

Final Progress Report

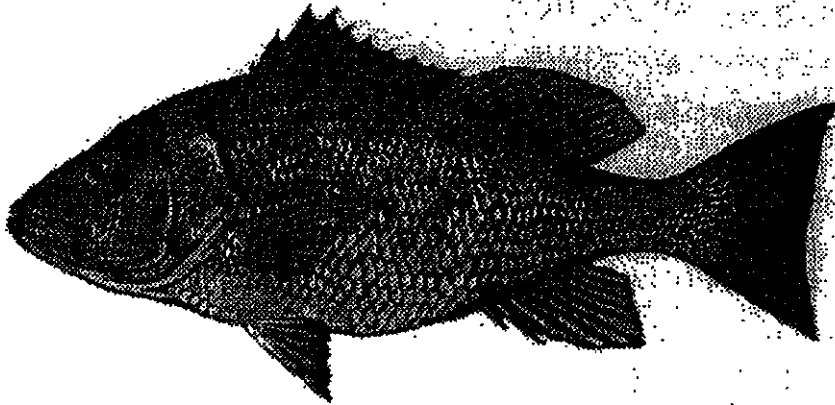
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MARFIN

Project title: Size-dependent spawning and egg quality of Red Snapper

Principal Investigator: Edward J. Chesney, Ph. D.

Report Authors: Edward J. Chesney and Richard San Filippo



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Table of Contents

I. Executive Summary.....	1
II. Introduction.....	2
III. Purpose of Study.....	2
A. Problem.....	2
B. Objectives.....	3
IV Study Approach.....	4
A. Methods.....	4
B. Project Management.....	6
V. Findings.....	6
A. Results.....	6
Significant Problems in the Research.....	9
VI. Evaluation.....	11
A. Original Goals.....	11
B. Project accomplishments.....	11
Other Accomplishments.....	12
C. Benefits to the Fishery.....	12
D. Economic Benefits to the Fishing Industry.....	13
E. Need for Federal Assistance.....	13
VIII Conclusions.....	14
A. Research Conclusions.....	14
B. Project Success.....	14
Acknowledgments.....	15
References.....	15
Figure Legends.....	17

**Final Progress Report
Submitted to MARFIN**

Grant Number: NA37FF0048-01

Project title: Size-dependent spawning and egg quality of Red Snapper

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I. Executive Summary

The project goal was to study the reproductive biology of red snapper in the laboratory emphasizing the size-specific spawning potential and the quality of female spawns. The objective was to determine the size-specific potential of young female red snapper to contribute to the spawning stock. Adult red snapper ranging in size from 305-579 mm TL (12.5-25 in.) for females and 298-521 mm TL (11.75-20.5 in.) for males were captured by hook and line at weekly intervals during a period from the end of May through the middle of October. Fish were brought to the lab in a transport tank, held overnight then sexed by catheterization. Individual females were injected with HCG (Human Chorionic Gonadotropin; 1.1 IU per gram body weight) and paired with two ripe males injected with HCG (0.55 IU per gram). Fish were held at field temperatures and photo period in spawning tanks for up to 3 days or until they spawned. Spawning eggs were counted and a subsample measured. Spawning in the laboratory took place between 2300 and 0100 hours typically 30-36 hours after injection. Laboratory spawnability of red snapper was highest in June, July and August and decreased in September and October. Overall laboratory spawnability for age 2 fish was low (~20%) and increased significantly in older females (~70%). Batch fecundities ranged from several hundred eggs for a 317 mm, age 2 female to 255,000 for a 552 mm age 5 snapper. There were no discernible differences in egg diameter related to female size or age. Mean egg diameters ranged from 0.71 to 0.81 mm with an overall mean of 0.77 mm and showed no obvious size-dependent or seasonal trends when all size classes were plotted together. However, when spawns from age 3 fish were plotted by month a trend of declining egg size later in the spawning season was apparent. Based on results from this study it is reasonable to conclude that while some age 2 female red snapper are spawnable, age 2 fish are unlikely to contribute significantly to spawning stock biomass. Numerous age 2 (25%) and age 3 (33%) males with flowing sperm were captured, one as small as 298 mm TL (11.75 in.), suggesting that age 2 males may participate in spawning at a higher frequency than females.

II. Introduction

The commercial and recreational fishery for red snapper, *Lutjanus campechanus*, in the Gulf of Mexico and SE Atlantic is a valuable resource. Trends in commercial and recreational catches of red snapper indicate they have been declining in the Gulf of Mexico since the early 1970's (Goodyear and Phares 1990). Total commercial and recreational landings have declined from 14-15 million pounds in the early 1980's to 5-6 million pounds in 1987-1988. Red snapper CPUE as bycatch in the shrimp fishery has declined significantly, indicating a real decline in red snapper populations. Fishery independent statistics, such as the NMFS fall groundfish survey, indicate similar trends with CPUE of juvenile fish declining 3-5 fold since 1972. Although it is often difficult to identify specific cause and effect mechanisms for population declines, it appears that 1) overfishing by directed fisheries and 2) bycatch of juveniles in trawl fisheries are significant contributing factors. Over-exploitation of this important species has brought the need for management of red snapper resources to a critical stage. Both creel limits (7 fish per day) and size limits (currently 14 inches TL scheduled to go to 15 in. TL in 1995 and then 16 in. TL in 1996) have been instituted for the recreational fishery and a catch quota has been instituted for the commercial fishery.

III. Purpose of Study

A. Problem

An analyses of the red snapper fishery indicated that the previous size limit (13 in. TL) and numbers in the adult spawning stock may not be adequate to return the fishery to its former yields (Goodyear and Phares 1990). At present, spawning stock ratio (SPR) is far below the 20% level needed for a healthy fishery. Furthermore, the ability to model and manage the fishery relies upon the quality of the input data. Essential information includes accurate estimates of fishing and natural mortality along with data on spawning, fecundity, spawning frequency and age at maturity so that stock-

recruitment models can be refined.

More information on the reproductive biology of red snapper was needed for management purposes. Especially important is a better understanding of the size-specific contribution spawning females potentially make to new recruits so that the spawning stock can be estimated and managed. Studies by NMFS and LSU are defining spawning sizes and frequency from histology of field collected specimens from the Northern Gulf. Knowledge of the size/age specific potential for a given individual to contribute recruits to the population allow refinements to be made in population dynamic assessments. It also identifies the year classes that are the most significant contributors to recruitment.

Previously some age 2+ red snapper were assumed to be mature and contributing to recruitment based on their fecundity. While they may spawn, their early spawns may be of low quality and contribute little to recruitment. It is well established that size-specific fecundity does not necessarily reflect the size-specific reproductive potential of adult fish populations (Zastrow et al 1989). For many fishes, young "mature" females are precocious spawners with lower relative fecundities and lower quality eggs (Knutsen et al 1985; Hislop 1988). Egg size may also be related to size of the female, which can affect the probability of producing new recruits to the population (Hislop 1988). Smaller eggs can result in smaller larvae which can reduce their chances for survival (Ware 1975; Rana 1985; Houde 1987). For multiple or serial spawners (i.e. red snapper), egg size and quality may decline later in the spawning season after females have spawned multiple times.

B. Objectives

The objective of the proposed research was to provide additional information on the reproductive biology of red snapper. Establishing better data on the relative

reproductive contribution of adult red snapper would allow better estimates of the potential for a given population structure to produce recruits and highlight the age classes most important to spawning and recruitment. More sophisticated stock assessment and management strategies could then be employed in the management of red snapper. The proposed research had the following objectives:

- 1) Determine the size/age-specific reproductive output of female red snapper, including egg quality, hatchability and viability.
- 2) Examine the changes in egg quality, hatchability and viability of red snapper eggs throughout the spawning season.

IV Study Approach

A. Methods

Adult red snapper ranging in size from 318-635 mm TL (12.5-25 in.) for females and 298-521 mm TL (11.75-20.5 in.) for males were captured by hook and line at weekly intervals during a period from the end of May through the middle of October. Upon capture over-inflated swim bladders were deflated with a syringe needle. This procedure was essential to the healthy transport and survival of the fish. Fish were transported to the lab in a live tank (120 gallons) aerated with oxygen. At the laboratory fish were held overnight in a temperature controlled recirculating seawater system. This system was made up of four 550 gallon spawning tanks with a central reservoir fitted with four egg collection devices. This allowed us to process up to four females simultaneously.

