]Colin 1992, [2]García-Cagide and García 1996; [3]Heemstra and Randall 1993; [4]Sadovy et al. 1992, [5]Sadovy et al. 1994; [6]Sadovy and Colin 1995; [7]Sadovy and Eklund 1999, [8]Shapiro et al. 1994, [9]Starr et al. 2007, [10]Froese and Pauly 2010.

Table 1.2. Fishery independent sighting frequencies (SF %) for *Epinephelus guttatus* (red hind), *E. striatus* (Nassau grouper) and *Mycteroperca venenosa* (yellowfin grouper) from selected locations throughout the Tropical Western Atlantic ocean from the Reef Environmental Education Foundation (reef.org) database. Percent sighting frequency was the percentage of all survey dives in which the particular species was recorded. Data from January 2000- May 2010 was queried.

	***Epinephelus guttatus* (red hind)**		***Epinephelus striatus* (Nassau grouper)**		***Mycteroperca venenosa* (yellowfin grouper)**	
	Site	**SF%**	**Site**	**SF%**	**Site**	**SF%**
1	US Virgin Islands	45.2	Turks and Caicos	67.1	Cayman Islands	16.2
2	Puerto Rico	33.4	Cayman Islands	53	Mexican Caribbean	14.2
3	Turks and Caicos	29.2	Central Bahamas	52.1	Cuba	13.1
4	Cuba	28.7	Belize	43.3	Turks and Caicos	6.8
5	Belize	25.3	Cuba	34.2	Central Bahamas	5.6
6	Central Bahamas	22.3	North Bahamas	33.5	Belize	4.8
7	Cayman Islands	21.4	US Virgin Islands	11.6	North Bahamas	3.9
8	North Bahamas	19.2	Florida (E. Coast and Keys)	10.8	US Virgin Islands	2.3
9	Mexican Caribbean	17.2	Mexican Caribbean	6.8	Florida (E. Coast and Keys)	2.1
10	Florida (E. Coast and Keys)	4.8	Puerto Rico	2.6	Puerto Rico	0.6

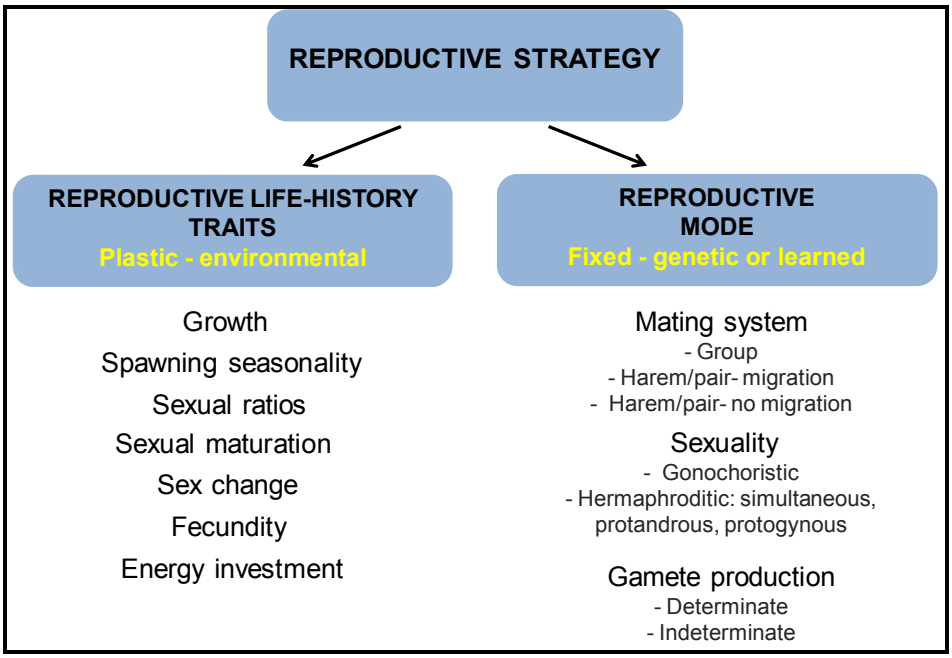


Figure 1.1. Outline of the reproductive strategy classification used for dissertation studies. The reproductive strategy of a species consists of 'plastic' life-history traits which vary between and among populations, and the 'fixed' reproductive mode factors which do not vary between and among populations.

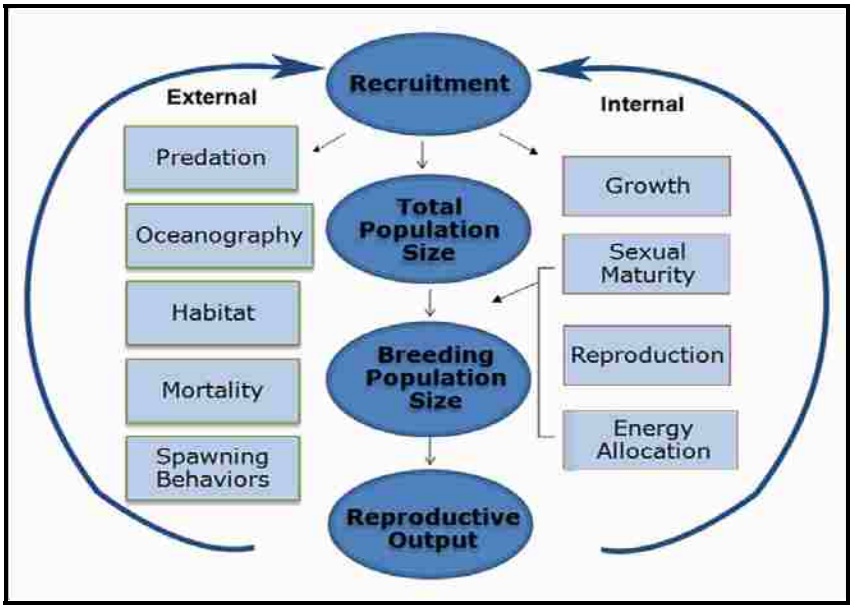


Figure 1.2. Outline of reef fish population regulation factors. The focus of the present study was on the internal factors for *Epinephelus guttatus, E. striatus,* and *Mycteroperca venenosa.*

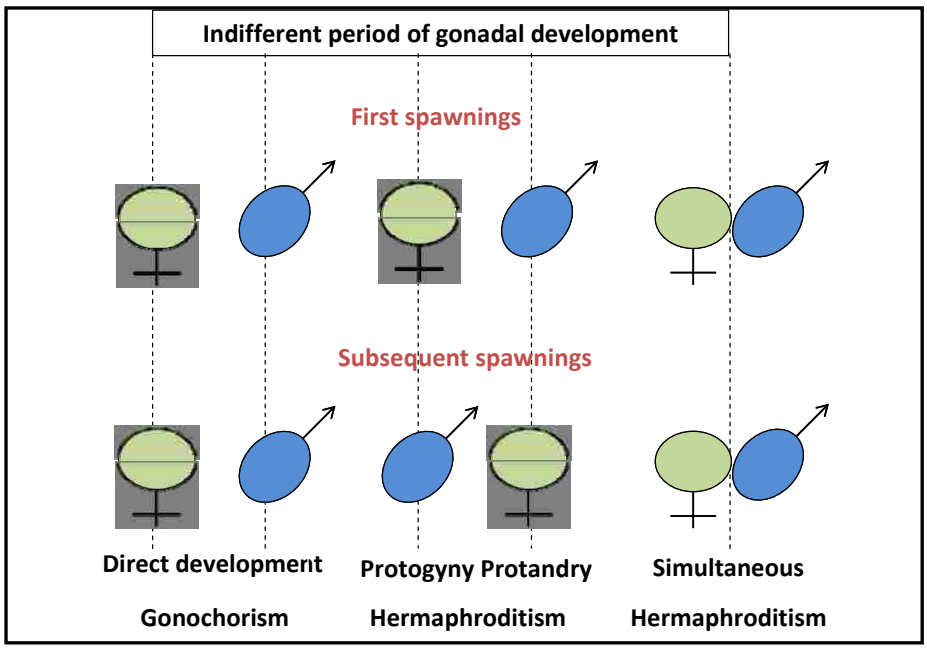


Figure 1.3. Diagram of the sexuality pathways found in the family Serranidae, subfamily Epinephelinae**.** *Epinephelus guttatus* and *Mycteroperca venenosa* are protogynous hermaphrodites. *Epinephelus striatus* has been classified as gonochoristic, but a small portion of some portions of a population may be protogynous (Sadovy and Colin 1995). Protandry has only been induced in controlled conditions.

CHAPTER TWO

AGE AND GROWTH OF *EPINEPHELUS GUTTATUS* (RED HIND), *E. STRIATUS* (NASSAU GROUPER) AND *MYCTEROPERCA VENENOSA* (YELLOWFIN GROUPER) HARVESTED FROM THE BAHAMAS

Background

Distinct growth patterns exist amongst tropical western Atlantic (TWA) species in the family Serranidae, subfamily Epinephelinae (Baldwin and Johnson 1993, Heemstra and Randall 1993). These distinct growth patterns are important to understand, because they reveal species-specific evolutionary adaptations and eco-physiological niches (Winemiller and Rose 1992, Jobling 2002). Epinephelinae species of the genera *Epinephelus* and *Mycteroperca* are commonly subdivided into hinds and groupers based on morphologically (Heemstra and Randall 1993) and physiologically different growth patterns (Sullivan and DeGarine 1990).

Hinds are mainly of the genus *Epinephelus* and reach a smaller maximum size than groupers. Groupers, which include all *Mycteroperca* species and the majority of *Epinephelus* species, also differ in their growth patterns. *Mycteroperca* species have relatively faster growth rates than *Epinephelus* species are more cylindrical and tapering and appear to be more agile swimmers, while *Epinephelus* species generally live longer (Heemstra and Randall 1993). The contrast is clear by noting that, *E. adscensionis* (rock hind) reaches a maximum size of ~610 mm and 4,080 g (Claro et al. 1994), while *E. itajara* (Goliath grouper), may reach over 2000 mm and 450,000 g and has been documented to reach 37 years old (yo) (Bullock et al. 1992). *Mycteroperca*

bonaci (black grouper), with a maximum reported size of 1500 mm, is one of largest TWA *Mycteroperca* species (Froese and Pauly 2010).

Growth is one component of a group of co-evolved life-history traits (LHTs) which can be evaluated at evolutionary and contemporary scales. Variable inter- and intra-species growth patterns provide the basis for tackling basic questions in evolutionary biology, while growth variations may also have profound management implications (Winemiller and Rose 1992, Bullock and Murphy 1994, Morgan 2008). Growth is the only process that replenishes the biomass taken from a fish population either by natural mortality or harvest, and age and growth studies provide the background for life-history studies and population estimates (Pauly 1994). Species and population-specific growth information is critical because it provides the foundation for understanding population production capabilities which are determined by the rates of mortality, reproduction and growth (Jobling 2002). For example, sexual maturation occurs at different ages and sizes for species and populations that follow different growth trajectories or are subjected to different size-selective harvesting regimes (Stearns and Crandall 1984, Hamilton et al. 2007).

Accordingly, the species *E. guttatus, E. striatus* and *M. venenosa* from the Family Serranidae, subfamily Epinephelinae provide an interesting system for examining growth patterns amongst closely related species (Craig and Hastings 2007) and how these differences relate to species-specific niches and population production capabilities. Also, because previous studies have been done throughout the TWA, comparative growth data are available to investigate intra-species population growth variation. *Epinephelus guttatus* (red hind) is a hind that has been reported to reach the maximum size of 760

total length millimeters (Tlmm) and may live to be over 20 yo (Luckhurst et al. 1992). *Epinephelus guttatus* is a protogynous hermaphroditic (female to male sex change) and is found typically in shallow reefs and rocky bottoms. *Epinephelus striatus* (Nassau grouper) is a grouper which has reached a maximum reported size of 1220 Tlmm and age of 29 yo (Sadovy and *Eklund* 1999). *Epinephelus striatus* is primarily gonochoristic (separate sexes) (Sadovy and Colin 1995), is an ambush predator associated with caves and occurs on reefs from the shoreline to at least 90 m depth (Sadovy and Eklund 1999). *Epinephelus striatus* was historically the most abundant TWA grouper, but is presently over-fished throughout most its range and is listed *Endangered* by the International Union for Conservation of Nature (IUCN) (Cornish and Eklund 2003). *Mycteroperca venenosa* (yellowfin grouper) has a maximum reported size of 1000 Tlmm (fishing tournament record, fishbase.org) and may live to 15 yo (Manooch 1987). *Mycteroperca venenosa* is a protogynous hermaphroditic grouper (Garcia-Cagide and Garcia 1996), and adults are usually found on rocky and coral reefs in depths of 2-137 m (Manooch 1987). *Mycteroperca venenosa* is listed as *Near threatened* by the IUCN (Brule and Garcia-Moliner 2004).

Length-at-mass relationships and growth rates are two common indices used to measure fish growth. The length-at-mass relationship (LMR) for a species is a morphometric measurement of how a species allocates mass allometrically and can be used to estimate standing crop biomass (Jennings and Polunin 1997). When a population is assessed over time or compared to others, seasonal variations in fish growth can be tracked and inferences can be made concerning condition and fitness (Bolger and Connolly 1989). Growth rate defines how fast a fish lengthens and is a defining LHT

that corresponds to when a fish transitions from juvenile to adult, the age or size of sexual maturity and overall life span (Jobling 1994, He and Stuart 2001). Growth rates decelerate as fish transition from juvenile to sub-adult to adult due to energy being allocated to reproduction as opposed to somatic growth and because greater amounts of energy are required for somatic growth. Growth rates are most commonly modelled by the von Bertalanffy growth function (VBGF) (Pauly 1984, Jobling 2002).

The present study was undertaken to ascertain age and growth data for Bahamian populations of *E. guttatus*, *E. striatus* and *M. venenosa* to elucidate species-specific growth patterns between closely related species of the family Serranidae, subfamily Epinephelinae. Specifically the LMRs and growth rates were determined from fishery-dependent samples landed in New Providence and Abaco, and Long Island, The Bahamas. The hypotheses that the LMR and VBGF growth rates would differ for each species were tested. This information is critical because all the study species are of considerable economic importance and are heavily harvested throughout the TWA. In addition, quantitative estimates of life-history parameters are scarce for each species, limiting formal assessment of long-term harvest rates and conservation risk, and the design of robust management measures (Cornish and Eklund 2003, Sadovy 2005).

Methods

Sample Acquisition

Epinephelus striatus samples were obtained from fish harvested off the coasts of Abaco and Long Island, The Bahamas during December 2000 and January 2001 and 2002. *Epinephelus guttatus* and *M. venenosa* and additional *E. striatus* samples were collected from December 2006 through April 2008 at Montague ramp fish market in New

Providence, The Bahamas. These samples were harvested from fishing grounds in the central Bahamas. Both sample sets were obtained from local fishermen. The *E. striatus* samples from Abaco and Long Island were primarily harvested using fish pots and the New Providence samples were taken were typically using spear and scuba-based fishing practices. Ungutted fish were measured for maximum total length (Tlmm) (snout to end of tail midpoint) to the nearest mm, and total body mass to the nearest 0.1 g.

Age Estimates

For grouper ageing and growth studies, otoliths are the primary skeletal structure used (Claro and Garcia-Arteaga 2001). For the ageing studies, all fish were numbered and both sagittal otoliths were removed, rinsed in water to remove all surrounding membranes, and stored dry for later processing. A sagittal otolith was mounted and cross-sectioned into 0.5-mm sections using a Buehler® Isomet 1000 digital sectioning saw. Typically four cross-sections per otolith were sectioned. Sections were permanently mounted in Histomount®. All samples were processed at the University of Florida ageing laboratory.

Samples were viewed using a stereomicroscope (20–45×) with transmitted light and/or a dissecting scope. Annuli in sections viewed under transmitted light appear as opaque brown rings (opaque zone) against an otherwise translucent background (translucent zone). For all species, annuli formation was considered annual. This has been confirmed for *E. guttatus* (Sadovy et al. 1992), and for *E. striatus* (Claro et al. 1990) by using marginal-increment analysis and/or an oxytetracycline (chemical) tagging method. Annual ring formation has been confirmed in multiple *Mycteroperca* species (e.g. *M. microlepis,* Hood and Schlieder, 1992; and *M. interstitialis*, Manickchand-

Heileman and Philip, 2000) and was assumed to hold true for the present study for *M. venenosa*. Ages were assigned based on the number of opaque zones. All otoliths were aged by a two readers. If ages between the experienced readers differed, both readers independently aged the otolith for a second time. An age was assigned only when three out of the four ages agreed. Otoliths were discarded if ages did not agree.

Data Analyses

All data were tested for normality and homogeneity of variance. Nonparametric tests were conducted if there were large violations of these assumptions. Results were considered significant if $P \leq 0.05$.

Length and Mass Relationships

The length (mm) and mass (g) measurements were used to construct size frequency graphs and to analyze the LMR for each species. The LMR is an allometric relationship typically calculated through a log-transformed linear regression (Zar 1996):

$$\text{Mass} = aL^b \quad \text{(equation 2.1)}$$

where M = total wet mass in *g*; L = maximum total length in mm; a = the y-axis intercept of the regression; and b = the slope of the regression. Parameter *b* is also known as the allometric coefficient, which measures the relative gains in mass for a given fish length. A log10 transformation was performed to normalize the data.

This linearizes the relation to:

$$\log10\,(\text{Mass}) = a + b^* \log10\,(\text{Length}) \quad \text{(equation 2.2)}$$

The intercept value *a* is the linearized form of *a* from the equation $M = aL^b$, and the slope *b* is the *b* power function in the equation $M = aL^b$.

Ageing and Growth

For aged fish, age-length frequency histograms were produced for each species. A Kruskal-Wallis test was used to test the null hypothesis that there was no difference in the mean length (mm) and mass (g) per age of each species (ages 4-11). Multiple Mann Whitney *U* tests were used to test for significant differences between species. Growth curves, based on the observed total lengths and respective ages, were modeled using a standard von Bertalanffy growth function (VBGF) (1938) (Haddon 2001).

$$\text{VBGF: } L_t = L_\infty (1-e^{-k(t-t0)}) \text{ (equation 2.3)}$$

where L_t = total length at time t (age); L_∞ = asymptotic length; k = growth coefficient which expressed the curvature of the growth function; t_0 = theoretical age when length would be 0; *e* = exponent for natural logarithms. Model parameters were fitted by minimizing the least squares and using the Solver routine in Microsoft Excel (Haddon 2001).

To determine if the VBGF gave a good fit to the length-at-age data, the curves were overlain on the length-at-age data for each species and expected lengths-at-age were calculated with the parameters. Growth curves were statistically compared using a likelihood ratio test for coincident curves (Haddon 2001) and the independent parameters ($L\infty$ and k) were compared between each species using Kimura's likelihood ratio tests (LRT) (Haddon 2001). The LRTs test the hypothesis that the curves are independent (referred to as the base case) verses the alternate hypothesis that the curves are samples from a single population (coincident curve) (Haddon 2001). Multiple pair-wise comparisons were used to test for significant differences ($P \leq 0.05$). Statistical analyses

was conducted using Fishery Analyses and Simulation Tools (FAST) Auburn University (2001), SPSS 17.0 and Microsoft Excel.

Results

Length and Mass Relationships

A total of 637 fish (*E.* guttatus, 190; *E. striatus*, 277; *M. venenosa*, 170) were measured and weighed from Abaco, Long Island and New Providence, The Bahamas (Figure 2.1). The average sizes for each species were: *E. guttatus:* 325.24 Tlmm, *E. striatus:* 616.98 Tlmm, and *M. venenosa*: 643.62 TLmm (Table 2.1). The modal lengths for *E. guttatus*, *E. striatus* and *M. venenosa* were 350, 650 and 750 Tlmm, respectively (Figure 2.2).

The relationships between total length (L) (Tlmm) and body mass (g) were: a) $M = aL^b$; b) log10 (M) = a + b* log10 (L) (Figures 2.3, 2.4):

E. guttatus a. $M= 6.76*10^{-4} (L)^{2.81}$

b. $Log_{10}(g) = -3.17 + 2.81* Log_{10}(Tlmm)$ ($r^2 = 0.75$, n =189)

E. striatus a. $M= 4.00*10^{-6} (L)^{3.20}$

b. $Log_{10}(g) = -5.38 + 3.20* Log_{10}(Tlmm)$ ($r^2 = 0.86$, n =265)

M. venenosa a. $M= 6.80*10^{-4} (L)^{2.75}$

b. Log10 (g) = -3.17+ 2.75* Log10 (Tlmm) ($r^2 = 0.76$, n =170)

The R-square values for each species indicated that there was a reasonable fit to the data.

Ageing and Growth

A total of 369 fish (*E. guttatus*: 114*, E. striatus*: 176, *M. venenosa*: 79) were aged. Opaque bands were identified and counted on 96% of *E. guttatus*, 97% of *E. striatus*, and 89% of *M. venenosa* otoliths (Figure 2.4). The maximum ages were similar to those found in other studies in the TWA (Tables 2.1, 2.2). The average ages for each species

were: *E. guttatus*, 7.06 yo; *E. striatus*, 9.06 yo; and *M. Venenosa*, 6.74 yo. Over 50% of the samples were between the ages of 5-8 for *E. guttatus*, 6-12 for *E. striatus* and 6-10 for *M. venenosa*. The modal ages were five and six for *E. guttatus*, ten for *E. striatus*, and six for *M. venenosa* (Figure 2.2).

Statistical analyses were confined to ages 4-11 because all species had adequate samples within this age range. For all ages between 4-11, *M. venenosa* had the greatest length-at-age, as compared to *E. guttatus* and *E. striatus*. A significant difference in the mean length-at-age (ages 4-11) was detected (Kruskal-Wallis, P=0.01) and multiple Mann Whitney *U* tests detected significant differences between the independent groups *E. guttatus* and *E. striatus* ($p<0.001$), *E. guttatus* and *M. venenosa* ($p<0.001$), and *E. striatus* and *M. venenosa* ($p=0.01$). The overall large standard deviations for the mean lengths-at-age highlight the variability in growth for each age (Figure 2.6, Table 2.2).

Von Bertalanffy growth curves were fitted to length (Tlmm)-at-age (years) data for each species (Haddon 2001) (Figures 2.6, 2.7 Tables 2.2, 2.3). The resulting curves were:

$$E.\ guttatus = 571\ (1 - e^{(0.11(t+1.2)})$$

$$E.\ striatus = 932\ (1 - e^{(0.10(t+1.7)})$$

$$M.\ venenosa = 977\ (1 - e^{(0.14(t+1.5)})$$

The parameter estimates for the VBGF indicate all species grow slowly (parameter *k*) relative to their potential maximum size ($L\infty$). Resulting theoretical asymptotic lengths were 571, 932 and 977 Tlmm, respectively (Figures 2.6). *Epinephelus striatus* was estimated to have the slowest and *M. venenosa* the fastest growth rate. *Epinephelus guttatus*, a hind, was estimated to grow at a faster rate than its grouper congener, *E.*

striatus. Theoretical lengths-at-age were calculated for each species using the calculated von Bertalanffy parameters. For all species, growths rates declined as fish transitioned from sub-adults to adults (Table 2.2). Due to a small sample number for all species over the age of eleven, the theoretical estimated and observed sizes did not pair tightly after age fourteen.

