Aspects of the Ecology and Reproduction of Nassau Grouper, *Epinephelus striatus*, Off the Coast of Belize, Central America

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ABSTRACT

Aspects of the life history of Nassau grouper, *Epinephelus striatus*, from Belize in the western Caribbean are described, including food habits, movements, protogyny, sexual maturation, seasonality, periodicity of spawning, fecundity, and population sex ratios. Additional information is provided on the potential effect overfishing spawning aggregations has on future reproductive potential of the population.

Gonad structure and ontogenetic development patterns are described and evidence for sex—reversal is given. Seasonal variation in the percentage of sexually active fishes indicate the majority of fishes aggregated and spawned along the shelf edge at the reef promontories around the full moon in December and January. A comparison of sex ratios and length frequency distribution among three sites indicates that there is a marked increase in the number of females relative to males at heavily fished sites, as well as a significant decrease in the size of both sexes relative to the general population outside of the spawning banks and at pristine spawning aggregation sites.

Spawning of Nassau grouper is discussed in relationship to environmental conditions (*e.g.*, reef geomorphology, temperature, photoperiod, current, etc.). Finally food habits of Nassau grouper are described and related to foraging strategy and daily patterns of movement.

KEYWORDS: Belize, ecology, protogyny, reefs, reproduction, serranidae, Nassau grouper.

INTRODUCTION

The Nassau grouper, *Epinephelus striatus* (Bloch), is one of the most common large serranids in the Caribbean (Randall, 1968) and forms a major portion of the commercial and sport catch of coral reef fishes. (Munro, 1973; Thompson and Munro, 1978, 1983). *E. striatus*, occurs in a broad area between the (23°C) isotherm that stretches from Bermuda and Florida, throughout the Yucatan Peninsula to Venezuela (Bardach *et al.*, 1958; Smith, 1961, 1971). The Nassau grouper is primarily insular and most abundant throughout its range in the West Indies. It appears to be absent from the Gulf of Mexico where it is replaced by its closest relative, the Red grouper, *E. morio*, a species which prefers to inhabit broad continental shelf areas (Moe, 1969). In northern latitudes, the young of a few have been taken north of these limits. Smith (1971) suggest such distributional patterns of larval Nassau groupers are the result of passive transport of pelagic larvae by ocean currents. Nassau grouper of all size and age are most abundant in relatively shallow waters, less that 50 meters, in and about coral reefs, and adjacent sea grass beds (Robins *et al.*, 1986). Unlike many gregarious "nuclear" coral reef species (Robertson *et al.*, 1976), Nassau grouper lead solitary lives often seeking shelter in abundant reef crevices and caves and rarely venturing far from cover (Bohlke and Chaplin, 1968; Smith, 1971; Carter, 1986, 1988, 1989). Nassau grouper are bottom-dwelling solitary predators consuming large quantities of small fishes and invertebrates (Randall, 1965; Randall and Brock, 1960). Although it is adept at rapidly changing colors to match its' background (Carter, 1986), Nassau grouper most frequently exhibit five irregular dark brown bars that circle the body, a broad black saddle patch near the base of the tail fin, and a prominent dark streak running from the snout through the eye.

Published accounts to date have focused primarily on adults and very little is known regarding the ecology of the early life history stages of Nassau grouper. Much of Nassau grouper early life history (i.e., growth rates, settlement time, etc.) has been inferred from studies on related species of epepheline groupers common to Pacific Ocean waters (Ukawa, *et al.*, 1966; Chen *et al.*, 1977; and Hossain and Higuchi, 1980). Guitart and Juarez (1966) reported briefly on spawning by *E. striatus* in the Havana Cuba public aquarium. Additional information has been provided by a recent series of several extensive field studies focused on the early life history of Nassau grouper, particularly as it relates to environmental (oceanographic) conditions (Colin, 1990). Finally, investigators have recently successfully spawned wild stock Nassau grouper and are currently rearing approximately 90 individuals (J. Tucker, pers. comm.).

Nassau groupers are recognized as one of the most commercially important food fishes inhabiting tropical western Atlantic reefs and offer a valuable source of income to local fishermen (Munro, 1973). In Belize, from 1972-1984, groupers, primarily *E. striatus*, constituted the second most commonly caught

and most valuable family of marine fishes. In 1984, 200,000 pounds of grouper were caught, worth $546,650 (Vasquez, 1984). In Belize, Nassau grouper are taken primarily with handlines, spear guns, and fish pots throughout the year, but the most intensive fishing in Belize (Carter, 1986, 1989) and probably generally throughout the Caribbean area (Olsen and La Place, 1979), is over localized spawning aggregations during one or two weeks each year. Localized spawning aggregates of *E. striatus* have been known to local fisherman throughout the Caribbean for generations and in Belize have provided the basis for a flourishing folk fishery (Craig, 1969). Smith (1972) observed such a spawning aggregation, estimated to consist of 30,000-100,000 individuals during late January off Cat Cay in the Bahamas. Similar spawning aggregations have also been observed in waters off Jamaica (Olsen and La Place, 1979), Cayman Islands (Colin *et al.*, 1987; Tucker, pers. comm.), Roatan, Honduras and Mexico's Yucatan (pers. obs.), and the Bahamas (Colin, 1990).

Intensive fishing over aggregations are thought to have potentially severe detrimental effects on future fishing yields (Olsen and La Place, 1979; Shapiro, 1984a.). Aggregation fishing directly removes reproductively active fish from the spawning ground and thus may have severe detrimental effects on future fishing yields. For example, in the 1950's local Belizean fishermen landed grouper catches well in excess of 100,000 pounds annually (Craig, 1969). In striking contrast fishermen landed less than 30,000 pounds in 1986 (Chief fisheries officer for Belize, pers. comm.). According to Shapiro (1987) the effect of intensive fishing on future reproductive potential cannot be fully evaluated until we gain a better understanding of grouper population structure, social organization, breeding behavior, and proximal cause of sex change.

Nassau grouper are believed to be protogynous hermaphrodites (Smith, 1959; Thompson and Munro, 1978; Carter, 1989) wherein most individuals function first as females and later as a male. Changing sex via protogyny not only occurs in all groupers carefully studied thus far (Shapiro, 1987; Sadovy and Shapiro, 1987) but is widespread among coral-reef fishes in general (Atz, 1964; Reinboth, 1970; Smith, 1975; Robertson *et al*, 1982; Chan and Yeung, 1983). According to Shapiro (1987), protogyny in Nassau groupers challenges our understanding of the evolutionary basis and selection advantage (if any) of sex change (Ghiselin, 1969; Warner, 1975). In theory, protogyny should evolve whenever an individual can produce more offspring by changing sex than by remaining strict gonochore. Nassau grouper, as well as many other species of large epinephelines, stand as an enigma since the "so-called" adapted behaviors common to nearly all protogynous coral-reef fish (Robertson and Choat, 1974; Thresher, 1984) are not seen in these fishes.

Despite obvious academic and commercial interests, there is insufficient published information at the present time, to describe the life history of this valuable commercial and recreational species in the Caribbean in sufficient

detail for the development of "workable" fisheries management models (Bannerot *et al*, 1987).

Studies of its reproductive biology, with particular attention to protogyny, are few. In response to this need, this report describes aspects of the life history of *E. striatus* from Belize in the western Caribbean, including food habits, daily and seasonal movements, protogyny, sexual maturation, seasonality, periodicity of spawning, fecundity, and population sex ratios. Additional information is provided on the potential effect overfishing spawning aggregation has on future reproductive potential of the population.

MATERIALS AND METHODS

Study Areas

The majority of specimens were taken from coral reef habitats along the Belize barrier reef and atolls by spear gun, handlines, and a very few were collected by antillean style wire mesh fish traps. Figure 1 illustrates the general areas from which the fish were taken. Systematic sampling was begun in august 1984 and continued on a monthly basis through July 1987 at various localities throughout the reef complex. During the spawning seasons, our sampling effort was focused on two widely separated localized aggregation sites or "grouper banks" as they are locally known. Cay Glory, situated about mid-way along the main frame of the barrier reef, has been extensively fished commercially since the early 1920's (Craig, 1969) and functioned in our experimental design as the "exploited" aggregation. In comparison, the spawning aggregation site Northern Two Cay, located at the northeast tip of Lighthouse reef atoll, had been only recently commercially fished by fishermen (*ca* 12 years ago) and served as a control or our "unexploited" aggregation site. Both spawning aggregation sites were similar in bottom morphology. Typically each is at or near the seaward end of a broad, relatively low relief coral shelf extending approximately 100-200 meters eastward of the exposed reef crest. The shelf slopes gently to about 27-30 meters. From this depth, the shelf drops away steeply to another "ledge" at approximately 50 meters. Beyond this point the narrow rocky shelf ledge drops away vertically to abyssal depths. The geomorphology of both spawning aggregation sites are further distinguished by the fact that each forms a distinct promontory or chevron-shaped wedge profile, clearly evident from aerial photographs.

Field and Laboratory Procedures

Epinephelus striatus were weighed to the nearest gram and total length (TL) and (SL) lengths were recorded to the nearest millimeter. Sagittae and scales were removed and stored dry in envelopes for subsequent age and growth studies determination. Whenever possible each fish was dissected and its stomach excised if not conspicuously empty. Each stomach was labeled,
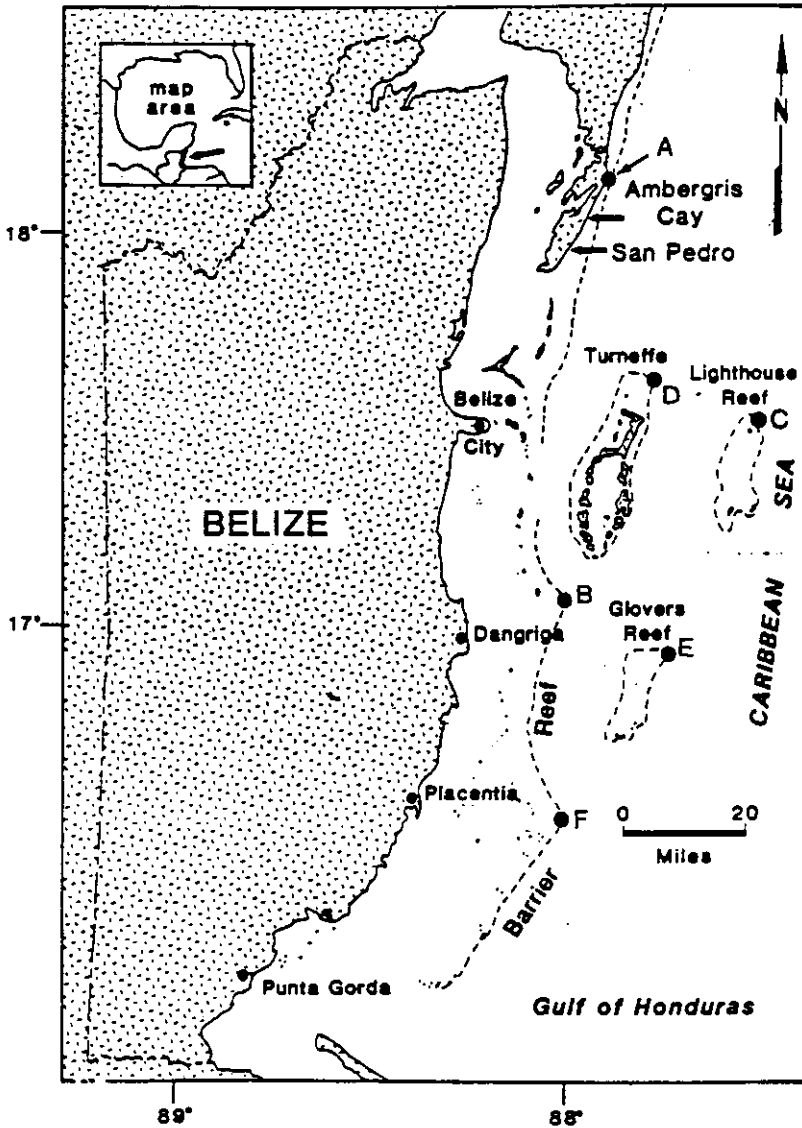
Figure 1. Map of Belize barrier reef and offshore atolls, showing Cay Glory exploited grouper bank (B), Northern Two Cay unexploited grouper bank (C) and other reported grouper banks (A,D,E,F) where breeding aggregations occur along the 30m shelf break.

wrapped and fixed in 10% seawater formalin. Following fixation, stomachs were transferred to 40% isopropanol. Stomachs were cut open and gut contents were sorted by lowest possible taxa and counted. Wet weight of the food item was measured by using pan and/or analytical balance.