All captured fish were anesthetized (MS222), sexed by catheterization and weighed. Samples of the catheterized female gonads were preserved for later analysis. Individual females were injected with HCG (Human Chorionic Gonadotropin; 1.1 IU per gram body weight) and

paired with two mature males which were also injected with HCG (0.55 IU per gram). Fish were incubated in temperature-controlled recirculating spawning tanks for up to 3 days or until they spawned. Temperatures, salinities and photoperiods were maintained at conditions approximating those in the field at the time of capture, typically 24-27C and 32-34 ppt. Eggs from spawners were collected in an egg trap fitted to the overflow of the spawning tank. Eggs from each spawn were counted, a subsample of eggs measured immediately (20-40), a subsample preserved in formalin and a second subsample frozen for lipid analysis.

Groups of eggs ranging in size from 14-43 eggs per sample were analyzed for lipid content. Only clear buoyant eggs were selected for analysis. Lipid analysis for snapper eggs followed familiar and established methods for the analysis of intact lipids in biological tissues (Harvey and Patton, 1981; Harvey and Kennicutt, 1992). Following extraction of groups of eggs with organic solvents, the Iatroscan TLC-FID system was used to separate and quantify intact lipid classes. With the Iatroscan, small aliquots of the total lipid extract (typically <0.5fg/lipid class) were chromatographically separated in an appropriate solvent system and quantified by flame ionization detection. This technique has been successfully applied to a number of different environments and organisms including oceanic water columns (Parrish and Ackman 1983), marine sediments (Harvey et al, 1984), copepods (Hakansen, 1984) and larval fish (Fraser, et al., 1987) and fish eggs (Fraser et al 1985).

Four classes of lipids (phospholipids, free fatty acids, triacylglycerols, and sterols) were quantified in 16 egg samples from different size females and used in an attempt to estimate egg quality . The addition of free fatty acids to the three lipid classes typically used as a condition index in fish larvae (Fraser 1989) were used here as an indicator of sample quality, allowing any storage artifacts to be quantified. In most living tissues, the concentration of free fatty acids is low (< 1% total lipid classes), with higher levels often a result of the release of fatty acids from other lipid classes.

B. Project Management

The research for this project took place at the Marine Center of the Louisiana Universities Marine Consortium in Cocodrie, Louisiana under the direction of Dr. Edward Chesney. Richard San Filippo served as the technician for the project. Lipid samples were analyzed by Dr. Roger Harvey of Chesapeake Biological Laboratory.

V. Findings

A. Results

Sixty seven female red snapper ranging in size and weight from 305 to 597 mm total length (TL) and from 0.474 to 2.862 kg, were tested between late May and the beginning of October. Sizes of the fish tested were selected to give a distribution of sizes and ages in the analyses emphasizing the youngest potentially mature size classes. All references to ages and age classes are approximations based on the following convention taken from Nelson and Manooch (1982) for Louisiana collected snapper: age 2= 267-378 mm TL, age 3= 379 - 468 mm TL, age 4= 469 - 545 mm TL, age 5= 546 - 613 mm TL.

Inspection of the egg traps at 2 hour interval on four occasions indicated spawning in the laboratory took place between 2300 and 0100 hours typically 30-36 hours after injection. Spawnability of red snapper was highest in June, July and August decreasing in September and October (Figure 1). It should be noted that weather-related sampling difficulties prevented testing significant numbers of females in the month of May. Of the 67 females tested between May and October, 40% spawned in captivity after hormone injection. Spawnability was strongly related to female age with 2 yr. old fish much less likely to spawn than other age classes tested ($p=0.0002$, One-way ANOVA, LSD range test). Overall spawnability was about 20% for 2 yr. old fish and increased significantly to about 70% in 3 and 4 year old fish (Figure 2). It should be noted that it is impossible to infer spawning frequency from our spawning data since the fish

were induced to spawn with hormones. Batch fecundities ranged from a few hundred eggs for a 317 mm age 2 fish to 255,000 for a 552 mm age 5 snapper (Figure 3). Batch fecundities were very low in some cases for age 2 fish. For example, the seven age 2 fish that spawned were 318, 337, 375, 362, 368, 330 and 362 mm TL (12.5, 13.25, 14.75, 14.25, 14.5, 13.0 and 14.25 inches, respectively). They produced batch fecundities of 196; 23,220; 5,063; 23,460; 22,290; 2,850; and 7,970 eggs respectively. Although spawnability of age 2 fish was low and some of their batch fecundities were quite low, it is notable that a 337 mm female produced a batch size of 23,460 eggs.

Male red snapper were categorized as ripe if sperm could be readily extracted with the catheter. However, for the purpose of describing male condition, males with flowing sperm were defined as those that emitted sperm after gently squeezing their abdomen. Of the males captured 25% of the age 2 and 33% of the age 3 males had freely flowing sperm, one as small as 298 mm TL (11.75 in.). This suggests that age 2 males may participate in spawning at a higher frequency than females.

Spawnability was correlated ($p=0.10$, correlation analysis) with phase of the moon and was lower in the new phase of the lunar cycle, although not significantly lower (One way ANOVA, $p=0.05$) (Figure 4a). Mean weight specific batch fecundity (expressed as eggs per gram of fish) was much lower during the full moon averaging only 13.8 eggs per gram female weight versus 27.3, 33.9 and 34.6 in the other moon phases (Figure 4b). This trend was also not statistically significant (One way ANOVA, $p=0.05$). We examined the distribution of ages among the moon phases and concluded that it is unlikely that the trends were not caused by an uneven distribution of age classes. Although these spawning trends were not statistically significant, the high degree of variability in spawn size and frequency and the low number of samples may have obscured all but the strongest trends in the data.

We attempted to relate the size frequency distribution of the female gonad tissue samples to their spawnability by hormone injection. The goals were two-fold. First we wanted to relate the condition of the female gonads at the time of capture to the general trend in our spawning studies. Our other goal was to establish criteria to predict potential spawnability from catheter sampling and staging of the gonads. The catheter gonad samples are not strictly quantitative because a fixed amount of tissue was not removed by this process. However the samples do seem to reflect the general condition of the female gonads. Examination of the egg diameters from the catheter samples of the female gonads supports the spawnability data from our laboratory studies, indicating that the probability of spawning in our lab experiments probably reflected the general spawning condition of the population in the field. No ovulated eggs nor oocytes larger than 0.5 mm were seen in any females captured during the new moon while females with ovulated eggs were present in the other lunar quarters and were most abundant during the last quarter of the moon (Figure 5). Those same data plotted seasonally showed that spawning dropped off abruptly at the beginning of October (Figure 6).

There were no discernible differences in egg diameter related to female size or age for the age classes tested (Figure 7 a, b). Mean egg diameters ranged from 0.71 to 0.81 mm with an overall mean of 0.77 mm and showed no obvious size-dependent or seasonal trends when all size classes were considered together (Figure 7a, b). However, when spawns from age class 3 fish were plotted seasonally and fitted by linear regression, a statistically significant trend ($P < 0.01$, One-way ANOVA) of declining egg sizes later in the spawning season was apparent (Figure 8). No similar trend could be discerned for other size classes because the total number of spawned females in other size classes were too few to detect a clear trend.

We were unable to determine size-dependent hatchability or viability of the eggs that were spawned because none of the eggs developed beyond the early gastrula stage for any of the spawned batches. We tested several possible causes for the lack of egg viability including water

quality, lack of fertilization, damage in the egg traps and disease and eliminated those as potential causes. Another possibility which we have been unable to test so far is that the hormone level recommended from strip-spawning studies causes over-ripening of eggs for tank spawned fish. We were unable to test different hormone levels in 1993, but hope to do this at some time in the future.

We did not detect size-dependent differences in egg quality based on total lipid content or lipid class analysis (Figure 9). However, the free fatty acids component of the eggs indicated a problem in the quality of the egg samples (Figure 10). Free fatty acid levels were inversely correlated with total lipids per egg and concentrations were above the level acceptable for intact tissue samples. This was not caused by mishandling of the samples or an analytical problem. The problem was undoubtedly due to the arrested development and the subsequent decline in quality of the biochemical constituents of the eggs. The high concentrations of free fatty acids in the samples and the range of concentrations is indicative of over-ripening of the eggs and subsequent decline in quality.

Significant Problems in the Research

The most significant experimental problem we encountered was difficulty in producing spawns of viable eggs. This prevented us from looking at the size-dependent hatchability of the eggs and viability of the larvae. As discussed earlier in this report we thoroughly investigated potential causes for this problem including water quality, adult condition, lack of fertilization, disease/parasites and handling. We believe that our physical setup and procedures were sound and more than sufficient to produce quality spawns. We are currently spawning anchovies using a similar physical setup and egg collection system with no problems or damage to the eggs. Our trouble shooting has led us to suspect that the level of hormones used were possibly too high for tank spawning red snapper that were in season. We followed protocols outlined in Minton et al.