Significant differences were detected between the von Bertalanffy growth curves between each species (*E. guttatus*/*E. striatus*: $\chi 2=362.26$, $P<0.01$; *E. guttatus*/*M. venenosa*: $\chi 2=391.47$, $P<0.01$; *E. striatus*/*M. venenosa*: $\chi 2=311.35$, $P<0.00$). Kimura likelihood ratio tests (LTR) (multiple pair-wise comparisons) were conducted to determine which parameters significantly differed between the two species. Significant differences were detected between the VBGF curves of *E. guttatus*, *E. striatus* and *M. venenosa* ($p < 0.01$). Individual VBGF parameter testing revealed significant $L\infty$ differences between *E. guttatus* and *E. striatus* ($p < 0.01$) and between *E. guttatus* and *M. venenosa* ($p < 0.01$). Significant growth rate differences were also found between *E. guttatus* and *M. venenosa* ($p = 0.02$) and *E. striatus* and *M. venenosa* ($p = 0.05$). Although significant differences were detected between curves, no significant individual parameter differences were detected between *E. striatus* and *M. venenosa*, suggesting that specific parameter differences were too slight within the truncated age range (4-11) to be detected (Haddon 2001). Parameter *t0* (size at age 0) was not tested because the datasets did not include juvenile samples.

Discussion

The comparative-species framework of the present study elucidated evolutionary derived growth differences between *E. guttatus*, *E. striatus* and *M. venenosa*. Also, this

is the first sizeable age and growth dataset for Bahamian populations of *E. guttatus*, *E. striatus* and *M. venenosa*; and the first formal *M. venenosa* age and growth study. All species are generally harvested and marketed as "grouper", but the results of the present study point out the seemingly obvious, but often overlooked fact, that "not all groupers are created equal." There are key differences in the growth patterns of *E. guttatus*, *E. striatus* and *M. venenosa* which can affect population production rates and need to be considered for management (Hutchings 2003).

The hypotheses for the present study that *E. guttatus*, *E. striatus* and *M. venenosa* differ in growth forms and rates are thus supported by the LMR and VBGF analyses. In comparing the LMR for these three species, the relationship between *TL* and *M* was most isometric for *E. striatus*, which had the greatest b of 3.20, indicating that this species gains more mass (g) per mm of length growth as compared to *E. guttatus* (b= 2.81) and *M. venenosa* (b=2.75). Parameters derived from LMR are especially important for populations for which no published parameter estimates exist because they may be applied to fishery independent methods, such as underwater visual census surveys, to estimate biomass. The application of population- and species-specific parameters greatly improves fishery assessments (Manooch 1987). Significant differences were detected between all VBGF curves and parameters using Kimura likelihood ratio tests. The finding that *E. striatus* and *M. venenosa* may grow to be significantly larger than *E. guttatus*, was expected because the maximum size obtainable by *E. guttatus* is inherently smaller than *E. striatus* and *M. venenosa* (Heemstra and Randall 1993). The differing growth patterns determined by the LMR and VBGF for *M. venenosa* and *E. striatus* highlight the utility of using both indices. *Mycteroperca venenosa* lengthen

faster than *E. striatus*, while *E. striatus* gains more girth than *M. venenosa* as it grows. The finding that Bahamian *M. venenosa* grow at a significantly faster rate than *E. guttatus* and *E. striatus* should be associated with the life span of these species. The oldest *M. venenosa* and *E. guttatus* and *E. striatus* for the present study are 13, 17 and 22, respectively. Thus, from an evolutionary fitness perspective, *M. venenosa* would need to grow comparatively faster to reach sexual maturity sooner, so as to have a reproductive potential similar to *E. guttatus* and *E. striatus* (Stearns 1998).

Inter-species growth and morphology variability among reef fish species is a major means by which species avoid direct overlap in resource use (Werner and Gilliam 1984, Wainwright 1996). The study species are all carnivores (Randall 1965, 1967), but their ecological niches differ. Adult *E. guttatus* occupy shallower reef habitats as compared to *E. striatus* and *M. venenosa* (Sluka and Sullivan 1996, Sadovy and Eklund 1999, Dominici-Arosemena and Wolff 2005, Froese and Pauly 2010). *Epinephelus striatus* and *M. venenosa* have different morphologies which relate to their different predation techniques, daily activity patterns and swimming modes (Heemstra and Randall 1993, Heemstra et al. 2002). Large, wide- bodied *Epinephelus* species are typically ambush predators which demonstrate high site fidelity and are more sedentary. Comparatively *Mycteroperca* species are more slender and elongated and are more agile swimmers and may be pelagic hunters (Heemstra and Randall 1993, Heemstra et al. 2002).

Reef fish growth is typified by rapid growth during pelagic and juvenile stages followed by a decelerated growth as the fish transitions into adulthood and begins to allocate energy to reproduction (Jobling 1994, Claro and Garcia-Arteaga 2001, Hutchings

2003). In laymen's terms fish may either "get bigger faster or make babies" (Froese and Pauly 2010). It is important to ascertain the particular size and age range when the juvenile to sub-adult transition happens for a given species and population, because this is when a species becomes sexually mature and begins to contribute to future generations (Jobling 1994). Charnov (2008) emphasized that the VBGF parameter *k* is closely related to meaningful biological parameters, particularly those processes which underlie the reproduction energy-allocation transition. He and Stuart (2001) compiled growth data from 85 species and determined that the average size (or age) of first reproduction is related to the decrease growth rate when fish transition from juvenile to sub-adults. Thus, if the size or age of first reproduction is unknown, the size or age where the VBGF curve starts to plateau and the rate decreases can be used to estimate the approximate sizes and ages of sexual maturity (Beverton 1992, He and Stuart 2001 and 2002, Charnov 2008). For the present study, the growth rate of *E. guttatus* decelerated from ~30 mm yr^{-1} to ~20 mm yr^{-1} after age three (~280 Tlmm). After age three, *E. striatus* growth decelerated from ~60 mm yr^{-1} to ~45 mm yr^{-1}. For *M. venenosa*, the average growth for ages 2-4 (~378-525 Tlmm) is ~65 mm yr^{-1}, then decelerated to ~45 mm yr^{-1} between ages 5-7 (~584-680 Tlmm).

Species-specific population growth variability reflects the interaction of internal (genetic) and external (environmental) factors (Werner and Gilliam 1984, Sale 1984). Local environmental factors, such as temperature, pH and food availability affect fish growth most obviously at relatively small spatial and short temporal scales (e.g., Conover 1990, Lombardi-Carlson et al. 2008, Munday et al. 2008). Because of temporal variation, growth parameter comparisons between the present study and previous studies must

consider that previous research may have been conducted fifteen or more years ago. Since then, the populations have likely been subjected to continual, possibly intense, harvest regimes, which have impacted their growth parameters (Conover and Munch 2002). The LMR estimates for *E. guttatus* and *E. striatus* from the present study differ slightly from other estimates throughout the TWA (*E. guttatus*: Potts and Manooch 1995; *E. striatus*: Claro et al. 1990, Sadovy and Colin 1995). The LMR estimates from St. Thomas and St. John (Bohnsack and Harper 1988) and SW Cuba (Claro and Garcia-Arteaga 1994) show that Bahamian *M. venenosa* apparently weigh less at a given length than specimens from these areas. These differences may be due to differing sample sizes or local environmental variables. In Puerto Rico and St. Thomas (Sadovy et al. 1992) and the southeastern US (Potts and Manooch 1995) larger mean lengths-at-age were recorded for all ages of *E. guttatus* as compared to the present study. The comparatively smaller mean lengths-at-age found by the present study could possibly be associated with counter- gradient variation in growth across the different regional temperature regimes. Counter -gradient variation is an adaptation to grow faster and possibly to a smaller maximum size, in areas characterized by colder temperatures and shorter growing seasons (Conover 1990). Sadovy and Eklund (1999) consolidated mean length-at-age data for *E. striatus* from five studies throughout the TWA. For all ages the mean lengths are highly variable. For example, age one ranged from 160-293 Tlmm and age ten from 542-800 Tlmm. *Epinephelus striatus* mean lengths-at-age from the present study are generally in the midrange of these studies. The size frequency sampled by Tuz-Sulub et al. (2006) of *M. venenosa* from commercial catches from the Mexican Yucatan was likewise similar to the range of the present study.

The maximum reported ages for *E. guttatus* and *M. venenosa* are 22 yo (Luckhurst et al. 1992) and 15 yo (Manooch 1987), respectively. Thus, the oldest *E. guttatus* (17 yo) and *M. venenosa* (13 yo) from the present study are close to the maximum reported in other TWA studies. The oldest reported *E. striatus* is 29 yo (Sadovy and Eklund 1999, Bush, Ebanks and Lane, unpublished data). Sadovy and Colin (1995) aged 22 *E. striatus* in The Bahamas and individuals ranged from 3-21 (295–695 standard length mm), while the oldest *E. striatus* from the present study was twenty-two. The VBGF parameter estimates for the study species are highly variable throughout the TWA. The slowest estimated growth for *E. guttatus* is in St. Thomas (Sadovy et al. 1992) and the fastest is in the southeastern US (Potts and Manooch 1995). Potts and Manooch (1995) stated that *E. guttatus* from the southeastern US "are shorter lived and faster growing than those from Bermuda, Jamaica, the Virgin Islands or Puerto Rico". This is likewise true for *E. guttatus* populations in the Bahamas, as compared to those of the southeastern US. For *E. striatus*, Claro et al. (southwest Cuba, 1990) estimated a slower growth rate as compared to the present study. Using size-classes (no ageing data), Munro (1983) estimated a faster growth rate for *M. venenosa* in Jamaica than the present study.

There are two major challenges for managing tropical fisheries. First, tropical fisheries generally comprise of multiple species and management is thus complicated by the enormous diversity of life-histories (Pauly 1994). A second challenge is being able to collect consistent data. Tropical fisheries are largely under the jurisdiction of developing countries that do not have ample resources to allocate to fisheries management (Johannes 1998). The results of the present study provide species-specific growth information

which is critical for managing Bahamian and other Caribbean populations of the study species. The study species grow relatively slowly compared to other TWA fish families (Froese and Pauly 2010). Slow growth is a characteristic important to the fishery potential and management of species. Slower growing fish have lower yields (because of a lower production/biomass ratio) and less survivorship of breeding fish than faster growing fish (Buxton 1993). Typically, fisheries based on slow growing animals are subject to growth overfishing, in which yield is poor as a result of catching the fish before they have had an opportunity to grow to sexual maturity, thereby further reducing spawning stock biomass and yields (Buxton 1993, Harris and McGovern 1997). Management initiatives should consider the species-specific size at which the growth rates begins to decline, and thus possibly has had an opportunity to contribute to future generations.

In addition, size-selective fishing (SSF) of the largest and oldest fish has been shown to have detrimental consequences on growth parameters after only a few decades. This is because SSF targets the fastest-growing individuals of a population, thereby selecting for smaller sizes and slower growth rates (e.g., Harris and McGovern 1997, Rochet 1998, Chiappone et al. 2000, Fromentin and Fonteneau 2001, de Roos et al. 2006, Hamilton et al. 2007). The study species are subjected to SSF in The Bahamas (Cushion and Sealey 2007). Thus the smaller mean lengths-at-age of *E. guttatus* as compared to the other populations in the TWA may be related to fishing pressure and catch size regulations may be warranted.

The present study highlights the utility and value of using a fishery-dependent sampling regime to collect consistent sub-adult to adult samples on multiple species. For

example, Erhardt and Deleveaux (2007) produced population estimates for Bahamian *E. striatus* using size-class analyses (which are less precise than age-based estimates due to cohort length variability) because "any hard parts (e.g., otoliths) to age Nassau grouper landings are very difficult to obtain". Fishery-dependent sampling is non-random, samples by age are generally unequal and there is typically a paucity of samples in the lower and upper ages of the dataset (Lombardi et al. 2008). However, this method of sampling is advantageous in that it is generally non-destructive and relatively inexpensive. It allows for consistent, long-term age-based datasets to be gathered on species that are vulnerable to over-harvesting and are extremely difficult to sample otherwise. The results of the present study represent the combined efforts of the researchers, their respective academic and governmental institutions' and the Bahamian government, and thus highlight the importance of collaboration for collecting consistent data. As managers move towards holistic reef management plans, individual species, timely information, and collaboration are essential because a lack of basic management data on many of the species still remains the major obstacle to successful management (Johannes 1998, South Atlantic Fishery Management Council 2005).

Table 2.1. Summary data for length and mass measurements and age ranges for *Epinephelus guttatus*, *E. striatus* and *Mycteroperca venenosa*. *Epinephelus guttatus*, *E. striatus* and *Mycteroperca venenosa* harvested from The Bahamas (*E. striatus* 1999-2002, 2006-8; *E. guttatus* and *M. venenosa* 2006-8). (Tlmm= total length mm.)

Species	Lengths (Tlmm) (SD) (n)	Mass (g) (SD) (n)	Ages (year) (n)
E. guttatus	209.55 - 565.15 (47.96) (190)	136.05 - 1496.55 (66.24) (190)	2 – 17 (118)
E. striatus	325.26 - 925.71 (84.01) (277)	594.08 - 12632.55 (97.91) (277)	4 - 22 (183)
M. venenosa	330.20 - 939.80 (97.59) (170)	1111.30 – 10248.74 (107.59) (170)	3 – 13 (81)

Table 2.2. Compilation of mass and length coefficients (a,b) (Mass=aLength^b) and von Bertalanffy growth parameters (L, k, t_o) ($L_t = L_\infty (1-e^{-k(t-t0)})$) from studies throughout the tropical western Atlantic for *Epinephelus guttatus*, *E. striatus* and *Mycteroperca venenosa*. FL= fork length, SL= standard length, TL= total length.

	Max age (yr)	a, b	L_∞ (mm)	k	t_0 (yr)	Location	Length/Source
E. guttatus	17	-	507	0.180	-0.440	Bermuda	(TL) Burnett-Herkes 1975*
	-	0.0176, 2.96	520	0.18	-	Jamaica	(TL) Thompson & Munro 1978**
	18	-	601	0.071	-4.69	St. Thomas	(FL) Sadovy et al. 1994*
	17	-	515	0.101	-2.944	Puerto Rico	(FL) Sadovy et al. 1994*
	11	$1.8*10^{-7}$, 2.61	471	0.200	-2.397	US (NC to FL)	(TL) Potts & Manooch 1995
	17	**$6.76*10^{-5}$, 2.81**	**571**	**0.11**	**-3.1**	**Bahamas**	**(TL) This study**
E. striatus	-	0.0107, 3.11	900	0.09	-	Jamaica	(TL) Thompson & Munro 1978**
	16	0.0097, 3.23	970	0.185	0.488	Virgin Islands	(SL) Olsen & LaPlace 1979 (SL)
	17	0.0198, 2.98	760	0.117	-1.12	Cuba (NE)	(TL) Claro et al. 1990
	17	0.0052, 3.30	940	0.063	-3.27	Cuba (SW)	(TL) Claro et al. 1990
	-	0.0107, 3.08	-	-	-	Belize	(SL) Carter et al. 1994
	-	$2.14*10^{-5}$, 3.03	-	-	-	Bahamas	(SL) Sadovy & Colin 1995
	22	**$4.0*10^{-7}$, 3.20**	**932**	**0.100**	**-1.70**	**Bahamas**	**(TL) This study**
M. venenosa	-	-	860	0.17	-	Jamaica	(TL) Munro 1983
	-	0.0069, 3.14	-	-	-	St. Thms/ John	(FL) Bohnsack &.Harper 1988
	-	0.0132, 3.04	-	-	-	SW Cuba	(TL) Claro & Garcia-Arteaga 1994
	13	**$6.8*10^{-4}$, 2.75**	**977**	**0.14**	**-1.5**	**Bahamas**	**(TL) This study**

* used length-frequency back calculation
**L_∞ assumed based on tagging, not ageing data from Randall 1962, 1963

Table 2.3. Observed and predicted mean total lengths mm (Tlmm), standard deviations (SD) and average growth (Tlmm/year) for ages 2-4, 5-7 and 8-10 for *Epinephelus guttatus*, *E. striatus*, and *Mycteroperca venenosa* harvested from The Bahamas (*E. striatus* 1999-2002, 2006-8; *E. guttatus* and *M. venenosa* 2006-8). Predicted values calculated from the von Bertalanffy growth equation ($L_t = L_\infty (1\text{-}e^{-k(t-t0)})$: *E. guttatus*: (Tlmm) = 571 $(1 - e^{(0.11(t+-3.1)})$; *E. striatus*: (Tlmm) = 932$(1 - e^{(0.10(t+-1.7)})$; *M. venenosa*: (Tlmm) = 977$(1 - e^{(0.14(t+-1.5)})$.

	E. guttatus			*E. striatus*			*M. venenosa*		
Age	Obs (SD)	Pred	Ave. growth (mm yr-1)	Obs (SD)	Pred	Ave. growth (mm yr-1)	Obs (SD)	Pred	Ave. growth (mm yr-1)
2	244.69 (24.41)	160.48		-	288.21		-	378.43	
3	279.61 (15.95)	203.24		-	349.47		445.90 (19.94)	456.62	
4	306.28 (45.05)	241.54	~35	380.5 (24.75)	404.9	~50	524.93 (52.75)	524.6	~65
5	297.24 (30.07)	275.86		452.83 (106.72)	455.05		593.78 (70.47)	583.7	
6	315.81 (29.73)	306.6		574.57 (62.75)	500.44		602.62 (39.65)	635.07	
7	332.87 (34.19)	334.14	~25	566.52 (80.38)	541.5	~40	648.41 (52.98)	679.74	~45
8	334.01 (38.34)	358.81		607.05 (73.41)	578.66		706.51 (42.42)	718.57	
9	334.14 (33.95)	380.91		633.00 (49.43)	612.28		741.68 (40.48)	752.33	
10	345.56 (41.22)	400.71	~15	635.94 (60.96)	642.7	~30	762.00 (35.92)	781.68	~35
11	379.98 (22.19)	418.44		699.64 (21.31)	670.23		822.96 (30.90)	807.19	
12	405.13 (55.68)	434.33		657.58 (48.11)	695.14			829.37	
13	404.50 (66.67)	448.57		651.75 (18.46)	717.68		939.8	848.66	
14	411.48 (118.54)	461.32		675.00 (26.00)	738.07			865.42	
15	-	472.74		-	756.52			880	
16	279.4	482.98		725.67 (139.27)	773.22			892.67	
17	-	492.15		682	788.33			903.69	
19	388.6	507.72		800	802			913.26	
22	-	525.5		792	814.37			921.59	

Figure 2.1. The Bahamas and sampling locations. *Epinephelus striatus* samples were collected at a landing ports on Abaco, Long Island, and New Providence, The Bahamas from 1999-2002. *Epinephelus guttatus*, *E. striatus* and *Mycteroperca venenosa* samples were collected at Montagu Ramp fish dock on New Providence from 2006-8. Base map from NOAA Office of Satellite Data Processing and Distribution (accessed April 2010).

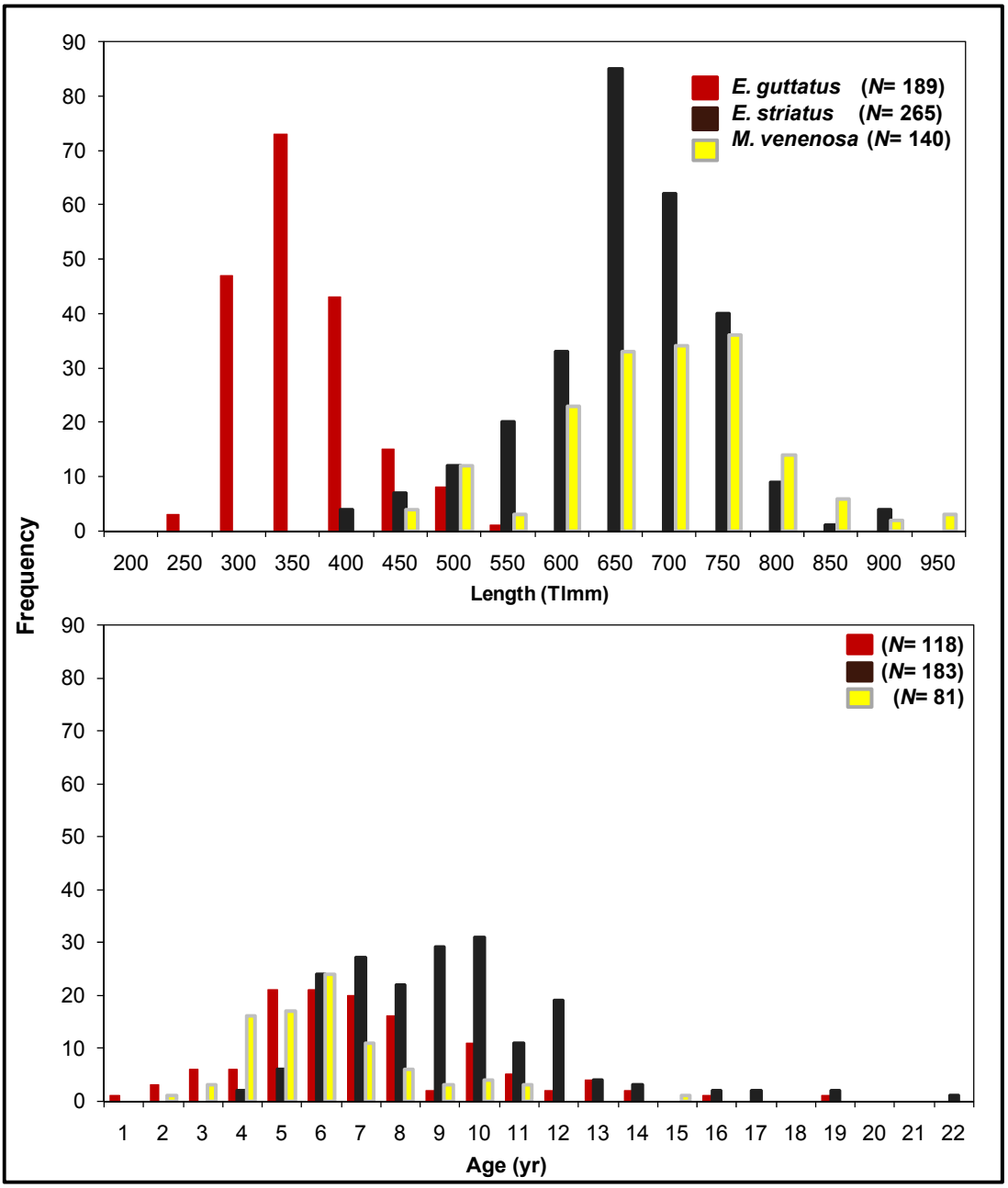


Figure 2.2. Length (total length mm) and age (year) frequency distributions of *Epinephelus guttatus*, *E. striatus* and *M venenosa* samples collected from The Bahamas. Samples were collected at a landing ports in The Bahamas from 1999-2002 (*E. striatus* 1999-2002, 2006-8; *E. guttatus* and *M. venenosa* 2006-8).