Reproductive organs from *E. striatus* were removed at sea, weighed to the nearest gram and fixed in 5% seawater formalin. After fixation the tissues were transferred to 50% isopropanol, processed through an Auto-Technician 2A Tissue Processor, vacuum infiltrated, and blocked in paraffin. Sections (7µm) were cut from each gonad by a rotary microtome, stained with Harris hematoxylin, and counter-stained with eosin-y. Histological sections from representative fish were read by at least two observers to develop agreement on sex and maturity stages; the remaining sections were then read by a single observer. Sex and maturity stages were modified from Smith (1965), Hilge (1977), Mercer (1978), and Wenner *et al* (1986) to determine size and inferred age at first maturity, spawning season, and sex composition (Table 1.). The stage of gametogenesis and terminology used in gonadal descriptions follow Smith (1965), Moe (1969), Wallace and Selman (1981), and Wenner *et al.*(1985). Males were defined as individuals whose gonads consisted entirely of testicular tissue, functional testicular tissue (active spermatogenesis) as well as traces of inactive ovarian tissue. Females were defined as either having entirely ovarian gonads or inactive testicular tissue in a functional ovary. Transitional gonads included only those with obvious proliferating testicular tissue within a nonactive, and/or regressing ovary.

For fecundity determination, gonads from selected maturing females were removed at sea, split open with a longitudinal incision, and placed in Gilson's solution (Bagenal, 1978). Oocytes were washed and stored in 70% isopropyl alcohol after digestion of the ovarian tunic and connective tissue and then diluted to 1L for enumeration. Three to five 1 mL subsamples were removed from suspension, transferred to a petri dish, and counted at a magnification of 10X. Total fecundity was estimated by expanding the mean of the subsamples to the total sample volume. Total fecundity was related to length by standard least squares linear regression (Sokal and Rohlf, 1981). Gonadosomatic index (GSI), i.e., gonad weight divided by body weight, multiplied by 100, was also plotted for selected specimens in monthly intervals.

Two methods were employed to observe the activities and movements of the fish: (1) ultrasonic tracking of individual fish and (2) direct underwater observations while using scuba. Fish were captured in chevron-shaped wire traps, baited with small fishes and set on the ocean floor in approximately 60 feet of water. Groupers caught in the traps were measured, weighed, and placed in a pen enclosure for 1-2 days prior to implanting the transmitter. The transmitter emitted pulsed signals at 75 KHz (kilohertz) and measures 16 x 60 mm (manufactured by &CTT-82 Sonotronics). The paraffin-coated,

Table 1. Histological criteria used in determining gonaldal condition of Nassau grouper, *Epinephelus striatus*. (Modified from Wenner *et al*. 1986)

Gonad Class	Testicular State	Ovarian State
Immature	Little or no spermatocyte development.	Small (100m) basophilic oocytes.
Developing	A few primary and secondary spermatocytes through lumina filled with spermatozoa.	Predominance of oocytes with yolk vesicle formation through late vitellogenesis
Ripe (running)	Predominance of spermatozoa, little active spermatogenesis.	Late vitellogenesis presence of hydrated oocytes.
Spent	No spermatogenic activity, some residual sperm present in tubules.	Unspawned, mature oocytes undergoing atresia.
Resting	Some mitotic regeneration of spermatogonia and interstitial tissues.	Predominance of small basophilic oocytes with residual traces of atresia.
Transitional	—	Inactive or regressing ovarian tissue with concurrent testicular proliferation.

cigar-shaped transmitter is inserted through a small incision in the fish's abdomen. After the cut is sutured and antibiotics administered, the fish were held in an enclosure for 24 hours to insure that the fish were responsive and that the transmitter was operating normally. Fish were released at sea near location of original capture and tracked from an 8 meter skiff. The unidirectional signal was monitored with hydrophone and sonic receiver (model DH-1 and USR-5 respectively, Sonotronics) following procedures described by Olla *et al* (1974). Fish location was recorded in relation to local landmarks. Fish were considered active whenever a change in transmitter signal was detected. Direct underwater observations confirmed that we were able to detect abrupt changes in fish orientation and straight line movement over 1 meter. The data were subsequently condensed to indicate movement of a fish for a period of time at a specific location. For each track, we recorded current direction, stage of tide, cloud cover, water temperature, water depth, and salinity. Temperature and

salinity were measured with a field thermometer and refractometer respectively. Cloud cover, water depth and tidal stage were visually estimated. In addition to our tracking, we directly observed fishes in the study area with scuba.

Additional field work was conducted at each Nassau grouper spawning locality covering two complete spawning seasons (December 1984 - February 1985 and December 1985 = February 1986. Observations of grouper distribution, courtship, and pre/post spawning were made using scuba. Diving operations were conducted from a series of small sailboats and skiffs anchored at the aggregation site. While sampling fishes we also conducted a series of interviews with local fishermen in an attempt to gather information regarding length of time of fishing, number of boats, catch per unit effort, and other "soft" data. On one occasion current speed and direction were measured using current meters deployed directly over the grouper spawning aggregation. Meters were on wire moorings with subsurface floats in bottom depths of 20-27 m. Concomitant with current measurements, an attempt was made to follow the immediate movement of eggs and larvae by deploying a florescent dye dumped into the water column and tracked using a portable navigation system over an eight hour period.

RESULTS
Gonad Structure and Ontogentic Development
The ovarian structure of *E. striatus* is similar to that of *E. fulva* (Smith, 1965), (*E. morio* Moe, 1969), *Hemanthias vivanus* (Hastings, 1981), *Centropristus striata* (Wenner *et al.*, 1985), and *E. cruentatus*(Nagelkerken, 1979). The bilobate ovary is suspended by mesenteries from the swim bladder in the posterior region of the body cavity. The lobes fuse posteriad, and their lumina form a common oviduct. Oocytes develop and mature within the folded germinal epithelium. Germinal epithelium does not occur in the common oviduct or on the ventral portion of the lumen, probably allowing for distension of ripe gonad without damage to the germinal epithelium. Although E. striatus is believed to be a protogynous hermaphrodite (Smith, 1959), the extent to which this species is protogynous is still unclear. Unlike the well-studied protogynous black sea bass *C. striata*, (Wenner *et al.*, 1985), testicular precursor cells were not evident bordering the lamellar regions of most female gonads. However, a very few individuals exhibited active proliferation of identifiable spermatogenic tissue concomitant with inactive and/or degenerating ovarian tissue. In sexually mature males, spermatozoa is produced and collected in sperm sinuses in the center of the lamellae and then pass to a central collecting sinus that extends along the dorsal surface of the gonad. A sperm duct forms between the urinary duct and the oviduct.

Histological sections of immature ovaries contained oogonia and previtelogenic oocytes about 20-200um in diameter. Maturing ovaries contained

oocytes about 150-550 um in diameter, in stages from early through late vitellogenesis. Ripe ovaries contained oocytes 450 um - 800 um in diameter with evidence of coalescence of yolk globules and oocyte hydration. Spent and resting ovaries contained atretic oocytes and empty ruptured follicles.

Immature males were characterized as young testes with proliferation of seminiferous tissue concomitant with the presence of inactive stage 2 oocytes. This stage is actually an extension of transitional female stage. Developing male gonads were characterized by the occurrence of primary and secondary spermatocytes concomitant with sperm sinuses filled with mature spermatozoa. Ripe male testes were characterized by the packing of sperm sinuses and ducts with tailed spermatozoa. At this stage, the entire gonad is distended. Spent testes were characterized by an absence of spermatogenic activity and presence of unspent sperm. Resting male testes exhibited evidence of mitotic proliferation of next seasons spermatogonia, primary and secondary spermatocytes, and atretic bodies. Seasonally related ontogenetic changes in gonad development in *E. striatus* are depicted in Figures 2, 3.

Pattern of Seasonal Maturation and Evidence for Protogyny

Development classes for e. striatus as defined earlier are plotted against season. The percentage of gonad classes are given for each month and by season as a percentage of all fishes collected (Figures 4-7). The data are derived from the gonads of 1,232 specimens collected during the years 1984-1987. Immature females are present in more or less constant numbers throughout the year. In December, January, and February, a decrease can be observed, due to the development of class 2 and the concentration of sampling effort at winter aggregation sites. Resting females decrease from December to February and increase again from March to November. Maturation begins late November - early December with greatest numbers of developing females occurring in late December. Ripe or "spawning" females are first observed in late December reaching a peak occurrence in early to mid-January. Post spawning or spent individuals appear first in early January reaching a peak in late January to early February. The latest spawning females are found in low numbers in early February.

Transitionals (i.e., individuals in the midst of sex change, as indicated by gonads containing degenerating ovarian and proliferating testicular tissue) are found in reduced numbers (N = 8) during July, August, and September. Transitionals appeared seasonally at a time when the population was dominated by immature and/or resting individuals of both sexes.

Immature males occurred exclusively during May through July. Developing males increased in the sample population from October, November, and December respectively, decreasing again in January and absent in February through September. Ripe individuals first appeared in reduced numbers in late
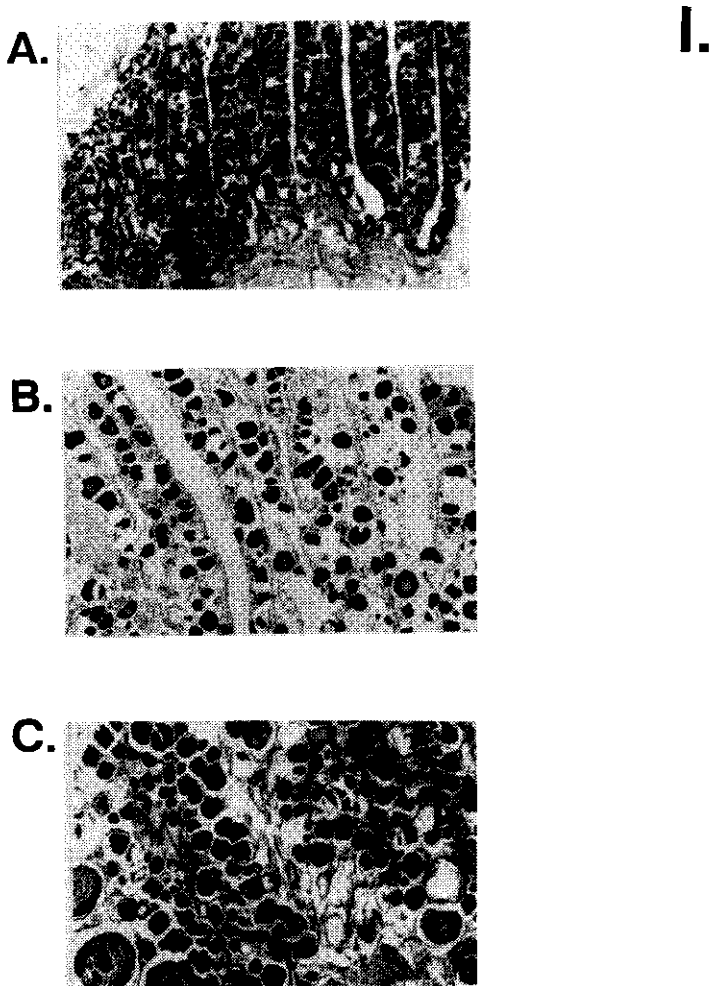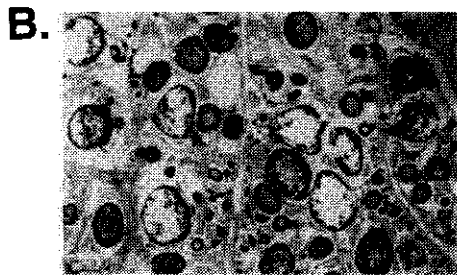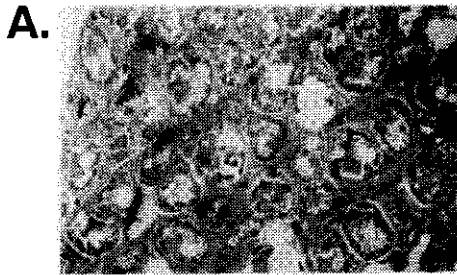
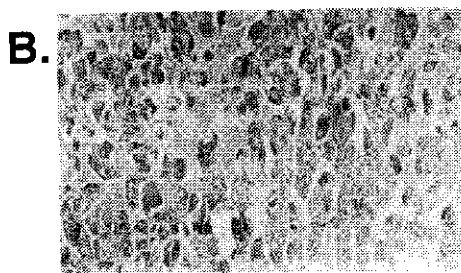**Figure 2I, II.** Photomicrographs of histological sections representative of gonad development stages from female Nassau grouper, *E. striatus*. I.A) Cross section of immature female ovary; 235mm SL; showing only oogonia and small basophilic, previtellogenic oocytes. B) Cross section of mature resting female ovary; 630mm SL; note thick gonad wall and internal muscle bundles. C) Cross section of developing female ovary; 539mm SL; many stage 3 and 4 oocytes with thin zona radiata; no post-ovulatory follicles present. II.A) Cross section of ripe female ovary; 626mm SL; note presence of hydrated oocytes and absence of post-ovulatory follicles. B) Cross section of spent female ovary; 619mm SL; note presence of degenerating stage 4 oocytes, contracted gonad walls and leukocytes. No post-ovulatory follicles.