(1983) for spawning red snapper in captivity, with the exception that we tank spawned rather than strip-spawned the fish. Tank spawning was preferable for our studies because with strip spawning we would not be able to detect whether the fish spawned on their own or not. Also strip-spawning can cause variability in the viability of eggs and larvae that is associated with handling of the adults and their eggs as well as skill in staging the eggs.

Some recent research with strip-spawned white bass showed that egg viability is markedly decreased (~20%) when dosages similar to the ones we used for red snapper are used to bring the fish into spawning condition (Kohler et al 1994). The study also showed that much lower levels can be used (i.e. 50 IU per Kg), resulting in much higher egg viability rates (66-89%). The standard dosage used for white bass was 1100 IU per Kg, but the research describe above got their best results with 50 IU per Kg. Prior to Kohler's research, efficacy of HCG had never been tested for white bass. As with white bass, efficacy of HCG dosages for the spawning of red snapper needs to be tested.

We believe that by injecting fish that were in their natural spawning cycle with high dosages of gonadotropin (the recommended dose of 1100 IU per Kg) resulted in over-ripening of the nearly mature or in some cases mature oocytes. In the near future we hope to test lower dosages of HCG to confirm our hypothesis and further refine tank spawning of red snapper.

Weather presented an occasional problem for sampling offshore. The fish were captured in water depths of 75-125 ft and distances of 30-45 miles offshore of Louisiana's barrier islands. Windy weather and rough seas prevented us from sampling as much as we would have liked in May and for two weeks during September, reducing the number of observations during those times. We also encountered problems in transporting larger fish during the warmest summer months. We captured significantly more age 4 and older fish than we were able to test in the laboratory because they were much more difficult to transport than smaller fish. The

combination of lower frequency in our catches and higher mortality rates in transport led to a lower frequency in our laboratory tests.

VI. Evaluation

A. Original Goals

The original project goals were to study the reproductive biology of red snapper emphasizing the size-specific spawning potential and the quality of female spawns. The objective was to determine the size-specific potential of young female red snapper to contribute to the spawning stock. This information will benefit the fishing industry by providing information needed to optimally manage red snapper resources in the Gulf of Mexico and SE Atlantic, eventually generating greater yields from the fishery.

B. Project accomplishments

This project added significantly to our understanding of spawning and reproduction of red snapper. Our results clearly show that age 2 female red snapper do not contribute significantly to the adult spawning stock. Although we were able to spawn some age 2 females in the lab, their batch fecundities tended to be very low as was their spawning frequency. Although age 2 females probably do not significantly participate in spawning, age 2 males probably do participate in spawning at a higher rate than females. Although we did not keep records of male conditions throughout the spawning season, for the 4 months where male condition was recorded there were a significant number of age 2 males (25%) with free flowing sperm.

Age 3 females were the first strong year class to show a high incidence of spawnability in our study. Although we were not able to detect any size-specific differences in egg size when all samples were considered together, a gradual decline in egg size was detected for age 3 females over the course of the spawning season, indicating female condition may decline in that age class.

Although we were not able to produce viable eggs using tank spawning procedures we learned much about spawning red snapper in captivity with hormones. The experience gained in this study has provided the opportunity to refine laboratory spawning techniques for red snapper in the future. The ability to routinely spawn and rear red snapper in the lab would provide numerous opportunities for red snapper research and management not currently available.

Other Accomplishments

Gonads were preserved from several of the larger females spawned in the lab for examination of the longevity of their post-ovulatory follicles (POF's). Although this was not a goal of this project and will not be reported here, this data will be useful to other MARFIN researchers studying reproduction, batch fecundity and spawning frequency of red snapper in the field. We are analyzing the POF samples as time permits. We made histological sections of some of those samples and made them available to MARFIN researchers at LSU (C. Wilson and J. Render) so that they can utilize the known age POF's to refine their estimates of spawning frequency.

We also made live red snapper specimens available to NMFS researchers for tag retention research. Working in collaboration with the Aquarium of the Americas, E. Prince (NMFS, Miami) and C. Jones (RSMAS, Miami) are testing a new tag for potential use in future NMFS tagging programs. As we completed our tests we passed many of our red snapper on to them for their research.

C. Benefits to the Fishery

These studies have expanded our understanding of red snapper spawning and gained experience that will be useful in future studies in the laboratory and field. The results from these

studies will be beneficial to future applied research by improving the technical knowledge needed to tank spawn red snapper in the laboratory. The results will also provide a broader base of knowledge for scientists seeking data that can be used to model population dynamics and for managers making recommendations for management of red snapper. With a sound basis for management and adequate management strategies the fishery will be able to gradually return to optimum populations and yields to the fishery.

D. Economic Benefits to the Fishing Industry

The fishing industry will benefit from this research indirectly through a better understanding of the reproductive biology of red snapper and through improved management of the fishery. With the high mortality rates juvenile snapper suffer as bycatch in trawl fisheries and the present low spawning stock ratio, it is important to know at what age red snapper recruit to the spawning stock and what their relative contributions of progeny is likely to be. These data will contribute to better management of the fishery and ultimately greater economic yields.

E. Need for Federal Assistance

Red snapper are under State and Federal management because of their recreational and commercial importance and declining catches. Red snapper are economically important for the entire Gulf and southeast Atlantic region. Ex-vessel value of red snapper are among the highest for finfish. Much of the catch and hence the remaining stock of red snapper in US. waters of the Gulf of Mexico is harvested in the Fisheries Conservation Zone (FCZ) off Louisiana's coast. In fact, of all red snapper harvested in the Gulf and SE Atlantic during 1992, 97% came from the Gulf and 73% were landed to Louisiana and Texas ports (NMFS, 1993).

Harvest of red snapper by commercial and recreational fisheries have surpassed sustainable yields for several years. With the many fishery and habitat loss issues the Gulf of Mexico faces, regionally important resources, such as red snapper, has and will require

cooperation between Federal and State governments to produce the needed research and management. With State resources dwindling because of budget deficits, Federal assistance was needed to provide management and research funds for this important national resource.

VIII Conclusions

A. Research Conclusions

Age 2 female red snapper do not significantly participate in spawning or contribute to spawning stock ratio. Although we were able to spawn some age 2 females in the lab, some of their batch fecundities tended to be very low as was their spawnability. No discernible differences in egg size was apparent for females of different sizes, however the mean size of spawned eggs declined throughout the course of the spawning season for laboratory spawned age 3 females, indicating female condition and egg quality may decline in that age class.

Although age 2 females probably do not significantly participate in spawning, age 2 males probably do participate in spawning at a higher rate than females. Although we did not keep records of male conditions throughout the spawning season, for the 4 months where male condition was recorded there were a significant number of age 2 males (25%) with free flowing sperm.

B. Project Success

Although this project did not meet all of its goals it was successful in refining our understanding of the spawning and reproduction of red snapper and provided information that will be useful to managers. In addition to meeting most of the project goals we were also able to provide useful information to other MARFIN researchers studying red snapper and provide live snapper specimens to NMFS researchers for tag development and retention research.

Acknowledgments

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Figure Legends

Figure 1. Rate of spawns per attempt for HCG injected female red snapper plotted by month. The numbers above each month represents the total number of females injected each month.

Figure 2. Rate of spawns per attempt for HCG injected female red snapper plotted by age class. The numbers above each age class represents the total number of females injected each age class. Ages are estimates based on length. See text for details.

Figure 3. Batch fecundities of laboratory spawned red snapper plotted against length (mm TL). On four occasions females spawned more than once while in captivity. Those females are denoted by the symbols for multiple spawners.

Figure 4 a, 4b. Rate of spawns per attempt (4a) and batch fecundity per gram of fish (4b) plotted by moon phase for HCG spawned red snapper. The numbers above each lunar phase represents the total number of females spawning in each quarter.

Figure 5. Size-frequency of oocytes diameters for field collected female red snapper plotted by moon phase. Samples were extracted by catheter within 24 hrs. of capture and prior to injection with HCG. Samples are from 69 females.

Figure 6. Size-frequency of oocytes diameters for field collected female red snapper plotted by month. Samples were extracted by catheter within 24 hrs. of capture and prior to injection with HCG. Samples are from 69 females.

Figure 7 a, 7b. Mean egg diameters from laboratory spawned red snapper plotted against live weight (g) (7a) and total length (7b) of the female. Vertical bars are ± 1 standard error.