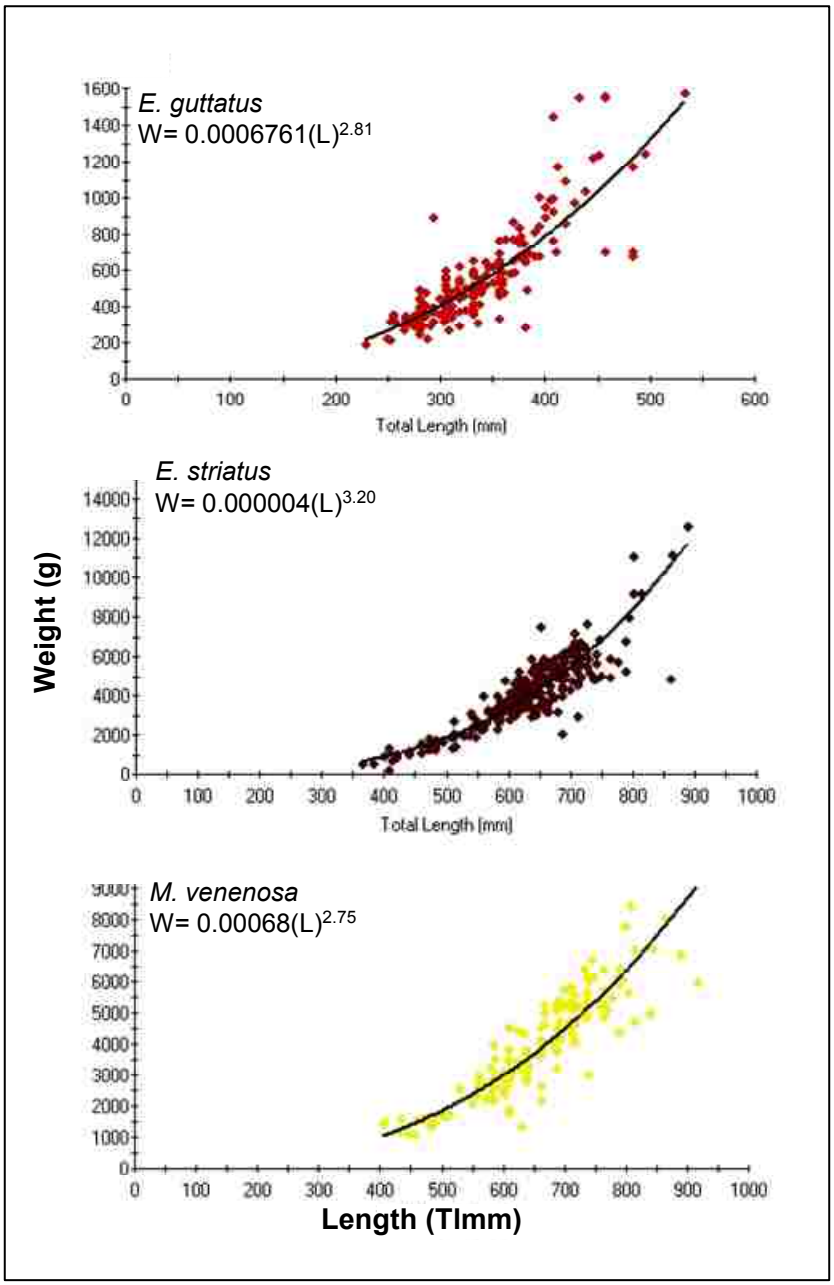


Figure 2.3. Length-to-mass relationships for *Epinephelus guttatus*, *E. striatus* and *Mycteroperca venenosa* harvested from The Bahamas. Relationships were calculated from the $\log_{10}$ transformation of $M = aL^b$, where M = mass (g) and L = total length (mm). Samples were collected at a landing ports in The Bahamas from 1999-2002 (*E. striatus* 1999-2002, 2006-8; *E. guttatus* and *M. venenosa* 2006-8). (Note the scales are different for each species.)

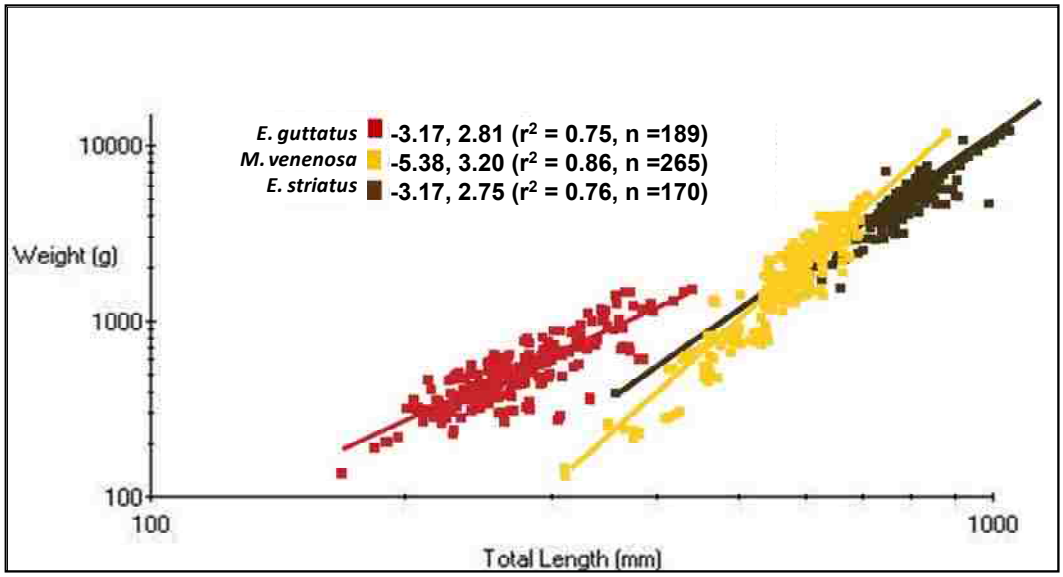


Figure 2.4. Length-to-mass relationships (log-log) for *Epinephelus guttatus*, *E. striatus* and *Mycteroperca venenosa* harvested from The Bahamas. Relationships were calculated from the $\log_{10}$ transformation of $M = aL^b$, where M = mass (g) and L = total length (mm). The constants a and b represent the intercept value (a) and the slope (b) of the linearized form of the equation (log10 (M) = a + b* log10 (L)). Samples were collected at a landing ports in The Bahamas from 1999-2002 (*E. striatus* 1999-2002, 2006-8; *E. guttatus* and *M. venenosa* 2006-8).

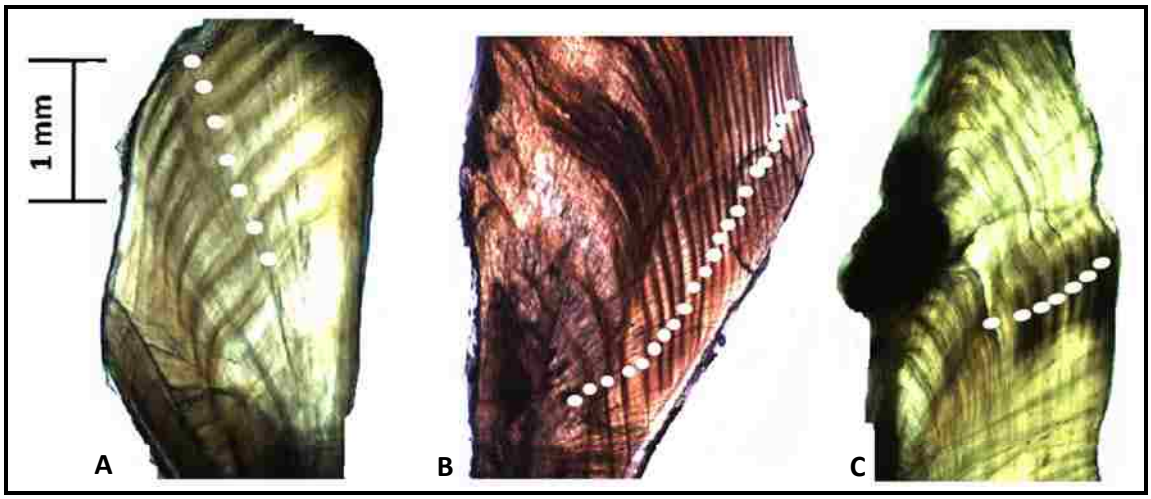


Figure 2.5. Photomicrographs of transverse sections through sagittal otoliths with annuli indicated by dots from (A) *Epinephelus guttatus,* 7+ year old = 338 Tlmm, total mass = 553 g, (B) *E. striatus,* 22+/1 year old = 792 Tlmm, total mass = 7994 g, and (C) *Mycteroperca venenosa,* 7+ year old = 665 Tlmm, total mass = 4990 g. (Tlmm= total length mm.)

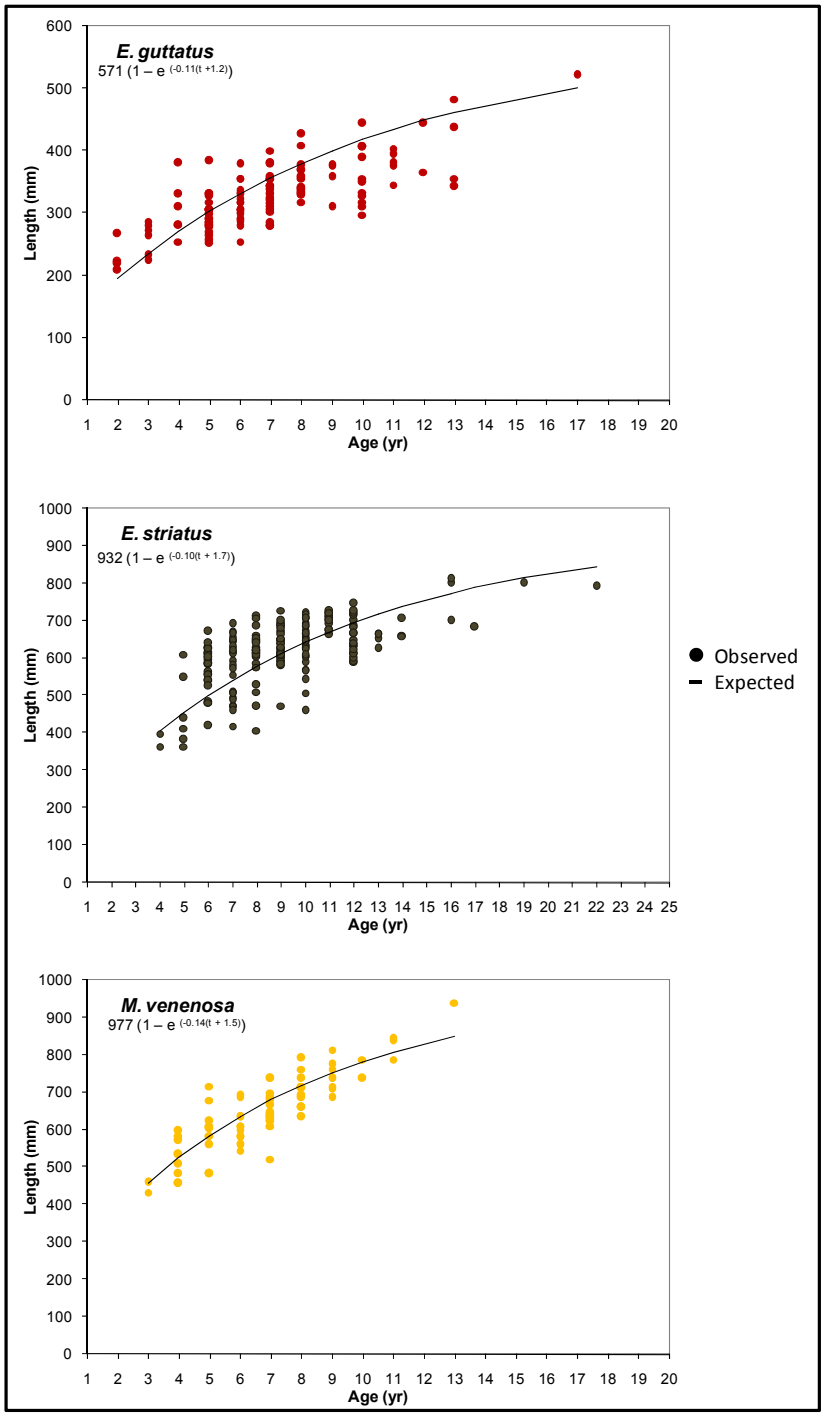


Figure 2.6. Data points of aged fish overlain with von Bertalanffy age (year) and length (Tlmm) growth curves, $L_t = L_\infty\ (1\text{-}e^{-k(t\text{-}t0)})$ for *Epinephelus guttatus*, *E. striatus* and *Mycteroperca venenosa*. *Epinephelus guttatus*: $571(1–e^{(0.11(t + -3.1)})$, *E. striatus*: $932(1–e^{(0.1(t + -1.7)})$ and *Mycteroperca venenosa:* $977(1–e^{(0.14(t + -1.5)})$. Samples were collected at a landing ports in The Bahamas from 1999-2002 (*E. striatus* 1999-2002, 2006-8; *E. guttatus* and *M. venenosa* 2006-8).

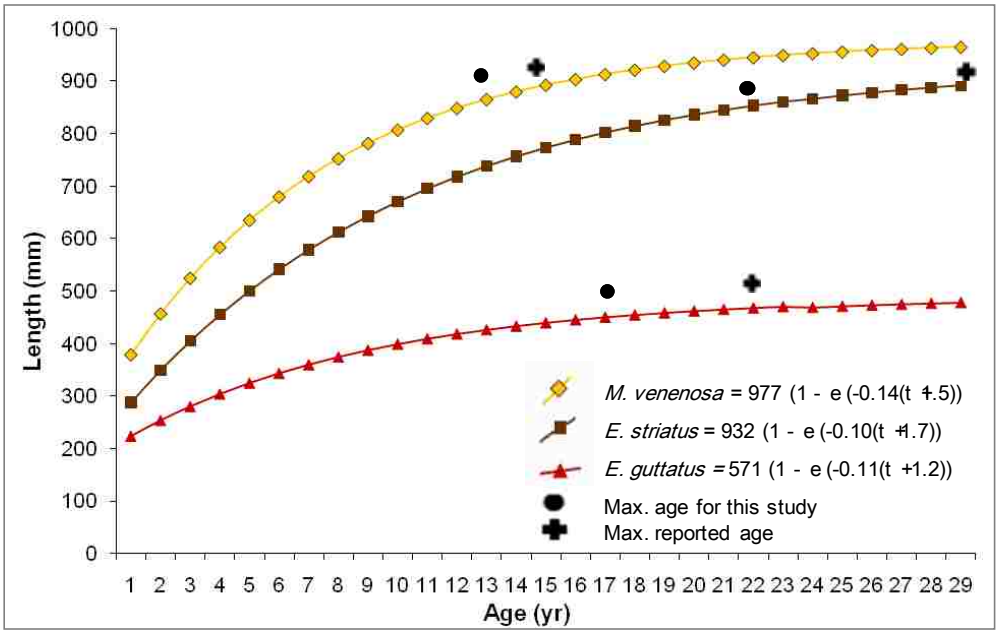


Figure 2.7. Fitted von Bertalanffy age (year) and length (Tlmm) growth curves, $L_t = L_\infty (1\text{-}e^{-k(t-t0)})$ of *Epinephelus guttatus*, *E. striatus* and *Mycteroperca venenosa*. *Epinephelus guttatus*: $571(1–e^{(0.11(t+3.1)})$, *E. striatus*: $932(1–e^{(0.1(t+1.7)})$ and *Mycteroperca venenosa:* $977(1–e^{(0.14(t+1.5)})$. Samples were collected at a landing ports in The Bahamas from 1999-2002 (*E. striatus* 1999-2002, 2006-8; *E. guttatus* and *M. venenosa* 2006-8). (Tlmm= total length mm.)

CHAPTER THREE

REPRODUCTIVE CLASSIFICATION AND SPAWNING SEASONALITY OF *EPINEPHELUS GUTTATUS* (RED HIND), *E. STRIATUS* (NASSAU GROUPER) AND *MYCTEROPERCA VENENOSA* (YELLOWFIN GROUPER) FROM THE BAHAMAS[1]

Background

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 to [1]allow for inter-species comparisons for ecosystem-based management and evolutionary life

[1][1]This chapter has been published. Citation: Cushion, N., Cook, N, Schull, J, Sullivan-Sealey, K. 2008. Reproductive classification and spawning seasonality of *Epinephelus striatus* (Nassau grouper), *E. guttatus* (red hind) and *Mycteroperca venenosa* (yellowfin grouper) from The Bahamas. Proceedings of the 11th International Coral Reef Symposium, Ft. Lauderdale, Florida (peer reviewed section).

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

Sample Acquisition

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 were 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, mass and gonad mass 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 Garcia (1996) (*M. venenosa*).

Reproduction Classification System

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 the present 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).

Data Analyses

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. 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 (regressed) 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 study 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 the present study provided the appropriate criteria for designation of 96% of gonad samples (n=675) into a class (all species combined) (Table 3.1 and Figure 3.1). The results of the present study corroborate previous reproductive biology studies on the study species. Gamete production in *E. striatus* is indeterminate and the species is functionally gonochoristic (Sadovy and Colin 1995). *Epinephelus 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 Garcia 1996).

Epinephelus 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 spermatigenic tissue was used to classify these fish, but 4% were classified as "bisexual" because no 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 study species was analyzed (Table 3.2). 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). *Mycteroperca 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 study 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 of *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 also was 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. The present 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 gonad 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., Cayman Islands: Whaylen et al. 2004, USVI: Nemeth et al. 2004). However, January and February are not the dominant spawning times for *M. venenosa*. Personal communications with fishermen in the present 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 composed of multiple 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.

Table 3.1. 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, skipped, 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 gonorchoristic 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.

Table 3.2. Spawning seasonality for *Epinephelus guttatus* (red hind), *E. striatus* (Nassau grouper), and *Mycteroperca venenosa* (yellowfin grouper) in The Bahamas. 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".

Species	**Spawning Years/ Months** *(Sample number in parentheses).*
E. striatus	November 2007 (21) January and February 2008 (27 and 23)
E. guttatus	February 2007 (17) January and February 2008 (22 and 30)
M. venenosa	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.	

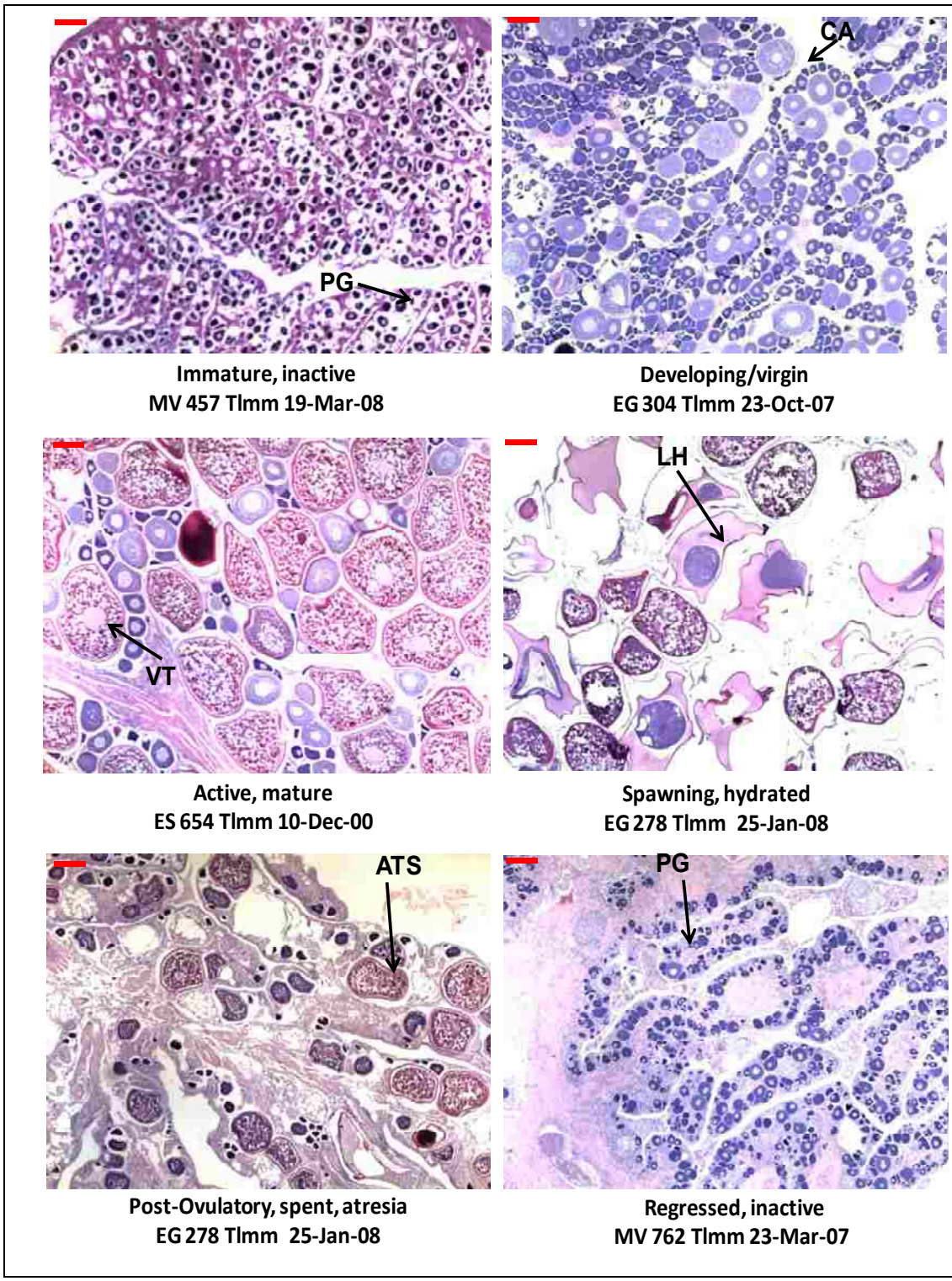


Figure 3.1a. Ovarian reproductive development in *Epinephelus guttatus* (EG), *E. striatus* (ES), and . (MV). Primary growth (PG), cortical alveolar (CA), vitellogenic oocytes (VT), late hydrated (LH) and atresia (ATS). Magnification=40x's, (–) = 100 µ. (Tlmm= total length mm.)

