INDUCED MATURATION AND SPAWNING OF STRIPED BASS, *Morone saxatilis*, EXPOSED TO 6-, 9-, AND 12-MONTH PHOTOTHERMAL REGIMES

by

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INDUCED MATURATION AND SPAWNING OF STRIPED BASS, *Morone saxatilis*, EXPOSED TO 6-, 9-, AND 12-MONTH PHOTOTHERMAL REGIMES

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(ABSTRACT)

This thesis was partitioned into three studies. In the first study, striped bass, *Morone saxatilis*, (n = 16) were monitored over a 13 month period to examine the efficacy of ultrasound to determine sex and to monitor sexual maturation. Four observers reviewed all ultrasound images (n = 208) of striped bass and were able to sex male and female striped bass with 99 and 95% accuracy, respectively throughout an annual reproductive cycle. Egg diameter was highly correlated with average (r = 0.89) and maximum (r = 0.92) ovarian diameter as measured by ultrasound. Spermiating males exhibited larger maximum testicular diameters (23.5 mm) than non-spermiating males (13.1 mm). Fully mature female and male striped bass exhibited maximum ovarian diameters > 30 mm and testicular diameters > 20 mm as measured by ultrasound. The results of this study indicate that ultrasound is an effective and non-invasive method that aquaculturists can use to quickly scan, sex, and assess the sexual maturation of adult male and female striped bass year-round.

In the second study, photothermal manipulation was used to induce spawning out-of-season and to increase the frequency of spawning in striped bass, *Morone saxatilis*. Forty adult striped bass, held in five environmental chambers, were exposed to compressed 6- and 9- month cycles and a 12-month reference cycle. Maximum egg diameters increased with cycle length, averaging 845 um, 946 um, and 1,073 um for females held on the 6-, 9-, and 12-month cycles, respectively. Females held on the 6-month cycle had smaller ovarian diameters (19.6 mm) than those held on the 9-month (27.4 mm) and 12-month (24.5 mm) cycles. Ovary diameter was highly correlated with egg diameter (r = 0.91). Spawning success (percent of females spawning) increased with cycle length, averaging 38%, 86%, and 100% in the 6-, 9-, and 12-month cycles, respectively. However, no differences in latent times, fecundity, or fertility were detected between cycles or between tank-spawned and strip-spawned females. Males held on the 6-month cycle had smaller testicular diameters (16.2 mm) than those held on 9-month (23.6 mm) and 12-

month (19.2 mm) cycles. Testicular diameter of spermiating males (15.7 mm) was significantly larger than that of non-spermiating males (6.9 mm) in all cycles. Mortality increased with cycle length, averaging 28%, 38%, and 88% for fish held on the 6-, 9-, and 12-month cycles, respectively. Maturation and spawning were advanced by five and three months for fish held in the 6- and 9-month cycles, respectively when compared to a natural 12-month reference cycle. Fish held on the 6-month cycle matured twice within a year. However, the relatively low number of spawning females and diminished egg, ovarian, and testicular diameter of fish held on the 6-month cycle suggest that an abbreviated cycle length (< 9 months) may inhibit maturation and constrain spawning success.

In the third study, monthly blood samples were obtained from 20 fish (equal sexes) held in each cycle. Maximum vitellogenin (Vg) levels were similar in females held on the 6-, 9-, and 12-month cycles, averaging 0.78, 1.05, and 0.80 mg/ml, respectively. Maximum estradiol (E_2) levels were similar in females held on the 6-, 9-, and 12-month cycles, averaging 0.70, 1.40, and 1.35 ng/ml, respectively. Maximum testosterone (T) levels were similar in females held on the 6-, 9-, and 12-month cycles, averaging 0.40, 0.79, and 1.18 ng/ml, respectively. Maximum plasma levels of Vg, E_2, and T in females exposed to the photothermal treatments were temporally shifted to correspond with spawning (January, day 219; March, day 282; and June, day 373) in the 6-, 9-, and 12-month cycles. Egg diameter was correlated with Vg ($r = 0.83$), E_2 ($r = 0.76$), and T ($r = 0.76$) levels. Maximum T levels of males held on the 9-month cycle (0.60 ng/ml) were lower than those in the 6-month (1.08 ng/m) and 12-month (1.24 ng/ml) cycles. Maximum 11-keto testosterone (11KT) levels were similar (1.36, 0.82, and 1.58 ng/ml) in males held on the 6-, 9-, and 12-month cycles. Maximum levels of T and 11KT occurred during spermiation in January (day 219), March (day 282), and June (day 373) for males held on the 6-, 9-, and 12-month cycle. Compression of the annual cycle (by six and three months) accelerated the rate of change (timing), but not the absolute concentrations of plasma Vg and reproductive hormones. Vg and hormone profiles of male and female striped bass were temporally advanced and reflected the maturational status of fish held in the 6-, 9-, and 12-month photothermal regimes.

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CHAPTER I:

USE OF ULTRASOUND TO DETERMINE SEX AND SEXUAL MATURATION IN STRIPED BASS, *Morone saxatilis*, BROODSTOCK.

ABSTRACT

Striped bass, *Morone saxatilis*, (n = 16) were monitored over a 13 month period to examine the efficacy of ultrasound to determine sex and monitor sexual maturation. Ultrasound scans of the entire gonad and cross-sectional images were recorded to determine sex and measure the maximum and average gonadal diameter of male and female striped bass year-round. Presence of eggs or sperm validated ultrasonic sex determination. Four observers reviewed all ultrasound images (n = 208) of striped bass and were able to determine sex with 99 and 95% accuracy for male and female striped bass, respectively throughout an annual reproductive cycle. Egg diameter, the conventional index of maturity, was highly correlated with average (r = 0.89) and maximum (r = 0.92) ovarian diameter estimated by ultrasound. Maximum, rather than average, gonadal diameter proved to be a consistent and simple measurement that was highly correlated with egg diameter and is recommended for similar future studies. Spermiating males exhibited larger maximum testicular diameters (23.5 mm) than non-spermiating males (13.1 mm). Fully mature female and male striped bass exhibited maximum ovarian diameters > 30 mm and testicular diameters > 20 mm measured by ultrasound. The results of this study indicate that ultrasound is an effective and non-invasive method that aquaculturists can use to quickly scan, sex, sort, and assess the sexual maturation of adult male and female striped bass year-round.

INTRODUCTION

Development of domesticated broodstocks to support aquaculture production requires improved methods to determine sex and assess the reproductive status of commercially-important species such as striped bass, *Morone saxatilis*. Sex determination early in a reproductive cycle is necessary to select broodstock for induced maturation. Assessing the reproductive status of broodstock is critical to spawning success.

Sex determination in striped bass and other sexually monomorphic species by external examination is possible only during the spawning season when males are spermiating. Several methods currently used for sex determination of live fish include urogenital catheterization to sample and visually examine gametes (Lewis 1962, Rees and Harrell 1990), analysis of plasma lipophosphoprotein levels (Thurston 1967, Craik and Harvey 1984), and plasma vitellogenin concentrations (Sullivan et al. 1991, LeBail and Breton 1981). These methods are intrusive. They require egg or blood sampling which may damage the urogenital tract, introduce pathogens, stress broodstock, and decrease reproductive performance.

Ultrasound has been used to determine the sex of mature coho salmon, *Onchorynchus kisutch*, within one month of spawning (Martin et al. 1983), and in mature Atlantic salmon, *Salmo salar*, and rainbow trout, *Onchorynchus mykiss*, five months prior to spawning (Reimers et al. 1987). Bonar et al. (1989) found that ultrasound was 100% accurate for sex determination in Pacific herring, *Clupea harengus*, within weeks of spawning when their gonadosomatic index (GSI) exceeded 12.0 and 4.5 for females and males, respectively.

Ultrasound may prove a valuable method to assess maturational status and distinguish sex. Measurements of gonad and egg size and histological examination are the conventional techniques used to determine maturational status in fish (West 1990). Egg diameter has been reported to increase throughout the reproductive cycle and to peak at spawning in female striped bass (Lewis 1962). High correlations between gonadosomatic index (GSI) and egg diameter have been reported for channel catfish, *Ictalurus punctatus* (Markman and Doroshov 1983), and milkfish, *Chanos chanos* (Kuo et al. 1979).

The feasibility of using ultrasound imaging to determine sex and the maturational status of striped bass has not been evaluated. The purpose of this study was to examine the effectiveness of ultrasonic imaging to determine sex and to quantitatively track the maturational condition of male and female striped bass at monthly intervals throughout an annual reproductive cycle.

METHODS

Adult striped bass (n=16), (Chesapeake Bay stock), used in this experiment were obtained in 1984 as fingerlings from the National Fisheries Research Center at Leetown, West Virginia. They were reared in raceways at the Aquaculture Research Facility at Virginia Polytechnic Institute and State University. At age V, the experimental fish were relatively uniform in size, averaging 2.5 kg in weight and 54.5 cm in total length (Table 1).

The fish were stocked into 1.9 m circular, fiberglass tanks (2200 l) in two environmental chambers (4 males, 4 females in each chamber) at the beginning of May 1990. The environmental conditioning chambers (2.6 m x 2.6 m) were constructed with 10 cm thick styrofoam walls. Water quality was maintained using a recirculation system (Appendix B). Water flowed from the fish tank into a sump for solids removal, from there it was pumped up into a trickling filter for nitrification and aeration, and gravity-flowed back into the fish tank. The sump, used to remove solids, consisted of a 220 L plastic drum containing a multitube clarifier suspended near the bottom and a submersible pump (80 L/min) suspended on top of the sump. The trickling filter (60.9 x 60.9 x 121.9 cm tall) and the multitube clarifier consisted of corrugated plastic (BioDeck, The Munter Corp. St. Petersburg, FL) biofilter material.

All fish were exposed to an experimental photothermal regime (Figure 1). The artificial photoperiod cycle was based on the 1990 Farmers Almanac adjusted for the mid-Atlantic latitude. Maximum and minimum temperature limits were established at 13.5 and 23.5° C to correspond to winter and summer conditions (Bain and Bain 1982). Water temperature and photoperiod regimes were

Table 1. Initial length, weight, sex, and indentification numbers of striped bass (n = 16) used in this study.

Fish ID	Sex	Length (cm)	Weight (kg)
104	M	55.5	2.8
105	M	55.5	2.7
107	M	52.5	2.4
111	M	56.0	2.7
114	M	55.0	2.6
117	M	52.5	2.2
128	M	55.0	1.9
130	M	54.0	2.4
155	F	56.5	2.9
158	F	57.0	2.6
159	F	54.0	2.3
161	F	52.5	2.1
167	F	56.5	2.9
173	F	55.5	2.7
175	F	53.0	2.4
181	F	50.5	2.0

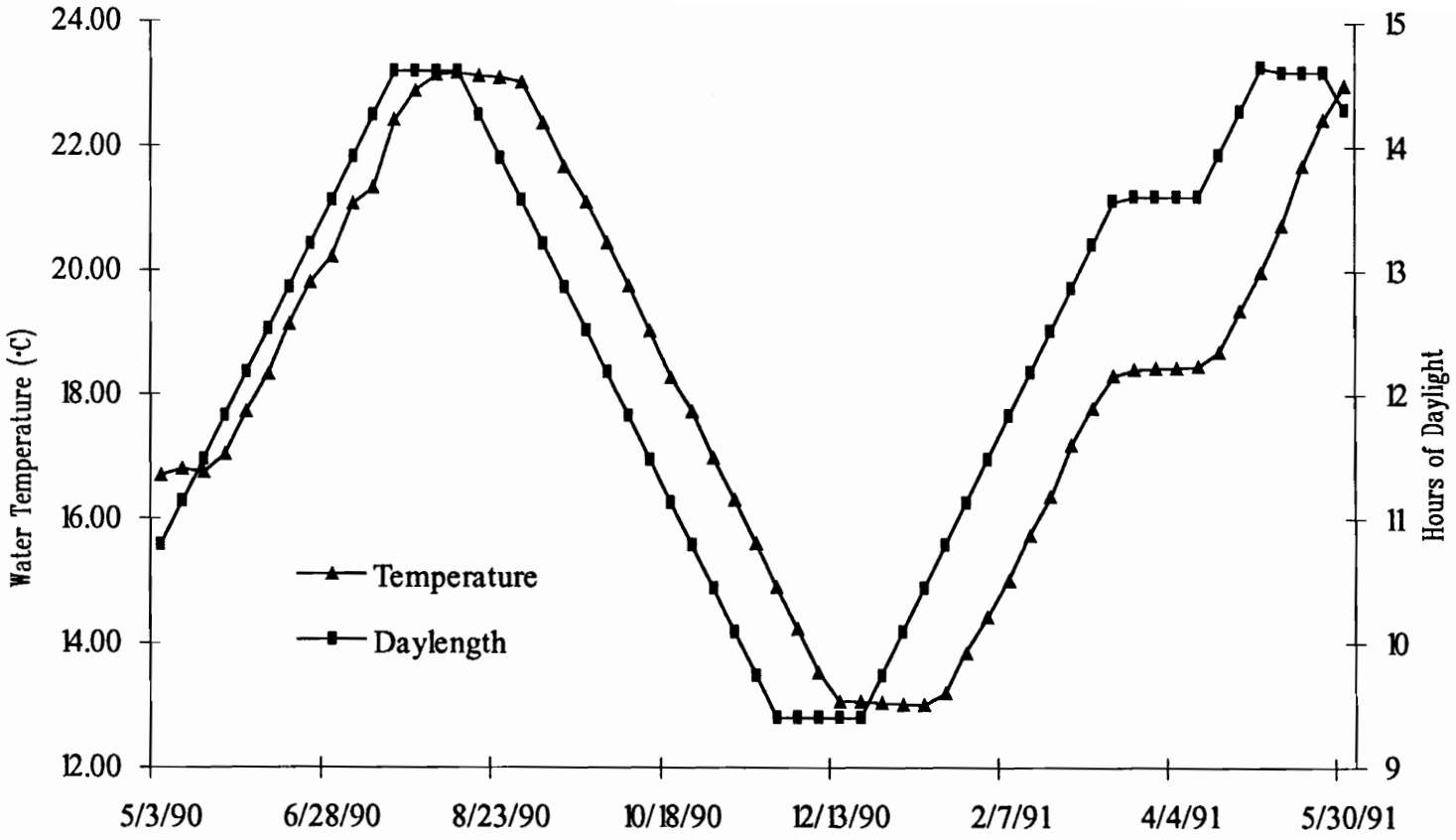

Figure 1. Experimental photothermal cycle from May 1990 through May 1991.

controlled simultaneously in each environmental chamber by a micro-computer network (Fly et al. 1991). Water temperatures were computer-adjusted by the addition of cold or hot water to maintain the desired temperatures in the tank. Photoperiod was supplied by three 60-watt incandescent lights suspended 2.5 m above the fish tank in each environmental chamber. Rheostatic controlled (G. C. Controls, Smithville Flats, N.Y.) crepuscular periods (1 hr in duration) simulated natural sunrise and sunset.