II.

A.

B.

I.

Figure 3I.II Photomicrographs of histological sections representative of gonad development stages from male Nassau grouper, *E. striatus.* I.A) Cross section of developing male testis; 595mm SL; note presence of all stages of spermatogenesis, sperm sinuses are full of spermatozoa and lobule walls broken down places. B) Cross section of ripe male testis; 631mm SL; gonad full of spermatozoa with many lobule walls broken down. C) Cross section of resting male testis; 535mm SL.II A) Cross section of transitional gonad; 560mm SL; note presence of scattered spermatogenic tissue, stage 2 oocytes and degenerating oocytes; B) Cross section of transitional gonad; 238mm SL.
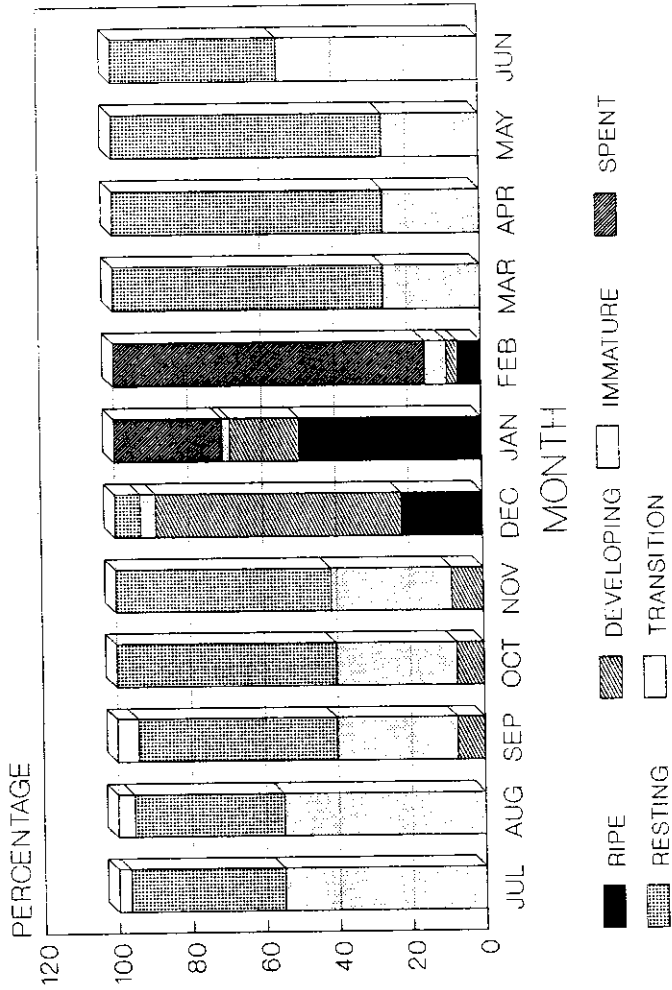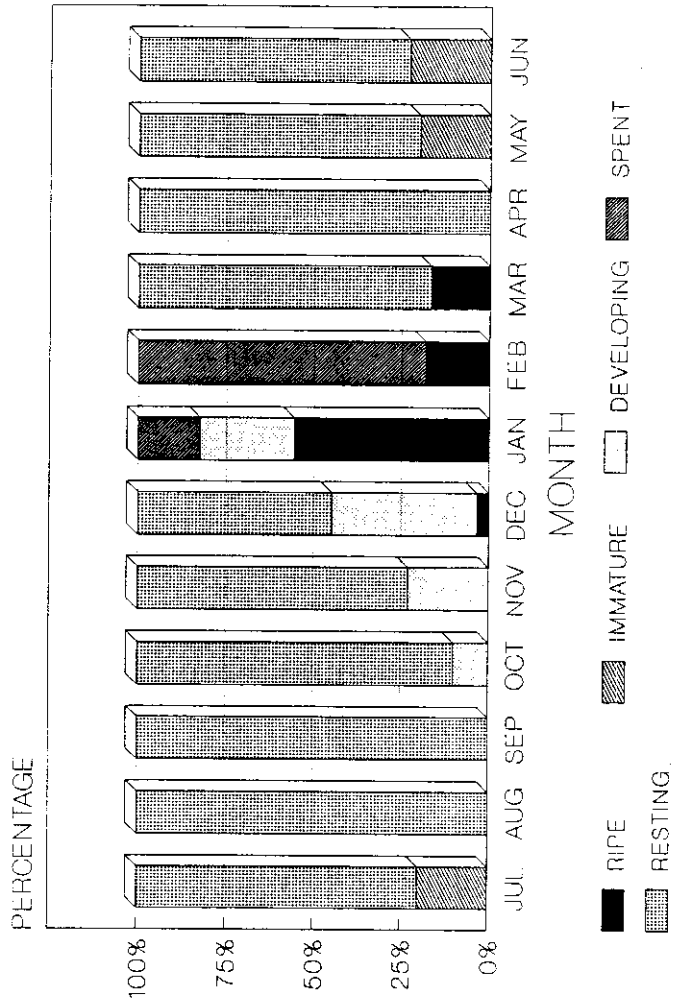
II.
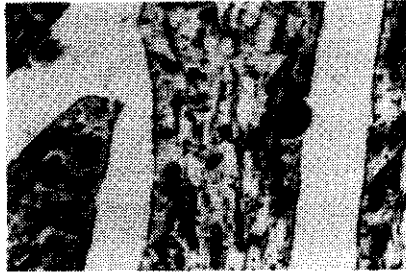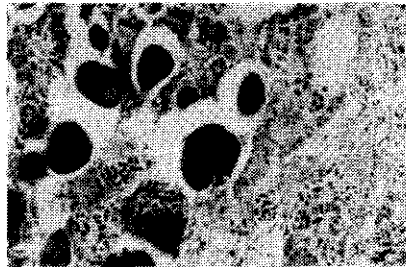
Figure 4. Percentage histograms of gonad development stages for female Nassau grouper, *E. striatus*, from each month.

Figure 5. Percentage histograms of gonad development stages for male Nassau grouper, *E. striatus*, from each month.
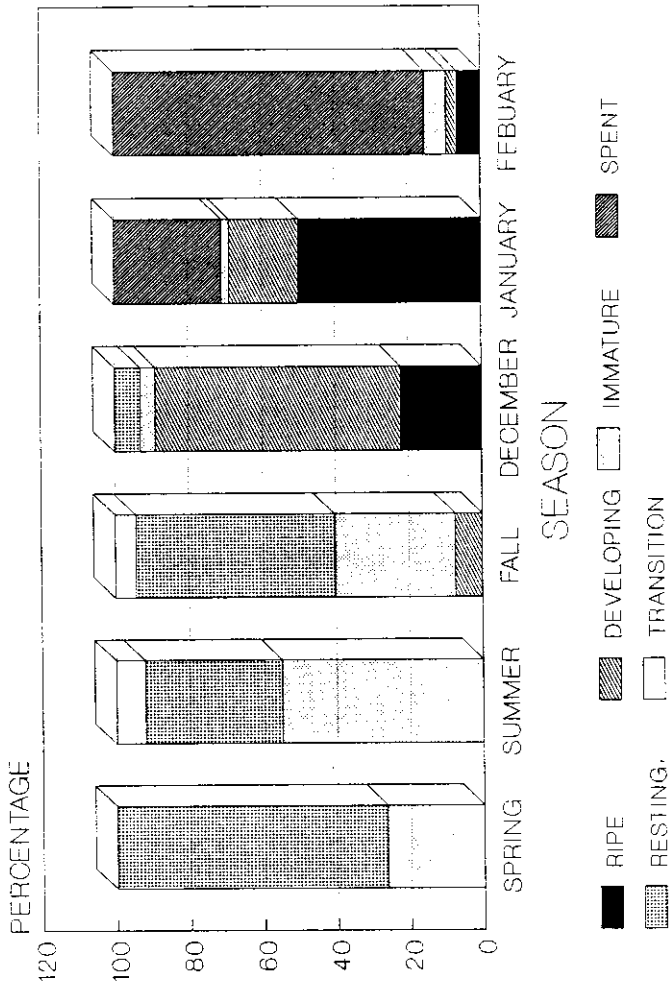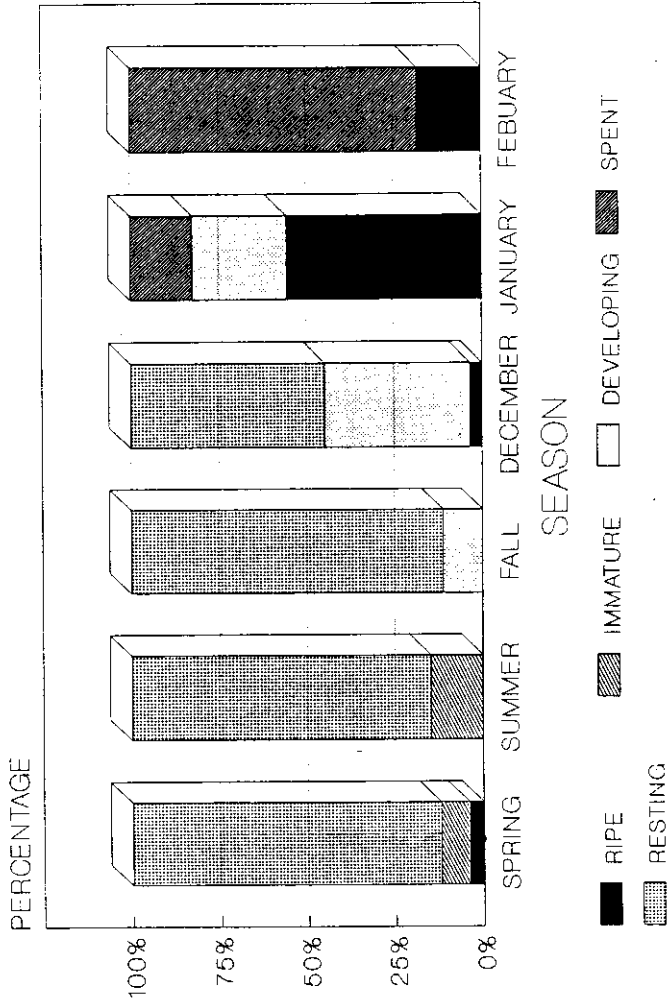
Figure 6. Percentage histograms of gonad development stages for Nassau grouper, *E. striatus*, for females by season.
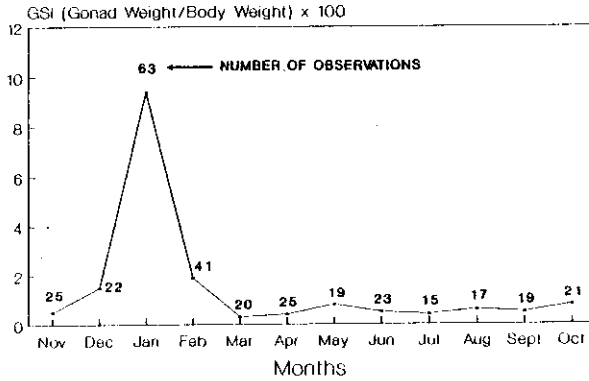
Figure 7. Percentage histograms of gonad development stages for Nassau grouper, *E. striatus* for males by season.