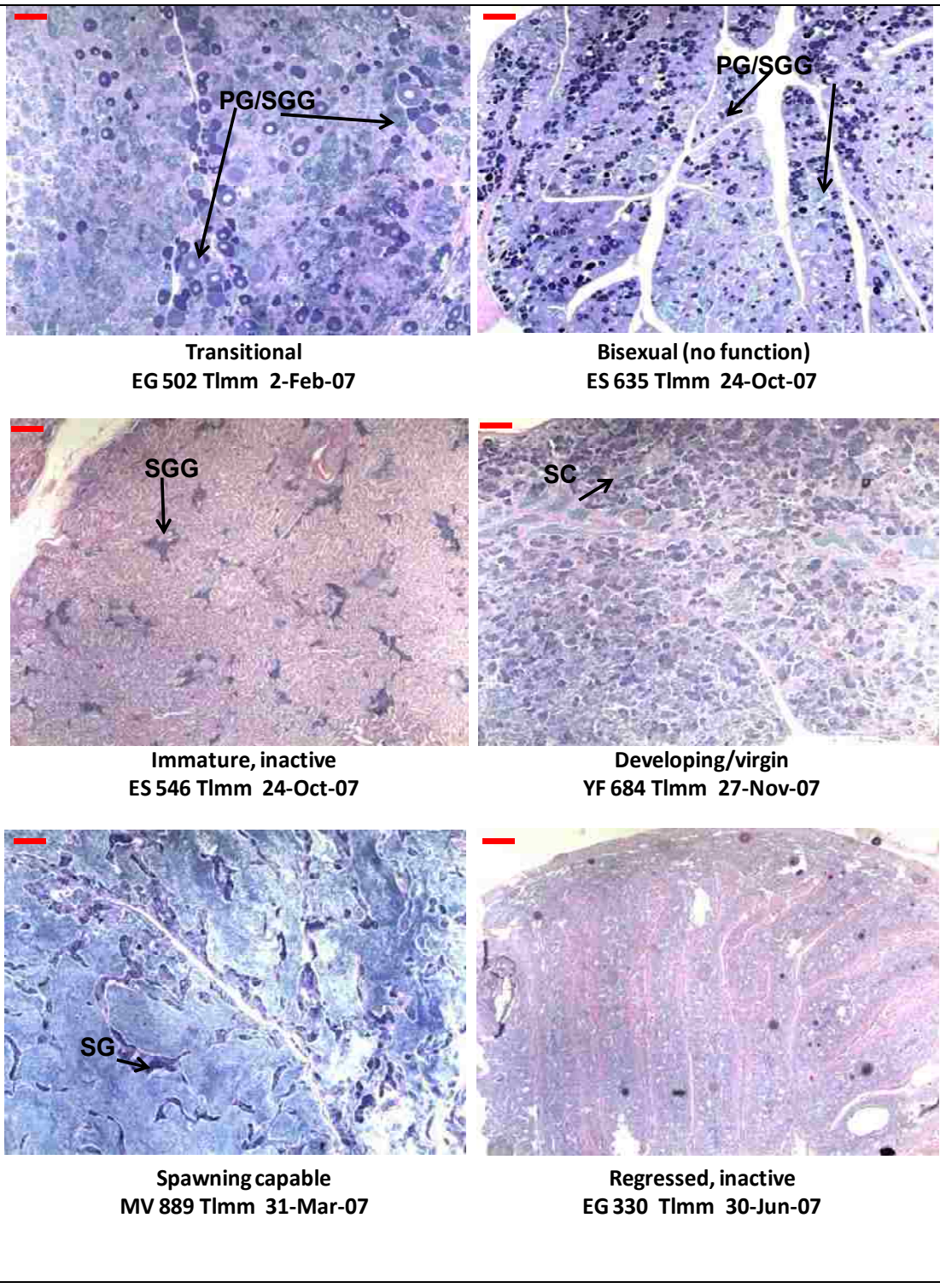


Figure 3.1b. Transitional and bisexual gonad and testes reproductive development in *Epinephelus guttatus* (EG), *E. striatus* (ES), and *Mycteroperca venenosa* (MV). Spermatogonia (SGG), spermatogenesis (SG), and spermatocytes (SC) are highlighted. (Spent male not pictured.) Magnification=40x's, (–) = 100 µ. (Tlmm= total length mm.)

CHAPTER FOUR

CONTRASTING REPRODUCTIVE LIFE-HISTORY TRAITS IN *EPINEPHELUS GUTTATUS, E. STRIATUS,* AND *MYCTEROPERCA VENENOSA* FROM THE BAHAMAS

Background

Life-history theory asserts that there is intra- and inter-species variation in reproductive life-history traits (LHTs) because LHTs are selected in a given environment to maximize the number of surviving offspring (Adams 1980, Stearns 1982 and 1992, Roff 2000). Stearns (1982) stressed the need for scientists to delve further into understanding reproductive LHTs for a given species when he stated the paradox, "More is known about what evolution ought to produce…than about what developmental systems can produce". For fish, determining what a population can produce requires information concerning extrinsic processes (recruitment, mortality and population estimates), and intrinsic reproductive LHT information, including spawning seasonality, sex ratios and the average age of sexual maturity (Jones and McCormick 2002). Extrinsic processes affect the present quantity of fish, while the internal reproductive LHTs determine the quality and quantity of gametes, and thus affect long-term population changes (Jones 1984, Morgan 2008).

Reproductive LHT variation among species and between populations is crucial to understand because the reproductive potential of a population is directly related to a populations' suite of LHTs (Tomkiewicz et al. 2003, Morgan 2008). Notably, Morgan (2008) reviewed multiple LHT studies of various fish, including northern pike (*Esox lucius*), Atlantic cod (*Gadus morhua*), and American plaice (*Hippoglossoides platessoides*), and found that changes or differences in the age of sexual maturity, sex

ratios and/or fecundity have frequently been linked with changes in population abundance and reproductive output. Reproductive biology information is greatly needed for most species in the family Serranidae, subfamily Epinephelinae (Shapiro 1987, Sadovy 1996, Morris et al. 2003). Globally most of the 159 Epinephelinae species are commercially important and they are heavily targeted in fisheries, especially fish of the genera *Epinephelus* and *Mycteroperca* in the Caribbean (Heemstra and Randall 1993, Morris et al. 2000). Epinephelinae species exhibit a wide variety of reproductive strategies, and thus species-specific information is needed. Accordingly, the two goals of the present study were to: 1) to obtain reproductive LHT information, including the sex ratios, size-frequencies, size and age of sexual maturation and sex change, spawning seasonality and the gonadosomatic indices (GSI) for populations of *Epinephelus guttatus*, *E. striatus* and *M. venenosa* from The Bahamas; and 2) contrast the findings in context of each species' reproductive strategy.

The reproductive strategy of a species is the overall pattern of reproduction common to the majority of individuals (Murua and Saborido-Rey 2003) and is characterized by variable and fixed intrinsic aspects (Winemiller and Rose 1992, Morgan 2008). For the present study, the fixed aspects of a strategy are collectively termed the reproductive mode, which is defined by the combination of the sexuality, gamete production system and typical spawning pattern exhibited by the majority of populations (Murua and Saborido-Rey 2003). The sexuality of most species of the genera *Epinephelus* and *Mycteroperca* is some form of protogyny (female to male sex change) (Shapiro 1987), while evidence of gonochorism (sexes fixed at sexual maturation) has been found in a few species including, *E. striatus* (Sadovy and Colin 1995) and

Mycteroperca rosecea (leopard grouper) (Erisman et al. 2008). The gamete production system of Epinephelinae species may be determinate, where one batch of eggs is produced per spawning bout within a season, or indeterminate, where multiple batches of eggs are produced within each spawning bout of a season (Murua and Saborido-Rey 2003). Three main spawning patterns have been classified for the genera *Epinephelus* and *Mycteroperca* (Sadovy et al. 1994): 1) non-migratory, single male with multiple females, pair-spawning species, 2) migratory, single male with multiple females, and, 3) migratory aggregating group spawning species. While aspects of a species' reproductive mode hold true for the majority of populations, reproductive LHTs vary temporally within populations, as well as spatially between populations (Shapiro 1987, Sadovy 1996, Morgan 2008).

Epinephelus guttatus, *E. striatus* and *M. venenosa* are closely related (Craig and Hastings 2007), but they have evolved unique reproductive strategies. Specifically, their sexualities, gamete production mechanisms and spawning patterns differ. *Epinephelus guttatus* is a monandric, protogynous hermaphrodite, which means that all juveniles are females and all males were once mature females that have changed sex (Sadovy et al. 1993). The spawning pattern of *E. guttatus* has been classified as migratory, and multiple groups consisting of one male with multiple females form across an aggregation site (Shapiro et al. 1993) and gametogenesis is classified as determinate batch spawning (Sadovy et al. 1994). The sexuality of *E. striatus* has been diagnosed as essentially and functionally gonochoristic (two separate sexes), with potential for sex change. Where and when sex change may be present, it is thought to be diandric protogynous, meaning that males may develop directly from immature or bisexual fish, or from sex-changing

females (Sadovy and Colin 1995). The spawning pattern employed by *E. striatus* is migratory and large aggregations of 100s-1000s of fish form at set points throughout the Caribbean (Colin 1992). Gametogenesis is thought to be indeterminate (Sadovy and Colin 1995). *Mycteroperca venenosa* has been diagnosed as a monandric, protogynous hermaphroditic species (García-Cagide and Garcia 1996), gametogenesis is indeterminate and the spawning mode is migratory, aggregating group spawning (Sadovy 1994, Nemeth et al. 2004). Spawning seasonality for all of the study species corresponds typically with the full moon during the winter-spring months, but may vary latitudinally (*E. guttatus*: Burnett-Herkes 1975, Sadovy et al. 1994; *E. striatus*: Sadovy and Colin 1995, Sadovy and Eklund 1996; *M. venenosa*: Garcia-Cagide and Garcia 1996, Nemeth et al. 2004, Tuz-Sulub et al. 2006).

This is the first reproductive biology study of Bahamian populations of *E. guttatus* and *M. venenosa*. The reproductive biology and spawning aggregations of *E. striatus* have been studied in The Bahamas by Colin (1992) and Sadovy and Colin (1995); however contemporary LHT information is valuable. I predicted that the different sexualities and spawning modes of *E. guttatus*, *E. striatus* and *M. venenosa* would correspond to significantly different sex ratios, size frequencies and GSIs. The reproductive LHTs are discussed in relation to other studies conducted throughout the tropical western Atlantic Ocean and are further related to ecological and spawning behaviors.

Materials and Methods

Sample Acquisition

Fishery dependent *E. guttatus*, *E. striatus* and *M. venenosa* samples were collected during

spawning and non-spawning seasons. *Epinephelus striatus* samples from spawning sites throughout The Bahamas were obtained in November and December 1999 (Montague ramp fish market, New Providence), in December 2000 and January 2002 (Long Island) and in January 2001 (Marsh Harbour). *Epinephelus guttatus*, *E. striatus* and *M. venenosa* samples were collected monthly from December 2006 through April 2008 at Montague ramp. The 2006-8 sampling was conducted monthly, corresponding with the full moon phase. Ungutted fish were measured for maximum total length (Tlmm) (snout to end of tail midpoint) and standard length (snout to caudal peduncle) to the nearest mm, and total body mass to the nearest 0.1 g. Gonad samples were taken and preserved in 10% neutral buffered formalin. Specimens obtained too long post-mortem for histology were sexed and staged by macroscopic examination when possible.

Gonad Histology

The histological samples were processed by the histology lab at the Louisiana State Veterinary School. Gonad sections were imbedded in paraffin, sectioned and stained using hemotoxylin and eosin following standard histological procedures. Initial gonad homogeneity tests were done to confirm that a subsample was representative of the entire gonad. Similar analyses were previously performed for each species by Sadovy and Colin (1995) (*E. striatus*), Sadovy et al. (1994) (*E. guttatus*), and García-Cagide and Garcia (1996) (*M. venenosa*). Subsequently, only a single, central transverse section was prepared for each gonad.

Histological Classification

The reproductive biology histological classification system for the present study was adopted (Cushion et al. 2007) with minor changes from Lyon et al. (2008) (See Figure

3.1 and Table 3.1 for complete classification system and pictures). The classification system is based on previous reproduction studies including Moe (1969), Wallace and Selman (1981), Shapiro et al. (1993), Sadovy and Colin (1995) and Brown-Peterson et al. (2007). Fish sex and reproductive state 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 (See Chapter 3, Figure 3.1 and Table 3.1). Ovaries were categorized histologically into nine stages, which incorporated ontogenetic changes in immature fish (1) immature, inactive, (2) inactive, uncertain, (3/4) developing virgin, developing, (5) active, mature, (6) spawning, hydrated, (7) post-ovulatory, spent, (8) regressed, inactive, mature, (9) regressed, skipped, mature. The gonads of inactive females, stages 1 and 2, were compact with tightly aligned lamellae and previtellogenic oocytes deeply embedded in connective tissue. Stage 3 or 4 females had cortical alveolar oocytes present; if there were no indicators of prior spawning (e.g. tissue muscle bundles, old hydrated oocytes, disorganized connective tissue and thick tunica albuginea) the fish were classified as stage 3. Stage 5 was diagnosed by the presence of vitellogenic oocytes, which indicates spawning will occur within days or weeks. Stage 6 was diagnosed by the presence of advanced vitellogenic oocytes (lipid and yolk coalescence), which represent the initiation of spawning, and fully hydrated oocytes that are indicative of actively spawning fish. Stage 7 females were diagnosed by the presence of post-ovulatory follicles. The end of the spawning season (i.e. regression, stages 8 and 9) was determined by massive cell atresia or the lack of vitellogenic oocytes. Stage 8 also included gonads that had markedly regressed cytologically between annual spawning periods and had

previtellogenic oocytes, but indicators of prior spawning were present. The smallest size class in which 50% of individuals are known to be mature (see below) also was used to distinguish between stage 1 and 8 individuals (Shapiro et al. 1993).

Fish were classified as transitional (10) if degenerating oogenic and proliferating spermatogenic tissue were present (Sadovy and Shapiro 1987). Fish were classified as bisexual (11) if comparatively equal amounts of oogenic and spermatogenic tissues were present, but it could not be determined if the fish would function as a female or male (Sadovy and Colin 1995). Male sexual maturity was indicated by initiation of spermatogenesis and formation of spermatocysts: (1) immature, inactive, (2/3) developing virgin, developing, (4) spawning capable, (5) spent, (6) regressed, inactive, mature. Males were classified as spawning capable when spermatozoa were evident and filling sperm ducts and lobules. All histological slides for each species were analyzed and classified by two readers and discarded if stage agreement could not be reached.

Ageing

Otoliths were collected from a subset of samples for each species. These data were used to construct growth curves using the von Bertalanffy growth equation (VBGE) (see Chapter 2 for complete analyses). Parameters estimated from the VBGE were used to extrapolate ages for samples without otoliths.

Data Analyses

All data were tested for normality and homogeneity of variance. Nonparametric tests were conducted if data were not normally distributed. Results were considered significant if $p \leq 0.05$.

Size and Sex Frequency and Sex Ratios

The female-to-male ratios (excluding transitional and bisexual-stage fish) were compared within species using a Pearson χ^2 two-sample test for independence. Frequency distributions for Tlmm and sex were constructed and assessed for normality with Q-Q plots. No linear relationships were observed between the observed values and the expected values for any species, so non-parametric statistics were used (Shapiro et al. 1993, Marino et al. 2001). For each species, sex by size-frequency distributions were compared by using the Kolmogorov-Smirnov test. Differences between mean female and male Tlmm were analyzed by using a one-tailed *z*-test (Sadovy and Colin 1995, Marino et al. 2001, Brule et al. 2003). Statistical analyses were conducted using SPSS v17.

Sexual Maturity and Sex Change

The estimated size and age at which 50% of females were sexually mature (L_{50}) (when 50% of the population is estimated to be mature) was determined by using binary logistic regression (Brule et al. 2003). Females in stages 5-9 (see Table 3.1) were considered as sexually mature individuals. The immature size range and the minimum size at which females became sexually mature were recorded. The size range of sex change (L_{sc}) and age range of sex change (A_{sc}) were estimated using the overlap of the largest females and smallest males (Shapiro 1987).

Reproductive Cycle and Gonadosomatic Index

The annual reproductive cycle for each species was determined by calculating the monthly distribution (% of total examined) of each reproductive stage. Monthly samples were grouped across all locations and years. Reproduction periodicity was also examined by the gonadosomatic index (GSI) values. The GSI is an index of the gonad mass

relative to body mass and was calculated as (Wootton 1990): GSI (wet mass, %) = 100 (wet mass of ovary or testis/ total body mass).

Results

Size and Sex Frequency and Sex Ratios

A total of 1151 histological samples were collected from the three species: *E. guttatus*, 312; *E. striatus*, 516; and *M. venenosa*, 323. The mean length of female *E. striatus* was slightly smaller than that of the males, but not significantly. *Epinephelus guttatus* and *M. venenosa* male mean lengths were significantly greater than those of females (one-tailed *z*-test, $P \leq 0.05$) (Table and Figure 4.1). The hypothesis that the sex by size-frequency distributions will significantly differ for each species was accepted for *E. guttatus* ($P \leq 0.00$) and *M. venenosa* ($P \leq 0.00$), but rejected for *E. striatus* (Kolmogorov-Smirnov, $P \leq 0.05$). The largest *E. guttatus* and *M. venenosa* were males and there was little male-female overlap in the upper size ranges. In contrast, mature female and male *E. striatus* overlapped throughout their size ranges (Figure 4.1). Sex ratios for all species differed from unity (χ^2, $P \leq 0.05$). *Epinephelus guttatus* had a male-biased sex ratio, in contrast, female-biased ratios were found for *E. striatus* and *M. venenosa* (Table 4.1).

Sexual Maturity and Sex Change

Immature fish ranged from 210-335 Tlmm for *E. guttatus*, 363-705 Tlmm for *E. striatus* and from 330-677 Tlmm for *M. venenosa* (Figure 4.1). The smallest mature *E. guttatus*, *E. striatus* and *M. venenosa* were 218, 406 and 407 Tlmm, respectively. The fifty-percent maturity (L_{50}) estimates from binary regression analyses were 235 Tlmm (2.05 year old, yo), 435 Tlmm (4.00 yo), and 561 Tlmm (4.66 yo) for *E. guttatus*, *E. striatus*

and *M. venenosa*, respectively (Figure 4.2). For all species, the L_{50} estimates and/or size ranges of maturity do not substantially differ for other studies throughout the Tropical Western Atlantic (TWA) (Table 4.1).

A few *E. guttatus* and *M. venenosa* and greater than 30% female and male *E. striatus* samples had both oogenic and spermatogenic tissue present (i.e. predominantly testicular gonads containing scattered stage 1-2 oocytes or vice-versa). The only bisexual *E. guttatus* sample was 254 Tlmm and was obtained in April 2008. Only three *E. striatus* were classified as transitional and ten as bisexual fish. The transitional fish contained stage 3-4 oocytes, indicating sexual maturation, and proliferating crypts of spermatogenic cells. The transitional *E. striatus* ranged in size from 458-813 Tlmm and were sampled in November 1999, January 2002 and May 2007.

The size and age range of sex change (L_{sc}, A_{sc}) for *E. guttatus* sex change were between 257-401 Tlmm and ~4-5 years old. *Mycteroperca venenosa* were determined to change sex between 716-871 Tlmm, ~8-9 yo and the L_{sc} and A_{sc} for *E. striatus* ranged from 521-890 Tlmm and ~7-8 years old. However, this species is thought to be functionally gonochoristic (Sadovy and Colin 1995) and protogyny is not the primary sexual pathway for this population.

Reproductive Cycle and Gonadosomatic Index

The three study species are reproductively inactive in the summer-fall, and begin spawning recrudescence in the winter through spring (November-April) (Figure 2.2). Female *E. guttatus* started early vitellogenesis in October, the process of yolk formation via nutrients being deposited in the oocyte, and by January more than 55% of the samples showed yolked oocytes. The peak spawning month was January. Sexually active

samples containing post-ovulatory follicles (POFs) were present through April. The majority of samples from May-October were regressed, inactive (determined by the presence of one or more indicators of prior spawning) or immature (see Chapter 3, Table and Figure 3.1). Spawning capable *E. guttatus* males were present December-April.

Female *E. striatus* were observed to have initiated vitellogenesis in October. The majority of samples from December- February were sexually active and/or spawning, and January and February were peak spawning months (Figure 4.3). No sexually active females were present in samples after March. Sexually active male *E. striatus* were found between November and April. No immature *E. striatus* males were sampled; however one developing virgin male and three developing males were sampled over the course of the present study. For *M. venenosa* samples with cortical alveolar and yolked oocytes were present from November-April. The peak spawning months were between February-April. All the samples between May-October were immature, inactive-uncertain or regressed (Figure 4.3). Spawning capable males were sampled December-April.

For all species, the GSIs for males and females in non-spawning months were less than 2%. The GSIs of sexually mature *E. guttatus* females and males ranged from 1.00-30.00% and 0.09-7.60%, respectively. Sexually mature *E. striatus* females ranged from 1.40-20.95%, and males from 0.20-9.08%. The GSI of sexually mature *M. venenosa* females ranged from 0.86-18.23% and males ranged from 0.72- 8.30% (Figure 4.5). Significant differences were found between the female and male GSIs for all species ($p \leq 0.05$, Mann-Whitney *U* test).

Discussion

For a given species or family, ample extrinsic information often exists in the form of environmental parameters, spawning behaviors and localities, habitat health and preferences. In contrast, intrinsic LHT information is typically scarce (Sadovy 1996, Tomkiewicz et al. 2003). The results of the present study provide important reproductive life-history information and insight into reproductive LHT variation between three closely related Epinephelinae species.

The sex-by-size-frequency distributions found corresponded to the sexuality classifications of *E. guttatus* and *M. venenosa* as monandric protogynous hermaphrodites (Shapiro et al. 1993 and Sadovy et al. 1994, Garcia-Cagide and Garcia 1996, respectively), and *E. striatus* as a functionally gonochoristic, with a small part of some populations thought be protogynous (Sadovy and Colin 1995). The variation in sex ratios, size ranges, and mean lengths between other studies in the TWA is possibly due to different sampling methods and time periods, and to inherent population differences. For example, the majority of the reproductive biology studies are fishery dependent (i.e. Sadovy et al. 1994, Sadovy and Colin 1995, Garcia-Cagida and Garcia 1996), while the spawning aggregation studies are fishery independent (i.e. Carter et al. 1994, Whaylen et al. 2004, Whiteman et al. 2005). Also, the study species are heavily harvested in The Bahamas and throughout the TWA (Morris et al. 2000) and fishing may greatly alter population LHTs (e.g. sex ratios, Coleman et al. 1996; size, Walters and Wilderbuer 2000 and Yemane et al. 2008). Thus differential fishing pressure and/or protection may influence LHT variation throughout the TWA.

A female-dominant sex ratio is characteristic of protogynous species such *E. guttatus* and *M. venenosa*. The sex ratios values obtained from the present fishery-dependent study did not corroborate the aforementioned notion for *E. guttatus*, which had a slightly male skewed sex ratio of 0.57: 1. Female and male *E. guttatus* only co-occur during spawning periods. During the non-spawning season, *E. guttatus* live isolated in shallow (<100 m depth), low-relief, patchy coral and rocky reefs (Burnett-Herkes 1975, Sadovy et al. 1994, Sluka et al. 1996). Thus, because the present study represents the annual ratio, versus that of a spawning aggregation, the male-skewed sex ratio may be attributed to the combination of a relatively shallow reef distribution and size selective fishing practices which target larger fish. In the case of *E. guttatus*, these larger would be predominately male. The female:male sex ratios of *M. venenosa* from Mexico (~3:1) (Tuz-Sulub et al. 1996) and St. Thomas (~1:1) spawning aggregations (Nemeth et al. 2004) greatly differ from those from annual fishery dependent studies such as the present study (12:1) and those from Cuba (~9:1) (Garcia-Cagide and Garcia 1996). Previously determined sex ratios for *E. striatus* sex ratios tend to be close to 1:1, confirming the gonochoristic sexuality of the species first described by Sadovy and Colin (1995).

Key factors regulating gametic output and reproductive potential are average size or age of sexual reproduction and sex change (Shapiro 1987). Sexual maturation is a critical transition point because it represents the period during which individuals enter the reproductive population and may contribute to future generations. The age or size of sex change for a given population dictates the proportion of female and male gametic output. Theoretically, individual fitness is maximized such that the average age or size of sexual change should maximize reproductive output. Thus, these LHTs may vary between

populations in close proximity which experience different environmental circumstances. Cowen (1990) found the age of sexual maturity and sex change differed in populations of *Semicos-syphus pulcher* (California sheephead) off of the coast of Californa. However, the plasticity of the average L_{sc} and A_{sc} may be somewhat limited by body size. Allsop and West (2003) found that the relative timing of sex change is "surprisingly invariant" across all taxa and observed that 91–97% of the variation in size at sex change across species can be explained by the simple rule that individuals change sex when they reach 72% of their maximum size. In general, larger, slower growing species tend to mature over a larger size range as compared to faster-growing and shorter-lived species (Sadovy 1996). Although *E. guttatus* has a faster growth rate than *E. striatus* (Chapter 2), for the present study, this sexual maturity pattern holds true when comparing the "dwarf" hind *E. guttatus* to the "giant" groupers, *E. striatus* and *M. venenosa*.