The environmental control system consisted of a Jameco/286 or DTK 386 personal computer linked with Campbell (CR10) data loggers (Campbell Scientific Inc. Logan, UT) regulated light and temperature in tanks in the five environmental chambers. The host computer routinely queried each data logger to retrieve data, control water temperature, and alter photoperiod and temperature as programmed.

The fish were fed to satiation twice per day (morning and afternoon) using 1.2 cm pelleted "Bass Grower" feed (Biosponge Aquaculture Products Co. Sheridan, WY.) This feed consisted of a minimum of 44% crude protein, 8% crude fat, and a maximum of 3% crude fiber and 13% moisture (Appendix C). The dry weight of feed consumed was recorded for all fish in each environmental chambers at each feeding.

Water temperature and dissolved oxygen concentrations were measured daily with a YSI model 58 meter. Water quality parameters (pH, total ammonia nitrogen (TAN), and nitrite) were measured periodically using standard procedures (Hach Chemical Co. Loveland, CO); throughout the study pH ranged from 8.8 to 8.0, TAN ranged from 1.0 to 0 mg/l, and nitrite ranged from 2.0 to 0 mg/l.

Ultrasonography was performed monthly on all fish using a real-time, B-mode, Aloka 500Z ultrasound unit (Aloka Company, Ltd. Japan), equipped with a 5.0 Mhz linear array transducer. Adult striped bass (n=16) were anesthetized in a 180 L tank containing 5000 mg/L of NaCl and 100 mg/L of MS-222, and held submerged in

swimming position against the tank wall. Fish were scanned mid-laterally along the entire length of the gonad. Cross-sectional ultrasound images taken anteriorly (at the base of the pelvic fin), posteriorly (at the isthmus of the dorsal fin), and at the maximum gonadal diameter were recorded. Recorded ultrasound images of the gonads (N = 208) were later examined independently by four observers to evaluate sex determination and the use of ultrasound to assess maturity in broodfish.

After ultrasonography, abdominal pressure was applied to all fish in an attempt to express sperm from males. Suspected females (i.e. those fish which did not release sperm) were catheterized using surgical tubing (1.2mm ID) attached to a 5 ml syringe. The tube was inserted into the urogenital pore and a slight vacuum was applied to collect the eggs. All eggs collected were placed in Cortland's solution to fix egg size prior to measurement (Hoar 1969). The mean diameter of twenty of the largest eggs collected were measured using a dissecting microscope equipped with an ocular micrometer.

Categorical chi-square analysis was used to distinguish any significant differences in sex determination among observers and months. Multiple analysis of variance was used to evaluate the relationship of egg and gonad diameter using fish length as a covariate. Simple correlations of egg and gonad diameter were calculated. Analysis of variance procedures were used to assess differences in gonadal diameter between spermiating and non-spermiating males throughout the study (Ott 1988).

RESULTS

Sex Determination

The sex of female striped bass females was readily distinguished (95% accuracy) throughout the 13-month study by four observers viewing recorded ultrasound images (Table 2). During the post-spawning period (July, August, and September), when egg and ovarian diameters were smallest, sex determination was the least accurate. However, sexing accuracy was similar throughout the reproductive cycle ($P > 0.1$). Average ovarian diameter ranged from 11.4 mm in September to 26.3 mm just prior to spawning. Only 5.3% of female striped bass were incorrectly sexed, and no differences were detected among observers ($P > 0.1$).

Males were as easily distinguished (99% accuracy) by ultrasound as females (Table 3). Male sex determination accuracy was lowest (91%) in September when testicular diameter was minimal. Sexing accuracy did not differ significantly ($P > 0.1$) throughout the study. Only 1.2% of male striped bass were sexed incorrectly, and no differences were detected among observers ($P > 0.1$). Sex determination was completely accurate (100%) during those months when at least one male was spermiating (May through June 1990 and March through May 1991). All males were spermiating only during April. Male testicular diameter ranged from 4.6 mm in July to 22.1 mm prior to spawning in March.

Table 2. Accuracy of sex determination based on all ultrasonographic images (n = 104) of female striped bass, as a function of date, mean egg diameter, average ovarian diameter, and maximum ovarian diameter (standard errors).

Date	Egg Diameter (um)	Average Ovarian Diameter (mm)	Maximum Ovarian Diameter (mm)	Sexing Accuracy (%)
5/3/90	911 (177)	22.0 (2.4)	-	96.9
6/5/90	340 (51)	17.3 (2.5)	-	100.0
7/10/90	364 (56)	13.2 (2.2)	-	87.5
8/7/90	307 (84)	11.9 (3.5)	-	78.1
9/5/90	332 (35)	11.4 (1.8)	-	81.3
10/4/90	318 (21)	11.7 (3.0)	16.6 (0.7)	96.9
11/7/90	382 (25)	13.3 (1.4)	19.6 (2.3)	100.0
12/5/90	472 (29)	14.3 (0.8)	19.6 (1.2)	100.0
1/2/91	569 (49)	15.0 (0.9)	22.7 (3.0)	96.9
2/7/91	668 (88)	19.5 (1.5)	27.2 (3.3)	100.0
3/6/91	861 (81)	26.3 (3.2)	35.4 (3.1)	100.0
4/11/91	731 (250)	17.8 (2.8)	25.7 (4.7)	96.9
5/8/91	336 (66)	15.0 (0.9)	20.4 (4.0)	100.0

Table 3. Accuracy of sex determination based on all ultrasonographic images (n = 104) of male striped bass, as a function of date, percent spermiation, average and maximum testicular diameter (standard error).

Date	Spermiation (%)	Average Testicular Diameter (mm)	Maximum Testicular Diameter (mm)	Sexing Accuracy (%)
5/3/90	63	11.1 (0.6)	-	100.0
6/5/90	13	6.9 (0.5)	-	100.0
7/10/90	0	4.6 (0.5)	-	96.9
8/7/90	0	5.7 (0.4)	-	100.0
9/5/90	0	5.1 (0.5)	-	90.6
10/4/90	0	5.3 (0.6)	-	100.0
11/7/90	0	6.4 (0.4)	-	96.9
12/5/90	0	7.4 (0.7)	10.9 (2.6)	100.0
1/2/91	0	7.3 (1.1)	11.9 (1.6)	100.0
2/7/91	0	11.2 (1.3)	14.9 (2.8)	100.0
3/6/91	88	22.1 (1.5)	25.2 (5.4)	100.0
4/11/91	100	16.8 (1.3)	23.3 (6.2)	100.0
5/8/91	43	12.3 (1.1)	17.2 (6.9)	100.0

Maturational Index

Average ovarian diameters (mean sum of anterior and posterior cross-sections) in striped bass, corrected for body size, were significantly ($P < 0.01$) correlated with egg diameters, $r = 0.89$ (Figure 2). Fish body length was a significant ($P < 0.07$) covariate, but the correlation between egg and ovary diameters without the correction of fish body size ($r = 0.89$) was identical to that when body size was considered.

Maximum gonadal diameter calculated with fish length as a covariate ($P < 0.02$), was highly correlated ($r = 0.92$) with egg diameter for fish from October through May (Figure 3). Correlations between maximum ovary and egg diameter calculated without fish length as a covariate ($r = 0.92$) were similar to those when fish length was considered.

During post-spawning egg diameters decreased rapidly, whereas average gonadal diameters declined slowly (Table 2). When using data only from the period of significant egg growth, September 1990 to March 1991, the correlation between egg and ovarian diameter is improved ($r = 0.95$) (Figure 4). Maximum gonadal diameter during this same period was similarly correlated to egg diameter ($r = 0.94$) (Figure 5).

Males exhibited a pattern of decreasing and then increasing testicular diameter corresponding to the seasonal pattern of ovarian changes in females (Figure 6). Testis diameter increased up until the initiation of spermiation in March. Spermiating males exhibited a larger ($P < 0.01$) average testis diameter (16.21 mm) than non-spermiating males (7.03 mm) throughout the study. Maximum testicular diameters of spermiating males (23.53 mm) were larger ($P < 0.01$) than those of non-spermiating males (13.05 mm).

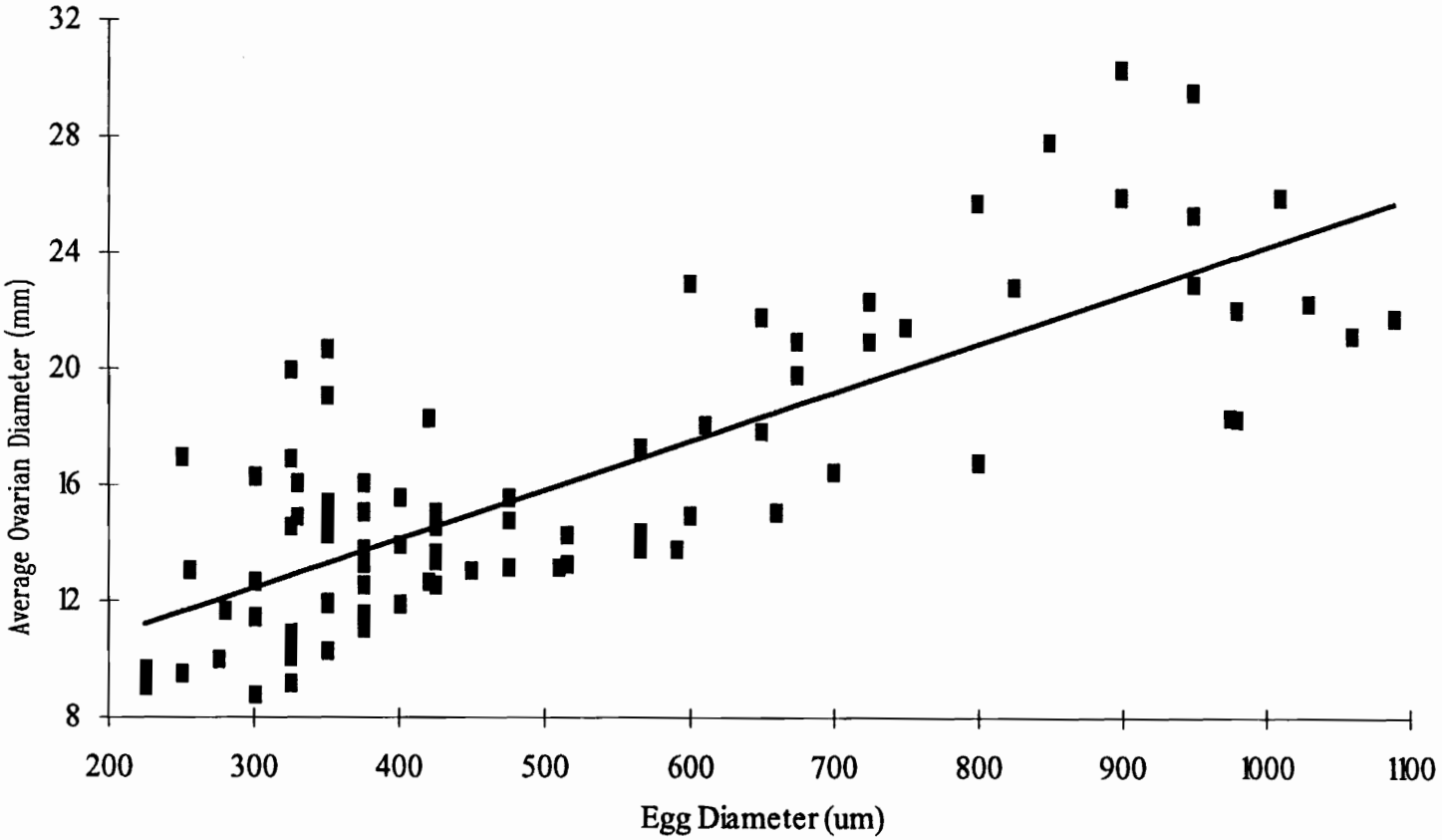

Figure 2. Relationship of egg and average ovarian diameters (r = 0.89) in striped bass (n=8) throughout an annual reproductive cycle (May 1990 to May 1991).