81

December, reaching a peak high in January, and decreasing in February to March respectively. It is interesting to note that although both female and male schedules for ripeness correspond closely as expected, males run ripe earlier and extend later into the season than females, that in effect translated into an apparent "wasted" reproductive effort. Spent males appear first in reduced numbers in mid to late January, peak in February, and are absent from February through December.

Reproductive season was further corroborated by examining the gonadosomatic index (i.e., gonad weight - body weight x 100 of 575 specimens taken monthly from the years 1984-1986. GSI remained at a very low level throughout the year (Figures 8a, b) except for a single, high peak during late December the first year and early January the second year (full moon occurred on December 22, 1984, January 21, 1985, and January 10, 1986). These peaks in GSI were associated with ripeness of ovaries and testes, as indicated by gross morphological appearance of gonads. Histological sections confirm gonadal ripeness during this time (Figures 2, 3). Time of occurrence of the spawning aggregation (Discussed more fully below) in each year sampled also agreed with the peaks of GSI.
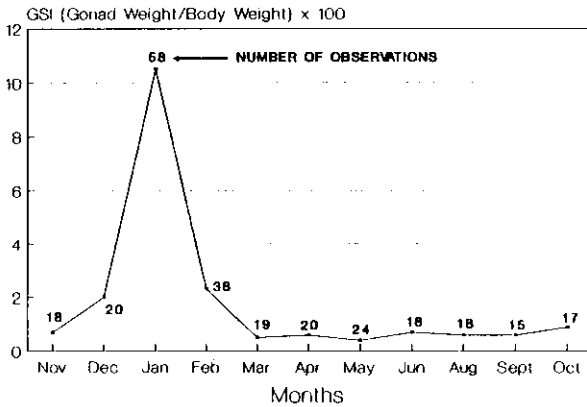
Monthly distribution of mean maximum oocyte diameter and standard deviation on either side of the mean for a total of 442 individuals are plotted for each month (1985-1986) compared with photoperiod and bottom temperatures (Figure 9). Standard deviation is a good index to monthly gonadal activity since it measures the dispersion of oocyte diameters. The time of peak spawning, December and January, and the time of post spawning and recovery, February through March are reflected by fluctuations of the mean. Given the concern over the impact aggregation fishing may have on population reproductive potential, it is important to note that no secondary, out of season gonadal activity is evident at other times of the year. Annual water temperature ranged from 24°C to just over 30°C. Spawning of *E. striatus* occurred at temperatures of 25.5 - 26.0°C during periods of gradually decreasing temperatures, about 1-2 months before the annual minimum in late February to early March. Photoperiod also correlates with gonadal activity in *E. striatus*. Initial gonadal development occurs in October and November to culminate in spawning in late December and early to mid-January. Recovery and resting phases follow in February through March and beyond. Spawning of E. striatus occurred at the low point of the photoperiod curve (Figure 9). The period of time prior to spawning is characterized by decreasing daily photoperiods, whereas the period of time immediately following spawning is characterized by increasing photoperiod values (Figure 9). Interestingly, these results are opposite of those reported by Harrington (1956) for the centrarchid fish, *Enneacanthus obesus* and by Moe (1969) for the closely related red grouper *E. morio*. In both these species, photoperiod elevation was correlated with out-of-season spawning activity.

A.

Gonadosomatic Indices (*E. striatus*)
Females Sampled by Month

GSI (Gonad Weight/Body Weight) x 100

63 ◄─────── NUMBER OF OBSERVATIONS

25 22 41 20 25 19 23 15 17 19 21

Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sept Oct

Months

1984 1985

B.

GSI (Gonad Weight/Body Weight) x 100

68 ◄─────── NUMBER OF OBSERVATIONS

18 20 38 19 20 24 18 18 15 17
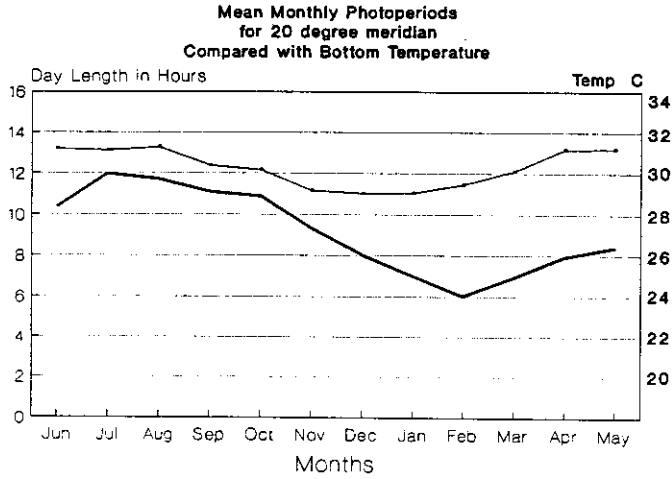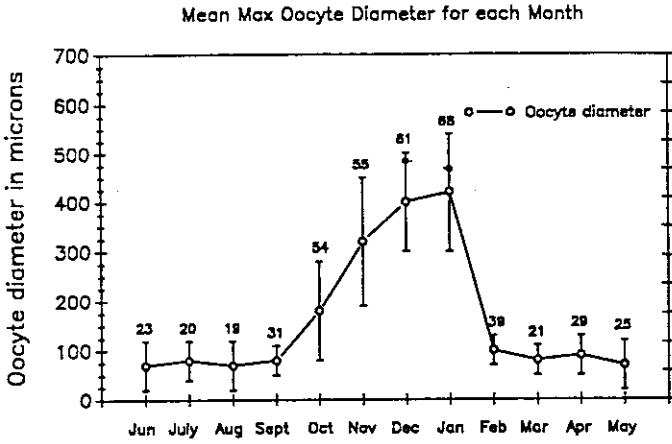
Nov Dec Jan Feb Mar Apr May Jun Aug Sept Oct

Months

1985-1986

Figure 8. Gonadosomatic indices by month for female Nassau grouper, *E. striatus* from November 1984 to October 1985 (A) and from November 1985 to October 1986 (B). The figure listed above each mean indicates the number of observations.

83

Mean Max Oocyte Diameter for each Month

Mean Monthly Photoperiods
for 20 degree meridian
Compared with Bottom Temperature

1985/1986

Figure 9. Mean maximum oocyte diameter for each month compared with photoperiod and bottom temperatures. The figure listed above each mean indicates the number of observations and the vertical bar represents the standard deviation.

Sex-Composition and Population Structure

Sex ratios and standard length frequency distributions for *E. striatus* are given by sex, for non-aggregating populations (excluding spawning sites), exploited spawning aggregation population (Cay Glory), and non-exploited spawning aggregation population (Northern Two Cay) (Figs. 10-12). Data is combined for years 1984-1986.

Total sample size for non-aggregating population was 717 individuals. Females numbered 496 individuals with a mean standard length of 418 mm, and of range 104-760 mm. Males numbered 221 individuals with a mean standard length of 420 mm, and range of 270-702 mm. An overall sex ratio for *E. striatus* was 2.2:1. Data did not show a distinct differentiation in size with sex. Furthermore, length frequency data for transition also indicates sex change can occur over quite a broad range of sizes (N=8, range=270-582mm SL).

Total sample size for the unexploited spawning aggregation was 694 individuals. Females numbered 420 individuals with a mean standard length of 517 mm, and a range of 240-780 mm SL. Males numbered 274 individuals with a mean standard length of 521 mm, and a range of 310-802 mm SL. An overall sex ratio for *E. striatus* was 1:5:1. Data did not show a distinct differentiation in size with sex.

Total sample size for the exploited spawning aggregation was 485 individuals. Females numbered 344 individuals with a mean standard length of 418 mm, and a range of 101-650 mm SL. Males numbered 141 with a mean standard length of 420 mm, and a range of 280-690 mm SL. An overall sex ratio for *E. striatus* was 2.4:1. Data did not show distinct differentiation in size by sex for sexually mature fish. Interestingly, the data indicates several smaller sexually immature females were present in the spawning aggregation (Figure 11). This is in marked contrast to the unexploited bank (Northern Two Cay) where immature, small-sized females were noticeably absent from the sample population.

A comparison of sex ratios and length frequency distribution among the three sites indicates that there is a marked increase in the number of females relative to males at the exploited spawning aggregation (Cay Glory), as well as a significant decrease in the size of both sexes relative to the general population outside of the spawning banks and at pristine spawning aggregation sites (Figures 10-12).

Fecundity and Egg and Larval Stages

The egg of the Nassau grouper, *Epinephelus striatus* is pelagic, spherical in shape, measuring less than 1 mm in diameter. The yolk is colorless, transparent, and contains a single oil globule. This description agrees with that of eggs of other similar grouper species (Guitart and Juarez, 1966; Moe, 1969; Olsen and La Place, 1978). Fecundity counts from 30 ovaries (90 subsamples) give a mean
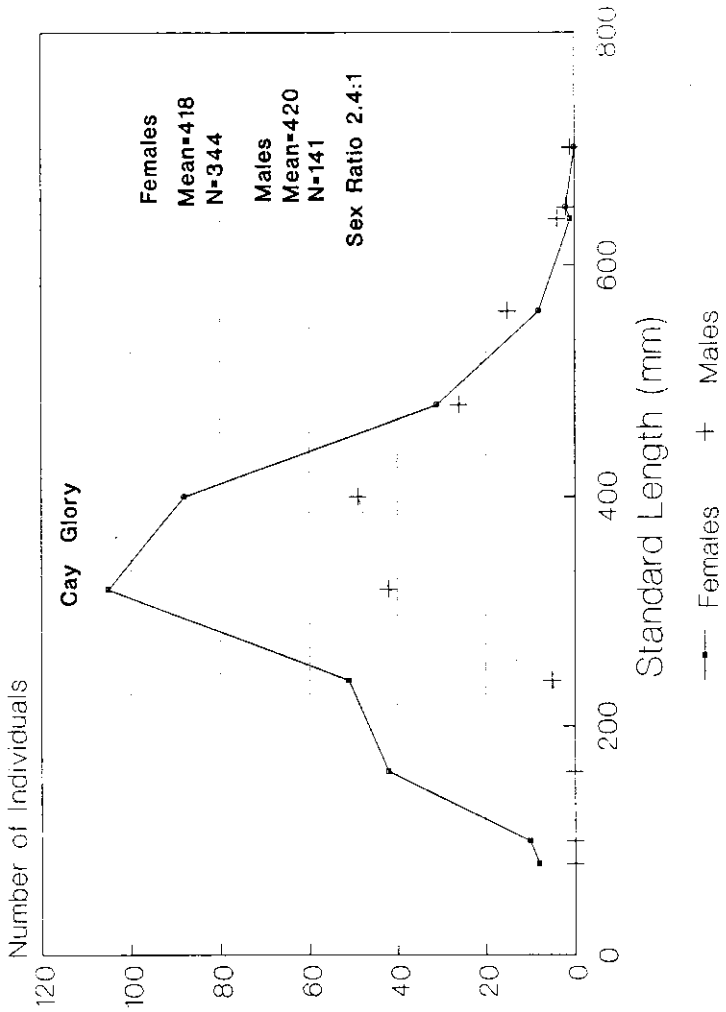
85