The fecundity of Epinephelinae groupers and other species increases with fish size; thus, a decrease in the average size of sex change may substantially reduce egg production and the potential number of new recruits (Sadovy 1996, Sullivan-Sealy et al. 2002, Whitemen et al. 2005). Many samples in the present study contained both oogenic and spermatogenic tissue, but this is a common occurrence in many Epinephelinae species and is not a determinant for sex change (Shapiro and Sadovy 1987, Shapiro et al. 1993, Sadovy and Colin 1995, Mackie 2006). The average L_{sc} for *E. guttatus* is within the range estimated by Sadovy et al. (1994) for *E. guttatus* in Puerto Rico. There is a notable size difference in the L_{sc} for *M. venenosa* from the present study as compared to data from Cuba (Garcia-Cagide and Garcia 1996).

Spawning for the study species is typified by discreet annual spawning seasons, during which the species migrate to spatially and temporally predictable aggregations during the full moon period throughout the TWA (Sadovy and Colin 1995, Nemeth et al. 2004, Starr et al. 2007). Yet, species and site-specific seasonality can vary slightly because spawning typically corresponds to variable environmental parameters (e.g. temperature) and population density (Colin 1992, Nemeth 2004, Whaylen et al. 2004, Starr et al. 2007). However, *E. guttatus* in Puerto Rico (Sadovy et al. 1994) were found also to also spawn during the new moon phase and Nemeth et al. (2007) found *E. guttatus* spawning in St Thomas and St Croix, the US Virgin Islands, to be correlated with the winter solstice. Sampling for the present study was conducted solely during the full moon phase, so there may be additional spawning periods throughout December-February in The Bahamas. The peak spawning season of *E. guttatus* in The Bahamas is similar to that found in Puerto Rico, St. Thomas USVI, the Netherlands Antilles and Jamaica (Sadovy et al. 1994, Whitemen et al. 2005, Munro et al. 1973 and Thompson and Munro 1978, respectively), while *E. guttatus* spawning in Bermuda has been documented to occur much later (May-July) (Burnett-Herkes 1975). The specific aggregation sites of *E. guttatus* in The Bahamas have not been formally documented. The capture of fully hydrated females with POFs in January and April indicates that the fishers were harvesting directly from aggregation sites (Appendix B). The spawning seasonality peak of *E. striatus* samples from New Providence, Abaco and Long Island was January-February. Cushion et al. (2008) analyzed samples solely from New Providence and found a similar pattern, thus *E. striatus* peak spawning seasonality does not appear to greatly vary throughout the Bahamian archipelago, or throughout the TWA (Sadovy and

Eklund 1999). The peak spawning period of Bahamian and other TWA populations of *M. venenosa* is slightly later than for *E. striatus* (Cuba: Garcia-Cagide and Garcia 1996; USVI: Nemeth 2004; Jamaica: Thompson and Munro 1978; and Mexico: Tuz-Sulub et al. 2006). The co-occurrence of the two species at spawning aggregations has been widely documented (Nemeth 2004, Whaylen et al. 2004, Starr et al. 2007), and thus *M. venenosa* likely spawn in similar areas as *E. striatus* in The Bahamas.

To most accurately estimate (so as to not over-estimate) the reproductive potential of a population, it is critical to know not only the potential fecundity, but how frequently a fish species actually releases eggs and what behavioral and/or size relationships are related to the frequency and amount of eggs released (Hunter and Macewicz 1985, Tomkiewicz et al. 2003). Also, Sadovy (1996) noted that it is important to distinguish peak spawning months in which 50% or more of females contain yolked eggs, so as not to overestimate the duration of the spawning season. Oocyte maturation is a protracted process and actual egg release is coupled with the specific spawning period and reproductive behaviors associated with the study species. Additionally, spawning capable males often mature prior to females and remain so longer than females. Histological or visual evidence is thus necessary to confirm a fish has actually spawned. For the present study, POFs were present in ten *E. guttatus*, thirty *E. striatus* and six *M. venenosa* samples. While this information and other indicators of prior spawning (e.g. muscle bundles) confirm temporally discrete spawning events, the number of spawning episodes per period within a spawning season (also termed the spawning frequency) is difficult to estimate. The sampling method used for the present study does not allow for the assessment of spawning frequency and moreover estimating spawning frequency for

indeterminate spawners such as *E. striatus* and *M. venenosa* is fraught with difficulties (Hunter and Macewitz 1985). Additionally, many studies have found evidence of frequent "skipped spawning", where fish mature, but resorb the gametes due to unfavorable physiological or spawning conditions (Rideout et al. 2005). Behavioral and population data, however, may be useful for inferring spawning frequency. Semmens et al. (2005 and 2007) used size-frequency and tagging data to assess *E. striatus* spawning aggregation dynamics and approximate spawning frequency in the Cayman Islands. Their findings suggest that older, larger fish stay at aggregation sites longer and they attend more spawning periods per season than smaller fish.

The GSIs for males and females coincided with the reproductive histology findings. Female GSIs for *E. guttatus*, *E. striatus* and *M. venenosa* peaked at 30%, 20.95% and 18.23%, respectively. These percentages highlight the substantial investment in egg production over a short time, and indicate that GSIs increased with size (data not shown). The average size of a hydrated egg is ~ 1mm. Thus, gonad size can be extrapolated to estimate potential fecundity. Thompson and Munro (1978) estimated that a sexually mature *E. guttatus* of ~260 Tlmm produces ~97,000 eggs, while a 410 Tlmm fish could produce ~379, 000. Whiteman et al. (2005) used ultrasound, fish size and mean oocyte diameter to estimate *E. guttatus* fecundity in Puerto Rico. They estimated average potential fecundity for a female within the spawning aggregation to be 978,620 oocytes (based on an average female size of 367 Tlmm fish), and found that fish greater than 370 Tlmm had significantly higher potential fecundities than those less than 370 Tlmm. Sullivan-Sealey et al. (2002) estimated the fecundity of *E. striatus* using the mean oocyte diameter of stage 5 oocytes and approximated there to be ~475 eggs/gram of gonad

tissue. By extrapolating this estimate to fish size, it was estimated that the smallest ripe female could develop approximately ~200,000 eggs, while the largest individual could produce over 1 million eggs (a GSI of ~20%).

The peak GSIs of 17.07 % for male *E. striatus* and 8.30% for *M. venenosa* highlight an interesting finding. Although the male and female GSIs differed significantly, *E. striatus* and *M. venenosa* GSIs are similar to those of the females, and differ from most other TWA *Epinephelus* and *Mycteroperca* species (Appendix A). The comparatively high male GSIs found for *E. striatus* have been previously documented (Carter et al. 1994, Sadovy and Colin 1995). Sadovy and Colin (1995) postulated the high male GSIs are indicative of sperm competition, because the mating pattern of *E. striatus* is typified by large group spawning aggregations, with hundreds to thousands of fish, and the males compete to fertilize ripe females (Colin 1992). The mating system of *E. guttatus* is characterized as pair-spawning, where multiple small, single-male, haremic aggregations form at an aggregation site and male territorial behavior is displayed (Shapiro et al. 1993), but sperm competition is likely not present. The comparatively larger male GSIs of *M. venenosa* was documented in Jamaica by Thompson and Munro (1978) who collected a 695 Tlmm with a GSI of 6.3% and by Garcia-Cagide and Garcia (1996) in Cuba who had a specimen with a GSI of 6.8%. However, the investigators did not speculate as to the importance of these values. In contrast to other *Mycteroperca* species, Brule et al. (2003) found a maximum GSI of 0.43% for *M. bonaci* (black grouper) in the Gulf of Mexico and Coleman et al. (1996) did not perform GSI analyses on *M. microlepsis* (gag grouper) or *M. phenax* (scamp) from the Gulf of Mexico because "active testes in each of these species were small". However, Erisman et al. (2007) found male

GSIs in *M. rosacea* (leopard grouper) in the Gulf of California ranged from 0.20 to 7.21%, and the mean GSI of mature males was 2.34%. *Mycteroperca rosacea* may be functionally gonochoristic, like *E. striatus*, and *M. rosacea* has a similar group-spawning mating system, which suggests sperm competition is present. Assuming ripe male testes vary in accord with mating systems (Sadovy et al. 1994), *M. venenosa*, although protogynous, may have evolved a spawning pattern similar to *E. striatus*.

Because the study species are relatively slow-growing, long-lived and spawn at predictable, limited periods, all are susceptible to overexploitation (Shapiro et al. 1987, Coleman et al. 1996, Sadovy et al. 1996, Morris et al. 2000). Contemporary reproductive LHT information is greatly needed because fishing generally selects for the largest individuals, leading to high adult mortality. High adult mortality has been shown to lead to negative changes in LHTs, such as decreased growth rates and an earlier age of sexual maturity (e.g., Buxton 1993, Rochet 1998, Conover and Munch 2002, de Roos et al. 2006).

Precautionary management tools, such as seasonal fishing closures can be merged with market-based management measures, such as size limits and gear restrictions, to manage key life-history stages (Rhodes and Tupper 2007). In The Bahamas, a mass-based catch limit exists for all groupers (less than 3 lbs, ~ 1360 g, is prohibited), and fishing is closed seasonally for *E. striatus* during its peak reproductive period. By using the formula $mass=aL^b$, and the species-specific a and b values determined for each species (Chapter 2), three pounds equates to approximately 434 Tlmm for *E. striatus* and 435 Tlmm for *M. venenosa*. These sizes are below the estimated average size of sexual maturity for these species (435 Tlmm, *E. striatus*; 561 Tlmm, *M. venenosa*), and thus fish

are being harvested before they have had an opportunity to reproduce. Also, the single-species *E. striatus* closure may be inadvertently leading to excessive harvest of other species. Cushion and Sealey (2007) documented commercial fish landings at Montagu ramp and noted the pattern of increased grouper landings, especially *M. venenosa*, during the *E. striatus* closure period. Rhodes and Tupper (2007) found a similar scenario when they analyzed Epinephelinae landings in Pohnpei, Micronesia. During a seasonal fishing ban on certain Epinephelinae, fishing pressure (measured as total fish mass) increased for parrotfish and emperor fish. The peak spawning months of the study species are similar in The Bahamas, thus, ceasing the harvest of all Epinephelinae during the winter-spring spawning period may be a sound management strategy.

The size and age-based findings of the present study can also be used to modify the mass-based limit into size-based regulations that may be more applicable for maintaining sufficient breeding populations. The size-related life-history parameters including L_{sc} and A_{sc}, L_{50}, GSI, are necessary for determining appropriate capture sizes. The exponential increase in fecundity in Epinephelinae (Sadovy et al. 1993, Sullivan-Sealey et al. 2002, Whiteman et al. 2005) may warrant the protection of larger, versus smaller females (all else equal) (Sadovy 1996, Birkeland and Dayton 2005). For *E. guttatus* and *M. venenosa*, the size of the most fecund females overlaps the size range of sex change. So, a closure of fishing within the respective size ranges for each species could likewise mediate the loss of males.

The sexuality of the study species may also lead to differential susceptibility to fishing (Huntsmand and Schaaf 1994, McGoverrn et al. 1998, Heppel et al. 2006). Huntsman and Schaaf (1994) modeled the effects of fishing on protogynous and

gonochoristic stocks. Protogynous stocks lost reproductive capacity as fishing mortality increased and failed reproductively (less egg production) at a lower fishing mortality rate than gonochoristic stocks. In heavily harvested gag grouper (*Mycteroperca microlepis*) populations in southeastern United States McGovern et al. (1998) found that the percentage of males decreased from 19.6% (1976-82) to 5.5% (1994-1995) and the average size of sexual maturity decreased from 644 mm to 622 mm. Thus, *M. venenosa* and *E. guttatus* may be more susceptible to over-harvesting as compared to *E. striatus* because these species have comparatively shorter life spans, and *M. venenosa* reaches sexual maturity at a larger size and is protogynous. As compared to *E. striatus*, *M. venenosa* has a much smaller female reproductive output "window", and size-selective harvesting may lead to the removal of mainly males, thus creating a possible sperm shortage. The results of the present study provide critical information for sound management of these ecologically and economically important species in The Bahamas, as well as information for future population and reproductive potential research.

Table 4.1. Compilation of population and reproductive biology information for *Epinephelus guttatus*, *E. striatus* and *Mycteroperca venenosa* harvested from The Bahamas (*E. striatus* 1999-2002, 2006-8) (*E. guttatus*, *M. venenosa* (2006-8) and from other studies throughout the tropical western Atlantic ocean. (Tlmm= total length mm, Flmm= fork length mm, Slmm= standard length mm, yo= years old).

	Length range and mean					Spawning months	Location/
	Female	Male	Sex ratio F:M	50% Maturity	Sex change length/range	(Peak)	References
E. guttatus	**210-407 (294) Tlmm**	**257-565 (354) Tlmm**	**0.57 : 1 (annual)**	**235 Tlmm (~2.05yo)**	**257-401 Tlmm (~4-5 yo)**	**Dec-Feb (Jan)**	**This study**
	110-480 Flmm	245-510 Flmm	4.9:1 (spawn.)/10.9:1	215 FLmm	273-345 Flmm	Dec-Feb	Puerto Rico[10]
	367 ± 33 Tlmm	354 ± 28 Tlmm	(inshore 1:1.4 (spawn.)	-	-	Jan-Feb	St. Thms USVI[15]
	318 ± 25 Tlmm	420 ± 32 Tlmm	2.9:1 (spawn.)	-	-	Jan-Feb	Netherlands Antilles[6]
	329 Tlmm	390 Tlmm	1.7:1 (spawn.)	-	-	May-July (June)	Bermuda[1]
			2.8:1 (annual)	-	-	Dec-Apr (Feb)	Jamaica7,[12]
E. striatus	**330-890 (637) Tlmm**	**521-953 (660) Tlmm**	**2.96 : 1 (annual)**	**435 Tlmm (~ 4yo)**	**581 (± 9.19) Tlmm**	**Dec-Feb (Jan-Feb)**	**This study**
	~174-650 Slmm	~280-724 Slmm	2·2 : 1 (spawn.)	425/402 Slmm (f/m) (3-6 yo)	-	Dec-Jan	The Bahamas[9]
	502 Tlmm	589 Tlmm	1.9:1 (spawn.)	400-450 Slmm (4+ yo)	-	Jan	Cayman Islands[4]
	675 Tlmm	723 Tlmm	1 :1.6 (spawn.)	-	-	Jan-Feb	Cayman Islands[14]
	- 554 (m/f)	-	0.72:1 (annual)	480 Tlmm	270-582 Slmm	Feb-Apr	Jamaica[7,12]
	104-760 (418) Slmm	270-702 (420) Slmm	2.2:1 (annual), 1.5:1 (unexpltd spawn.), 2.4:1 (expltd spawn.)			Dec-Feb (Jan)	Belize[2]
M. venenosa	**330-871 (632) Tlmm**	**716-940 (838) Tlmm**	**11.96: 1 (annual)**	**561 Tlmm (~4.7yo)**	**716-871 Tlmm (~8-9 yo)**	**Jan-Apr (Feb-Apr)**	**This study**
	~250-950 Tlmm	~750-1050 Tlmm	9.3:1 (annual)	-	~650 Tlmm	Jan-June (Jan, Mar-Apr)	Cuba[5]
	430-853 Tlmm	390-920 Tlmm	3.03:1 (spawn.)	-	-	March to May,	Mexico[13]
	645 Tlmm	754 Tlmm	~1:1 (spawn.)	-	-	Feb-Apr (March)	St. Thms USVI[8]
	-	-	0.85:1 (Unexploited)	~510	-	Feb-Apr	Jamaica7,12
		-	-	-		Mar-Aug	Florida (USA)[11]

*= fishery dependent, **= fishery independent. [1]Bumett-Herkes 1975*; [2]Carter *et al.*. 1994**; [3]Colin 1992**; [4]Colin *et al.*. 1987*; [5]Garcia-Cagide and Garcia 1996*; [6]Kadison *et al.*. 2009**; [7]Munro *et al.*. 1973** ; [8]Nemeth *et al.*. 2004**; [9]Sadovy & Colin 1995*; [10]Sadovy *et al.* 1994**; [11]Taylor and McMichael 1983*; [12]Thompson & Munro 1978**; [13]Tuz-Sulub *et al.*. 2006*; [14]Whaylen *et al.*. 2004**; [15]Whiteman *et al.*. 2005**

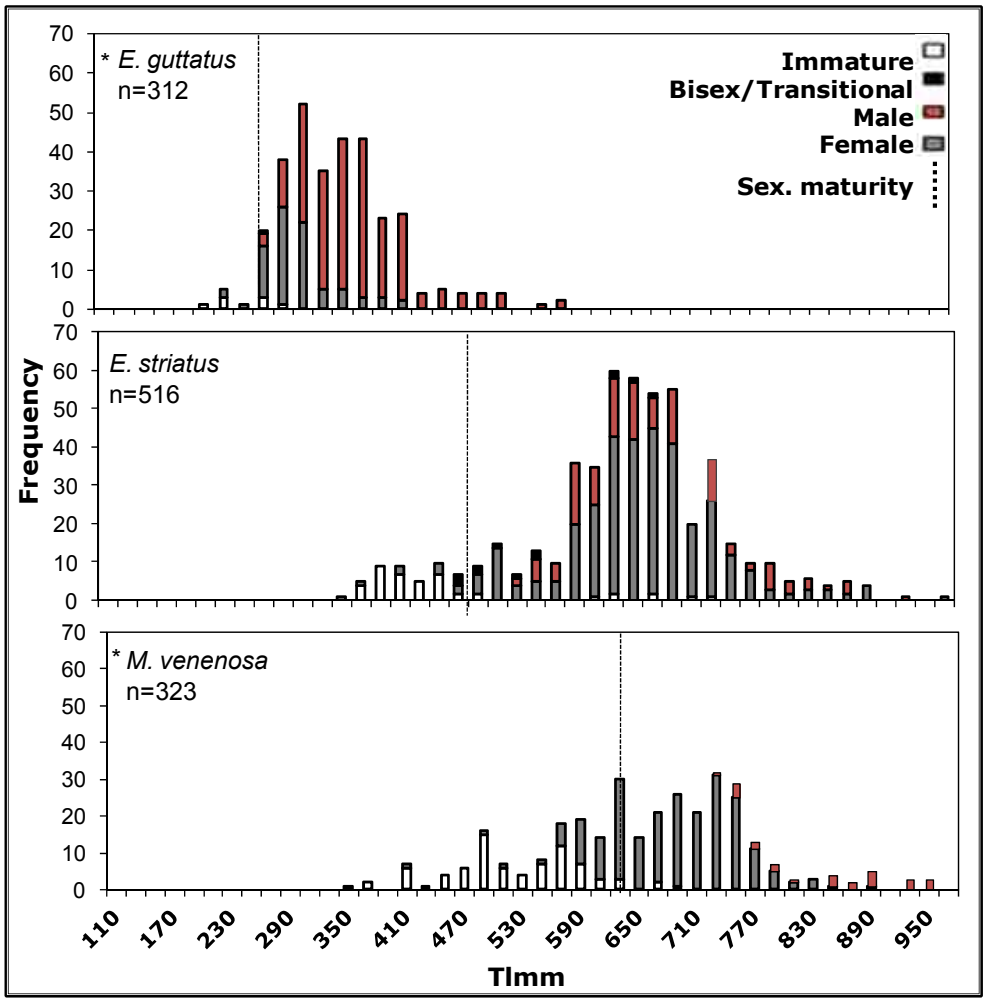


Figure 4.1. Size-frequency distribution for sexually immature, mature females and males and bisexual or transitional *Epinephelus guttatus*, *E. striatus* and *Mycteroperca venenosa* from The Bahamas (*E. striatus* 1999-2002, 2006-8; *E. guttatus*, *M. venenosa*, 2006-8). Sex by size-frequency distributions differed significantly for *E. guttatus* and *M. venenosa* (Kolmogorov-Smirnov, $P \leq 0.05$).

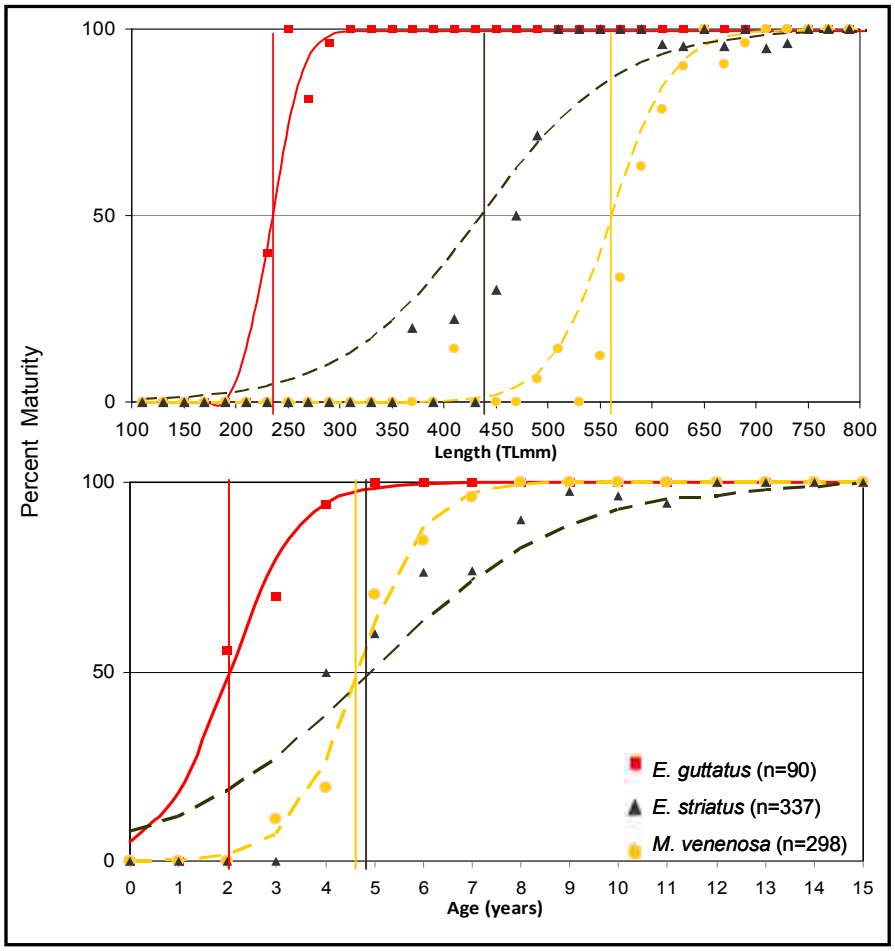


Figure 4.2. Percent of mature females at length (top) and age (bottom) for *Epinephelus guttatus*, *E. striatus* and *Mycteroperca venenosa* from The Bahamas (*E. striatus* 1999-2002, 2006-8; *E. guttatus*, *M. venenosa*, 2006-8). Proportions of sexually mature females within each size class and age are plotted with a binary logistic regression. Perpendicular lines indicate the length and age of 50% maturity for each species.