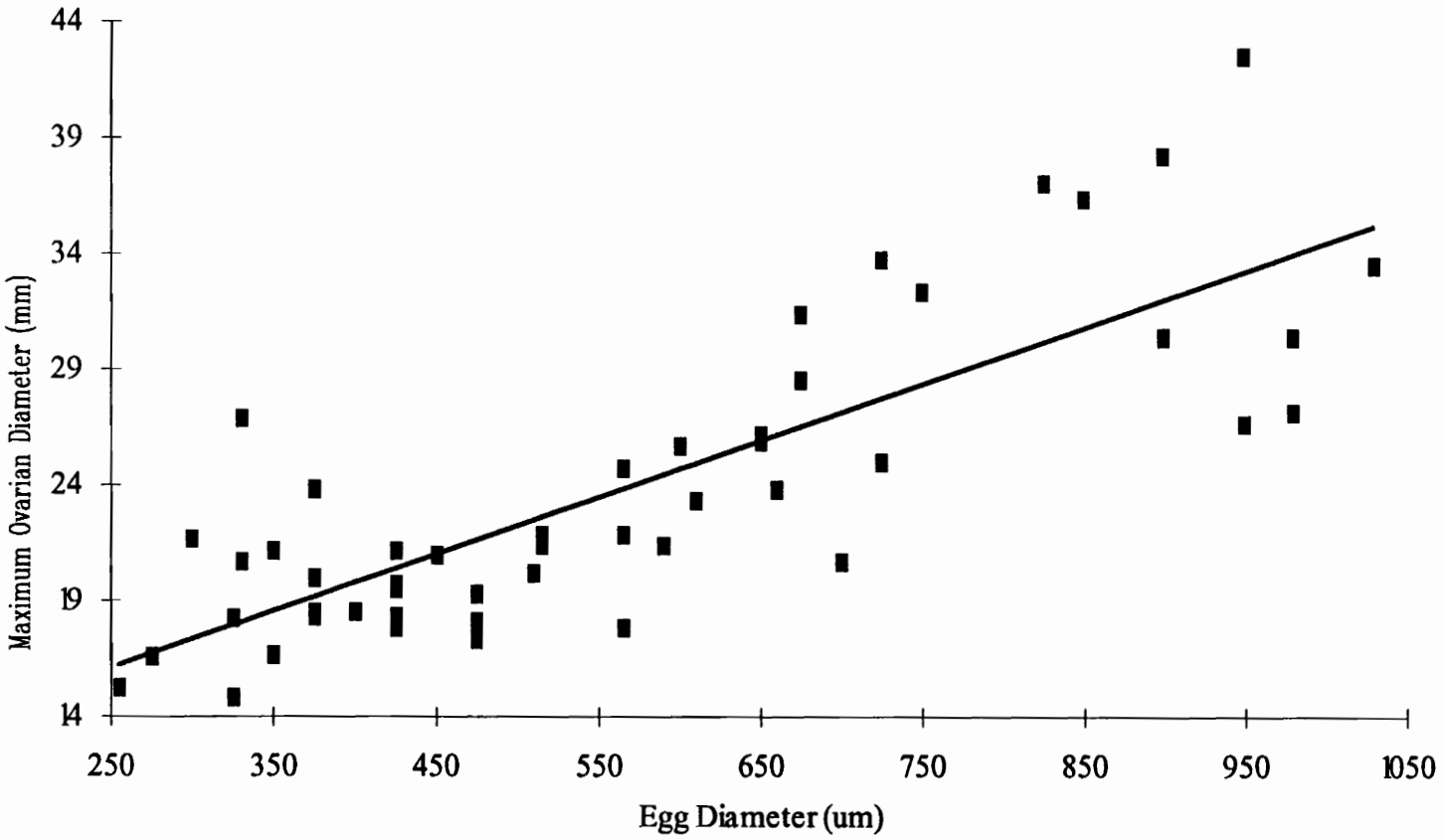

Figure 3. Relationship of egg diameter and maximum ovarian diameters (r = 0.92) from October 1990 to May 1991 for eight females.

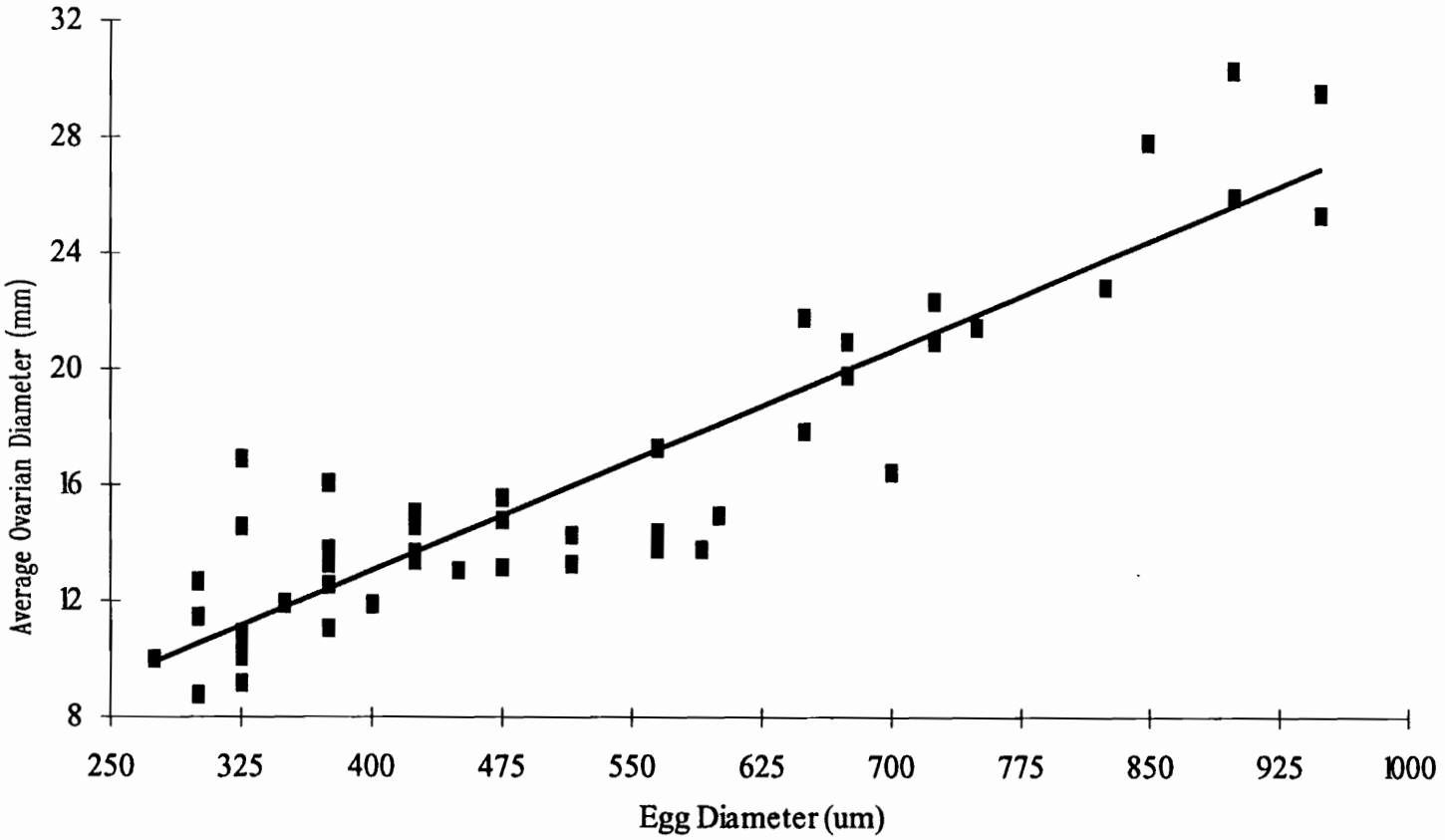

Figure 4. Relationship of egg and average gonad diameters (r = 0.94) September 1990 to March 1991 for female striped bass (n=8).

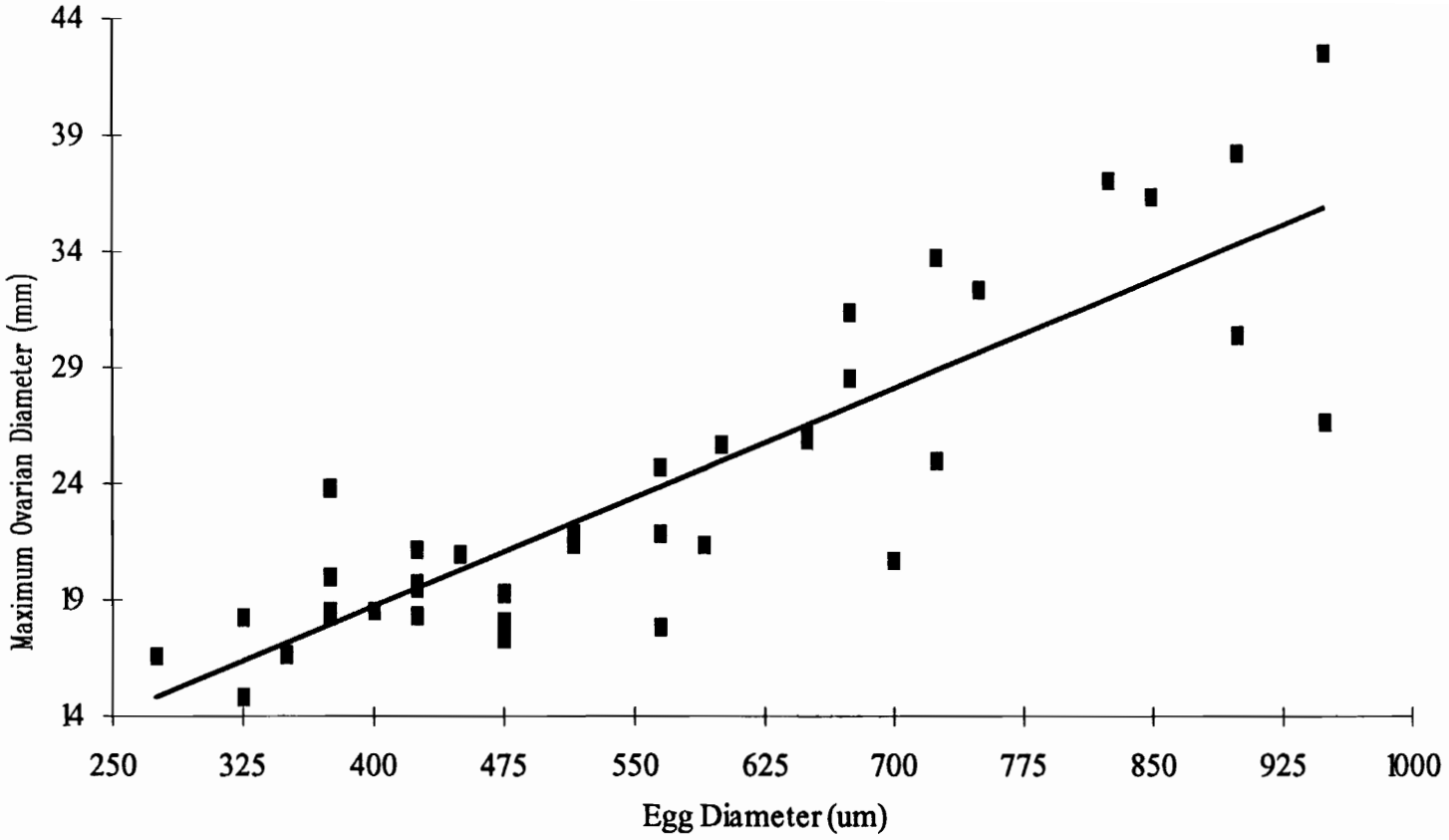

Figure 5. Relationship of egg and maximum gonad diameters (r = 0.94) for female striped bass from September 1990 to March 1991.

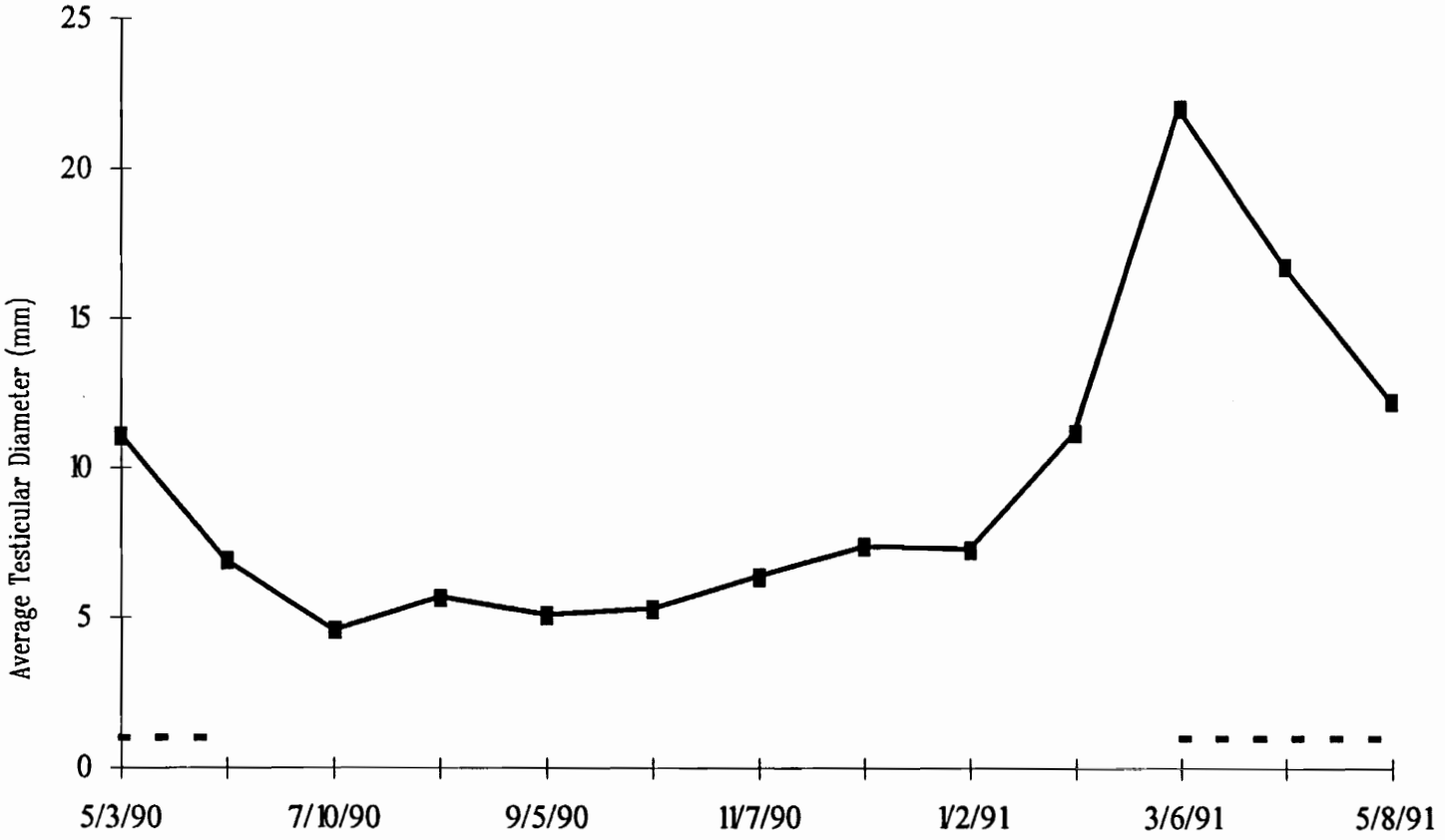

Figure 6. Average testis diameter throughout the annual reproductive cycle (n=8). Dotted lines represent periods of spermiation.

DISCUSSION

Sex Determination

The results of this study indicate that ultrasound is a quick, non-invasive, and highly accurate method to determine sex of striped bass broodstock at any time throughout the annual reproductive cycle, regardless of size or condition of gonads. Ultrasonic sex determination accuracy averaged 95% for females and 99% for males year-round. Minimal sex discrimination of female fish (79 to 88%) occurred during the post-spawning period (July through September). Males were easily identified (99% accuracy) by ultrasound year-round.