Figure 8. Mean egg diameters from laboratory spawned red snapper plotted by month for age 3 females only. The regression and the slope of the regression were significant (ANOVA, $p=0.01$). Vertical bars are ± 1 standard error.

Figure 9. Mean egg diameter verses total lipid content per egg for selected spawns ($n=16$ females).

Figure 10. Total lipid content per egg verses free fatty acid content for selected samples of red snapper eggs ($n=16$ females).

Figure 1.

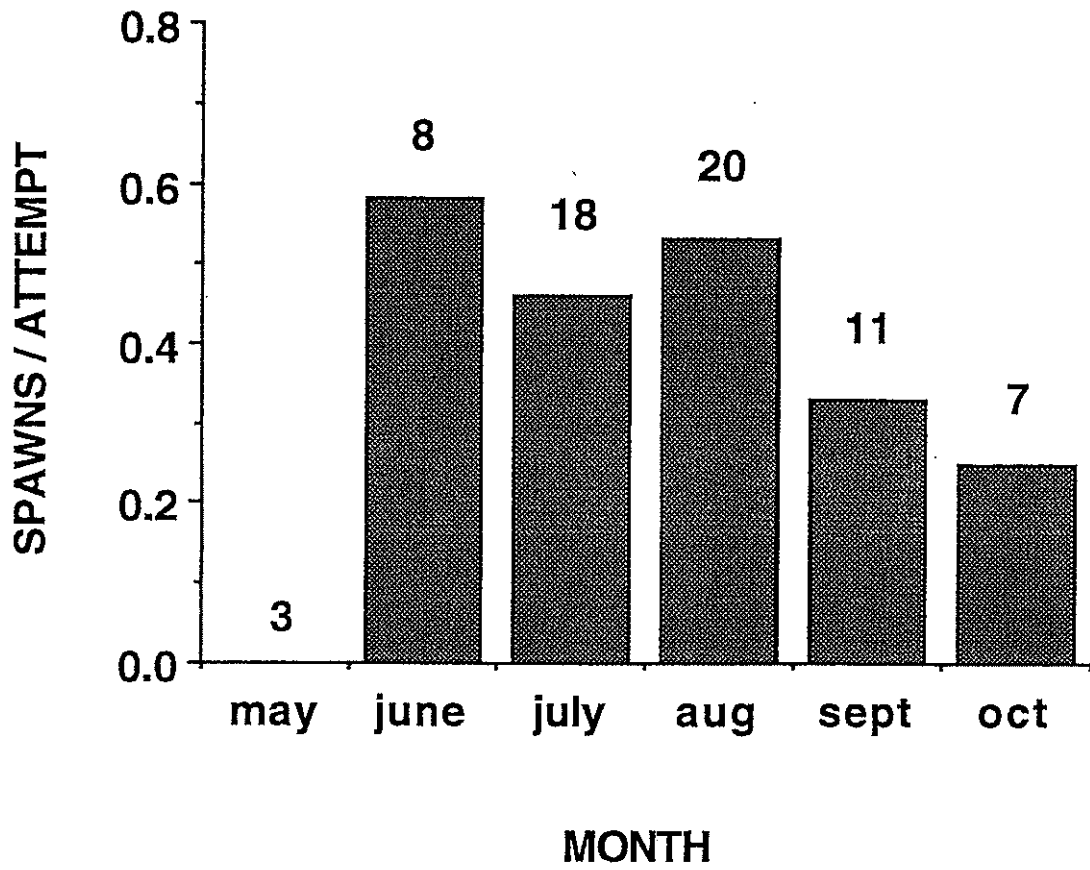


Figure 2.

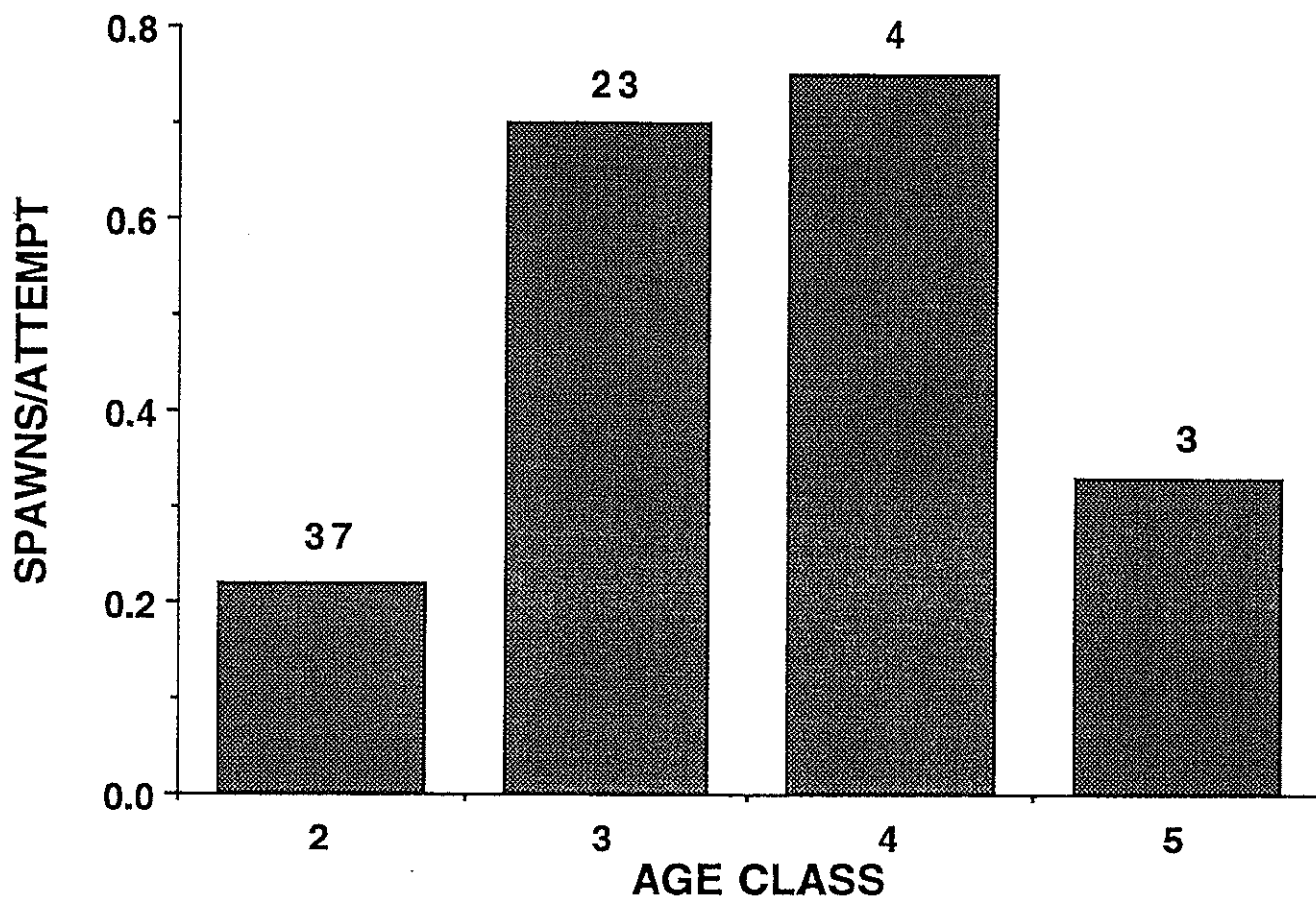
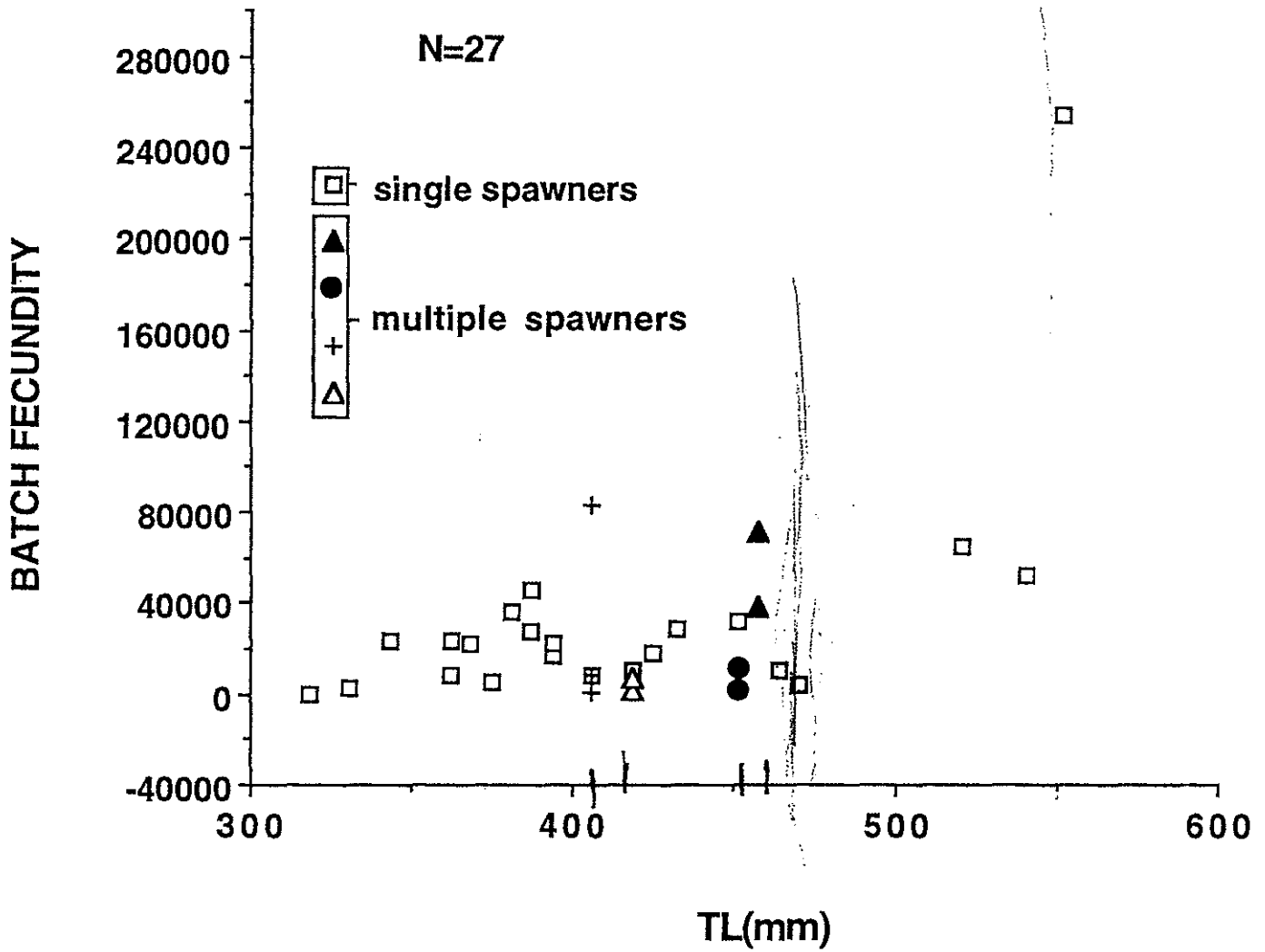