A:

Standard Length Frequency Distribution
Nassau Grouper, *Epinephelus Striatus*
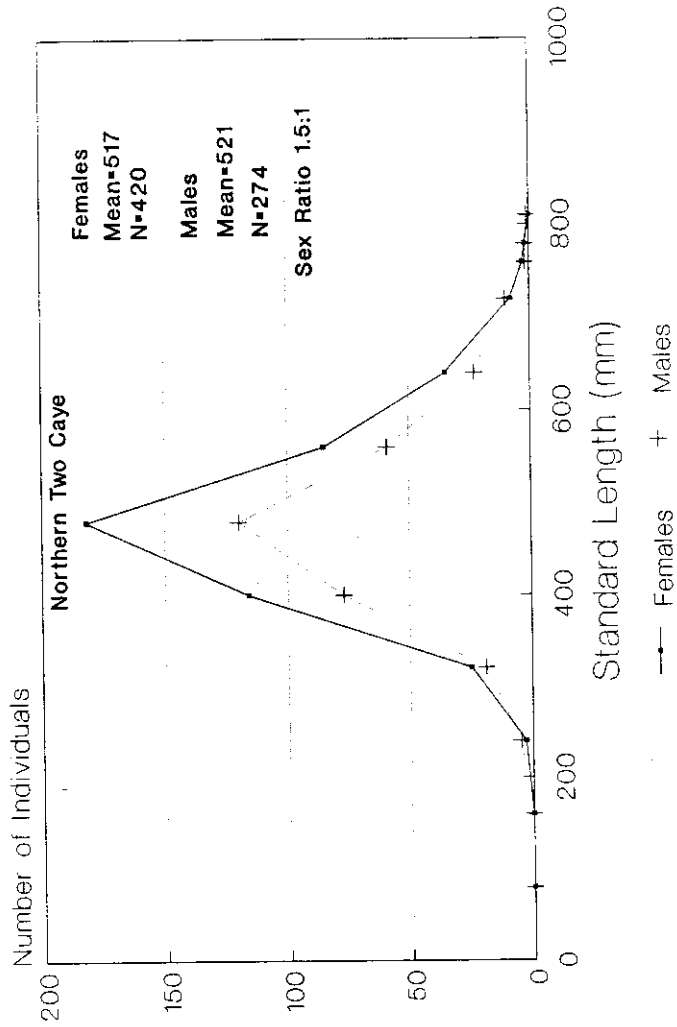
Figure 10. Standard length frequency polygon for male and female Nassau grouper, *E. striatus* from Northern Two Cay breeding aggregations (unexploited bank).

Figure 11. Standard length frequency polygon for male and female Nassau grouper, *E. striatus* from Cay Glory breeding aggregations (exploited bank).
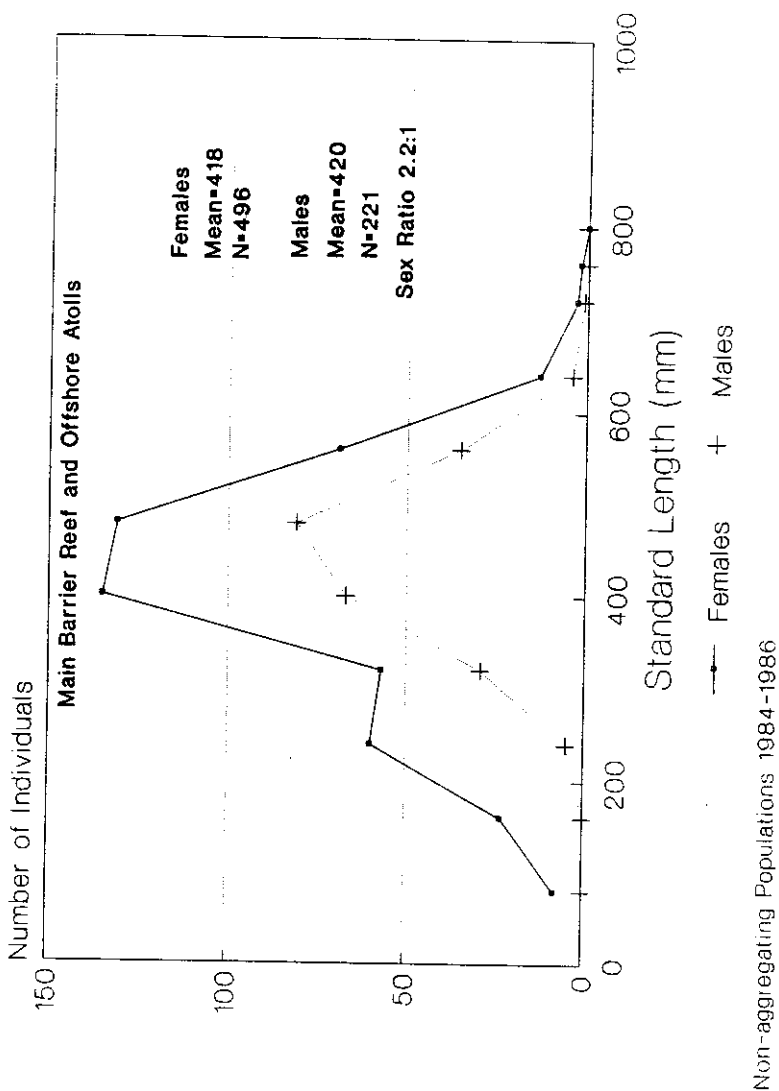
87

Figure 12. Standard length frequency polygon for male and female Nassau grouper, *E. striatus* from barrier reef and atoll non-aggregating populations.

value of 4.1 eggs/mg. The mean total number of eggs was 4.2 x 106 eggs with a range of 350,000 to 6,500,000. A scatterplot depicting the relationship between standard length and egg production potential is shown in figure 13.

Length-Weight Relationship
The regression of log10 (weight) on log10 (standard length) indicate that for 930 individuals (both sexes combined) ranging from 180-802 mm SL, the length-weight relationship equation expressed exponentially was (q) = .0107 x SL3.0812. In figure 14, a scatter plot of individual length against weight clearly suggests that Nassau grouper weight is probably a function of the cube of the length.

Effect of Fishing Aggregation
Interviews with local fishermen (Craig, 1969) have revealed that the Cay Glory (exploited) grouper bank has been the focus of a well-organized, seasonal handlined folk fishery since the early to mid 1920's. In those early years of the fishery, only a few fishermen sailed fishing smacks to the banks on the first full moon of January to catch grouper. However, by the mid 1960's as many as three hundred boats, each with a crew of 4-6 men, converged on the bank. It was not uncommon for an experienced crew to catch and process between 1,200 and 1,800 fish. Over one hundred short tons were harvested annually, most of which was sold as "salted" fish to neighboring Honduras and Guatemala for Lenten season. Since this time the fishing pattern has changed dramatically.
During the 1984 and 1986 seasons respectively less than 20 long boats (dugouts) fished on the Cay Glory grouper bank. A similar number of boats worked the unexploited aggregation site at Northern Two Cay, as well as the other aggregation sites along the reef. Although fishermen arrived weeks prior to the January full moon, catch during both seasons (Figure 15) followed a lunar trend. Over eighty percent of the catch came between the week prior to the full moon and ten days following. The handline fishery accounted for nearly the entire catch in 1985 and 1986, however in recent years Antillean styled fish traps have also appeared on the bank. Finally, comparison of the Cay Glory commercial catch with that of samples collected by spear (N=35) randomly within the aggregation indicates that there was no statistical difference in sex ratio or size distribution (Chi-square=0.083, df=1, NS).

Spawning Aggregation Observations
Aggregation sites-location, bathymetric, and current patterns
Our study identified six widely scattered spawning sites off the coast of Belize (Figure 1). Three of the grouper banks lie along the main spine of the barrier reef, about eight to twenty miles offshore. Farther out, the spawning banks are located on northeast promontories on the seaward slope of the outer
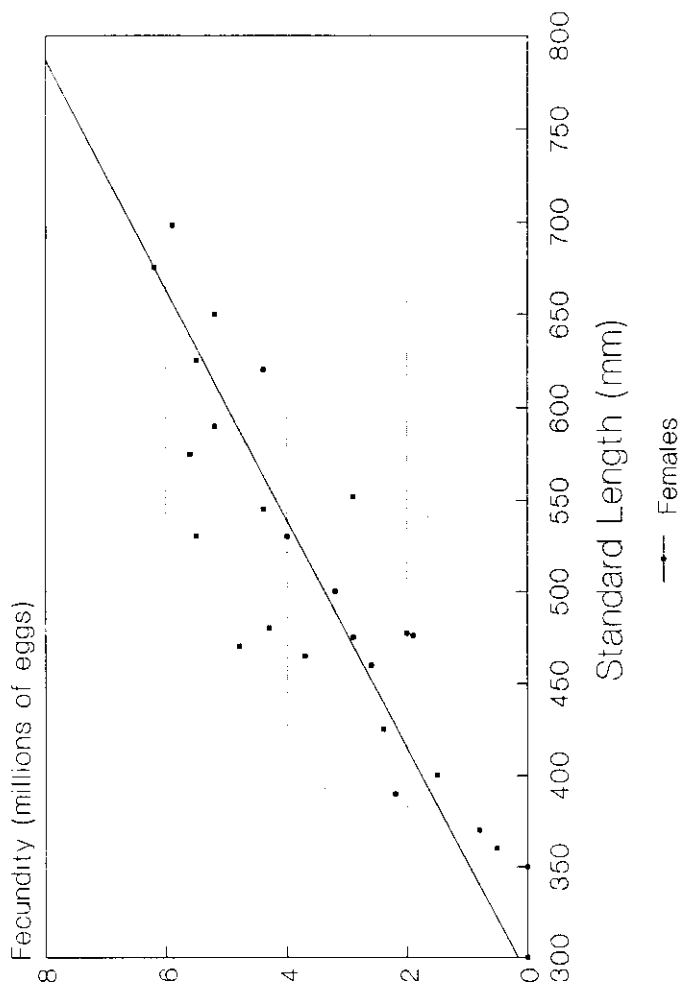
Figure 13. Relationship between number of ripe eggs in the gonads and standard length.