This study is the first to employ ultrasound to distinguish sex in striped bass throughout the reproductive cycle. Previously published ultrasound studies have reported qualitative observations on fish scanned only at one time during the year, primarily in the spawning season. Martin et al. (1983) found that mature coho salmon, *Onchorynchus kisutch,* could be sexed accurately with ultrasound one month before spawning, but they examined only five adult fish (two females). Reimers et al. (1987) were able to distinguish the sex of rainbow trout, *Onchorynchus mykiss*, and Atlantic salmon, *Salmo salar*, five months before spawning, but reported that for accurate sexing the fish needed to be starved for three days. Feed was withheld from the striped bass broodstock in this study for only one day prior to sampling and the digestive tract did not obscure ultrasonic images. Bonar et al. (1989) were able to determine sex in Pacific herring, *Clupea harengus*, captured in March in mature condition, but only if their gonadosomatic indices were over 4.5 for males and 12.0 for females.

The nearly perfect sex determination in this study may be attributed to a number of factors, including the specific techniques and equipment employed, the expertise and

experience of the ultrasound operator and interpreters, and the echogenic properties of striped bass gonads. Complete gonadal scans of live, anesthetized striped bass were used in this study. In contrast, Bonar et al. (1989) was less successful (38% accuracy) distinguishing the sex of dead Pacific herring that had been held on ice for several days prior to ultrasonic examination. Reimers et al. (1987) developed a sorting technique based on ultrasonic images of salmonids as they swam by a stationary ultrasound transducer, but no data on the accuracy of this technique was provided, and fish not in spawning condition would be difficult to sex using their method.

Some expertise and experience in ultrasonography is essential for interpretating the images and accurately sexing the fish, especially during periods when gonad sizes are minimal. In this study, the interpreters were familiar with the shape and location of striped bass gonads as displayed by ultrasound. The acoustical properties and large gonad size of striped bass may have further facilitated sex identification. Pacific salmon and Pacific herring, especially those not sexually mature, may have smaller or less echogenic gonads than striped bass, accounting for less accurate sex determination noted by Martin et al. (1983) and Bonar et al. (1989).

Maturation Index

Because egg diameter is the conventional measurement of reproductive status for many species of fish (West 1990), including grey mullet, *Mugil cephalus* (Shehedah et. al. 1973), milkfish, *Chanos chanos* (Kuo et al. 1979), channel catfish, *Ictalurus punctatus* (Markmann and Doroshov 1983), and striped bass (Lewis 1962), it was compared to ultrasound estimates of gonadal size.

Monthly ultrasound estimates of gonad diameter were highly correlated ($r = 0.89$) with egg diameter throughout the annual reproductive cycle. Correlation of egg diameter

with average ($r = 0.89$) or maximum ($r = 0.92$) gonad diameter was high regardless of whether fish length was used in the model. Correlations were highest during October 1990 to March 1991, when egg diameter was increasing. Results of this study indicate that females with maximum gonad diameters exceeding 30 mm are fully mature. Predicted egg size from an ovarian diameter of 30 mm is greater than 700 um, the minimal size needed for hormone injection (Henderson-Arzapalo and Colura 1987).

Expression of sperm by applying abdominal pressure is a common technique for determination of sex and maturity of male fish, but the method is ineffective for identifying immature and non-spawning males or females. Spermiation in striped bass and most temperate zone teleosts is seasonal and brief, corresponding to a short, discrete spawning period. Results indicated that synchronous spermiation of all males occurred only in April, 1991, ultrasonic measurement of maximum testicular diameters were larger in spermiating (23.5 mm) than in non-spermiating (13.1 mm) males, and males with a maximum testicular diameter greater than 20 mm when spermiating.

Ultrasound has distinct advantages over other techniques used to determine sex and maturity in striped bass and other species that lack secondary sexual characteristics. In this study, ultrasonography proved to be a simple, rapid (< 1 min/fish) method for determining sex and maturity of live striped bass throughout their annual reproductive cycle. Ultrasound is less stressful than the use of ovarian catheterization to collect eggs, blood sampling for vitellogenin and steroid analyses, or gonadal tissue extraction for histological analyses. External ultrasound examination can minimize the possibility of disrupting maturation and spawning or damage to the ovarian duct resulting from invasive use of syringes and catheterization tubes for bleeding and egg collection. Moreover, the immunodiffusion assay for vitellogenin (Mancini et al. 1965) requires a complex and lengthy analysis that is limited seasonally and specific to females. Plasma

vitellogenin was only detectable five months before spawning in female brown trout, *Salmo trutta* (LeBail and Breton 1981), and Atlantic salmon, *Salmo salar* (Idler and Ng 1979), and seven months prior to spawning in female striped bass (Sullivan et al. 1991).

The use of ultrasound for sex and maturational determination has some inherent disadvantages. Fish still are subjected to stresses from netting and anesthezation during ultrasound examination. The technique for scanning fish and interpreting the ultrasound images requires experience, especially at post-spawning when gonads are small. When the fish are near spawning, urogenital catheterization may represent a more precise index of gonadal development, which is necessary to induce ovulation with hormones and predict spawning. Ultrasound equipment is costly ($10-15,000), although many veterinarians, universities, and some consultants now have ultrasound units.

Results of this study suggested that maximum (rather than average) gonadal diameter (as measured by ultrasound) was a good index of reproductive condition. Maximum gonadal diameter (determined by a scan of the entire gonad) proved to be a consistent measure of maturity and was highly correlated with egg diameter. Gonad size and shape was highly variable within and between individuals and seasons, therefore measurement at arbitrary points in the gonad may be a less consistent measurement of maturity than maximum gonadal size determined by a complete gonadal scan. Correlations of egg and gonad size calculated with and without fish length as a co-variable were similar. Body size range of broodfish used in this study was small (10 cm), but other populations with highly variably-sized fish may require a consideration of body sizes.

The results of this study indicate that ultrasound is an accurate and effective method for sexing and assessing maturity of striped bass year-round. In addition to its aquaculture applications, ultrasound technology has significant implications for the

management of wild stocks. For example, sex ratios of striped bass and other sexually monomorphic fish species, including sport, forage, and endangered fishes, could by determined for populations with little sampling effect. Ultrasound may be especially useful in describing the reproductive condition of endangered and threatened species, with minimal disturbance and mortality.

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CHAPTER 2:

INDUCED MATURATION OF STRIPED BASS, *Morone saxatilis*, EXPOSED TO 6-, 9-, AND 12-MONTH PHOTOTHERMAL REGIMES.

ABSTRACT

Photoperiod and temperature manipulation was used to induce spawning out-of-season and to increase the frequency of spawning in striped bass, *Morone saxatilis*. Forty adult striped bass, held in five environmental chambers, were exposed to compressed 6- and 9- month cycles and to a 12-month reference cycle. Maturational status was monitored by monthly egg and gonadal (ultrasound) diameter measurements. Maximum egg diameters increased with cycle length, averaging 845 um, 946 um, and 1,073 um for females held on the 6-, 9-, and 12-month cycles, respectively. Females held on the 6-month cyclehad smaller ($P < 0.05$) ovarian diameters (19.6 mm) than those held on the 9-month (27.4 mm) and 12-month (24.5 mm) cycles. Ovary diameter was highly correlated with egg diameter ($r = 0.91$). Spawning success (percent of females spawning) increased with cycle length, totalling 38%, 86%, and 100% in the 6-, 9-, and 12-month cycles, respectively. However, no differences in fecundity or fertility were detected among treatments, averaging 58,452 eggs/kg and 38.6% among all females. Tank-spawned and strip-spawned females exhibited similar latent times, fertilities, and fecundities. Males held on the 6-month cycle had smaller testicular diameters (16.2 mm) than those held on 9-month (23.6 mm) and 12-month (19.2 mm) cycles. Testicular diameter of spermiating males (15.7 mm) was significantly larger than that of non-spermiating males (6.9 mm) for all cycles. Mortality of fish increased with cycle length, totalling 28%, 38%, and 88% for fish held on the 6-, 9-, and 12-month cycles,

respectively. Serial blood sampling did not significantly impact reproductive success or survival of domesticated striped bass broodstock. Maturation and spawning of male and female striped bass held on the 6- and 9-month cycles, respectively, were advanced by five and three months compared those held on the 12-month cycle. Fish held on the 6-month cycle matured twice within one year. However, the relatively low number of spawning females and diminished egg, ovarian, and testicular diameter of fish held on the 6-month cycle suggest that abbreviated cycle length (< 9 months) may inhibit maturation and constrain spawning success. The ability to induce maturation and spawning in striped bass broodstock by photothermal manipulation can provide a reliable, year-round source of larvae for commercial aquaculture.

INTRODUCTION

Reproductive control of commercially-important fish species such as striped bass, *Morone saxatilis*, is fundamental to the development of aquaculture (Parker 1988). The ability to manipulate maturation and spawning in domesticated broodstock reduces dependence on wild stocks, helps assure a reliable, year-round supply of eggs and fingerlings for aquaculture, and provides opportunities for genetic selection and improvement.

Water temperature and photoperiod are major environmental cues that control the reproductive cycle in fishes (Lam 1983, Zohar 1990). Photoperiod may be more important than temperature in regulating reproduction in salmonids and certain cold water marine species (Duston and Bromage 1986, Bye 1984). Water temperature may be a more important regulator than photoperiod in warm water fishes such as carp (Horvath 1986), but both factors likely play a role in maturation and spawning of most warm water species (Stacey 1984), including striped bass. In female striped bass decreasing photoperiod and temperature initiate vitellogenesis, incorporation of yolk into the oocytes, whereas increasing temperature and photoperiod stimulate spawning (Sullivan et al. 1991).

Photothermal manipulation has been used to induce maturation and out-of-season spawning in a variety of species including red drum, *Scianeops ocellatus* (Arnold 1988), sole, *Solea solea* (Devauchelle et al. 1987), rainbow trout, *Onchorynchus mykiss* (Bromage et al. 1982), and coho salmon, *Onchorynchus kisutch* (MacQuarrie et al. 1979). Prior attempts to control spawning of striped bass in laboratory tanks have met with limited success; some maturation but no spawning (Lasker 1974, Van Olst et al. 1982). Striped bass were successfully induced to mature and spawn when held in a 12-

month photothermal cycle (Henderson-Arzapalo and Colura 1987) and on a compressed 9-month photothermal cycle (Smith and Jenkins 1986). Photothermal manipulations of F_1 hybrid striped bass held on a 12-month cycle (Harrell 1984) and a compressed 9-month cycle (Smith and Jenkins 1984) have been used to induce maturation. In all of these studies, ovulation required hormone injection, usually human chorionic gonadotropin (HCG). Once injected with hormones, striped bass could either be tank or strip spawned. Tank spawning was recommended because it is more natural, less stressful, and avoids the need for precise timing required for strip spawning (Bishop 1975, Rees and Harrell 1990).

Although striped bass and *Morone* hybrids have been aquacultural candidates for two decades, the supply of seed fish remains dependent on the unpredictable acquisition of eggs from wild broodstock (Harrell 1984, Rees and Harrell 1990). Induced maturation and spawning by photothermal manipulation of striped bass broodstock could provide a consistent, year-round supply of larvae for commercial aquaculture, while decreasing reliance on wild broodfish. The objective of this study was to examine the influence of 6-, 9-, and 12-month photothermal regimes on the timing and reproductive success of striped bass broodstock.

METHODS

Adult striped bass (n=40), (Chesapeake Bay stock), used in this experiment were obtained in 1984 as fingerlings from the National Fisheries Research Center at Leetown, West Virginia. They were reared in raceways at the Aquaculture Research Facility at Virginia Polytechnic Institute and State University. At age V, the experimental fish averaged 2.5 kg in weight and 54.5 cm in length (Table 1). Sex was determined by ultrasonic imaging and validated by presence of sperm or ova in individually tagged fish.

The fish were stocked into 1.9 m circular, fiberglass tanks (2200 L) in five environmental chambers (4 males, 4 females in each chamber) at the beginning of May 1990. Early mortalities were replaced to maintain similar densities and sex ratios in each chamber (Appendix A). The environmental conditioning chambers (2.6 m x 2.6 m) were constructed with 10 cm thick styrofoam walls. Water quality was maintained using a recirculation system (Appendix B). Water flowed from the fish tank into a sump for solids removal, was then pumped up into a trickling filter for nitrification and aeration, and gravity-flowed back into the fish tank. The sump, used to remove solids, consisted of a 220 L plastic drum containing a multitube clarifier suspended near the bottom and a submersible pump (80 L/min) suspended on top of the sump. The trickling filter (60.9 x 60.9 x 121.9 cm tall) and the multitube clarifier located in the sump consisted of corrugated plastic (BioDeck, The Munter Corp. St. Petersburg, FL) biofilter material.

Three different photothermal cycles were used in the experiment. The compressed 6-month (Figure 1) and 9-month (Figure 2) cycles were duplicated in two environmental chambers. The 12-month (Figure 3) reference cycle was not replicated. The 6-and 9- month photothermal cycles were established based on the 1990 Farmers Almanac adjusted for the mid-Atlantic latitude and compressed from a 12-month natural

Table 1. Identification tag number, sex, cycle, environmental chamber, initial length, and weight data of the experimental broodstock.