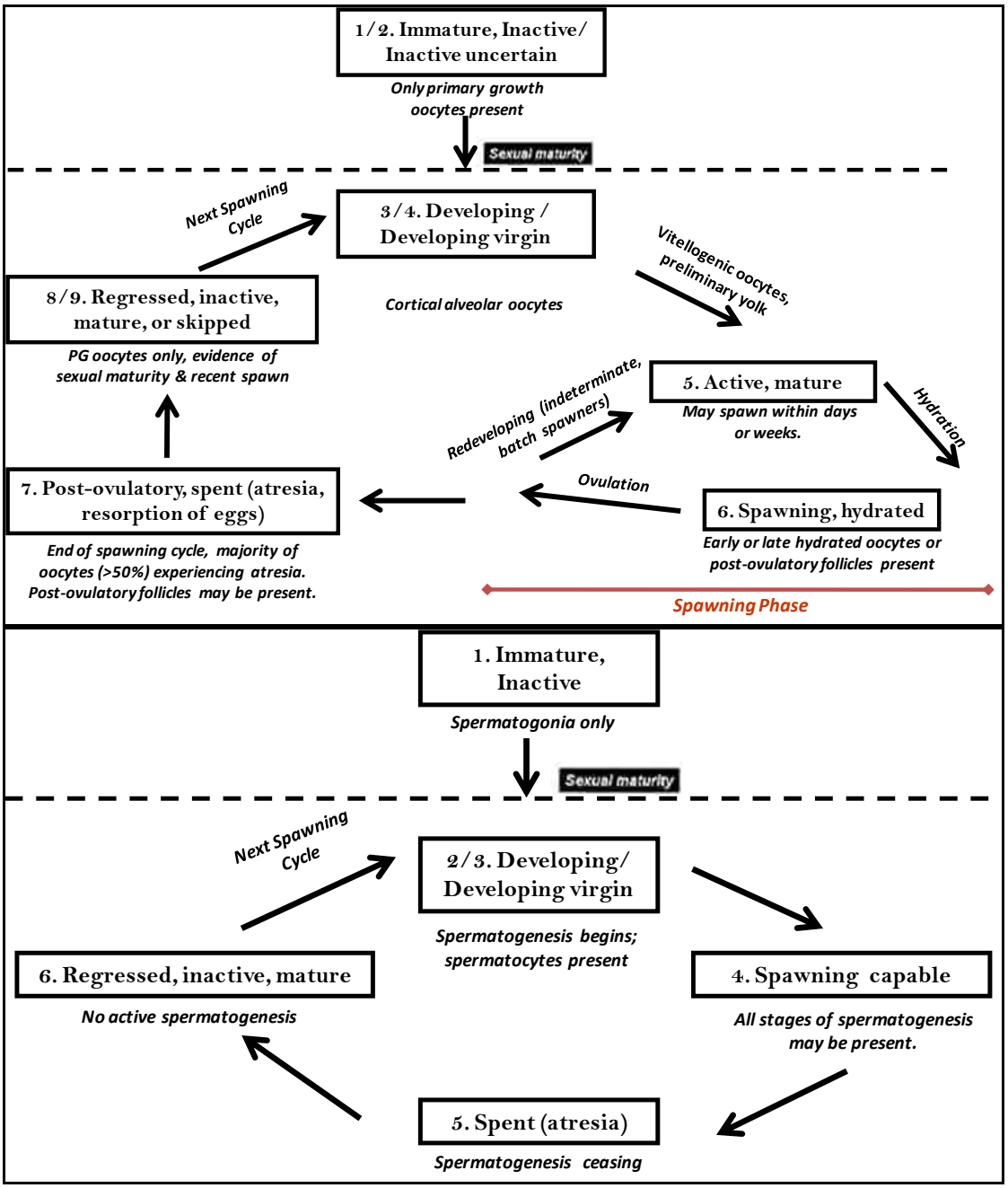


Figure 4.3. General diagram of the annual reproductive cycle and corresponding histological features of female and male Epinephelinae. The stages correspond to gamete development (see Table 3.1). Adapted from Brown-Peterson et al. (2008).

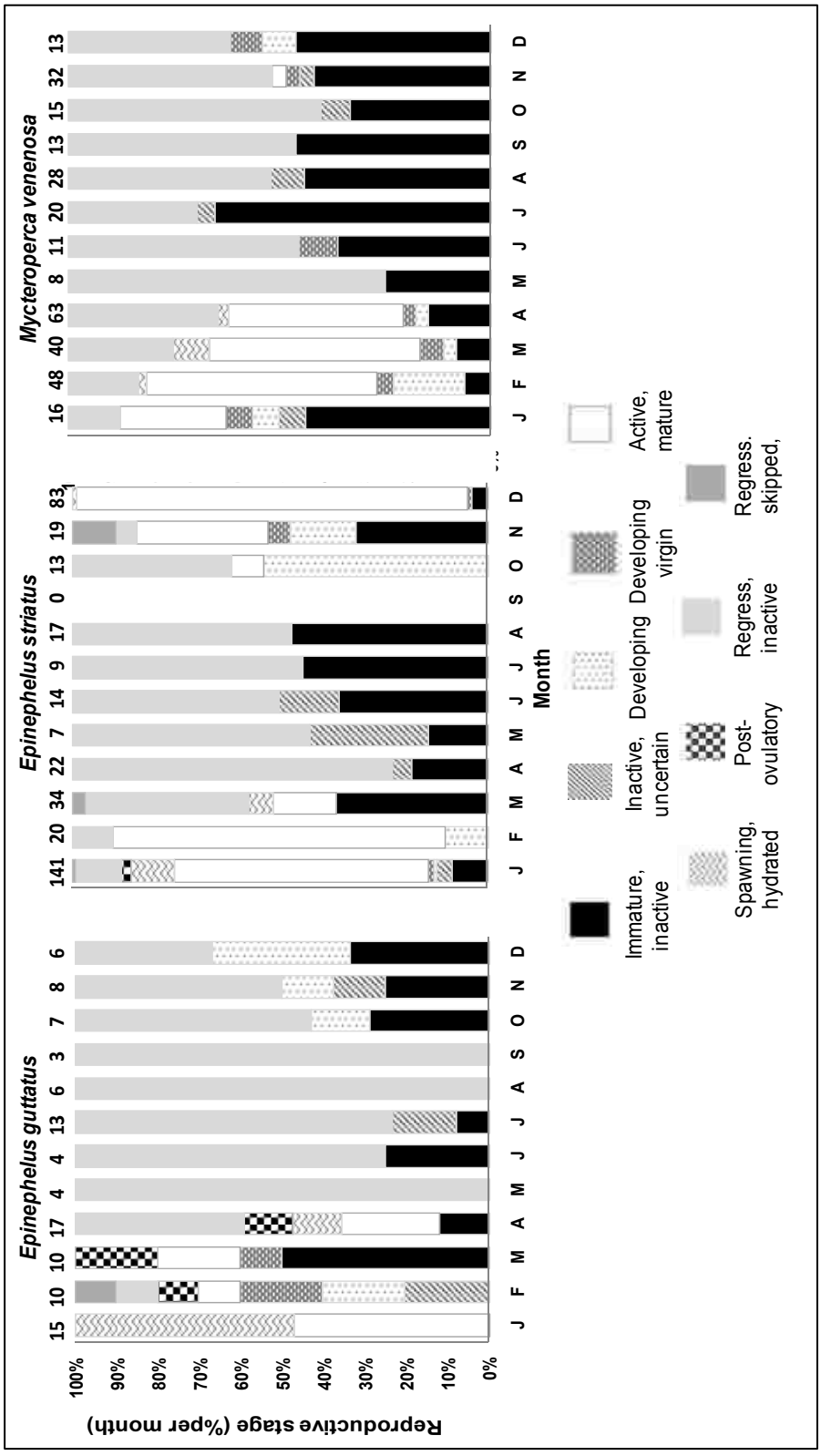


Figure 4.4. Monthly percent histology class frequencies of female *Epinephelus guttatus*, *E. striatus* and *Mycteroperca venenosa* harvested from The Bahamas (*E. striatus* 1999-2002, 2006-8; *E. guttatus* and *M. venenosa*, 2006-8). Samples were grouped by month across all years. Number of female fish sampled is given per month.

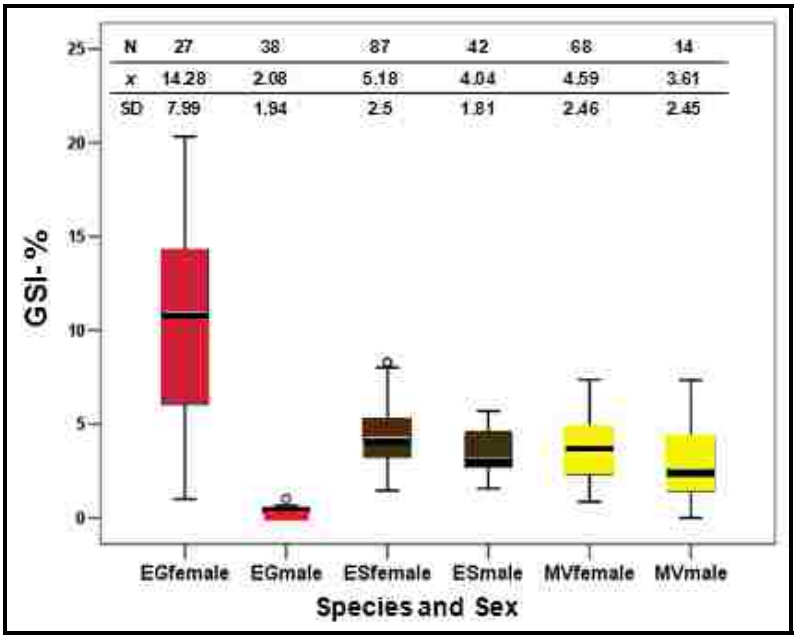


Figure 4.5. Box plots of mean gonadosomatic index (GSI) (%, ± SE) of female and male *Epinephelus guttatus*, *E. striatus* and *Mycteroperca venenosa.* The sample number (n), mean % (x) and standard deviation (SD) given above. Female and male *E. guttatus, E. striatus* and *M. venenosa* GSIs differ significantly (Mann-Whitney U, $p \geq 0.05$).

CHAPTER FIVE

ENERGY ALLOCATION TO GROWTH AND REPRODUCTION IN GROUPERS AND HINDS (FAMILY SERRANIDAE, SUBFAMILY EPINEPHELINAE)

Background

Growth and reproduction energy allocation patterns have been viewed as adaptive traits molded by natural selection to maximize the intrinsic rate of population increase (Calow 1985, Stearns 1993). A trade-off exists between allocating energy to growth or reproduction. Reproduction in fish typically entails large energy costs and correlates with a loss of somatic energy, slower growth rates, and possibly susceptibility to disease and predation post spawning. For physiologists and ecologists, important questions to thus answer are (Kamler 1992): 1) in terms of biomass production, when is fish growth maximum during its life-span and at what size or age does production begin to decrease, 2) how does reproductive energy allocation differ between sexes, and 3) how much energy is invested into reproduction annually and over a species life-span?.

Energy allocation studies are important because they provide specific quantifications of growth and reproduction costs which can then be related to life-history traits and reproductive strategies to understand species-specific differences (Henderson et al. 1995, Jonsonn and Jonsonn 1997, Aristizabal 2007). Life-history theory predicts that energy allocation patterns may differ among species to maximize reproductive success (Jonsson and Jonsson 1997). Accordingly, the purpose of the present study was to quantify and to contrast growth and reproduction energy allocation in three reef species

from the family Serranidae, subfamily Epinephelinae: *Epinephelus guttatus* (red hind), *E. striatus* (Nassau grouper) and *Mycteroperca venenosa* (yellowfin grouper). The reproductive strategies and energy allocation patterns of freshwater and temperate species have been well-documented and are known to differ from reef fishes (von Rooij et al. 1995, Bustamante et al. 2001). However, growth and reproduction energy allocation studies of reef fishes of the tropical western Atlantic Ocean are scarce (Bustamante et al. 2000).

The reproductive strategies of the majority of reef fish can be classified as (Winemiller and Rose 1992): 1) *opportunistic strategists*- small, rapidly maturing, short-lived fishes; 2) *periodic strategists*- larger, highly fecund fishes with longer life spans, and 3) *equilibrium strategists*- fishes of intermediate size that may exhibit parental investment and produce fewer, larger offspring. Most Epinephelinae species are periodic strategists and asynchronous spawners, meaning that gametes are recruited and ovulated over a protracted period during the spawning season. Research on the growth patterns and reproductive cycles of the study species has demonstrated that a large amount of energy is invested into annual reproductive cycles based on growth indices and gonad somatic index values (Chapters 2 and 4). There are however, notable variations in the overall reproductive strategies of *E. guttatus, E. striatus* and *M. venenosa*, such as their sexualities, gamete production modes and mating systems (Chapters 2 and 4), which may affect individual species energy allocation patterns. For example, spawning male *E. striatus* and *M. venenosa* were found to have considerably large GSIs as compared to other tropical grouper species (Chapter 4). The overall goals of the present study were to thus quantify and contrast between species: a) the annual energy investment into growth

(somatic), b) when each species transitions from allocating more energy to reproduction than growth, c) and to evaluate reproductive energy investment in gonad formation over an annual cycle in *E. guttatus, E. striatus* and *M. venenosa*. It was predicted that: 1) growth energy allocation would differ between the study species, 2) gametic energetic investment would differ between male and female *E. guttatus, E. striatus* and *M. venenosa* (each species tested independently) and 3) male *E. striatus* would invest more into sperm production as compared to *E. guttatus* and *M. venenosa*. For the present study gonad and muscle tissue samples were collected from male and female fish of varying sizes, ages, and reproductive development stages. The amount of energy invested into growth and reproduction was based on tissue proximate analysis (lipid and protein content).

Methods

Sample Acquisition

Monthly samples of muscle and gonad tissue were collected for *E. guttatus, E. striatus* and *M. venenosa* from Montague fish market in New Providence, The Bahamas over sixteen months from December 2006 until April 2008. This was a fishery-dependent, tissue-only collection from fishes landed by local fishers from the central Bahamas. Fish were weighed (g), measured (total length, Tlmm), the gonads were dissected to obtain total gonad weight (g), and gonad tissue samples were preserved in 10% neutral buffered formalin for histological processing (as per Cushion et al. 2008). Gonad and small somatic muscle tissue samples were frozen for proximate analyses. The Department of Marine Resources, Government of The Bahamas, and the US National Marine Fisheries Service (NOAA) provided additional samples and data on fish landed by fishers off the

coasts of Abaco and Long Island, The Bahamas during December 2000, January 2001, and January 2002. Histology slides of the gonad tissues to sex the fish and determine the reproductive stage of each sample (see Cushion et al. 2008).

Data Analyses

All data were tested for normality and homogeneity of variance. Nonparametric tests were conducted if data were not normally distributed. Results were considered significant if $p \leq 0.05$.

Proximate Composition and Energy-Content Determination

Water content was measured by drying approximately 1 g tissue samples at 60°C until constant weight was obtained (~30 hrs). Duplicate samples were taken from each tissue sample for both muscle and gonad tissue. Protein content (g $100g^{-1}$ wet mass) was determined by micro-Biuret method (Grodzinski et al. 1975) with Bovine Serum Albumin (BSA) as the standard. Samples were homogenized in 0.1 N NaOH to a 1:11 dilution using glass conical homogenizing tubes with approximately 0.5 grams of tissue for both muscle and gonad samples. The protein assay was run with 2 ml assay volumes with 50 and 100 µl aliquots run in duplicates. Tissue lipid content (g $100g^{-1}$ wet mass) was determined using a gravimetric method (Folch et al. 1953 and Hara et al. 1978). Approximately 0.3 grams of tissue samples were homogenized with 3-hexane/2-propanol (3:2) extraction solvent. Solutions were well mixed, then allowed to separate into water and organic solvent layers. The hexane liquid phase was extracted, evaporated and the lipid content was determined gravimetrically (weighed to the nearest milligram with an analytical scale). Duplicates were completed for each sample. Tissues were separated according to sex and reproductive state (spawning or non-spawning) (Chapter 4). The

percent composition (water, protein and lipids) wet weight (g $100g^{-1}$) was determined for the muscle and gonad tissues. Total gross energy content (kJ) of muscles and gonads was calculated from the following conversion factors: 39.6 kJ/g wet mass for lipids (Kamler 1992), and 20.1 kJ/g wet mass for proteins (Brett and Groves 1979).
The energy content (kJ $100g^{-1}$ wet mass) was estimated by adding the energy in protein and lipid of the tissues.

Somatic Growth and Production Energy ($P_{g,kj}$*)*

Somatic growth, the annual increase in biomass, was determined by estimating the biomass-at-age using the von Bertalanffy growth ($L_t = L_\infty(1-e^{-k(t-t0)})$) age-at-length estimates and then converting the lengths to mass using the mass-at-length relationships ($M=aL^b$) estimated for each species (Chapter 2).

	E. guttatus	*E. striatus*	*M. venenosa*
$M=aL^b$: (a,b)	$6.76*10^{-5}$, 2.81	$4.0*10^{-7}$, 3.20	$6.80*10^{-4}$, 2.75
VBGF: ($L\infty$, k, t_0)	571, 0.11, -1.2	932, 0.10, -1.7	977, 0.14, -1.5

The biomass-at-length estimates were a summation of cartilage, visceral contents, gonads and other non-somatic tissues. Since white muscle tissue, i.e., somatic tissue, accounts for 60-80% of finfish biomass (Hoffman et al. 1999, Sen 2005), biomass estimates were multiplied by 0.65, so as not to over-estimate biomass calculations. Somatic growth was calculated as:

Somatic growth = biomass for $year_2$ –biomass $year_1$, etc (equation 5.1)

The percent increase in biomass (g) was determined as:

Percent biomass increase = (total biomass for $year_2$ / total biomass $year_1$), *100, etc (equation 5.2)

Non-spawning, sex-specific protein and lipid proximate values were used to convert biomass into energy values (kJ g^{-1}). The energy (kJ) required to achieve the annual somatic growth, production energy ($P_{g,kj}$) (Kamler 1992), was then calculated as:

$$P_{g,kj} = \text{kJ } g^{-1} \text{ of muscle tissue} * \text{somatic growth (g)} \quad \text{(equation 5.3)}$$

Energy Investment into Reproduction

The gonadosomatic index (GSI) was used to express the gonad mass (g) to total the total body mass (g) (wet mass), and was calculated as (Wootton 1990):

$$\text{GSI (wet mass g, \%)} = (\text{wet mass of ovary or testis/total body mass}) * 100 \quad \text{(equation 5.4)}$$

Sex-specific proximate values of spawning fish were used for reproductive energy analyses. The GSI in energy (wet mass kJ, %) was used to express the energy ratio of gonads to somatic tissue (Wooton 1990) and was calculated as:

$$\text{GSI (kJ \%)} = (\text{energy of ovary or testis/total body energy}) * 100 \quad \text{(equation 5.5)}$$

Total body energy was calculated as body mass (g) of the spawning fish multiplied by 0.65.

Sex-specific GSIs (kJ %) of spawning fish were tested for significant differences between and within species with the Kruskal–Wallis test and Mann-Whitney *U* tests were used for pair-wise testing ($p \leq 0.05$). The mass (g) and energy (kJ) GSIs were tested for significant differences within sex (each species tested independently) with a Mann-Whitney *U* test ($p \leq 0.05$).

Reproductive effort (reproduction production, $P_{r,kJ}$, i.e. the energy released in gametes) is an estimate of the seasonal minimum energy invested into reproduction, based on the mass of the gonad (g) in actively spawning fish (Kamler 1992).

$P_{r,kJ}$ = kJ g^{-1} of gonad tissue * gonad biomass (g) of spawning fish (equation 5.6)

The net reproductive effort (NRE), i.e., the relative energy investment into gonadal tissue, was estimated as the relationship between the energy released into gonads ($P_{r,kJ}$) and the energy increment in somatic body growth ($P_{g,kj}$) (Mills and Eloranta 1985, Jonsson and B. Jonsson 1987, Kamler 1992) and was calculated as:

$$NRE\ (\%) = (P_{r,kj})/ (P_{g,kj} + P_{r,kj}) * 100 \text{ (equation 5.7)}$$

Results

Proximate Composition

An average of 96% of the tissue composition was measured by the protein, lipid and water content analyses (Table 5.1). The remaining constituents include carbohydrates and ash weight. Water was the greatest constituent for all tissue types. Gonad protein content (% wet mass) and lipid content (% wet mass) was greatest in spawning females. Non-spawning male muscle tissue contained the greatest average protein content (% wet mass). Male muscle protein content was similar between spawning and non-spawning samples. All muscle tissues contained less than two percent lipids (% wet mass). The total energy (% wet mass) of non-spawning female and spawning male gonads were approximately half as much as spawning ovaries (Table 5.1).

Somatic Growth and Growth Production ($P_{g,kj}$)

Somatic growth (g) per year was greater than 50% for all species between the ages of one to three years old (yo). *Epinephelus guttatus* had an average somatic growth of 53 (g) and $P_{g,kj}$ of 220 (kJ), and could grow to an estimated 1600 (g) during its maximum reported life-span of 19 years (Froese and Pauly 2010). During its maximum reported life-span of 29 years (Froese and Pauly 2010), *E. striatus* had an average somatic growth of 327 (g) and $P_{g,kj}$ 1360 (kJ) and could grow to an estimated 15,000 (g). *Myceteroperca venenosa* has a comparatively shorter life-span of 15 years (Froese and Pauly 2010), and had an average somatic growth of 345 (g) and $P_{g,kj}$ 1442 (kJ), and could grow to an estimated 10,000 (g) (Figure 5.2). The somatic growths (g) and $P_{g,kj}$ (kJ g-1 wet mass) values peaked at 7 yo for *M. venenosa*, 8 yo for *E. striatus* and 9 yo for *E. guttatus* and subsequently declined most rapidly annually in *M. venenosa*.

Energy Investment into Reproduction

For all species, males and females, the main spawning periods during which more than 50% of the histology samples were sexually mature coincided with the period when GSIs (g %) were at their annual maximum (Figure 5.2). For *E. guttatus*, the GSIs (g %) started to increase in November and female and male GSIs (g %) peaked in January. The GSIs (g %) for *E. striatus* started to increase in November and peaked January-February. For both female and male *E. guttatus* and *E. striatus*, GSIs (g %) decreased to less than 1% February- November. *Mycteroperca venenosa* female GSIs (g %) began to increase in November, females and males peaked in February and GSIs were less than 1% May-October. For all species, spawning females had significantly greater GSIs (g %) than their respective males (Mann-Whitney *U*, $p \leq 0.05$). Spawning male *E. striatus* had

significantly greater GSI (wet mass g, %) values than *E. guttatus* and *M. venenosa* and *M. venenosa* had significantly greater GSI (wet mass g, %) values than *E. guttatus* (Kruskal–Wallis, H= 25.221, Mann-Whitney *U* pair-wise comparisons, $p \leq 0.05$).

Epinephelus guttatus female GSIs (kJ %) were significantly greater than GSIs (g %) (Mann-Whitney *U,* $p = 0.04$); while *E. guttatus* male GSIs (g %) and GSIs (kJ %) were approximately equal (Table 5.2). *Epinephelus striatus* female and male GSIs (g %) were slightly greater than GSI (kJ %) values, but not significant. *Mycteroperca venenosa* female GSIs (kJ %) were significantly greater than GSI (g %) values (Mann-Whitney *U,* $p \leq 0.00$), while males GSI (g %) and GSI (kJ %) values were approximately equal (Table 5.2). *Epinephelus striatus* and *M. venenosa* males had significantly greater GSI (kJ %) values than *E. guttatus* (Kruskal–Wallis, H= 25.221, Mann-Whitney *U*, $p \leq 0.05$ for both pair-wise comparisons).

Regressed GSI (kJ %) values by length for each species and sex, show that only *E. guttatus* females had a positive increase in GSI (kJ %) as length increased (Figure 5.3). GSI (kJ %) values for all other species decreased with increasing length. NRE (%), the estimated gonad energy investment as a proportion of total tissue growth, slopes were the inverse of the GSI (kJ %) slopes for all species and sexes. NRE (%) effort increased with size for all species and sexes, with the exception of female *E. guttatus*.