Length-Weight Relationship
E. striatus (both Sexes)

Body Weight (grams in thousands)

Standard Length
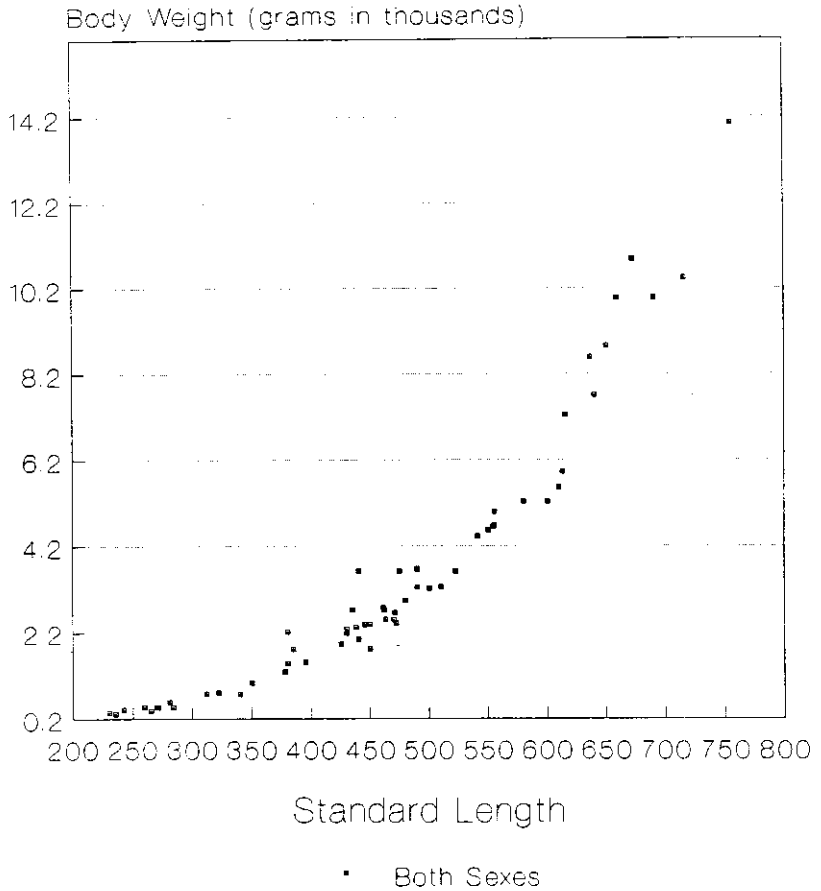
• Both Sexes

1984-1986

Figure 14. Scatterplot relationship for standard length to weight for sixty
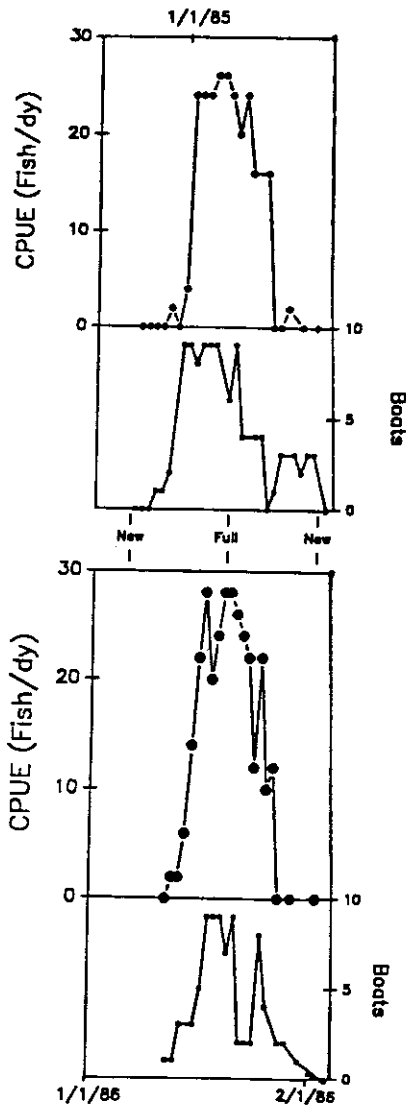Nassau grouper, *E. striatus* (both sexes combined).

Figure 15. Catch per unit effort and fishing effort for the 1984-85 (A) and 1985-86 (B) breeding aggregations of *E. striatus*. The two years' data are on a time axis aligned with lunar period.

reef. Localized current patterns associated with these reef promontories are primarily wind driven but poorly understood. On January 11-14, my colleague John Olney (VIMS) and I deployed an array of two S-4 current meters just above the grouper aggregation at Cay Glory approximate position 17o05 N., 89o58 W. (Fig. 16). Depth of meter deployment was approximately m (top) and 20m (bottom).

A short but novel data set of current speed and direction was generated (Fig. 17). This data, combined with information from a fluorescent dye released into the water to "mock" spawning track, indicated a fairly strong SSE current seaward with marked brief periods of slack water and current reversals. Despite evidence for localized and highly variable currents at aggregation sites, the overall long-term prevailing offshore current is in a NNE direction at approximately 2 kts with a weaker counter clockwise southerly flow along the western boundaries of the atolls and inside the barrier reef lagoon (James and Ginsburg, 1979).

Migration and occurrence of aggregation

Local fishermen in Belize report that Nassau grouper aggregations occur at all six localities simultaneously during the periods around the full moon during December and January. The exact time relative to the full moon that Nassau grouper appear at the aggregation site is not known. However, I have observed large schools of Nassau grouper (several dozen individuals) passing slowly along the 30-40 meter shelf break contour at several localities along the main reef by late October and early November. By the full moon of late December or early January several thousand individuals will have arrived at each aggregation site. I believe each aggregation site along the main reef attracts individuals from a broad area at least 20 to 30 miles wide given the average distance apart for each site in Belize. It is believed each offshore atoll has only one spawning aggregation site and hence, these sites presumably draw fish from the entire atoll complex. It is possible that other aggregation sites exist somewhere along the perimeter of these atolls but to date our efforts to locate additional sites have been unsuccessful. Furthermore, it is unlikely that fish are drawn from outside the atoll complex given the bottom dwelling nature of groupers and the sheer depths and distance that fish must negotiate to reach atolls from main barrier reef or other offshore localities. Just prior to the spawning run of 1985-86, I implanted ultrasonic tags into the abdomens of two local groupers off Ambergris Cay, a large island situated along the northern province of the barrier reef ecosystem. One individual was subsequently lost but the other was recovered by a local Mexican fisherman nearly two years later at another grouper bank, off the Central part of the Mexican Yucatan peninsula, more than 150 miles north of where I had released it.

93

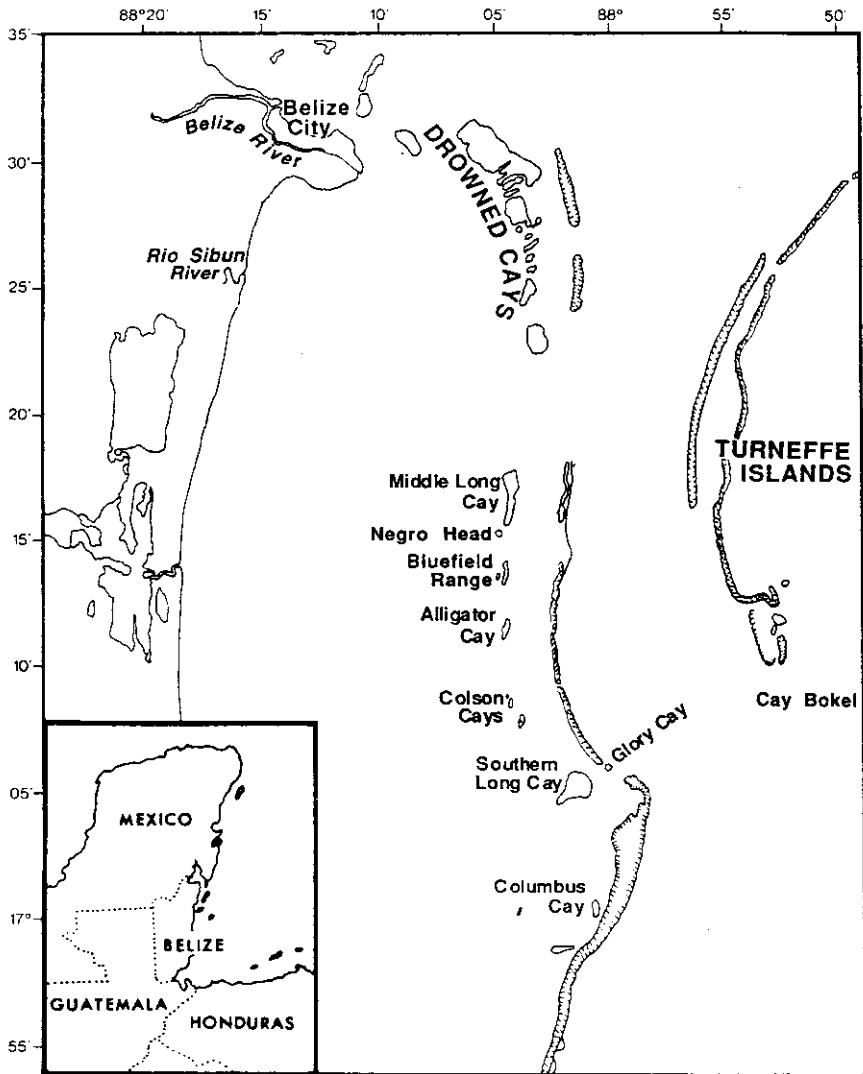**Figure 16.** Chart of the study area around Cay Glory, Belize, Central America. The reef promontory due east of Southern Long Cay is the historical Nassau grouper spawning site.
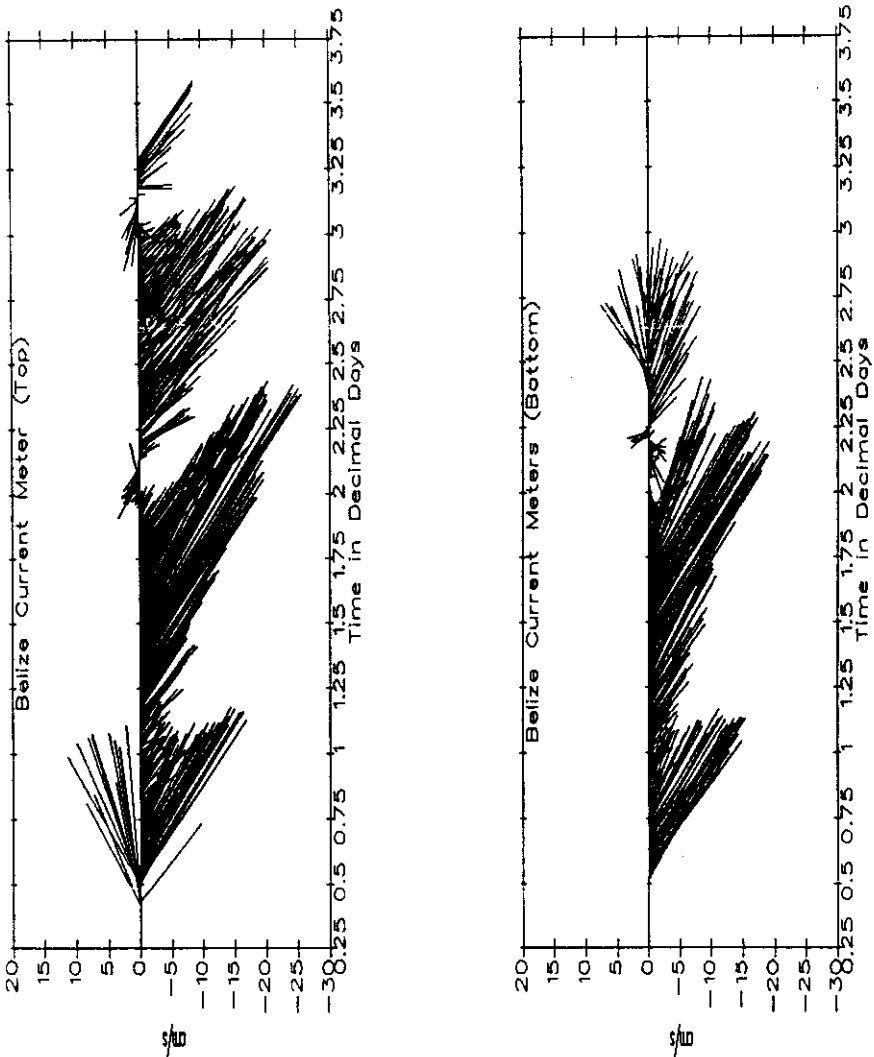
Figure 17. Unedited current meter records resulting from deployment of an array of two S-4 current meters during the period 11-14 January 1989. Depth of meter deployment was approximately 6m (TOP) and 20m (BOTTOM).

Apparently, several other species of fish are attracted to these grouper aggregation sites for spawning in addition to the Nassau grouper (Colin, 1990). I have observed courtship behavior but no spawning in smaller aggregations of *Mycteroperca bonaci. Epinephelus fulva,* and *Mycteroperca venosa.* In January 1988, at the Cay Glory site, Doug Perrine and I observed a densely packed ball of thousands of dog snappers, Lutjanus griseus, at about 100 feet. These fishes descended to a depth of more than 200 feet before suddenly turning and rushing back toward the surface, presumably releasing both milt and eggs. Interestingly several individual Nassau grouper were intermingled and participating in the dog snapper spawning aggregation (Carter and Perrine, in prep).