Figure 3.



Last Qtr.
3

Figure 4a.

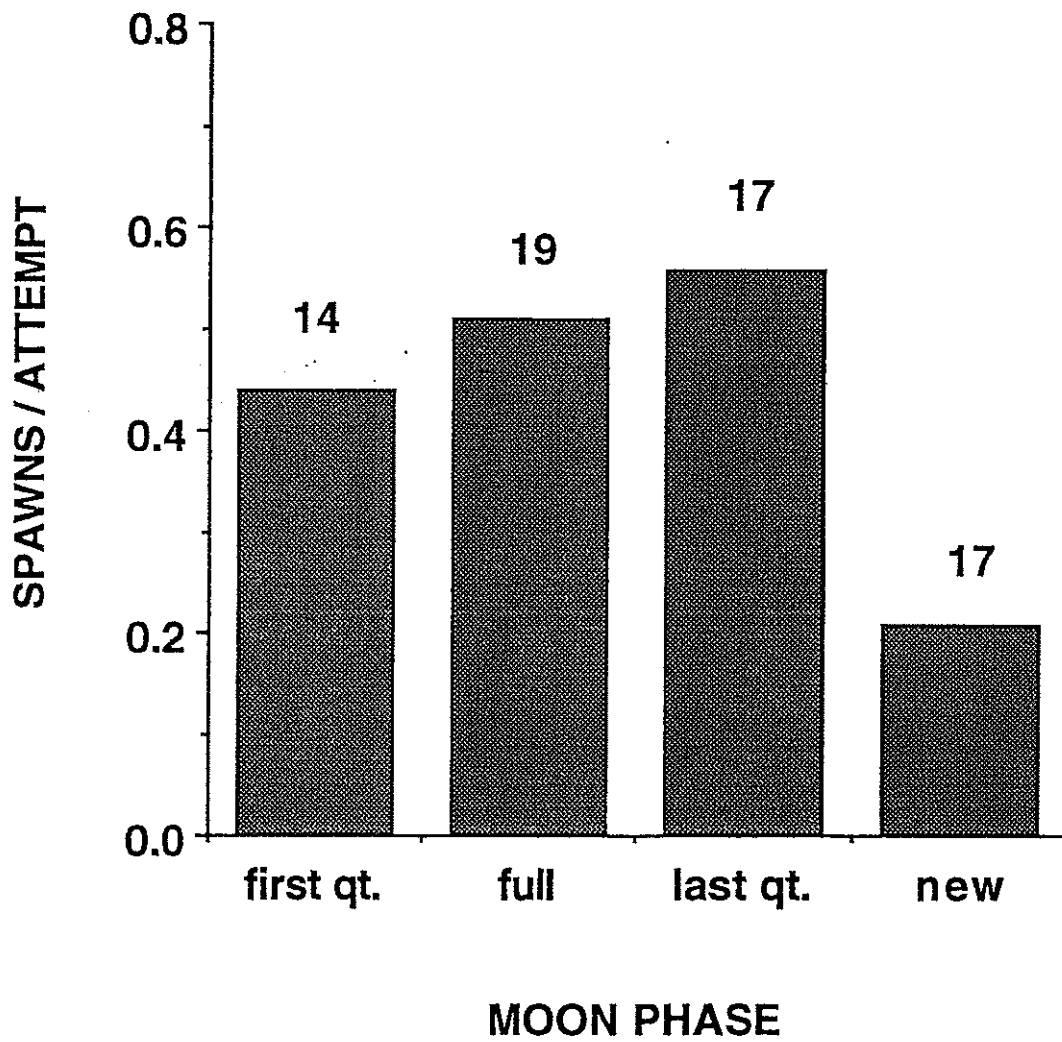


Figure 4b.