Tag #	Sex	Cycle	Chamber #	Length (cm)	Weight (kg)
102	M	6	2	56.0	2.7
104	M	9	3	55.5	2.8
105	M	9	3	55.5	2.7
106	M	6	4	56.5	3.1
107	M	9	3	52.5	2.4
111	M	9	1	56.0	2.7
112	M	6	4	51.0	2.1
114	M	9	1	55.0	2.6
117	M	9	1	52.5	2.2
119	M	6	2	50.0	1.8
120	M	12	7	57.0	2.6
121	M	6	2	56.5	2.5
122	M	6	4	56.5	2.9
124	M	6	4	54.0	2.2
126	M	6	2	53.5	2.5
127	M	12	7	53.5	2.8
128	M	9	3	55.0	1.9
129	M	12	7	55.5	2.5
130	M	9	1	54.0	2.4
131	M	12	7	51.0	2.0
152	F	12	7	54.5	2.4
153	F	6	2	50.0	2.0
156	F	12	7	50.5	1.9
158	F	9	1	57.0	2.6
159	F	9	3	54.0	2.3
161	F	9	1	52.5	2.1
166	F	6	4	58.0	3.0
167	F	9	1	56.5	2.9
168	F	6	2	52.5	2.3
170	F	6	4	56.5	2.9
171	F	6	4	51.0	2.1
172	F	9	3	50.0	1.6
173	F	9	3	55.5	2.7
174	F	6	2	55.5	2.6
175	F	9	3	53.0	2.4
178	F	6	4	50.5	2.0
179	F	12	7	54.5	2.6
180	F	12	7	56.5	2.9
181	F	9	1	50.5	2.0
182	F	6	2	54.0	2.5

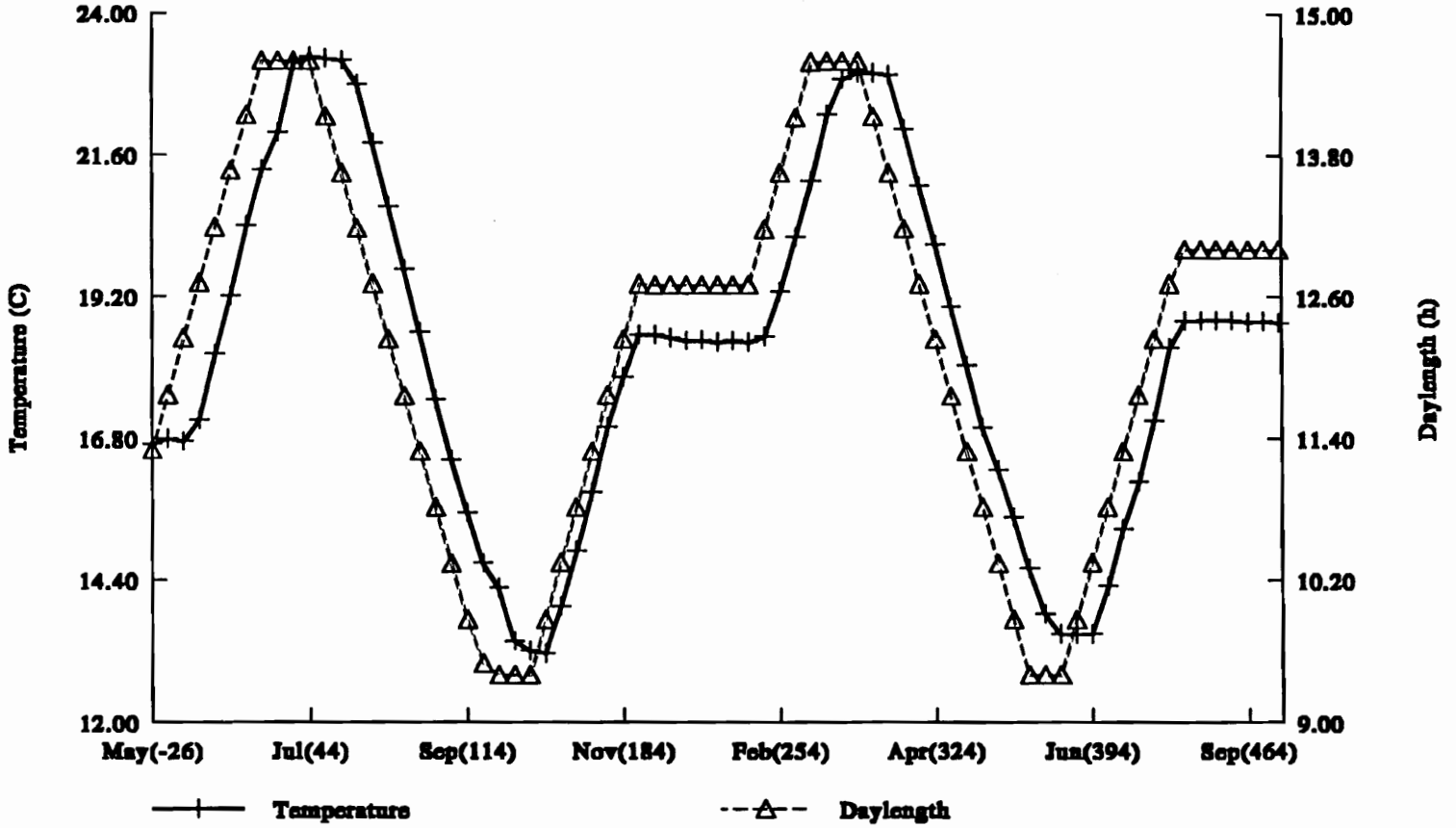

Figure 1. Six-month compressed temperature-photoperiod cycle from May 1990 through September 1991. Months shown with (experimental days).

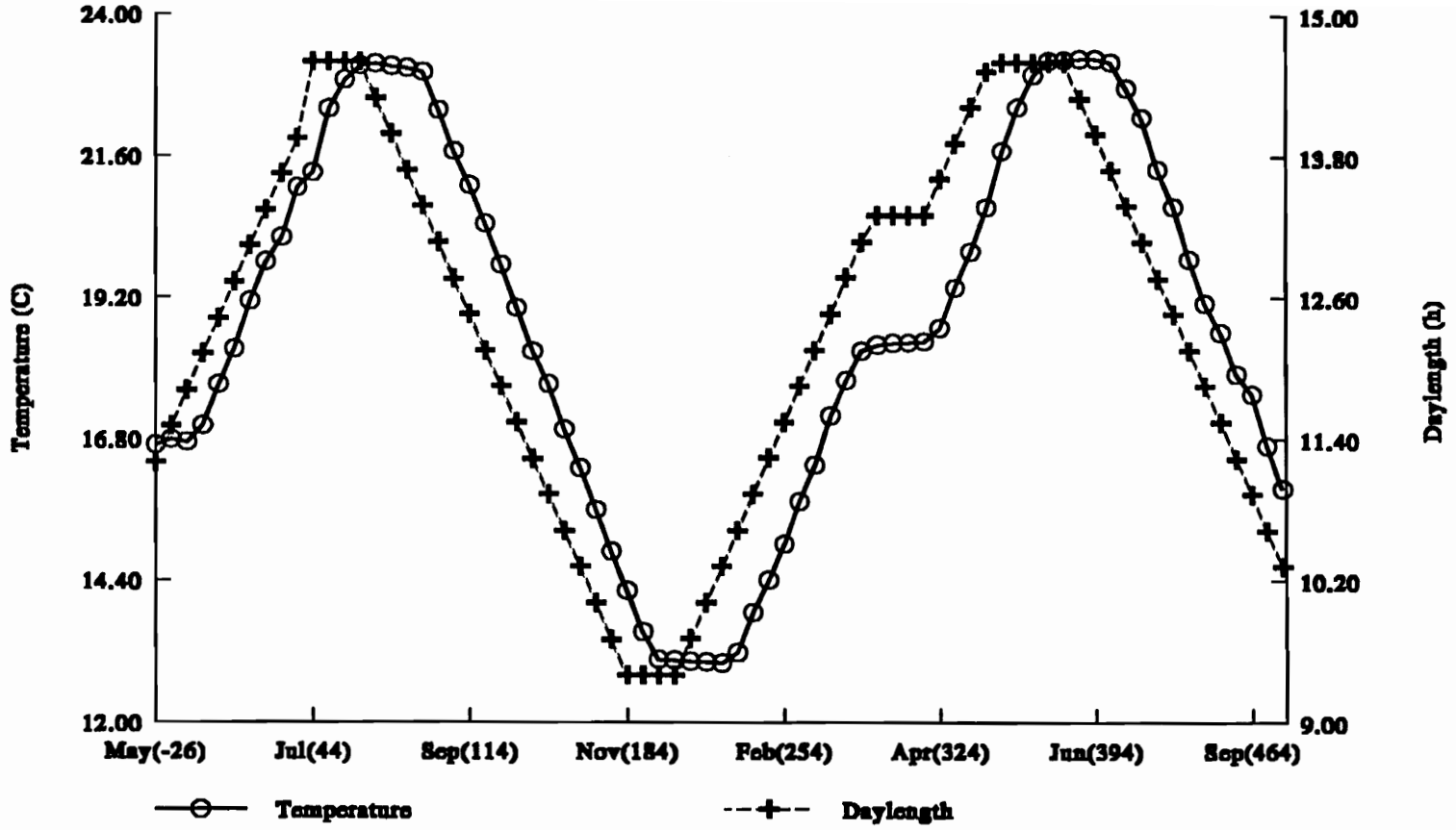

Figure 2. Nine-month compressed temperature-photoperiod cycle from May 1990 through September 1991. Months shown with (experimental days).

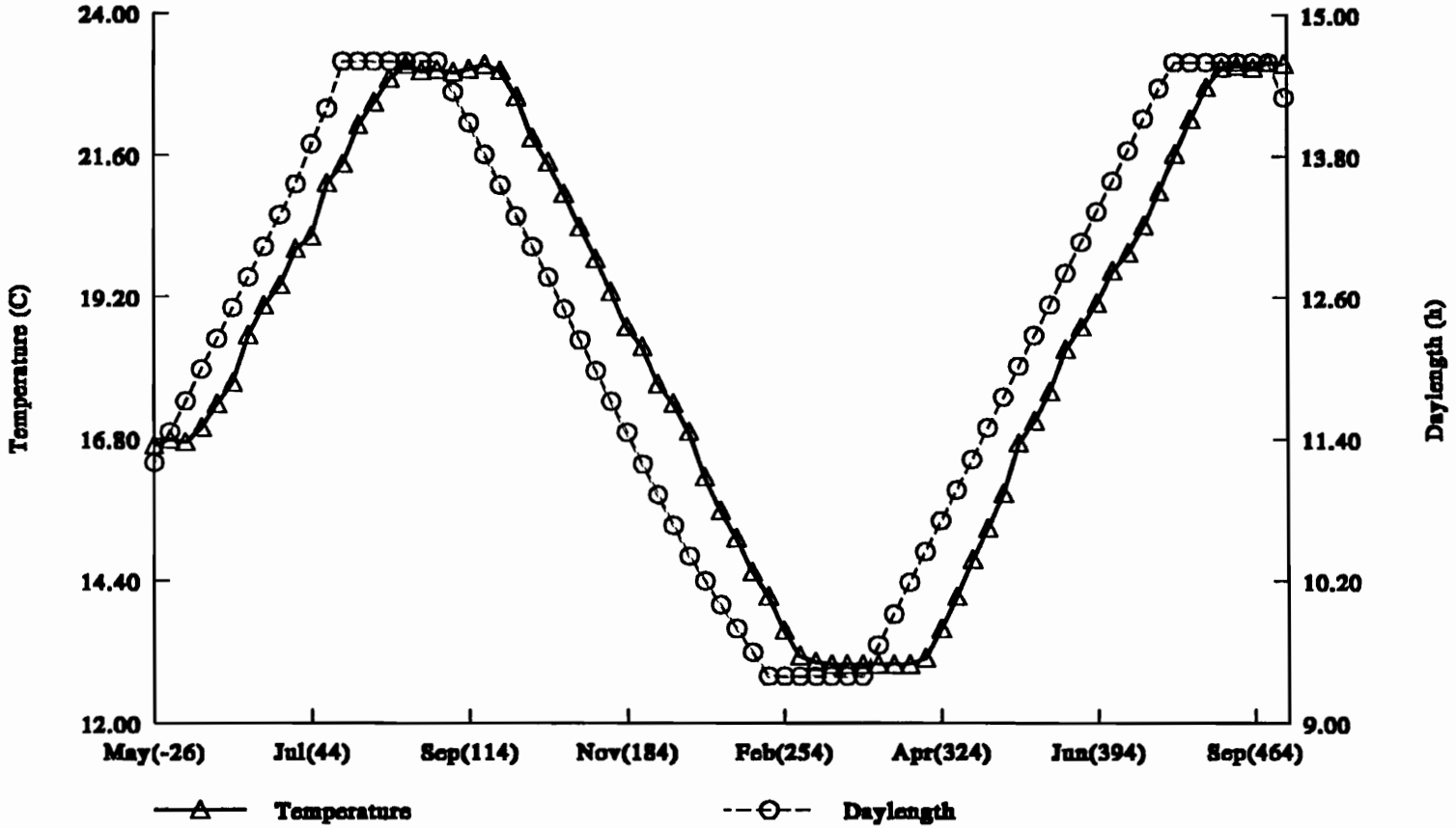

Figure 3. Twelve-month 'natural' photothermal cycle from May 1990 through September 1991. Months are shown with (experimental days).

cycle. Maximum and minimum temperature limits were established at 13.5 and 23.5° C to correspond to winter and summer conditions (Bain and Bain 1982).

Water temperature and photoperiod regimes were controlled simultaneously in each environmental chamber by a micro-computer network (Fly et al. 1991). Water temperatures were computer-adjusted by the addition of cold or hot water to the tank to maintain the desired temperatures. Photoperiod was supplied by three 60-watt incandescent lights suspended 2.5 m above the fish tank in each environmental chamber. Rheostatic controlled (G. C. Controls, Smithville Flats, NY) crepuscular periods 1 hr in duration simulated natural sunrise and sunset.

The environmental control system consisted of a Jameco/286 or DTK 386 personal computer linked with Campbell (CR10) data loggers (Campbell Scientific Inc. Logan, UT) regulating light and temperature in tanks in the five environmental chambers. The host computer routinely queried each data logger to retrieve data, control water temperature, and alter photoperiod and temperature as programmed.

All fish were fed to satiation twice per day (morning and afternoon), using 1.2 cm pelleted "Bass Grower" feed (Biosponge Aquaculture Products Co. Sheridan, WY). This feed was comprised of a minimum of 44% crude protein, 8% crude fat, and a maximum of 3% crude fiber and 13% moisture. The dry weight of feed consumed was recorded for all fish in each environmental chambers at each feeding.

Water temperature and dissolved oxygen concentrations were measured on a daily basis using a YSI model 58 meter. Water quality parameters (pH, total ammonia nitrogen (TAN), and nitrite) was measured periodically using standard procedures (Hach Chemical Co. Loveland, CO); throughout the study pH ranged from 8.8 to 8.0, TAN 1.0 to 0 mg/l, and nitrite ranged from 2.0 to 0 mg/l. Water was added to the system only to maintain water temperature control or to flush suspended solids from the sump tank.