Discussion

This was the first proximate analysis data set for muscle and gonad tissues of wild (non-aquaculture) tropical Epinephelinae species. These data are important because muscle and gonadal proximate composition values are highly variable between species and reproductive state, and data are scarce for tropical reef species (Bustamante et al. 2000).

All species are carnivores and have a similar sub-adult to adult diet which consists of crabs and fish (Froese and Pauly 2010). However, there are differences in how the study species allocate energy to growth and reproduction. For each of the three study species, the prediction that growth energy allocation would differ between the study species was supported by the values determined for annual somatic growth (g), energy to somatic growth ($P_{g,kj}$), and percent biomass increase. The differences in annual somatic growth between *E. striatus* and *M. venenosa* are indicative of their differing growth patterns. *Epinephelus striatus* lengthens at a slower rate, and allocates mass more width-wise as compared to *M. venenosa* (Chapter 2). Thus, *E. striatus* delegates more energy ($P_{g,kj}$) to somatic growth on average as compared to *M. venenosa*. In *M. venenosa*, somatic growth substantially declines after ~7 yo; whereas the same pattern does not occur in *E. guttatus* and *E. striatus* until ~ 10 yo. This is likely because *M. venenosa* has a shorter life-span (Froese and Pauly 2010).

The approximate ages and sizes when 50% of a population is sexually mature (L_{50}) are ~4.0 yo and 435 Tlmm for *E. striatus*; and ~4.6 yo and 561 Tlmm for *M. venenosa* (see Chapter 4). For both species, the percent increase in biomass begins to reduce to less than 50% around their respective L_{50} estimates. The same pattern was not found for *E. guttatus*, where there was a greater than 50% increase in biomass at L_{50} (~2.0 yo, 235 Tlmm). This may be related to the smaller maximum body size of *E. guttatus* compared to *E. striatus* and *M. venenosa*. Growing faster is a predator-avoidance strategy (Wooton 1995), thus *E. guttatus* may delegate more energy to somatic growth over a given timeframe than *E. striatus* and *M. venenosa*. Thus, young *E. guttatus* appear to allocate differently to growth and reproduction as compared to *E.*

striatus and *M. venenosa* and would need to consume comparatively greater amounts of food to increase body mass and invest so greatly in reproduction.

The proximate protein and lipid values for female and male gonads equate to females investing more energy into gamete production per gram of tissue. A 100 g female gonad equals approximately 600 kJ, while a male 100 g gonad equals approximately 360 kJ. The hypothesis that reproductive energetic investment will differ between males and females was thus accepted. The significant difference between female and male GSI (kJ %) is due to females having a greater percentage of lipids than males.

The proximate values also allowed for GSI (g %) to be quantified into energy GSI (kJ %). This is important, because while the GSI (g %) allows for the general determination of spawning seasonality and effort, it is not an accurate assessment of reproductive energy input. This is because GSI (g %) is based on wet weight values and water comprises the greatest proportion of fish tissues, but varies greatly between tissues and with age (Henderson et al. 1996, Lupatscha et al. 2003). GSI (g %) and GSI (kJ %) values were not equivalent for a given species and sex, emphasizing that caution should be used when interpreting GSI (g %) in terms of energy investment. Significantly greater GSI (kJ %) values for *E. guttatus* and *M. venenosa* demonstrate that traditionally used GSI (g %) underestimates reproductive effort in these species. The opposite is true for male *E. striatus* which have the comparatively greater GSI (g %) than GSI (kJ %) values.

The lack of substantial muscle lipid concentrations or changes, but increased gonad lipid concentration during reproduction, indicates that these fish make little use of muscle tissue as a depot for reproduction energy. The study species are asynchronous

spawners and may be considered ‘income spawners’, which eat throughout the reproductive season and utilize current ‘income’ for reproductive investment (Sibly and Calow 1986, Stearns 1989). This is in contrast to synchronous spawners that spawn only once a season, such as salmon, cod and herring (Aristizabal 2007). Synchronous spawners are often ‘capital spawners’ that build up a reserve of energy (capital) for the spawning season and usually reduce their food intake during the season and mobilize nutrients from endogenous reserves in muscle, adipose tissue and liver (Sibly and Calow 1986, Rijnsdorp 1989). Additional samples over an annual cycle of adipose tissue and liver tissues would be needed to more accurately assess the degree to which Epinephelinae species are ‘income’ spawners, because there is likely some energy storage prior to reproduction.

Except for female *E. guttatus*, GSI (kJ %) decreases or remains constant as the fish grow, while the NRE (%), the proportion of annual somatic energy investment ($P_{g,kJ}$) to reproduction energy investment ($P_{r,kJ}$) increases as the fish grow. The NRE slope for male *E. guttatus* and *M. venenosa* is steeper than the *E. striatus* NRE slope. Thus, while *E. striatus* and *M. venenosa* males had significantly greater GSI (kJ %) values than *E. guttatus*, the hypothesis that male *E. striatus* will invest more into sperm production as compared to *E. guttatus* and *M. venenosa* was not fully supported. This may relate to the sexuality of the males. *Epinephelus striatus* is gonochoristic species, and *E. guttatus* and *M. venenosa* are protogynous species. Therefore, there are proportionally more male *E. striatus* per population, as compared to *E. guttatus* and *M. venenosa*, and male *E. striatus* contribute gametes throughout the life-span of the species. In contrast, *E. guttatus* and *M. venenosa* populations have theoretically female-dominated sex ratios and males only

contribute during a portion of the life-span of the species, and thus need to increase their NRE as they grow. The decreasing NRE of female *E. guttatus*, but increasing NRE for female *E. striatus* and *M. venenosa* is a notable finding which may be related to *E. guttatus* having a different growth form and gamete production system. *Epinephelus guttatus* is a determinate spawner, and thus does not continually produce eggs, as *E. striatus* and *M. venenosa* do during a spawning bout. This fact along with the pattern of continual percent increase in biomass during the sub-adult to adult phases highlights a unique energy allocation pattern in *E. guttatus*, as compared to *E. striatus* and *M. venenosa*. Female *E. striatus* and *M. venenosa* have the steepest NRE positive slopes, which is typical for slow-growing species which invest more into reproduction as they grow (Winemiller and Rose 1992).

The present study was designed to increase understanding of how energy is allocated to growth and reproduction in Epinephelinae species and to estimate the gonad reproductive energy costs in these species. The estimates do not take into account gamete production, spawning frequency and reproductive behavior, all of which have energetic costs and greatly influence reproductive output (Kamler 1992, Sadovy 1996). The female reproduction energy estimates are likely conservative because they do not take into account spawning frequency, which can be multiple times a season for all species (Sadovy et al. 1993, Sadovy and Colin 1995, and Garcia-Cagide and Garcia 1996). Male estimates are not a comprehensive reflection of reproductive energy investment because males often expend a large amount of energy towards defending spawning territories and/or sperm competition behaviors (Sadovy 1996). The findings of the present study are nonetheless valuable for understanding general differences in life-

span growth and reproduction energetics of tropical marine species and for comparing energetics among the study species.

Allocation to reproduction earlier or later in life is likely to influence the entire demographic structure and population dynamics (Roff 2000). The reproductive potential output of a population may be well overestimated if size and sex-based reproductive investment is not considered. This factor has been cited to partially explain the often poor relationship between spawning stock size and recruitment (Henderson et al. 1996). This finding also stresses the reproductive value of mature females, and highlights the need for size-specific harvest regulations.

This is the first study to investigate reproductive and growth energy investment in Epinephelinae species in the TWA. The findings highlight that growth and reproductive energy investment differ between the species and these differences are partially related to unique aspects of their reproductive strategies.

Table 5.1. Mean (± SD) proximate composition for muscle and gonads tissues by reproductive state and sex of *Epinephelus guttatus*, *E. striatus* and *M. venenosa* (samples pooled). Calculated energy values (kJ/g) reported for g wet mass. Kilojoule energy conversions were 39.6 kJ/g for lipids (Kamler 1992) and 20.1 kJ/g for proteins (Brett and Groves 1979).

		Reprod. Stage	Protein (%)		n	Lipids (%)		n	Water (%)		n	Total (%)	Total energy (kJ/g)
Female	Ovaries	Active/ Vitellogenic	21.8	± 2.0	35	5.8	± 4.2	23	69.8	± 3.4	35	97.3	6
		Spawning/ Hydrated	16.1	± 5.4	6	4.9	± 1.5	6	75.2	± 3.8	8	96.3	4.7
		Regressed/ Inactive	15.7	± 4.0	18	1.6	± 0.5	9	79.5	±2.9	18	96.8	3.3
Male	Testes	Spawning capable	16.4	± 2.8	25	2.1	± 0.8	12	80.1	± 4.3	25	98.6	3.7
Female	Muscle	Active/ Vitellogenic	19.8	± 1.2	44	1.5	± 3.8	18	75.3	± 2.6	44	96.6	4
		Regressed/ Inactive	21.4	± 4.1	12	1.2	± 3.3	8	73.1	± 3.9	12	95.8	4.2
Male		Spawning capable	22.8	± 1.7	17	1.7	± 2.6	12	69.4	± 3.8	17	93.9	4.6
		Regressed/ Inactive	23.9	± 5.4	12	1.2	± 4.7	7	71.4	± 4.3	18	96.6	4.6

Table 5.2. Sex and size class (50 Tlmm, total length mm) gonadosomatic index (GSI) in percent mass (%, g) and percent energy (%, kj) for *Epinephelus guttatus*, *E. striatus* and *M. venenosa* harvested from The Bahamas. For each size-class the largest reproductively active fish was chosen. For GSI (kj, %), somatic muscle and gonad percentages from proximate analyses (Table 5.1) and energy conversions were 39.6 kJ/g for lipids (Kamler 1992), and 20.1 kJ/g for proteins (Brett and Groves 1979). * = significant differences between GSI (g %) and GSI (kJ %) (Mann Whitney *U*, $p \geq 0.05$). (Full data set not shown) (Tlmm= total length mm.)

	E. guttatus				***E. striatus***				***M. venenosa***			
	F*		M		F		M		F*		M	
Tlmm	**g**	**kJ**	**g**	**kJ**	**g**	**kJ**	**g**	**kJ**	**g**	**kJ**	**g**	**kJ**
250	20	31										
300	18	19	4	4								
350	31	34	5	2								
400			4	3								
450			8	3								
500					4	4						
550					13	11	13	11				
600					18	13	18	13	8	15		
650					14	11	10	8	10	18		
700					10	8	14	8	12	25		
750					16	12	5	3	11	25	8	9
800					4	3	14	11	6	15	4	5
850							3	3	4	7	5	5
900									4	10	4	4
950											1	2

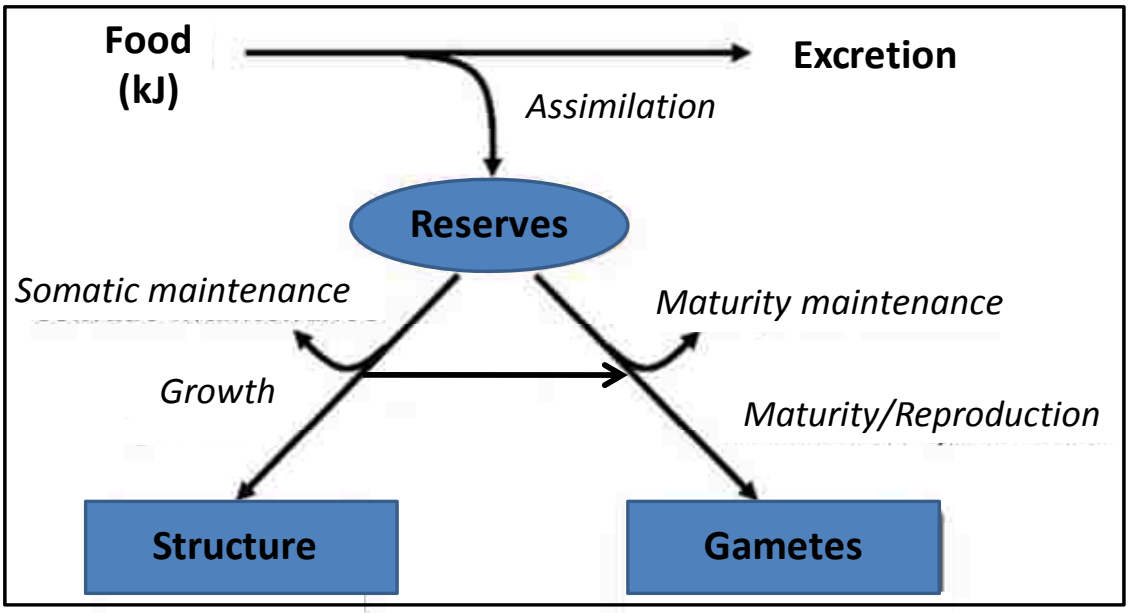


Figure 5.1. General diagram of fish energy allocation. After maintenance requirements are met, excess energy can be diverted to growth and/or reproduction (gametes) once a fish is sexually mature. Adapted from Kooijman (2000).

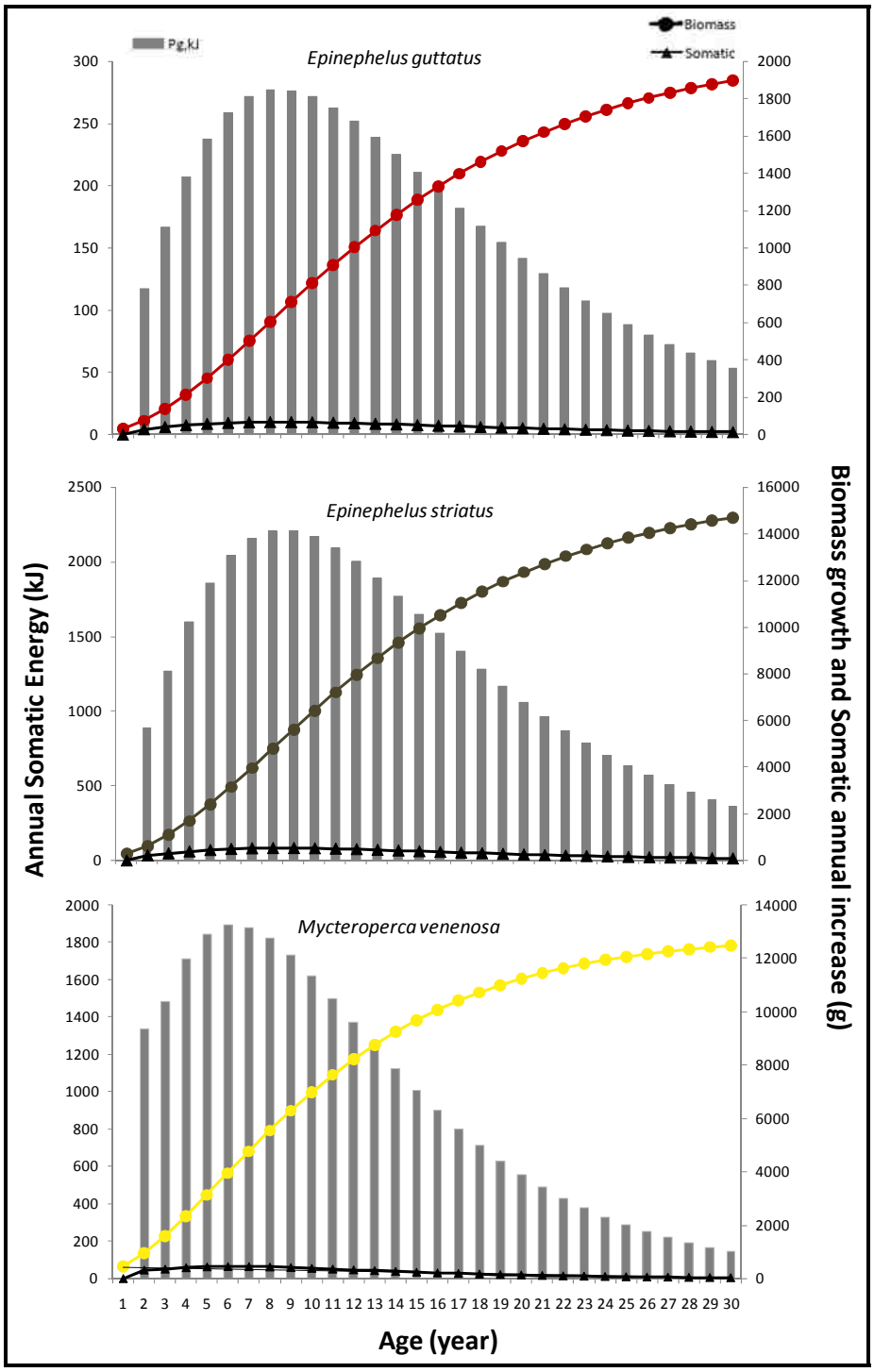


Figure 5.2. Lifespan growth energy allocation. Annual somatic energy input ($P_{g,kj}$) (kJ) (y-left) and somatic growth biomass (—●—) and annual biomass increase (g) (—▲—) (y-right) for *Epinephelus guttatus*, *E. striatus*, and *M. venenosa*. Somatic and annual growth (g) estimated from species-specific weight-length relationships and von Bertalanffy growth curves (Chapter 2). Calculated energy values (kJ g-1) calculated wet muscle. (Note y-axes scales differ.

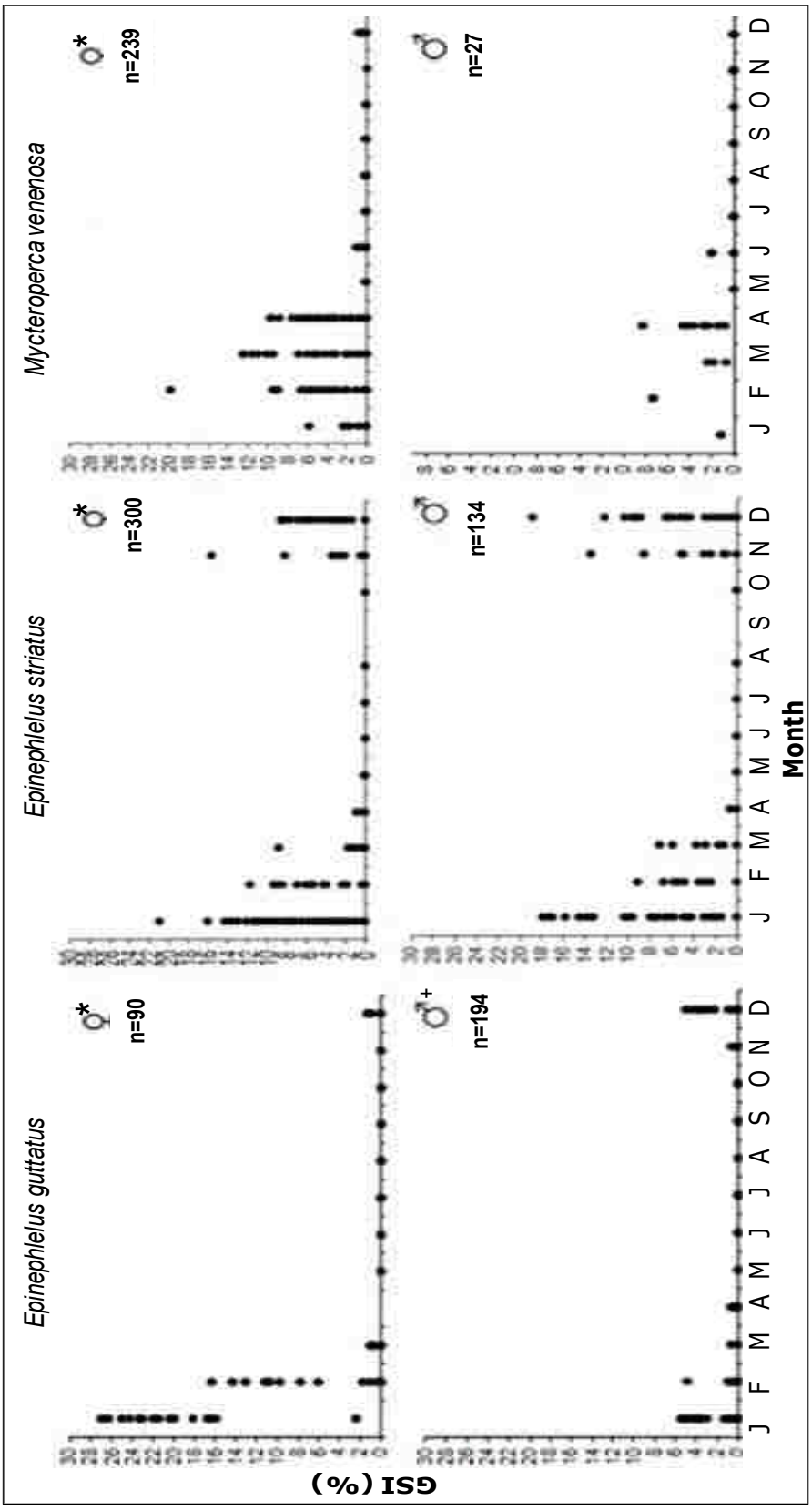
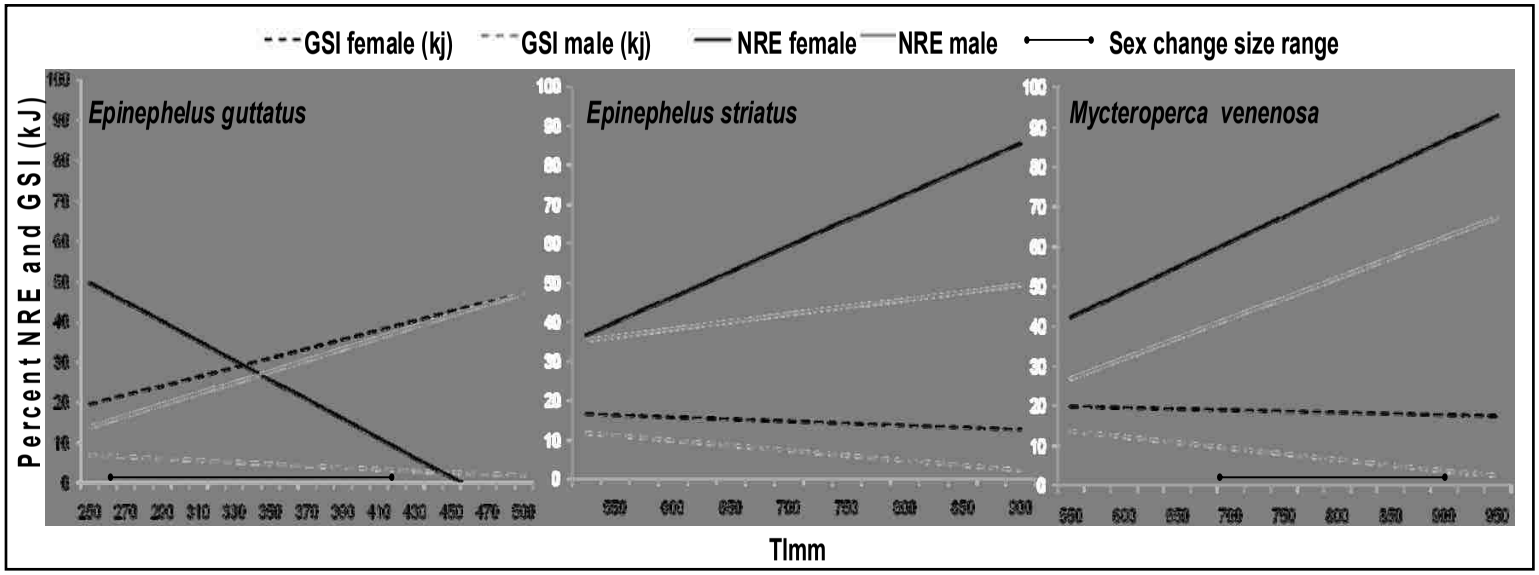


Figure 5.3. The annual gonadosomatic index cycle for *Epinephelus guttatus*, *E. striatus* and *M. venenosa* harvested from The Bahamas. *= significant differences between sexes, $p \leq 0.05$ (all species), Mann-Whitney *U* tests. + = significant differences between male GSIs, H= 25.22, p = 0.02, Kruskal–Wallis, NG/RH and YF/RH, Mann-Whitney *U*, $p \leq 0.05$.