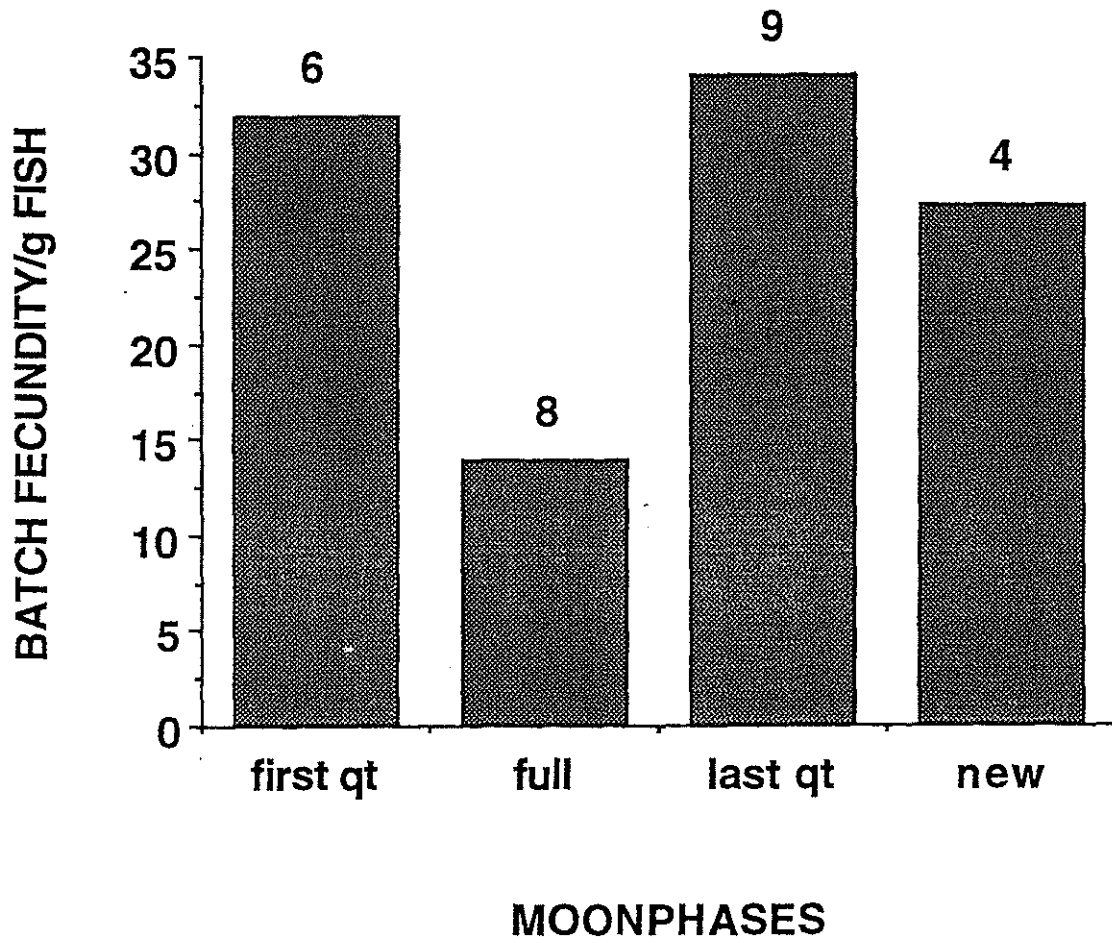
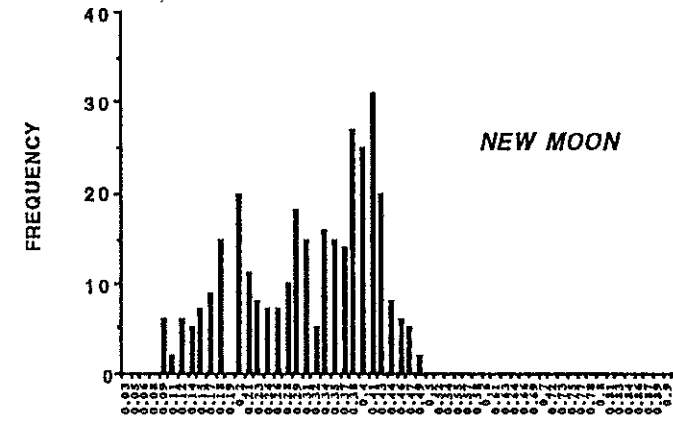
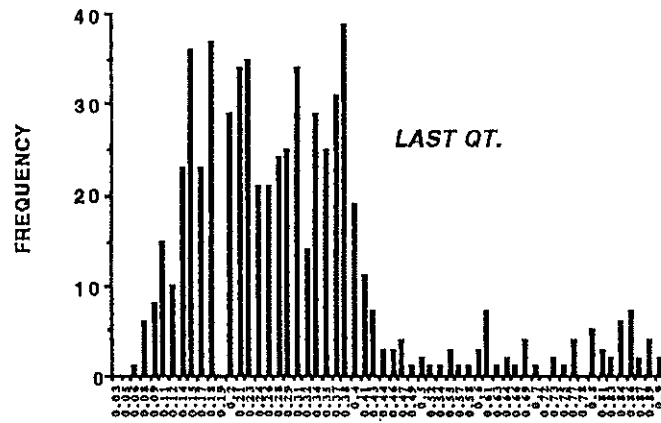
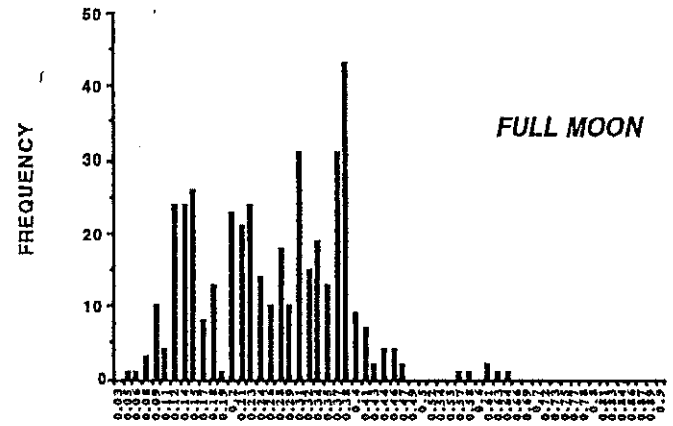
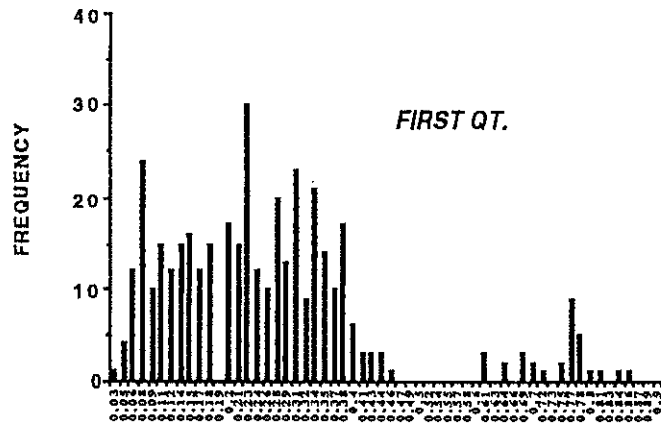


Figure 5.



OOCYTE DIAMETER (mm)

OOCYTE DIAMETER (mm)

Figure 6.

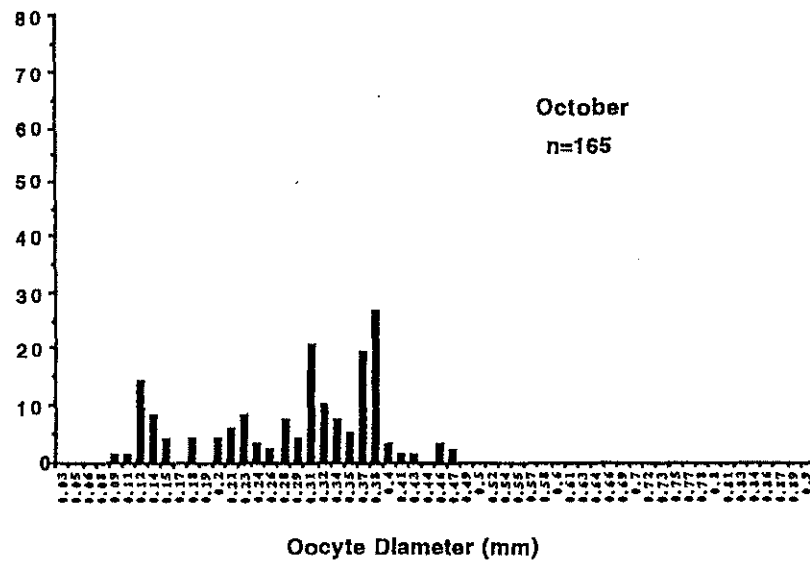
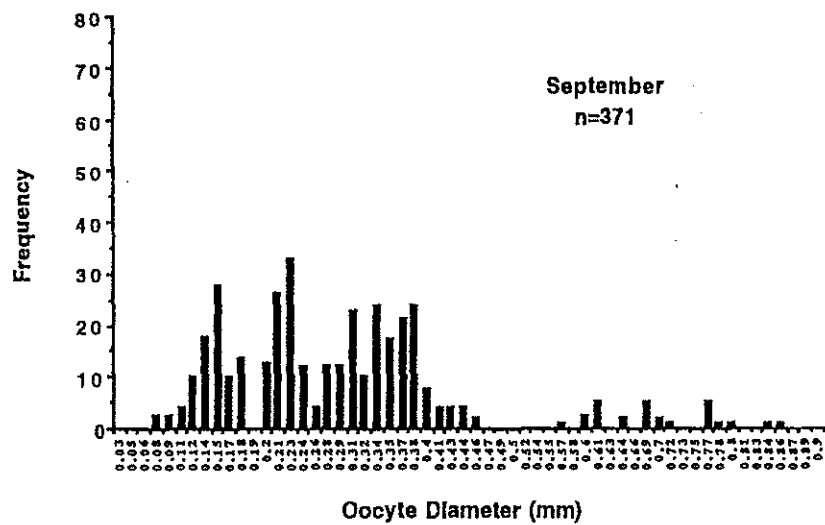
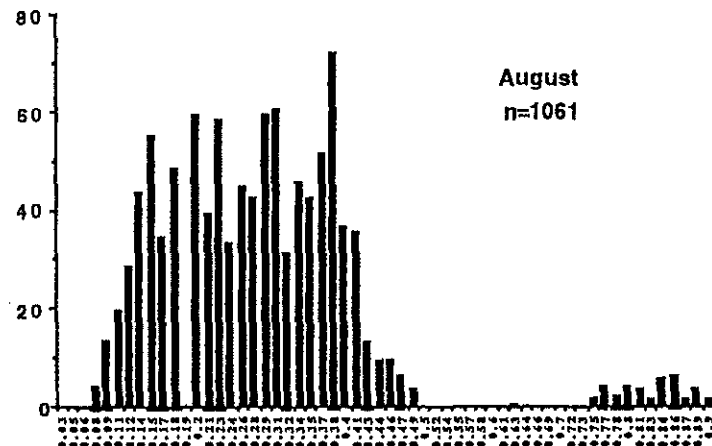
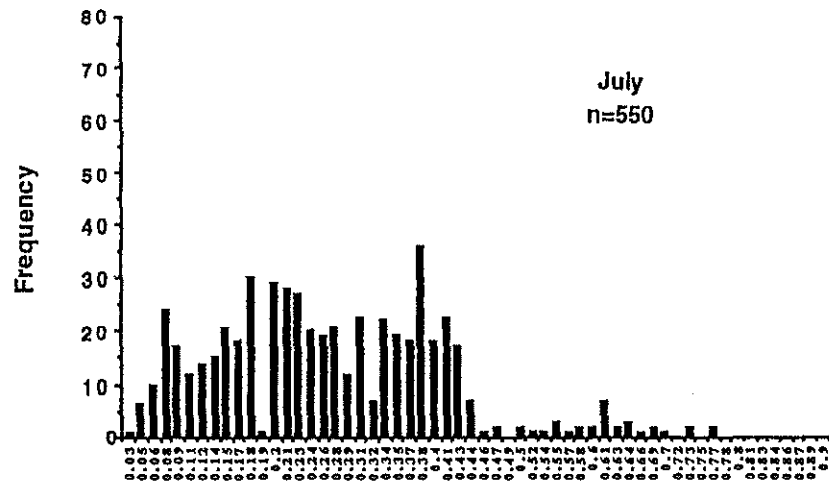