Ultrasonography was performed monthly on all fish using a real-time, B-mode, Aloka 500Z ultrasound unit (Aloka Company Ltd. Japan), equipped with a 5.0 Mhz linear array transducer. Adult striped bass (n=40) were anesthetized in a 180 L tank containing 5000 mg/L NaCl and 100 mg/L MS-222, and held submerged in swimming position against the tank wall. Fish were scanned mid-laterally along the entire length of the gonad. Cross-sectional ultrasound images were taken anteriorly (at the base of the pelvic fin) and posteriorly (at the isthmus of the dorsal fin). Ultrasound images of the gonads were recorded on videotape throughout the 18-month period and examined later to determine the gonadal size of individual fish.

After ultrasonography, abdominal pressure was applied to all fish in an attempt to express sperm from males. Females were catheterized using surgical tubing (1.2mm ID) attached to a 5 ml syringe. The tube was inserted into the urogenital opening and a slight vacuum was applied to collect the eggs. All eggs collected were placed in Cortland's solution to fix egg size prior to examination (Hoar 1969). Egg diameter was measured using a dissecting microscope equipped with an ocular micrometer, and mean diameter of twenty of the largest size eggs collected was calculated.

At optimal spawning temperature (19°C) and photoperiod (13L:11D); the fish were held under constant photothermal conditions until females developed egg diameters > 700 um. At egg sizes > 700 um, females were considered fully mature and ready for hormone (HCG) injection. Mature females were transferred into a special environmental chamber containing a spawning tank (1800 L) and two spermiating males one week before hormone injection. Females were injected with human chorionic gonadotropin (HCG) at 330 IU per kg of body weight, and males received 165 IU per kg body weight (Rees and Harrell 1990).

All fish held in the first 6-month cycle were permitted to spawn naturally (tank spawn) in the breeding tank. Equal numbers of females held in the 9-month and 12-month cycles were tank spawned and strip spawned in order to compare the effect on reproductive success. Strip-spawning was compared to tank-spawning and used to minimize demand on the single spawning chamber available. Ova were examined 24 hours after injection and the time of ovulation was estimated. Once ovulation occurred, the female was anesthetized (100 ppm MS-222) and eggs were stripped into a dish, where they were fertilized with sperm from one or both males in the spawning environmental chamber.

Methods used for tank-spawning were similar to those of Bishop (1975). Tank spawning was conducted in a circular fiberglass tank (1.9 m in diameter) housed in an environmental chamber. A circular aeration hose was placed at the bottom center of the tank to keep eggs suspended in the water column. Broodstock were removed from the tank immediately after spawning. Eggs were then moved to McDonald jars for incubation. Number of females spawning, latent time (interval between injection and spawning), fecundity (eggs/kg body weight), and fertilization rate (% fertilized eggs) were monitored during spawning.

Fecundity and fertility of tank-spawned fish were esimated by collecting five 1 L water samples from the spawning tank. The total number of eggs was then calculated by extrapolating from the average number per liter to the volume of the tank (Henderson-Arzapalo and Colura 1987). Egg numbers from the stripped fish were estimated by counting the number of eggs per ml and determining the volume of eggs stripped. Two hours after spawning, at least 500 eggs were randomly sampled and examined microscopically for cleavage to determine the percent fertilization. Tank spawned eggs

were either transferred to McDonald jars or left in the spawning tank for incubation. Incubation temperature was held at 19°C.

An analysis of variance (ANOVA) model was used to compare mean monthly and maximum egg, ovarian, and testicular diameters and spawning parameters (fertility, fecundity, and latent time) among fish held on different cycles. ANOVA was also used to ananlyze for differences in reproductive variables among blen and non-bled fish, and among tank and strip spawned fish. Multiple analysis of variance (MANOVA) models were used to correlate egg diameter to ovarian diameter with fish length used as a covariate (Ott 1988).

RESULTS

Maturation in Females

Egg diameters of female striped bass were at a minimum at the start of the study on May 24, 1990, but increased steadily for fish held in all three photothermal cycles until spawning in January, March, and June 1991 (days 225, 299, 381) for fish held in the 6-month, 9-month, and 12-month cycles, respectively (Figure 4). Females in each cycle developed maximum egg diameters within a month of their predicted median spawning time, days 200, 290, and 380 for fish held in the 6-, 9-, and 12-month cycles, respectively (Table 2).

Mean monthly egg diameters of females held on separate cycles differed significantly ($P < 0.05$) except during August and September 1990 and January and February 1991 (Table 3). Mean maximum egg size was directly related to cycle length. Peak egg diameters of fish held in the 6-month cycle (721 um) were smaller ($P < 0.05$) than those of females held in the 9-month (946 um) and 12-month (1,073 um) cycles. Only females with egg diameters in excess of 700 um were injected and subsequently spawned.

All of the females held in the 9-month (n=8) and 12-month (n=4) cycles matured (developed egg diameters > 700 um) as compared to only 50% (n=4) of those held in the 6-month cycle. Egg diameters for fish held in the 6-month cycle which matured were significantly larger ($P < 0.06$) (845 um) than those which did not mature (598 um). Females held in the 6-month cycle were maintained on the same photothermal regime for a second 6-month period starting in March 1991 (day 282). During the second cycle, they matured and reached spawning condition (mean egg diameter 744 um) in September 1991 (day 466, Figure 4). Peak egg diameters of females held in the second

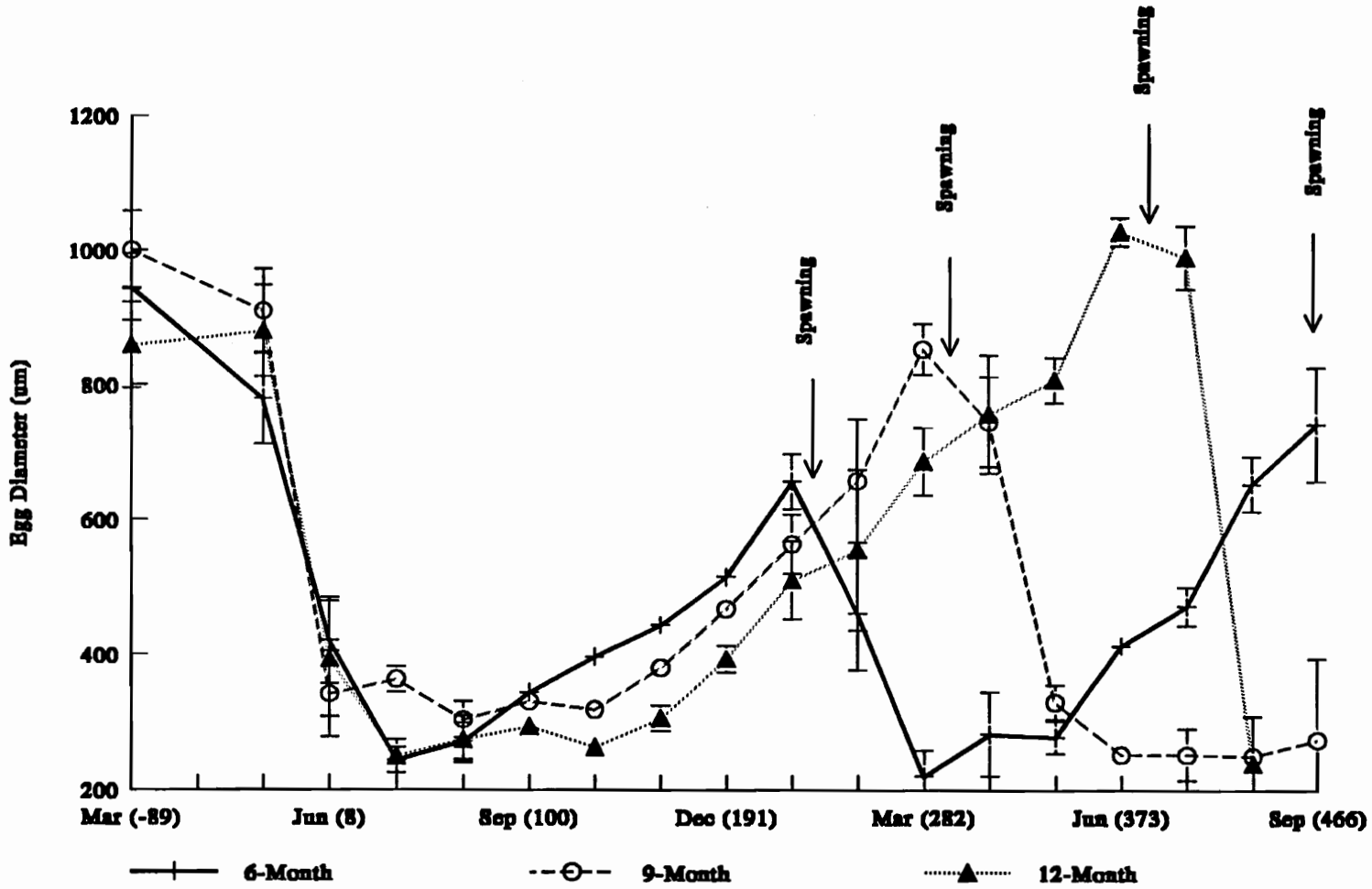

Figure 4. Mean egg diameters (standard error) for all females in the 6, 9, and 12 month cycles. Month shown with (experimental days).

Table 2. Spawning data (standard error) for females held in the 6-month (n=8), 9-month (n=7), and 12-month (n=4) cycles.

Parameter	Cycle			P-value
	6-Month	9-Month	12-Month	
Females Spawned (%)	37	86	100	<0.05
Spawning (day)	239 (9)	306 (6)	389 (7)	<0.05
Predicted Spawning (day)	200	290	380	
Latent Time (h)	38.4 (2.2)	36.5 (1.6)	35.4 (1.8)	0.60
Fertility (%)	35.8 (14.8)	47.8 (10.5)	32.2 (12.1)	0.61
Fecundity (eggs/kg)	55,260 (23008)	74,642 (16269)	45,454 (18786)	0.52

Table 3. Mean monthly egg diameters (standard error) of female striped bass held in the 6-month (n=8), 9-month (n=8), and 12-month (n=4) cycles. Different letters represent significantly different in-row values.

Month	Day in Cycle	Cycles			P value
		6-Month	9-Month	12-Month	
		Egg Diameter (um)	Egg Diameter (um)	Egg Diameter (um)	
March	-89	945 (50)	1000 (58)	859 (64)	0.31
May	-26	780 (67)	911 (62)	881 (88)	0.37
June	8	421 (64)	342 (64)	394 (86)	0.69
July	43	244 (19)^{a}	364 (19)^{b}	250 (25)^{a}	< 0.01
August	71	272 (27)	304 (27)	275 (35)	0.67
September	100	343 (12)	331 (13)	294 (16)	0.08
October	129	397 (11)^{a}	319 (12)^{b}	263 (15)^{c}	< 0.01
November	163	444 (13)^{a}	381 (14)^{b}	306 (19)^{c}	< 0.01
December	191	516 (14)^{a}	468 (16)^{b}	394 (20)^{c}	< 0.01
January	219	658 (41)	565 (44)	511 (58)	0.12
February	255	462 (85)	659 (91)	556 (120)	0.32
March	282	221 (38)^{a}	855 (38)^{b}	688 (50)^{c}	< 0.01
April	318	283 (62)^{a}	747 (67)^{b}	758 (88)^{b}	< 0.01
May	345	278 (24)^{a}	330 (26)^{a}	809 (34)^{b}	< 0.01
June	373	414 (15)^{a}	253 (17)^{b}	1031 (21)^{c}	< 0.01
July	406	473 (29)^{a}	253 (38)^{b}	993 (59)^{c}	< 0.01
August	441	655 (41)^{a}	251 (58)^{b}	240 (117)^{b}	< 0.01
September	466	744 (85)^{a}	275 (120)^{b}	---	0.01

6-month cycle (794 um) were similar ($P > 0.05$) to those held in the first 6-month cycle (721 um).

Ovarian Size

Monthly changes in ovary and egg size paralleled one another. In October (day 129), ovarian size increased rapidly for females held in all three photothermal cycles (Figure 5). Maximum ovarian diameters occurred near spawning on days 216, 291, and 372 for fish held in the 6-month, 9-month, and 12-month cycles, respectively. These dates corresponded closely to the time of maximum egg size and occurred within three weeks of the median predicted spawning times (days 200, 290, and 380 for the 6, 9, and 12-month cycles, respectively). Within cycles, maximum egg and ovarian sizes occurred at similar times (Table 4).

Mean monthly ovarian diameters of females held in different cycles were significantly different ($P < 0.05$) during October (day 129) and November (day 163) 1990, February (day 255) to June (day 373) 1991, and August (day 441) and September (day 466) 1991 (Table 5). As with egg size, maximum ovarian diameters were related to cycle length. Peak ovarian diameters of females held in the 6-month cycle were significantly smaller ($P < 0.05$) (19.6 mm) than those held in the 9-month (27.4 mm) and 12-month (24.5 mm) cycles. Maximum ovarian diameters of females held in a second 6-month cycle (22.8 mm) were similar ($P > 0.05$) to those maturing in the first 6-month cycle (20.3 mm).