Figure 5.4. Energy investment into reproduction. Regression lines of the energy gonadosomatic index (GSI, kJ, wet weight) (%) and Net Reproductive Effort (NRE) (%). NRE was estimated as the relationship between the energy released into gonads ($P_{r,kj}$) and the energy increment in somatic body growth ($P_{g,kj}$) and as was calculated as: $[(P_{r,kj})/ (P_{g,kj} + P_{r,kj}) *100]$. Values as a function of size are shown for spawning male and female *Epinephelus guttatus*, *E. striatus* and *M. venenosa*. (Tl= total length mm.)



CHAPTER SIX

CONCLUSION: THE EFFECTS OF FISHING ON LIFE-HISTORY TRAITS AND MANAGEMENT IMPLICATIONS

Summary and Conclusions

The main objectives of my studies were to determine growth parameters, reproductive life-history traits (LHT) and growth and reproduction energy allocation patterns for *Epinephelus guttatus* (red hind), *E. striatus* (Nassau grouper) and *Mycteroperca venenosa* (yellowfin grouper) from The Bahamas. The results were then related to their species-specific reproductive strategies. My research included the following novel contributions: a) the first comprehensive age and growth and reproductive biology LHT dataset for Bahamian populations of *E. guttatus*, *E. striatus* and *M. venenosa*; b) a detailed overview and comparative analyses of the reproductive strategies of the study species; and c) the first Epinenphelinae growth and reproduction energy allocation study. The findings of my studies allow for a greater understanding of how reproductive strategies can vary between closely related Epinephelinae species, and how the inter- and intra-species LHT variation may influence the reproductive potential of each species or a population. Moreover, valuable species- and population-specific LHT data was obtained for Bahamian populations the study species. The findings of this body of research should be considered in the context that the datasets are fishery-dependent obtained from a small-scale commercial fishery and the study species are heavily harvested in The Bahamas.

Five major conclusions were drawn from my dissertation studies:

1. There are key differences in the overall reproductive strategies of *E. guttatus*, *E. striatus*, and *M. venenosa*, i.e., "not all groupers are created equal";
2. The overall reproductive strategy of a species and intra-species LHT variation will affect the overall reproductive output of each species and population;
3. Fecundity and reproductive energy investment increase with age and/or size, thus the reproductive potential of the study species must be considered within relatively long life-spans of the study species;
4. The population-specific information presented for Bahamiam populations of the study species is critical for assessing the present population states, for monitoring population changes, and for assessing the success of management initiatives; and
5. To be effective, management initiatives need to consider the impact of harvesting on LHTs and species-specific reproductive strategies.

To address conclusion number five, a review of the effects of size-selective fishing on LHTs is presented, as well as, management implications and suggestions.

Over-fishing and the Effects of Size-Selective Fishing on Life-History Traits

Over-fishing of reef fish via artisanal, small- and/or large-scale fisheries is a global problem which can be segregated into two distinct phases (Pauly et al. 1998, Jackson et al. 2001). Initially, fishers primarily target the largest, most commercially valuable species, such as groupers and hinds, which detrimentally affects LHTs (Table 6.2) (Sadovy 2001, Conover and Munch 2002, de Roos et al. 2004, Yemane et al. 2008). Once the commercially valuable species become scarce, fishers target lower-valued species, such as parrotfish and surgeonfish, which is known as "fishing down the food web" (Pauly et al. 1998). Thus, top-level trophic species, such as the study species, may experience both direct top-down (via fishing mortality) and indirect bottom-up (via decreased prey) effects of fishing (Jackson et al. 2001).

Size-selective fishing (SSF) of the largest fish and the high mortality rates exerted on fish populations from commercial fisheries leads to detrimental, fisheries-induced evolution of LHTs such that population reproductive output and recruitment becomes diminished (Conover and Munch 2002, de Roos et al. 2004, Heino and Dieckmann 2009) (Table 6.2). Epinephelinae species are comparatively more susceptible to over-fishing than other reef fish species because of their slow growth rates, late sexual maturation and increased fecundity with size (Adams 1980, Shapiro 1987, Gilmore and Jones 1992, Coleman et al. 1996, Sadovy 2002). Additionally, aspects of a species reproductive mode, such as its sexuality and mating system (see Figure 1.2 and Table 1.1), may make one species more vulnerable than another to SSF (Table 6.2) (Gilmore and Jones 1992, Buxton 1993, Coleman et at. 1996, Sadovy 2002, Alonzo and Mangel 2004).

The size spectra, mean length, mean length-at-age, and maximum length and ages of a population are typically the first LHTs to be perceivably impacted by SSF due to the removal of the largest fish (Gislason and Rice 1998, Sluka and Sullivan 1997,Chiappone et al. 2000, Graham et al. 2005, Conover and Munch 2002, Yemane et al. 2008). As fishing intensity increases the size spectra of a population becomes more truncated due to a greater reduction of larger size classes than smaller size classes (Graham et al. 2005). The removal of the largest fish likewise leads to a decline in the mean length, mean length-at-age, and maximum length and ages of a population (Rochet 1998, Walters and Wilderbuer 2000, Yemane et al. 2008). Additionally, protogynous populations may have a greater decrease in the average male length as compared to gonochoristic populations because males are the largest fish and thus comparatively more males may be removed Buxton (1993) (Table 6.2).

Growth rates and spawning population biomass have been shown to be negatively affected by SSF, sometimes within only a few generations (Buxton 1993, Conover and Munch 2002). Buxton (1993) found significantly slower population growth rates in unprotected seabream populations (*Chrysoblephus cristiceps*¸ a protogynous reef species) in South Africa, as compared to protected seabreams. Conover and Munch (2002) tested the effects of three different harvest regimes (removal of small size classes only, random size removal and removal of large size classes only) on silverside fish (*Menidia menidia*) populations over multiple generations. Population-level differences in biomass were found due to increased juvenile growth rates in small-harvested populations and decreased juvenile growth in large-harvested lines. Thus, SSF selects for slower growing individuals, which leads to a decrease in the average weight-at-age (i.e., decline in condition-at-age). Slower growth rates could have large population impacts because the pelagic larval duration increases, which has been shown to lead to a decrease in survivorship and recruitment success (Suthers 1998, Bergenius et al. 2002). Also, slower-growing individuals are more susceptible to predators (Leggett and DeBlois 1994, Conover and Munch 2002).

Intense SSF also selects for a smaller length and/or younger age of sexual maturation (Jørgensen 1990, Buxton 1993, Tripple 1995, Conover and Munch 2002, de Roos et al. 2006), as well as, a smaller length and/or younger age of sex change (Buxton 1993) (Table 6.2). Tripple (1995) compiled analysis of historical records of Atlantic cod (*Gadus morhua*) and revealed substantial decreases in the size and age of sexual maturity (greater than three years for some populations) within the last four decades. Buxton (1993) found that the size at sex change was also significantly smaller for unprotected

seabreams (*C. cristiceps*) versus protected seabreams in South Africa. At the population level, a smaller length of sexual maturity and sex change can have large reproductive output and population growth implications. By allocating energy to reproduction at a smaller size, a growth is negated and theoretically predation risk increases. A decrease in the length of sex change in protogynous species limits the reproductive "window of opportunity" for females. Also, the relative fecundity of groupers fecundity increases exponentially with size (Shapiro 1987, Whiteman et al. 2005), thus smaller fish contribute far fewer gametes as compared large fish. Thus, a population has a much greater potential fecundity when it is composed of larger verses smaller individuals (Bohnsack 1999).

For species which aggregate to spawn, such as *E. striatus* and *M. venenosa*, the removal of the largest fish has been shown to have detrimental effects on spawning dynamics, and thus overall reproductive output (Gilmore and Jones 1992, Coleman et al. 1996, Whaylen et al. 2004, Kadison et al. 2009). Groupers return to the same spawning sites in consecutive years and newly recruited, inexperienced fish follow the older fish. Thus, by fishing the oldest, largest fish, critical spawning behavior knowledge is lost (Gilmore and Jones 1992, Coleman et al. 1996). At *E. striatus* spawning aggregations in the Cayman Islands, Whaylen et al. (2004) found that there was much less spawning activity (in terms gamete release) at aggregation sites where the population was severely depleted prior to protection, as compared to protected sites which had more and larger fish. Additionally, Pattengill-Semmens (2010) tagged *E. striatus* in the Cayman Islands and found that males leave the spawning aggregation sites and return multiple times during a spawning period, possibly in an effort to recruit younger fish to the spawning

site. Contrary, because *E. guttatus* spawn in smaller social groups (Shapiro et al. 1993), it is plausible that *E. guttatus* spawning populations are impacted less by fishing, and may show a comparatively more rapid recovery once fishing is reduced (Nemeth 2005).

The spawning dynamics of protogynous species may be differentially affected as compared to gonochoristic species, because the selective removal of the largest fish (i.e., males) may greatly alter sex ratios, leading to possible sperm limitation. Coleman et al. (1996) found that heavily-harvested gag grouper (*Mycteroperca microlepis*) and scamp (*M. phenax*) populations in the Gulf of Mexico suffered a drop in the proportion of males from 17-1 percent and 36- 18 percent, respectively, over a 20 year span. While, the sex ratios red grouper (*Epinephelus morio*) populations, which live solitarily and do not aggregate to spawn, showed little during the same timeframe (Coleman et al. 1996). Additionally, female fish may not spawn if there are not enough males to warrant the release of gametes, and resorb the eggs to keep the energy (Rideout et al. 2005).

Experimental and *in situ* studies of harvest restrictions and marine protected areas (MPAs) have shown that populations have an intrinsic capacity to recover from detrimental LHT changes caused by fishing (e.g. Bohnsack 1999, Chiappone et al. 2000, Nemeth 2005, Conover et al. 2009). Conover et al. (2009) experimentally removed only the largest silverside fish (*M. menidia*) and the populations evolved slower growth rates, smaller body sizes and reduced yield, but displayed reverse evolution back towards their original state after size-selective fishing was relaxed. The authors noted that recovery of large-bodied fish with long life-spans may take decades. Chiappone et al. (2000) examined grouper populations (nine species) in fished and protected areas of the Florida Keys, Bahamas and northern Caribbean. The density and biomass of larger grouper

species were significantly greater in the no-take marine reserve (Exuma Cays Land and Sea Park, The Bahamas).

In the USVI, Nemeth (2005) studied the effects of a spawning season fishery closure and a MPA with no extraction allowed on *E. guttatus* LHTs and population parameters. With only the seasonal closure, the average size of *E. guttatus* increased (100 mm over 12 yr), and the maximum total length of male red hind increased by nearly 70 mm following the establishment of the MPA with a permanent fishing closure. Also, the average density and biomass of spawning red hind increased by over 60% following permanent closure and maximum spawning density more than doubled (Nemeth 2005). The studies demonstrate that populations have an intrinsic capacity to recover from harmful evolutionary changes caused by fishing.

Management

The susceptibility of groupers to over-fishing has been widely documented (Shapiro 1987, Coleman et al. 1996, Sluka et al. 1997, Chiappone et al. 2000, Conover and Munch 2002, Sadovy and Domeir 2005, de Roos 2006). If management initiatives are to be successful, the life-histories of the species need to be considered (Tables 6.1 and 6.2). Present management initiatives in The Bahamas for groupers include a three pound catch minimum, some MPAs throughout the country with varying degrees of management, and a seasonal fishery closure of *E. striatus* was mandated from December 1- February 28 (2005-2008), but shorted to January 1- February 28 in 2009. Additionally, there are many public outreach campaigns which advocate the need to protect and conserve *E. striatus*. There is strong evidence that these regulations are insufficient and *E. guttatus*, *E. striatus* and *M. venenosa* populations are likely to collapse if fishing intensity remains

consistent or increases. The catch minimum of greater than 3 lbs (~1360 g), equates to approximately 434 Tlmm (total length mm) for *E. striatus* and 435 Tlmm for *M. venenosa*. These sizes are below the estimated average size of sexual maturity for these species (435 Tlmm, *E. striatus*; 561 Tlmm, *M. venenosa*), and thus fish are being harvested before they have had an opportunity to reproduce. In addition, fully hydrated, ready-to-spawn, *E. guttatus* were sampled during this study, confirming fishermen are targeting *E. guttatus* spawning areas (Appendix B).

The single-species *E. striatus* closure may be inadvertently leading to excessive harvest of other species. Cushion and Sealey (2007) documented commercial fish landings at Montagu ramp, New Providence, The Bahamas and noted the pattern of increased grouper landings, especially *M. venenosa* and *E. guttatus*, during the *E. striatus* closure period. Rhodes and Tupper (2007) found a similar scenario when they analyzed Epinephelinae landings in Pohnpei, Micronesia. During a seasonal fishing ban on certain Epinephelinae, fishing pressure (measured as total fish mass) increased for parrotfish and emperor fish. Additionally, Cushion and Sealy (2007) documented preliminary evidence of the second phase over-fishing from Montagu ramp landings. Landings were dominated by two families, Lutjanidae (snappers) and Serranidae (groupers and hinds), and the most abundant catch species were lane (*Lutjanis synagris*), Nassau grouper (*Epinephelus striatus*) and yellowtail snapper (*Ocyurus chrysurus*), respectively. However, a diversity of other families were also targeted including, Haemulidae (grunts and margates), Carangidae (jacks), Scaridae (parrotfish) and Sphyraenidae (barracuda), Balistidae (triggerfish), and Labridae (wrasse).

The precautionary approach to fisheries management needs to be taken before populations reach a reproductive "tipping" point and population recovery is unlikely (Richards and Maguire 1998). The precautionary approach as outlined by the Food and Agricultural Organization (1995) says, "States shall be more cautious when information is uncertain, unreliable or inadequate. The absence of adequate scientific information shall not be used as a reason for postponing or failing to take conservation and management measures and improved methods are required for dealing with risk and uncertainty."

Management initiatives should include market-based management measures, such as size limits and gear restrictions and seasonal fishing closures, which take into account the overall reproductive strategies of the species and manage key LHTs (Rhodes and Tupper 2007. Globally, fishing effort is increasing due to the incorporation of technologies such as outboard motors, handheld global positioning units and scuba. SCUBA and compressor (hookah) -based spear fishing emerged as a huge threat and has led to decimated fisheries where it has not been made illegal (e.g. American Somoa, Sabater and Tofaeono 2002; American Somoa, Sabetian and Foale 2006; China, Godoy et al. 2010). It is illegal to use SCUBA gear while fishing in The Bahamas, however compressor (hookah) spearfishing is allowed within a certain depth range. Cushion and Sealey (2007) documented many boats with and wide-use of compressors with multiple hookah hoses by fishermen. The landings of groupers, hinds and other species (e.g. hogfish, *Lachnolaimus maximus*) from these boats were significantly greater than those which used hand lines or nets and interviewed fishermen noted that they did not dive within the depth limits (Sealey and Cushion, unpublished data, Appendix B). Also,

compressor air is not safe to breathe while diving and many fishermen do not know or follow SCUBA depth and time limit guidelines. This has led to (Cushion, unpublished data), and will continue to lead to fishermen getting decompression illness, which may be fatal. Spear fishing with compressors should thus be made illegal because it is not a sustainable form of fishing and the depth regulations are impossible to monitor. For size limits, a precautionary approach would be to incorporate a size minimum that exceeds the minimum size at sexual maturity for smaller species, such as *E. guttatus*, while encompassing the minimum size at sexual maturity for some larger-bodied groupers, such as *E. striatus* and *M. venenosa* (Rhodes and Tupper 1997). Also, size restrictions should protect the larger female and male size classes of each species, versus the smaller size classes, to protect the most fecund, breeding portions of each population (Sadovy 2002). For more vulnerable or heavily targeted species, species-specific size restrictions may be necessary. Lastly, because the peak spawning months of the study species are similar in The Bahamas (Table 6.1), a seasonal fishing closure of all groupers (and hinds) during the winter-spring spawning period is a viable management strategy. Additionally, because many species utilize spawning aggregation sites, fishing of any species at a spawning site should be banned.

Lastly, all attempts to garner public or community support when implementing protected areas are beneficial. Education and outreach efforts to the fishermen are strongly encouraged. Collaborating with fishermen who have vast experience and local environmental knowledge can provide valuable information to researchers on historical and active aggregation sites (Bohnsack 1999, Whalen et al. 2004).

Table 6.1. Compilation of the reproductive strategy and life-history trait data from this study for *Epinephelus guttatus*, *E. striatus* and *Mycteroperca venenosa* harvested from The Bahamas (*E. striatus* 1999-2002, 2006-8) (*E. guttatus*, *M. venenosa* (2006-8) (*E. striatus* 1999-2002, 2006-8, *E. guttatus* and *M. venenosa*, 2006-8).

Species	Sexuality	Spawning style	Growth rate (von Bertalanffy), life-span, max length	Primary spawning season months	a. Size and age 50% sexual maturity and b. Sex change length and age range	Sex-specific GSIs	Reproductive effort with size increase
Red Hind (*E. guttatus)*	Protogynous	Small groups aggregation site, 10-100's fish	Slow (0.11), 17 yrs, 565 Tlmm	J-F	a. 235 Tlmm: ~2.05yo b. 257-401 Tlmm: ~4-5 yo	F > M, very small male GSIs	F decreases: M increases
Nassau grouper (*E. striatus*)	Gonochoristic	Large group spawning, 100's of fish	Slow (0.10), 22 yrs, 925 Tlmm	D-F	a. 435 Tlmm: ~ 4yo b. n/a	F ~> M, large male GSIs	F increases: M increases
Yellowfin grouper (*M. venenosa*)	Protogynous	Large group spawning, 100's of fish	Comparatively faster (0.14), 13 yrs, 939Tlmm	M-A	a. 561 TLmm, ~4.7yo b. 716-871 Tlmm: ~8-9 yo	F > M, but some large male GSIs	F increases: M increases

Table 6.2. Overview of the effects of size-selective harvesting on life-history traits.

Life-History Traits	Average Size and Age	Growth and Biomass	Sex Ratio	Age/Size Sexual Maturation and Sex Change	Fecundity and Spawning Stock Biomass
Selection	1. Removal of longest and oldest Protogynous species- removal of more fish. 2. males and largest females.	1. Removal of faster growing fish and heaviest fish. 2. Protogynous species- removal of more males and largest females.	1. Proportionally more males are removed[3], especially during spawning periods.[1,2] 2. Fishing methods may be male-slective.[2]	1. Removal of longest and oldest fish. 2. Protogynous species- removal of more males and largest females.	1. Removal of highly fecund larger individuals. 2. Protogynous species- selective removal of proportionally more males at spawning aggregations.[2]
Population Effects	1. Truncated size and age range.[3] 2. Decrease in the average populations size and /or age.[3,4] For protogynous fish, a greater decrease in the average male size.[4] 3. Decrease in the average size-at-age.[4] 4. Loss of spawning behavior knowledge and reproductive output. -Sub-optimal mating opportunities and conditions, loss of spawning behavioral knowledge.[6] - Fishing the oldest, largest fish leaves no experienced fish to train recruits.[3,4,] 5. In spawning aggregations where the largest fish have been harvested there is less reproductive activity.[3,8]	1. Decrease in the average weight-at-age (i.e., decline in condition-at-age).[2] 2. Decrease in growth rates. - Slower growth would lengthen larval duration, perhaps leading to increased risk of larval mortality.[2,3] - Decreased growth rates may also lead to increased sub- adult predation because of the smaller relative size.	1. For protogynous species, a lack of males could pose a possible sperm shortage. Also, females may not spawn if there are not enough males.[1,2] 2. The largest males arrive at aggregation sites before females and also leave aggregation sites to recruit and search for mates, thus they may be disproportionately harvested during spawning periods if only aggregation sites are protected (protogynous and gonochoristic species.[2,3]	1. Decrease in mean maturation length decrease.[1,5,6] 2. For protogynous species, a decrease in size of sexual change [1,2] 3. Both 1 & 2 will which lead to diminished reproductive output because fecundity increases with size.[2,4]	1. Decrease in spawning stock biomass.[4] 2. Allee effect, fish spawn less because the aggregation density is too small.[6,8] 3. Diminished reproductive output because fecundity increases with size.[2] 4. Egg sizes may become significantly smaller, which may affect embryo quality and viability.[2,7] 5. Protogynous more affected than gonochoristic species, because possible sperm limitation in protogynous fish. Also, females may not spawn if there are not enough males.[1,6]

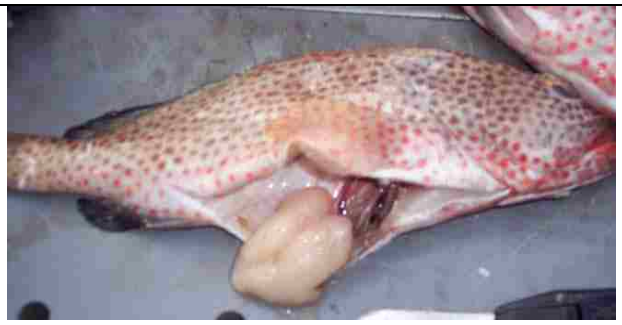
[1]Buxton 1993 [2]Coleman et al. 1996, [3]Conover and Munch 2002, [4]de Roos et al. 2006, [5]Fromentin and Fonteneau 2001, [6]Gilmore and Jones 1992, [7]Mikaela et al. 2002, [8]Whaylen et al. 2004.

Appendices

Appendix A. Photographs of sexually mature, ripe, male (top) and female (bottom) *Mycteroperca venenosa* gonads collected at Montagu ramp, New Providence, The Bahamas 2007-2008. The size of the male gonads is uncommon for Epinephelinae in the tropical western Atlantic Ocean. *Epinephelus striatus* males also have comparatively large ripe gonads.

	A) The gonad of a ripe male sampled 2/22/08. 716 Tlmm, 4611 g total mass, 315 g gonad mass = 7.3% GSI
	B) Female (top) and male (bottom) *M. venenosa* gonads. Male: 3/22/08, 889 Tlmm, 9086 g total mass, 240 g gonad mass, = 2.7% GSI.

Appendix B. Photos of landings from Montagu Ramp, New Providence, The Bahamas landings (2006-2008). The photos depict: landings from compressor spear-fishing, size-selective fishing, fishing of ready-to-spawn fish, sexually immature fish being harvested and the targeting of lower-valued species, such as parrotfish and surgeonfish. All of which indicate high-value species such as groupers are becoming less abundant in The Bahamas.

<table>
<tr><td>a. Landings from one day of compressor spear fishing. Multiple trophic levels are being fished, and the largest groupers and hinds are being targeted. The landings from compressor-based spear fishing far exceed non-compressor spear fishing landings.</td><td>  </td></tr>
<tr><td rowspan="2">b & c. A ready-to-spawn Epinephelus guttatus female and a sexually immature Mycteroperca venenosa. This highlights that E. guttatus spawning areas are being targeted. Also, size-restrictions need to protect sexually immature, and highly fecund fish.</td><td>  </td></tr>
<tr><td>  </td></tr>
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