Figure7a.

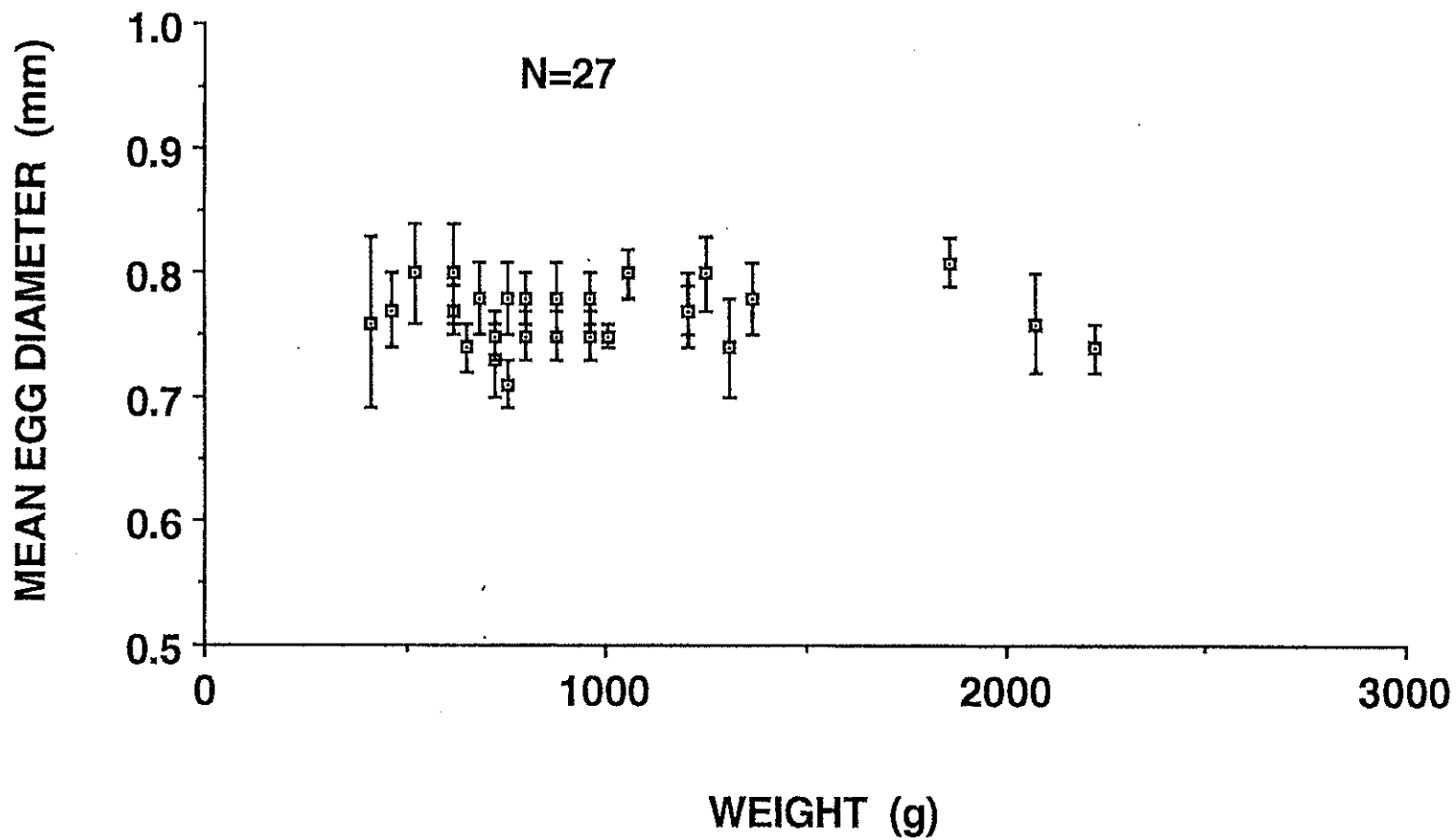


Figure 7b.

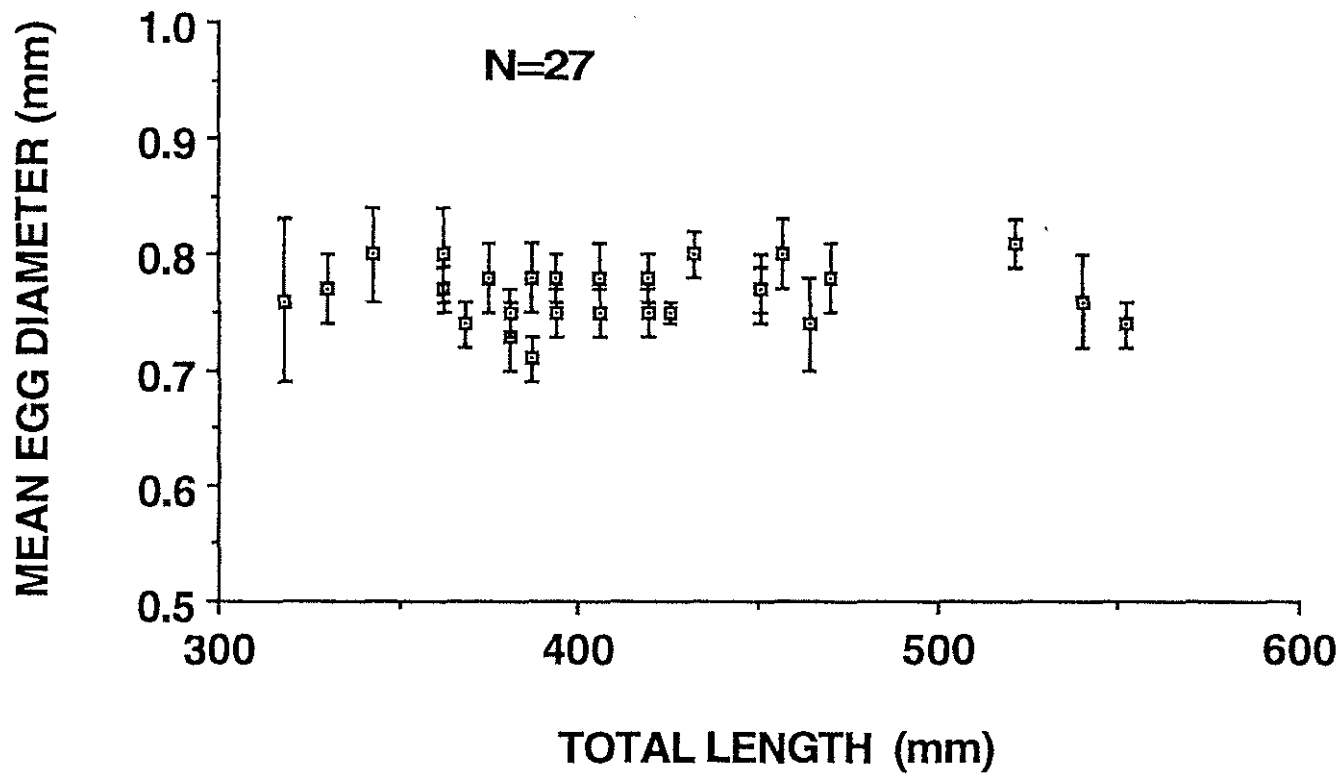


Figure 8.