Courtship and spawning-occurrence, coloration, and behavior
During December 1985, no spawning activity was reported. In January 1986, spawning occurred 1 day before the full moon (January 10) and continued for three days following. Although the actual release of eggs and milt underwater was not observed, fishes captured by hook and line were freely releasing hydrated eggs and sperm. There appears to be a strong lunar component to the occurrence of spawning. According to local fishermen, the occurrence of a full moon in early January results in a much greater aggregation of fish than if the full moon falls later in the month. Local fishermen also report that years ago Nassau grouper gathered twice annually, once in late December and early January, and then again, in the following full moon in late January and early February. While I suspect this information is historically correct, I saw no evidence of two well defined temporarily segregated aggregations during my studies.

Coloration of Nassau grouper in spawning aggregations off the coast of Belize falls into four different patterns as described in detail by Colin (1990). Colin defines these patterns as: 1) barred pattern (normal), 2) white belly, 3) bicolor, and 4) dark phase. It appears that all color phases are under direct nervous control, and rapid changes in color do occur. Patterns observed for groupers in Belize correspond well with those described for Nassau grouper in the Bahamas (Colin, 1990).

The barred pattern is "normal" among individuals during non-aggregating periods, and is common during mornings in fishes in the aggregation site. I also observed the white belly phase early in the morning in large individuals (presumable females), resting on the bottom. The bicolor phase can occur in both males and females. During the late afternoon, the major portion of fish within the aggregation assume a bicolored phase and move into deeper water, approximately 30-35 meters depth. The "Dark" phase was only seen occasionally throughout my observations on spawning sites. They were most common in deep water off the bottom in late afternoon.

Although we were unable to witness actual release of gametes, courtship behavior exhibited by Nassau grouper from Belize, follows closely that described for Nassau grouper in the Bahamas (see Colin, 1990 for details). In addition to confirming spawning behavior as described by Colin (1990), I observed what appeared to be an increase in intensity and duration of intraspecific (group) encounters on the grouper bank each day leading up to the spawning event. By "intraspecific encounters" I refer tot he frequent gathering off the bottom of small groups of fishes during the day. Fishes participating in these small group associations would often change from the normal barred pattern and assume primarily bicolored pattern and/or the "dark phase". The continual forming and then breaking apart of small groups could be interpreted as necessary to prepare or "ready" fish in a physiological sense for the upcoming spawning event. Alternatively, it could be tied to a mapping mechanism whereby fishes assess population structure (i.e., sex-ratios) through frequent encounters with each other prior to spawning and subsequent departure from aggregation site. Such a mechanism would enable individual groupers to compare spawning aggregation population structure with that encountered in their home sites throughout the rest of the year.

It is not known whether individuals no present at the aggregation sites spawn and contribute significantly to the propagules present in the pelagic environment. It is also not known what percentage of the entire grouper population actually comprises the spawning aggregation. Answers to these questions are critical for management decisions

Additional Life History Observations
Feeding Activities
Stomach contents were examined from a total of 100 Nassau groupers ranging in standard length from 185-730mm. These fish were almost all taken by spear fishing in less than 150 feet of water. Only half (50) contained identifiable food material. The diet of groupers by weight was 58.4% fish, 29.3% crustaceans, 6.4% cephalopods, 1% gastropods, less than 1% pelecypods, and 4.6% unknown. The crustacean category was broken down further as follows: crabs, 51.2%; lobster, 18.3%; shrimp, 10.2%; hermit crabs, 5.0%; stomatopods, 4.5%; and 10.8% unknown. Fish were broken down further to grunts, 21%; snappers, 18%; wrasses, 9%; squirrel fishes, 7%; parrotfishes, 6.0%; damselfishes, 5.0%; surgeonfishes, 4.0%; and 30% unknown. Data is presented in Figures 18 and 19.

Daily and Seasonal Patterns of Movement(Preliminary Observations)
Patterns of daily movement for Nassau grouper were determined for three sonically tagged individuals (two females, one male) on three separate occasions (Figure 20). Each hour the location of individually tagged fish was recorded
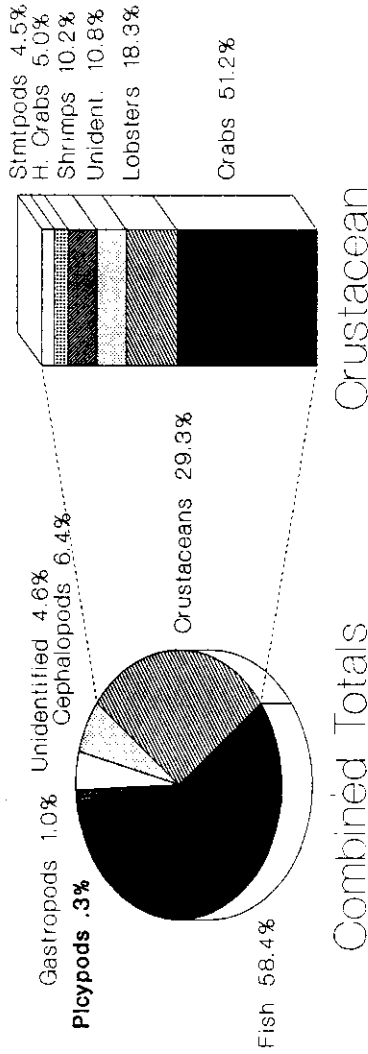
along with a qualitative assessment of its activity (presumably related to feeding forays but not confirmed by visual observations or gut content analysis). A character state of (1) equals inactive (no movement), (2) active (slow to moderate sustained swimming speed, and (3) rapid short-lived burst. The tracking record indicates Nassau grouper are most active in the hours just prior to and following sunrise and sunset. No movement was noted during the evening hours among individuals observed. However, given the small sample size this may not be the pattern for all Nassau grouper. An area of daily movement was described for two of the three groupers (Figure 21). Fishes moved randomly within a rectangle 160 meters by 80 meters during the day returning by evening to nearly the same spot from which they began their respective forays.

DISCUSSION AND CONCLUSION

Smith (1965) constructed a phylogeny of serranid fishes based on three types of protogynous hermaphroditism. According to Smith, the most advanced type was characterized by fishes of the genus *Epinephelus* where testicular tissue cannot be found prior to the onset of sexual transition. Subsequent studies on the reproductive biology of the red grouper, *Epinephelus morio*, (Moe, 1969) a close relative of the Nassau grouper supports Smith's interpretation of the reproductive history of this genus. Since then, a series of studies on the reproductive biology of other Serranids has added to the list of epinephelines confirmed as protogynous hermaphrodites (Brusle and Brusle, 1976a, b; Chen *et al.*, 1977). To this list we can add Nassau grouper, *Epinephelus striatus*, based on the combination of female-biased sex ratios, organization of testicular tissues into lamellae similar to ovarian lamellae, and atretic bodies in stages of oocytic atresia within testes. This evidence for protogyny complies with explicit criteria for diagnosing protogyny as discussed by Sadovy and Shapiro (1987). Despite the widely held belief that all members of this specious family serranidae are protogynous, only relatively few species to date (*ca.* 8) have been adequately diagnosed as changing sex (Shapiro, 1987). Sadovy and Shapiro (1987) caution that sole use of non-specific features such as size or age specific sex ratios to diagnose protogyny may lead to misinterpretations since these features may have other causes than protogyny.

The fact that Nassau grouper, *Epinephelus striatus* , exhibits some degree of protogyny is by itself not surprising given the evolutionary history of the family serranidae. The important and as of yet unanswered question is to what extent is sex reversal occurring within a given population of Nassau grouper and what purpose does it serve? In this study we observed a considerable number of small males and no significant difference in the mean size of male/female *E. striatus* from aggregation sites or in the general population outside the spawning season. In fact the smallest and largest fish measured from the pristine aggregation site (Northern Two Cay) was a running ripe male. Similar observations are reported
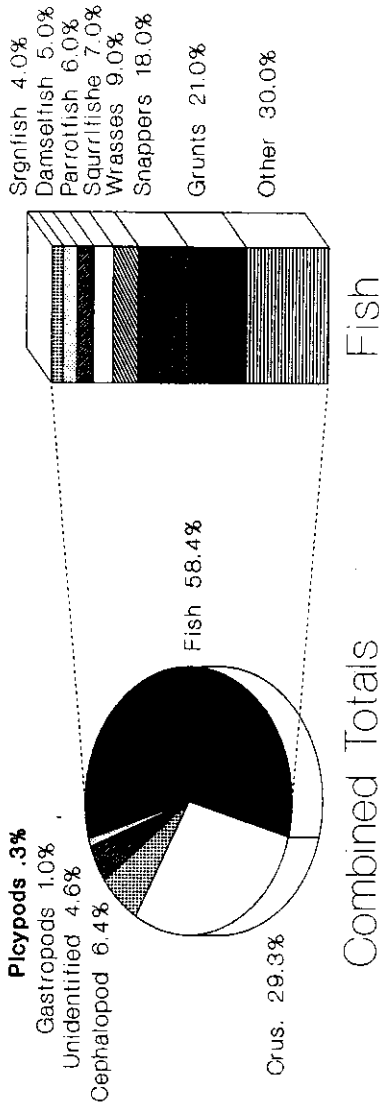
A:

Food Habits of Nassau Grouper
Epinephelus striatus

Stmtpods 4.5%
H. Crabs 5.0%
Shrimps 10.2%
Unident. 10.8%
Lobsters 18.3%

Crabs 51.2%

Crustacean

Unidentified 4.6%
Cephalopods 6.4%

Crustaceans 29.3%

Gastropods 1.0%

Combined Totals

Picypods .3%

Fish 58.4%

1985-1987 (N=50)

Figure 18. Percent frequency of occurrence of higher taxonomic groups of food and crustaceans in the diet of *Epinephelus striatus*.

B.

Srgnfish 4.0%
Damselfish 5.0%
Parrotfish 6.0%
Squrrlfishe 7.0%
Wrasses 9.0%
Snappers 18.0%

Grunts 21.0%

Other 30.0%

Fish

Fish 58.4%

Pisypods .3%
Gastropods 1.0%
Unidentified 4.6%
Cephalopod 6.4%
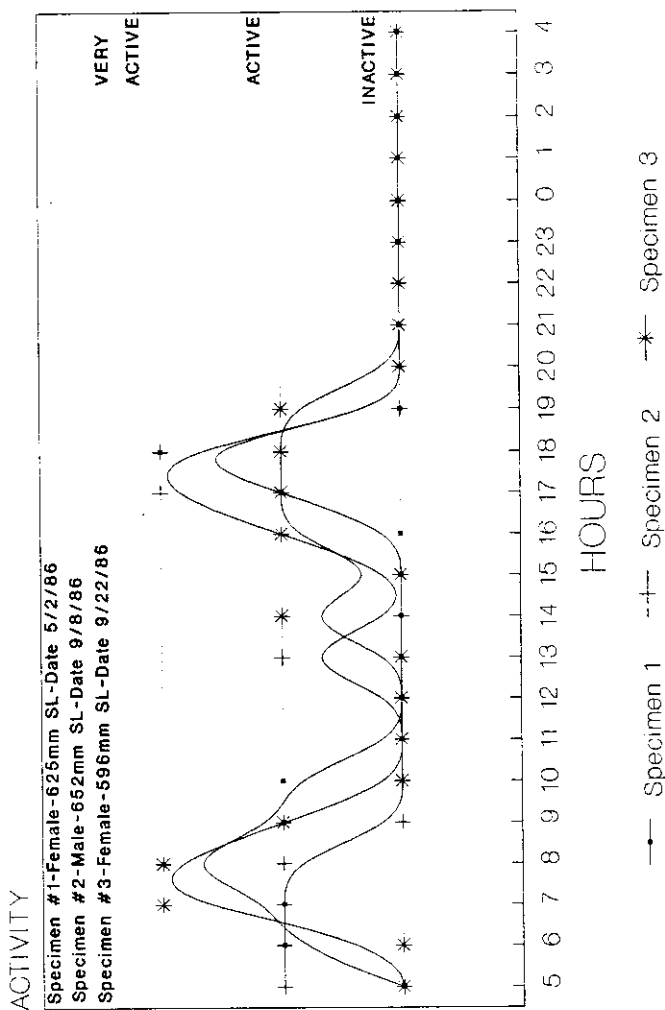
Crus. 29.3%