Mean ovarian diameters were positively ($r = 0.91$) and significantly ($P < 0.05$) correlated with egg diameter for all females throughout the study (Figure 6). Similar high correlations of ovarian and egg size were evident for females held in the individual 6-month ($r = 0.92$), 9-month ($r = 0.90$), and 12-month ($r = 0.91$) cycles. These

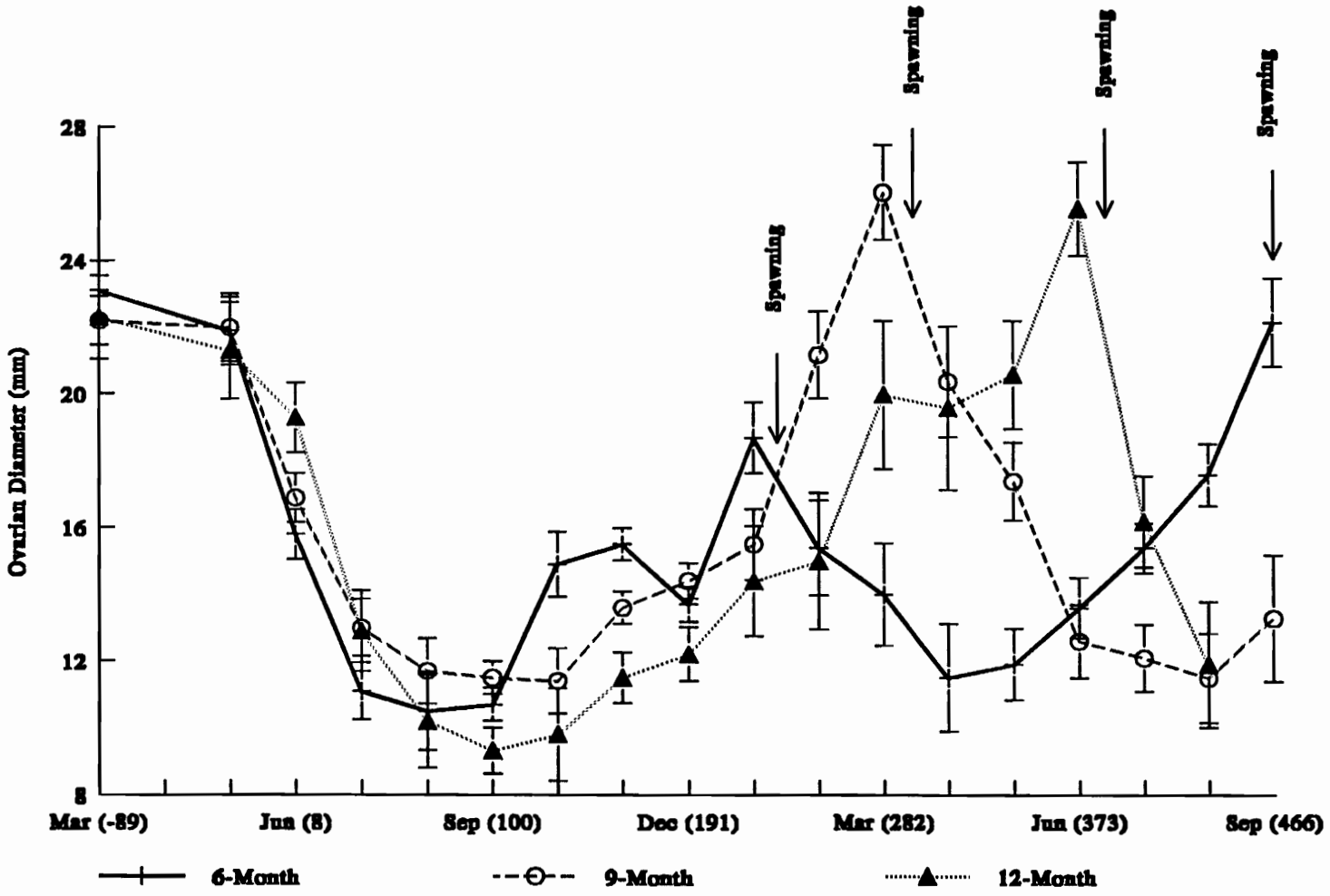

Figure 5. Mean ovarian diameter (standard error) as measured by ultrasound of all females in the 6, 9, and 12-month cycles. Months shown with (experimental days).

Table 4. Number of experimental days (standard error) to develop maximum egg, ovarian, and testicular diameters and spermiation period for fish held on each photothermal cycle.

Maturational Parameter	Cycles		
	6-Month (days)	9-Month (days)	12-Month (days)
Egg Diameter	225 (7)	299 (8)	381 (10)
Ovary Diameter	217 (8)	291 (7)	372 (12)
Testis Diameter	227 (5)	288 (5)	373 (7)
Spermiation	219-282	282-345	345-406

Table 5. Mean monthly ovarian diameters (standard error) of female striped bass held in the 6-month (n=8), 9-month (n=8), and 12-month (n=4) cycles. Different letters represent significantly different in-row values.

Month	Day in Cycle	Cycles			P value
		6-Month	9-Month	12-Month	
		Ovarian Diameter (um)	Ovarian Diameter (um)	Ovarian Diameter (um)	
March	-89	23.1 (0.9)	22.2 (0.7)	22.3 (1.3)	0.73
May	-26	21.9 (1.0)	22.0 (1.0)	21.3 (1.5)	0.91
June	8	15.8 (0.7)^{a}	16.9 (0.7)^{a}	19.3 (1.0)^{b}	0.05
July	43	11.1 (0.9)	13.0 (0.9)	12.9 (1.2)	0.29
August	71	10.5 (1.2)	11.7 (1.0)	10.2 (1.4)	0.62
September	100	10.7 (0.5)	11.5 (0.5)	9.3 (0.7)	0.08
October	129	14.8 (1.0)^{a}	11.4 (1.0)^{b}	9.8 (1.4)^{b}	0.02
November	163	15.5 (0.5)^{a}	13.6 (0.5)^{b}	11.5 (0.8)^{c}	< 0.01
December	191	13.7 (0.5)	14.4 (0.5)	12.2 (0.8)	0.13
January	219	18.7 (1.1)	15.4 (1.1)	14.4 (1.7)	0.07
February	255	15.4 (1.4)^{a}	21.2 (1.3)^{b}	15.0 (2.1)^{a}	0.02
March	282	14.0 (1.5)^{a}	26.1 (1.4)^{b}	20.0 (2.2)^{c}	< 0.01
April	318	11.5 (1.6)^{a}	20.4 (1.7)^{b}	19.6 (2.5)^{b}	< 0.01
May	345	11.9 (1.1)^{a}	17.4 (1.2)^{b}	20.6 (1.6)^{b}	< 0.01
June	373	13.6 (0.9)^{a}	12.6 (1.1)^{a}	25.6 (1.4)^{b}	< 0.01
July	406	16.4 (0.8)	12.0 (1.0)	14.5 (1.5)	0.06
August	441	17.6 (0.9)^{a}	11.5 (1.3)^{b}	11.3 (2.6)^{b}	0.02
September	466	22.2 (1.3)^{a}	13.2 (1.9)^{b}	---	0.01

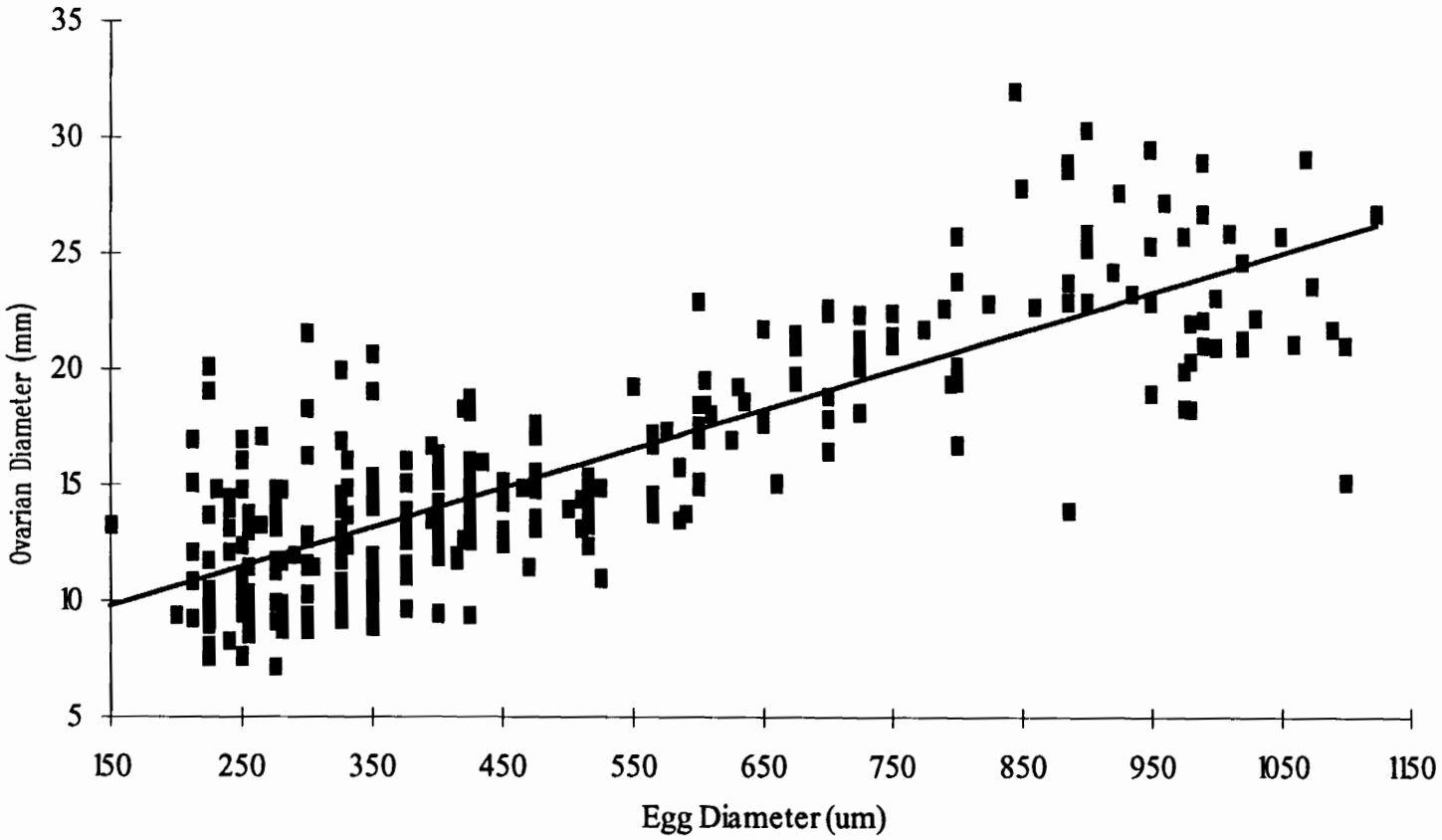

Figure 3. Relationship of ovarian diameter to egg diameter for females in all cycles throughout the 18 month experimental period, $r = 0.91$ ($P < 0.01$).

correlations were improved for females held in the 9 and 12-month cycles ($r = 0.93$ and 0.98, respectively) by excluding data from post-spawning to the initiation of vitellogenesis (March through October 1990 and April through September 1991 for the 9-month cycle, and March through October 1990 and July through September 1991 for the 12-month cycle), when ovarian size decreases more slowly than that of egg size.

Maturation in Males

The majority (95%) of males (n=20) in all three photothermal cycles spermiated. The number of males spermiating in the first 6-month (88%), 9-month (100%), and 12-month (100%) cycles were similar ($P < 0.05$). However, substantially fewer males (43%) matured when held for a second 6-month cycle.

Seasonal variation in male testicular size corresponded to the pattern of ovarian development in females. Testicular diameters were minimal at post-spawning in July 1990 (day 43), but gradually increased for males held in all photothermal cycles (Figure 7). Peak testicular diameter occurred on days 227, 288, and 377 respectively, which corresponded to the spawning period within the respective cycles (Table 4). All fish developed maximum testicular diameters within a month of their predicted median spermiation times, days 200, 290, and 380 for males held in the 6, 9, and 12-month cycles, respectively (Table 2).

Mean monthly testicular diameters of males held on different photothermal regimes differed significantly ($P < 0.05$) during March (day -89), August (day 71), October (day 129), and December (day 191) 1990, and during all months of 1991 (Table 6). Peak testicular diameters of males held in the 6-month cycle (16.2 mm) were significantly smaller than those of males held in the 9-month (23.6 mm) and 12-month

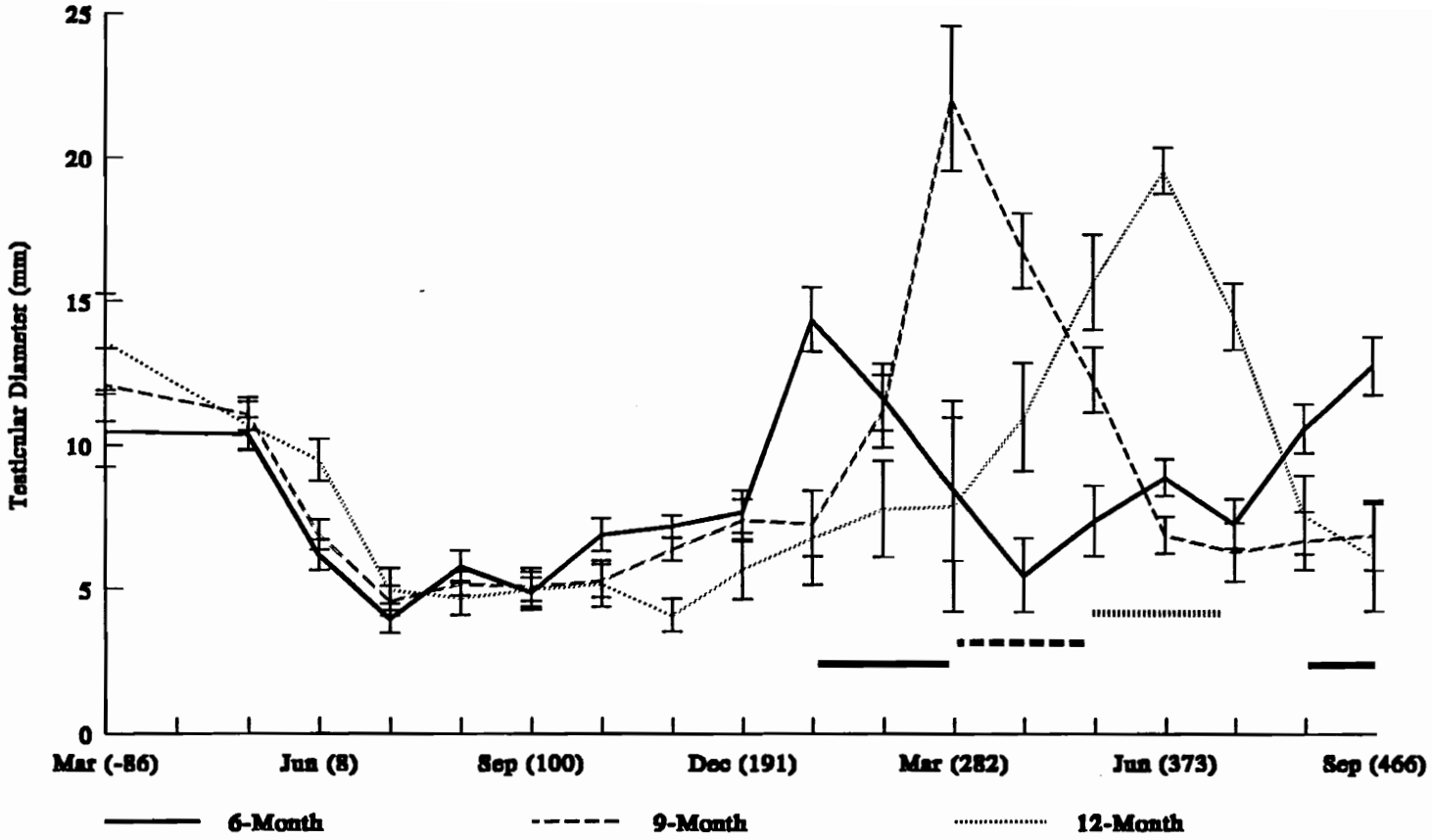

Figure 7. Mean testicular diameter (standard error) as measured by ultrasound for all males in the 6-, 9-, and 12-month cycles. Bottom lines represent periods of spermiation for representative cycles. Months shown with (experimenatl days).