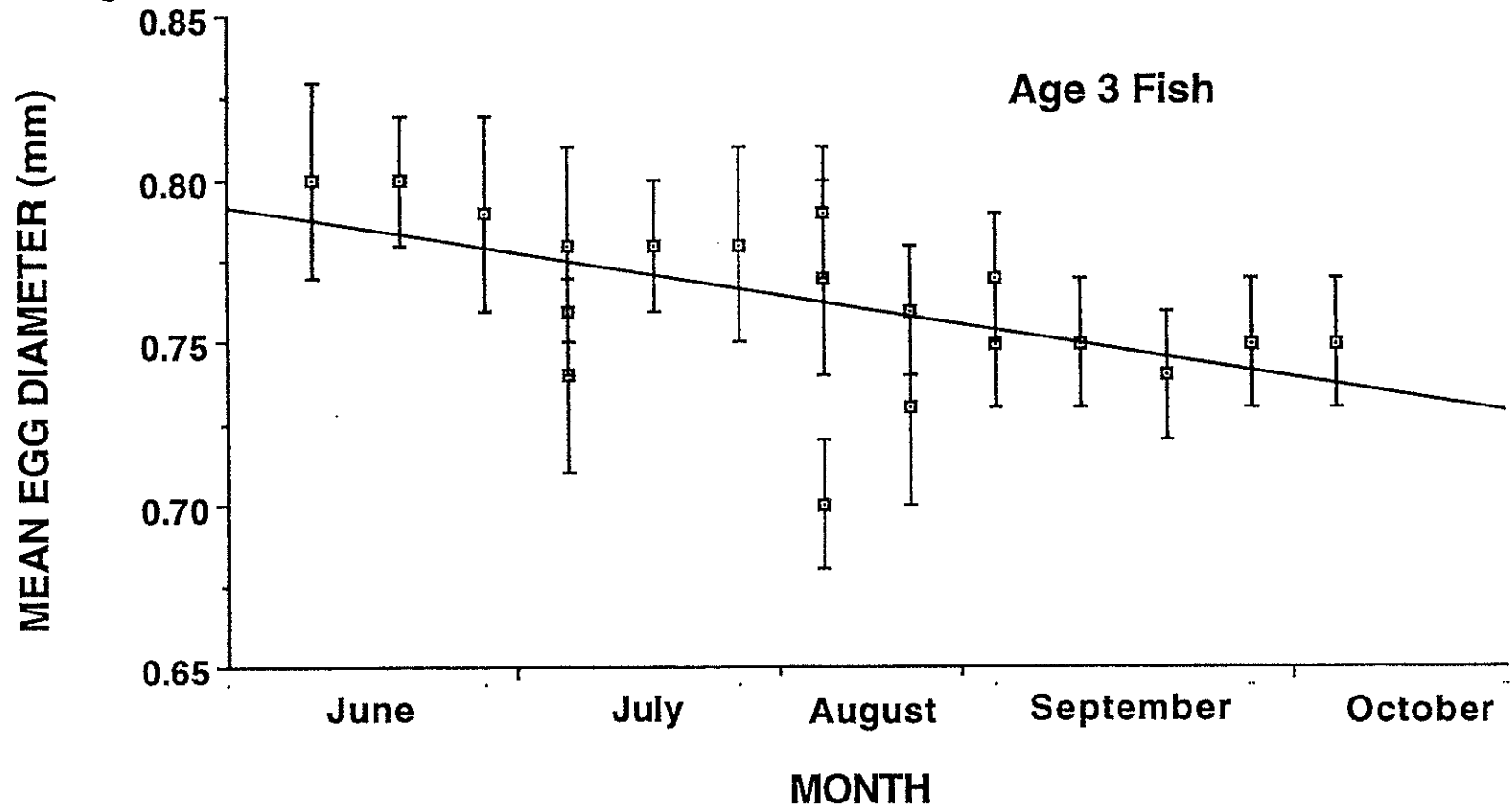


Figure 9.

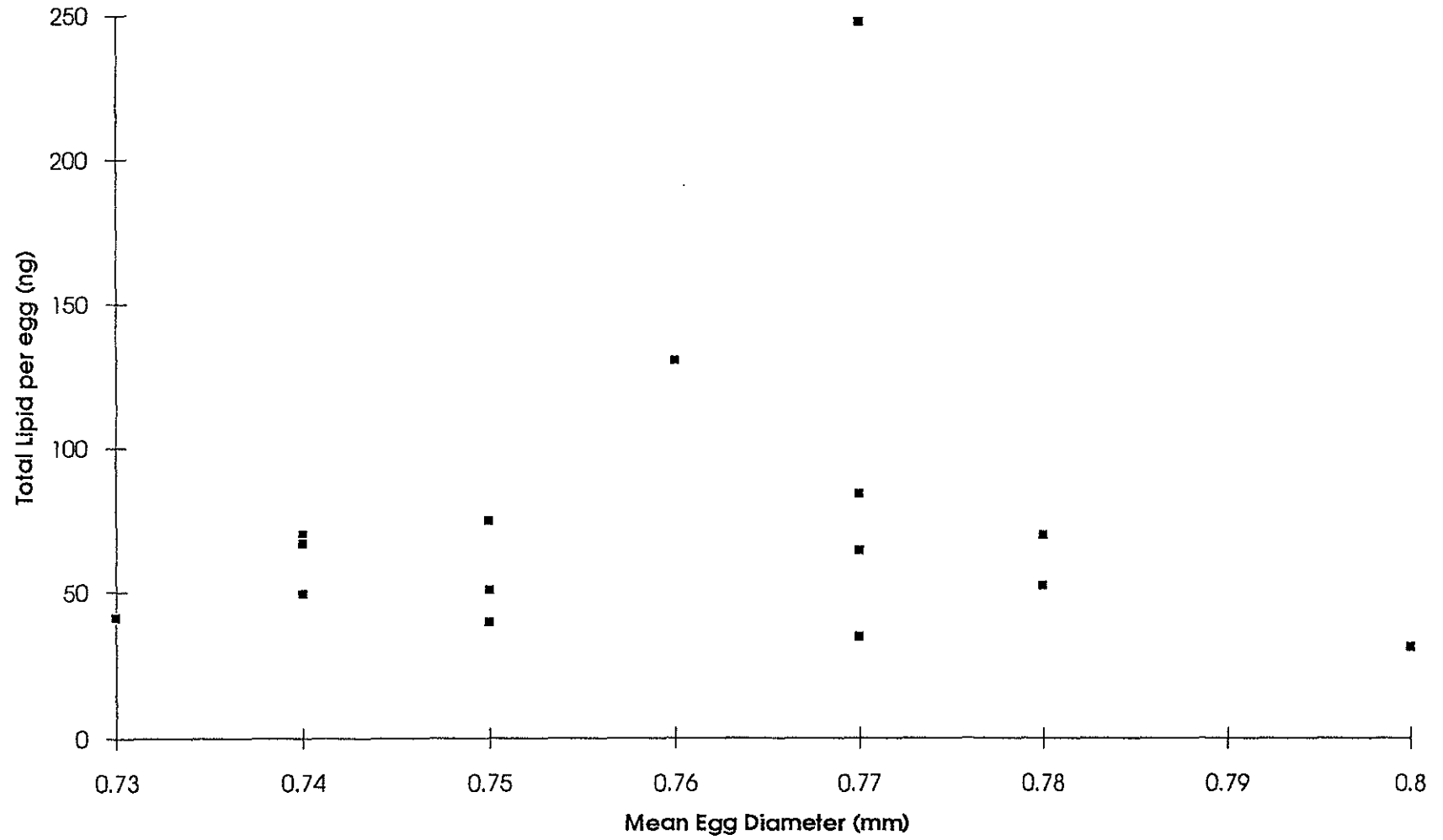


Figure 10.