Combined Totals

1985-1987 (N=50)

Figure 19. Percent frequency of occurrence of higher taxonomic groups of food and fish in the diet of *Epinephelus striatus*.
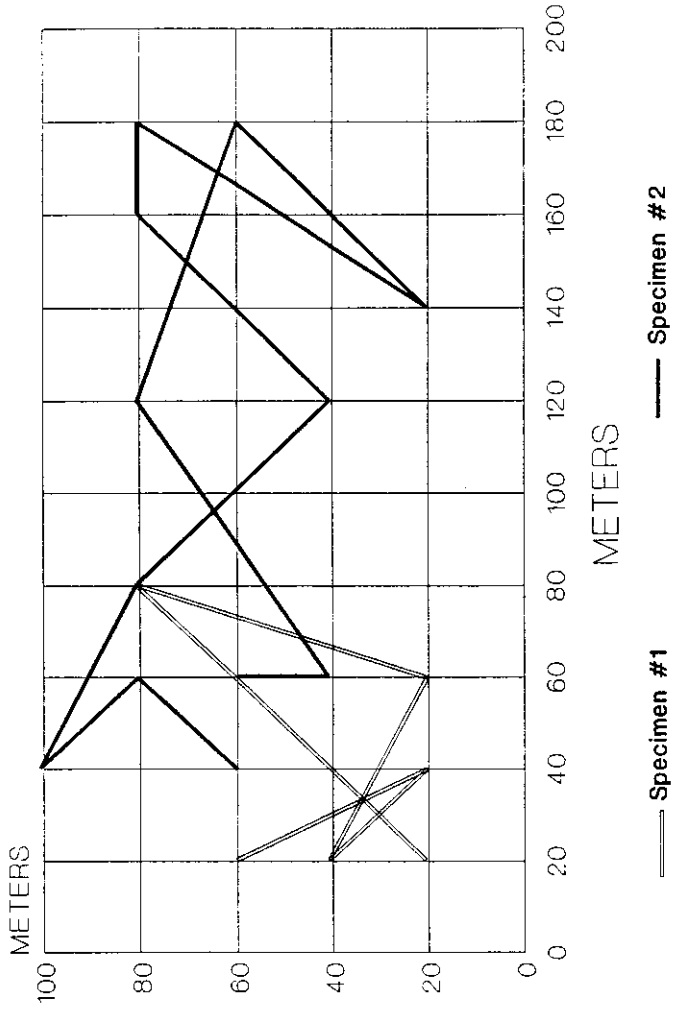
A:

Feeding Activity Periods
E. striatus

Figure 20. Activity patterns for male and female Nassau groupers, *E. striatus*, over a 24 hour period. Ultrasonic transmitters emitting a pulse frequency of 75 kH were surgically implanted within the abdominal cavity of all three fish.

Figure 21. Range of movement for sonically tagged male and female Nassau grouper, *E. striatus*, over a 24 hour period.

for Nassau grouper populations from the Virgin Islands (Olsen and La Place, 1978), Cayman Islands (Colin et al., 1987), and the Bahamas (Colin, 1990). Clearly the data for size and sex indicates sex reversal (when it occurs) in E. striatus is not simply related to size and sex, but probably influenced by socially controlled factors as of yet not well understood.

Changing sex is widespread among numerous fish taxa, particularly marine fishes (Atz, 1964; Reinboth, 1970; Cole and Robertson, 1988). In some species females become males (protogyny), and in others, males become females (protandry), and in still others, individuals can be both sexes at the same time. What advantages do these tactics provide for the survival of the species and why should natural selection favor their development are questions of considerable biological interest and importance.

Theoretically, protogyny can evolve whenever an individual can reproduce more successfully under particular environmental or social conditions by changing sex than by continuing as the same sex (Ghiselin, 1969; Warner, 1975; Shapiro 1984b). Following this train of thought, a number of scientists have proposed a size-advantage theory to explain many cases of sex change. For example, fishes that exhibit protogyny often live in social units in which larger males monopolize spawning females. Small males may not spawn at all but small females have little difficulty finding a mate. Natural selection favors sex reversal in this case because an individual that functions as a female when small and as a male after attaining a large size would produce more offspring than one that didn't change sex.

Sex change among Nassau groupers, however, is difficult to explain using this theory. The behaviors common to nearly all protogynous fishes - frequent social interactions, frequent spawning by large monopolizing males, and dense populations bring in separate colonies (Robertson and Choat, 1974; Thresher, 1984) - are not seen in this species. In fact, E. striatus exhibit the opposite characteristics. Male and female individual E. striatus are solitary and widely dispersed along the reef. Furthermore, as far as we know, they gather annually only to spawn during brief two-week intervals. One explanation recently proposed by Colon (1990) and Sadovy (pers. comm) is the Nassau grouper, like other large epinephelines that tend to aggregate, are not necessarily obligate protogynous hermaphrodites. Furthermore, direct observation of E. striatus actually releasing eggs and milt within the aggregation, indicates spawning is random with no evidence of haremic social structure (Colin 1990). Colin (1990) points out that species of groupers which appear haremic, even when aggregated, do not have the extreme color changes found in E. striatus. In these species males acquire additional dark pigment during reproductive activity that may facilitate territorial control (Colin et al. 1987; Shapiro, 1987). Coloration in Nassau grouper and other large epinephelines may serve different purposes unrelated to establishing haremic social structure (Colin, 1990). However, even

103

if the majority of large epinephelines function as gonochores, we're still left to explain the "exception" of protogyny!

The Effect of Aggregation Fishing

According to Atz (1964) protogynous hermaphroditism may be selectively advantageous as a mechanism of population control and/or as assurance of the presence of both sexes in isolated insular areas. Disturbances to an otherwise "stable" environment may be sufficient to stimulate sexual transition (Liem, 1963; Harrington, 1968). Intensive fishing of grouper populations over localized spawning aggregations may stimulate sexual transition in males similar to the stressful effects overcrowding and food scarcity may have on populations. Since each transformation is equivalent to the death of a female, the cumulative effect of transition may alter the reproductive potential of the population Comparison of Bardach *et al.* (1958) Nassau grouper data and Moe's (1969) red grouper data with this study lends further support to the idea. At the time of Bardach's study the Bermuda grouper fishery on offshore banks was in its infancy whereas Florida west coast groupers had been heavily exploited for many years. The ratio of females to males was 1:1 for large Nassau groupers and 2:1 for large red groupers off Florida. In this study the grouper handline fishery off Northern Two Cay along the northeast point of lighthouse reef atoll was also in the initial stages of exploitation. It had only been known to local fishermen for eight years. In contrast, the Cay Glory aggregation site along the main barrier reef had been heavily exploited since the early 1920's! In this study, the ratio of females to males was 1.5:1 for groupers at Northern Two Cay and 2.4:1 at Cay Glory. According to Moe (1969) an intensive fishery would increase the number of females by preventing overcrowding by inducing a drop in the transition rate. A rate normally functioning to maintain balanced sex ratios in the face of slow and predictable long-term environmental change. Initially this would result in an increase in reproductive potential by increasing the number of females. Continued exploitation however could reduce the male population below a critical threshold, causing a rapid decline of the population and disappearance of the spawning aggregation from its historic site. This is particularly alarming given the fact that several traditional Nassau grouper aggregation sites have already disappeared throughout the Caribbean and several more are presently threatened with extinction (Bannerot *et al.*, 1987).

Ocean Currents, Spawning Sites and Larval Dispersal

How Nassau grouper manage to locate the same few aggregation sites each year and what is the ecological advantage of this behavior is not clearly understood. One common explanation is that reef fishes spawn at times and locations which insure their eggs are swept quickly away from the reef to offshore waters where predation by benthic predators is reduced (Johannes,

1978) while maximizing larval dispersal to facilitate chances of finding food in patchy environments (Barlow, 1981). Colin and Clavijo (1988) suggest that spawning times and locations serve better the needs of the adults, to coordinate their reproductive activities.

Clearly in the present study, the six widely scattered grouper aggregation sites off the coast of Belize are distinct geomorphological structures. They are all reef promontories or projections to Nassau grouper with a northeast orientation. The attraction of such features (if any) remains unclear. It has been reported that Nassau grouper in the Bahamas, and elsewhere in the Caribbean, do not necessarily gather at such distinct geomorphological features, and appear to prefer only a shelf break, regardless of its orientation (Colin, pers. comm.). Based on my understanding of overall surface circulation patterns for the region (James and Ginsburg, 1979), I suggested in an earlier paper (Carter, 1986) that fish need only to swim to the shelf edge and continue down current until they reach the end of the island shelf where they simply begin to stack up. Then one could envision a variety of communication modes to further attract fish (sound production, pheromones, etc.). Colin *et al.* (1987) suggested the same system earlier, only with the fish moving always up current to find the aggregation. These scenarios are now unlikely given the limited results from our current studies on the Cay Glory site, and more detailed observation in the Bahamas (Colin, 1990). It is clear localized aggregation site currents are too highly variable and unpredictable on a daily and/or seasonal basis to be of major value in locating the sites.

Whether the six spawning sites off the Belize coast favor the entrainment of Nassau grouper larvae into localized mesoscale eddies (Lobel, 1989) is unknown. Studies indicate a larval life of 35-40 days for *E. striatus* (Colin, 1990; Tucker, pers. comm.). They are also noticeably absent from Icthyo-plankton collections in offshore waters but have been reported as recruitment into the Bahamas banks via tidal channels (Colin, pers. comm.). One possible approach to addressing these questions would be to examine gene flow (via mitochondrial DNA analyses) within populations restricted within atolls, populations between widely scattered spawning sites, and finally with geographically distant populations from the eastern Caribbean or Bahamas.

Food, Feeding Habits, and Movement

Optimal foraging theory (Schoener, 1971) predicts that a prudent predator will prey upon the most common prey species available but will switch to alternative preys when the common prey species becomes scarce (Slobodkin, 1961; Werner and Hall, 1974). Nassau grouper conform to this pattern. Generally, *E. striatus* can be characterized as an unspecialized, top-level carnivore on the reef feeding on a variety of food items including such different prey as fish, crustaceans, and cephalopods. However when we examine the kind

105

of food selection within major categories, data indicates that grunts (Pomadysidae) were the most common fish taken. This data agrees well with fish community structure data from Belize which indicates that grunts rank high both in numerical abundance and by weight (Barrick, 1990; Sedberry and Carter, 1990). In comparison, Randall (1965) reported in the food habits of Nassau grouper from the Virgin Islands and Puerto Rico where he found similar percentages for major food categories but listed parrotfish as the most common family of fish taken, possibly reflecting this species relative abundance in the region and/or its low rate of digestion. Similar results were reported in Curacao for the graysby, Epinephelus cruentatus, which is common in the region (Nagelkerken, 1979).

Groupers exhibit four main feeding tactics: bottom ambush; midwater ambush; cave ambush; and nuclear predator association (Diamant and Shpigel, 1985). Bottom ambush is by far the most common pattern employed by these normally solitary fishes. However, grouper are also known to alter their behavior and follow other primary predators such as octopus or eels around. Prey that escapes the primary predator is then captured secondarily by groupers (Diamant and Shpigel, 1985). I have observed Nassau grouper on several occasions associating with eels and other nuclear predators in the numerous limestone sinkholes and caves that distinguish the Belize seafloor karst topography. The daily track observed for sonically tagged individuals may reflect movement related to these nuclear predator associated feeding forays.

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