Table 6. Mean monthly testicular diameters (standard error) of male striped bass held in the 6-month (n=8), 9-month (n=8), and 12-month (n=4) cycles. Different letters represent significantly different in-row values.

Month	Day in Cycle	Cycles			P value
		6-Month	9-Month	12-Month	
		Testicular Diameter (um)	Testicular Diameter (um)	Testicular Diameter (um)	
March	-89	10.5 (1.2)	12.1 (1.3)	13.6 (1.7)	0.36
May	-26	10.4 (0.6)	11.1 (0.6)	10.7 (0.8)	0.64
June	8	6.2 (0.5)^{a}	6.9 (0.5)^{a}	9.5 (0.7)^{b}	0.01
July	43	4.0 (0.5)	4.6 (0.5)	5.0 (0.7)	0.49
August	71	5.8 (0.6)	5.2 (0.4)	4.7 (0.6)	0.45
September	100	4.9 (0.5)	5.1 (0.5)	5.0 (0.7)	0.96
October	129	6.9 (0.6)	5.3 (0.6)	5.2 (0.8)	0.13
November	163	7.2 (0.4)^{a}	6.4 (0.4)^{a}	4.1 (0.6)^{b}	< 0.01
December	191	7.7 (0.7)	7.4 (0.7)	5.7 (1.0)	0.32
January	219	14.4 (1.1)^{a}	7.3 (1.1)	6.8 (1.6)	< 0.00
February	255	11.7 (1.2)	11.2 (1.3)	7.8 (1.7)	0.19
March	282	8.5 (2.5)^{a}	22.1 (2.5)^{b}	7.9 (3.6)^{a}	< 0.01
April	318	5.5 (1.3)^{a}	16.8 (1.3)^{b}	11.0 (1.9)^{c}	< 0.01
May	345	7.4 (1.2)^{a}	12.3 (1.1)^{b}	15.7 (1.7)^{b}	< 0.01
June	373	8.9 (0.6)^{a}	6.9 (0.6)^{b}	19.6 (0.8)^{c}	< 0.01
July	406	7.3 (0.9)^{a}	6.3 (1.0)^{a}	14.5 (1.2)^{b}	< 0.01
August	441	10.6 (0.9)^{a}	6.7 (1.0)^{b}	7.6 (1.4)^{b}	0.05
September	466	12.8 (1.0)^{a}	6.9 (1.2)^{b}	6.1 (1.9)^{b}	0.01

(19.9 mm) cycles. Testicular diameters of males held in the second 6-month cycle (12.8 mm) were similar ($P > 0.05$) to those held in the first 6-month cycle (16.2 mm).

Males exhibited significantly ($P < 0.05$) larger mean testicular diameter (14.6 mm) at spermiation than during non-spermiating periods (6.9 mm). The period of spermiation for males in all cycles occurred at spawning and the time of maximum testis size coincided with development of maximum egg and ovarian diameters in females held in corresponding photothermal regimes (Table 4).

Feeding and Maturation

Mean daily feed consumption of fish held on the 6-month (9.9 g/day), 9-month (10.3 g), and 12-month (10.0 g) cycles were similar. Fish held on all cycles were fed at approximately the same rate (0.5% body weight per day) throughout the study. Total feed consumed from the start of the experiment to spawning increased with cycle length, 1.96, 2.95, and 3.93 kg for fish held on the 6-, 9-, and 12-month cycles respectively (Table 7).

Maturation and Spawning

Spawning success (# of females spawning) was directly related to cycle length (Table 2). Only 37% of the females held on the 6-month cycle spawned, whereas 86 and 100% of those held on the 9-month and 12-month cycles, respectively, spawned. Spawning dates differed ($P < 0.05$) between cycles. Latent time (interval from injection to spawning), fertility, and fecundity were similar among females held in all treatments ($P > 0.05$).

Table 7. Mean daily and total feed consumption (standard error) of fish held on the 6-, 9-, and 12-month cycle.

Parameter	Cycle			P-value
	6-Month	9-Month	12-Month	
Daily Ration (g/day)	9.87 (0.52)	10.33 (0.52)	9.97 (0.58)	> 0.10
Total Ration (kg/cycle)	1.96	2.95	3.93	< 0.01

Table 8. Comparison of the spawning method (stripped vs tank) as a function of latent time, fertility, and fecundity (standard error) of females pooled over all cycles.

Spawning Parameter	Spawning Method		P-value
	Strip (n = 7)	Tank (n = 10)	
Latent Time (h)	35.4 (1.8)	36.8 (1.4)	0.62
Fertility (%)	41.8 (8.7)	38.8 (8.2)	0.93
Fecundity (eggs/kg)	53,411 (17659)	67,605 (13834)	0.54

The method of spawning (tank vs stripping) did not influence spawning success (Table 8). No notable differences in latent time, fertility, and fecundity of female striped bass were evident between artificially (stripped) and naturally (tank) spawned fish.

In the second 6-month cycle, spawning success (29%) was not significantly lower than in the first 6-month cycle. However, none of the three females that spawned in the first 6-month cycle spawned in the next 6-month cycle. In the first 6-month cycle one female died after spawning, her replacement, and one female which did not mature during the first 6-month cycle, spawned during the second 6-month cycle.

Blood Sampling and Maturation

Maturational characteristics (ovary, egg, and testis size) for fish that were bled monthly did not differ ($P > 0.05$) from those that were not bled . Spawning characteristics such as latent time, fertility, and fecundity were similar for bled and non-bled fish (Table 9).

Mortality and Maturation

A substantial number of mortalities (41%) occurred during the 18 month experimental period. Twenty-six percent of these occurred during the first two months (Table 10). About half (52%) of these were attributed to disease (bacterial and fungal infections) associated with the elevated summer water temperatures, remaining mortalities resulted from suffocation from jumping out of the tank (13%), handling stress (22%), and stress associated with spawning (13%). Almost equal numbers of females (13) and males (10) died. Mortality was related to cycle length; mortalities of fish held in the 6-month (28%) and 9-month (38%) cycles were lower than in the 12-month (88%) cycle.

Table 9. Comparison of maturational and spawning parameters (standard error) between bled and non-bled females pooled over all cycles.

Parameter	Treatment		P-value
	Bled (n = 8)	Non-Bled (n = 9)	
Egg Diameter (um)	829 (53)	871 (49)	0.28
Latent Time (h)	38.2 (1.6)	35.3 (1.5)	0.24
Fertility (%)	42.5 (10.7)	34.6 (9.9)	0.60
Fecundity (eggs/kg)	54,182 (16567)	62,723 (15339)	0.72

Table 10. Number and causes of mortalities as a function of sex and cycle length during the experimental period.

Mortality Type	Cycles						Total	
	6-Month (n=22)		9-Month (n=24)		12-Month (n=10)			
	Female	Male	Female	Male	Female	Male	Female	Male
Jumpers	1	1	1	-	-	-	2	1
Handling Stress	1	1	-	1	2	-	3	2
Spawning Stress	1	1	-	1	-	-	1	2
Disease	-	-	3	3	4	2	7	5
Total	3	3	4	5	6	2	13	10

DISCUSSION

In this study, female and male striped bass were induced to mature and to spawn in laboratory tanks under three photothermal regimes. The number of females that spawned was directly related to cycle length, averaging 37%, 86%, and 100% for fish held in the 6-, 9-, and 12-month cycles, respectively. Cycle length appears to be an important consideration affecting reproductive status of striped bass. Fish held on a 6-month cycle matured and spawned after 7 months of exposure, whereas those on the 9-month and 12-month cycles matured and spawned on schedule after 9 and 12 months. Differences between the observed and predicted spawning times were minimal, averaging 39, 16, and 9 days in the 6-, 9-, and 12-month cycles, respectively. Actual and estimated spawning times of fish held on all three regimes were remarkably similar considering the fact that this was the first attempt to reset the reproductive period on a number (n=40) of relatively small (2.5 kg), young (age V), adult striped bass in only their first spawning season.

Induction of out-of-season spawning by compressing the annual cycle has been reported for certain species, including the red drum, *Scianeops ocellata* (Arnold 1988), rainbow trout, *Onchorynchus mykiss* (Whitehead 1978, Bromage et al. 1982), and pink salmon, *Onchorynchus gorbuscha* (MacQuarrie et al. 1979). F_1 hybrid striped bass and striped bass have been induced to mature and to spawn on a compressed 9-month cycle (Smith and Jenkins 1984, Smith and Jenkins 1986). In this study, striped bass maintained on a 6-month cycle matured and spawned twice within a 14-month interval. Those broodstock, maintained on a compressed 9-month cycle, spawned after a 9-month interval.

The minimal time necessary for gametogenesis and spawning in most species is unknown. At least 5 to 7 months are required for complete reproductive development in salmonids and flatfish (Bye 1984). Certain species such as the red drum, subjected to a constant photoperiod (12L:12D) and varying thermal regime, have been induced to spawn regularly without entering a period of recrudescence (Arnold 1988). Red drum, when manipulated under these conditions, become fractional spawners, capable of ovulating mature eggs every two to three months indefinitely (Arnold 1988). Other marine species with similar asynchronous oogenesis such as the gilthead seabream, *Sparus aurata*, can ovulate and spawn at 24 hour intervals over a prolonged two to three month period (Zohar 1990). In contrast, striped bass exhibit group synchronous oogenesis (Specker et al. 1987) in which the ovary contains both large and small oocyte populations representing the reproductive products for the next two successive reproductive cycles. These fish generally spawn once per year during a limited breeding season.

Compressing the annual reproductive cycle of striped bass into a 6-month period affected reproductive success in this study. The reduced number of ovulating females (<50%) and diminished mean egg diameter (<800 um) observed suggests that oocyte development may have been compromised by this abbreviated cycle. However, ovarian diameter, fecundity, and fertility of fish held on the 6-month cycle that spawned (at 7 months) were similar to those of fish held on the 9- and 12-month cycles. Smith and Jenkins (1984) reported that compression of the annual reproductive cycle into a 9-month period caused a reduction in the average oocyte size and the number of fish spawning, but they found spawning success (fecundity and larval survival) of F_1 hybrid striped bass or striped bass was not affected (Smith and Jenkins 1986). Photothermal compression of

the natural 12-month reproductive cycle in striped bass to less than 9 months may inhibit reproductive development and reduce overall spawning success.

Nutritional deficiencies also may have impacted reproductive development, especially for fish held on the compressed, 6-month cycle, 50% of which did not mature. Fish on all cycles were fed daily on artificial diet to satiation, averaging about 0.5% body weight per fish per day over the experimental period. This relatively low ration may not have been entirely adequate to support rapid and complete maturation of all fish. Typical feeding rates for maturing broodstock should exceed 2% body weight (Luquet and Watanabe 1986). Henderson-Arzapalo and Colura (1987) fed captive striped bass held on a 12-month cycle a diet of cut fish, frozen shrimp, and beef liver at 12.5% (wet weight) body weight per fish per day and reported successful spawns for both females. Feeding the broodstock more frequently (> 2 times/day) or perhaps a higher quality diet may have improved nutrition and reproductive development.

Sustaining striped bass on an second 6-month compressed cycle had little effect on reproductive success. The number of ovulating females (n = 2), ovarian and egg sizes, fecundity, and fertility were similar for fish held on two successive 6-month cycles. Mature oocytes were present in those females that failed to ovulate in the second 6-month cycle, suggesting that 6 months is sufficient for maturation, if not ovulation and respawning. Accelerating the spawning frequency of striped bass broodstock to every six months for two successive spawnings without greatly diminished reproductive success was achieved, but the reproductive life expectancy (total number of spawns) and overall spawning success of individual fish sustained continuously on such an abbreviated cycle is unknown and may be diminished.

The methods used here for successfully inducing out-of-season maturation and spawning and increaing the frequency of spawning in striped bass were based on

compressing the natural 12-month photothermal regime into 9-month and 6-month intervals. However, simply compressing a 12-month period into uniformly shorter seasons may not be the most expedient way to elicit control of the reproductive cycle in commercial aquaculture. Shortening the warm, summer period while prolonging fall and spring may improve maturation and reduce the duration of stressful non-productive seasons. For example, a disproportionately shorter summer period than used in this study may reduce mortality and stress on broodstock subjected to high water temperatures. Sixty-six percent of the mortalities reported in the 9- and 12-month cycles occurred during the summer period shortly after spawning. In the 6-month cycle, the post-spawning summer period was proportionately shorter and no mortalities occurred during this time. A condensed summer can accelerate the start of vitellogenesis, which is initiated by a shortened fall photoperiod in some fishes (Kuo et al. 1974, MacQuarrie et al. 1979, DeVauchelle et al. 1987). Sullivan et al. (1991) documented an increase in vitellogenin levels in striped bass with decreasing daylengths in October, and suggested that a summer photothermal period inhibits gonad development in striped bass.

A warmer winter season than that used in this study may accelerate oocyte development and further improve reproductive success. Although temperature does not initiate vitellogenesis, it does regulate this process by controlling the metabolism. Vitellogenesis has been inhibited by temperatures below 17°C for grey mullet (Kuo et al. 1974) and above 12°C in sole (DeVauchelle et al. 1987). Although Smith and Jenkins (1986) speculated that low temperatures (<10°C) may be necessary for maturation in striped bass, a minimum temperature of 13.5°C in all experimental cycles in this study did not inhibit oocyte maturation. It is likely that an even warmer winter period than used here may foster more rapid reproductive development. The spring season for fish held in the 6- and 9-month cycles may have been too short, because optimal spawning

temperatures (17-19°C) were reached before some fish were fully mature; a prolonged maintenance of spawning conditions (19°C, 13L:11D) was necessary to complete maturation.

An alternative to compressed seasonal cycles is the use of phase-shifted cycles to induce off-season maturation and spawning. This type of photothermal manipulation has been used to delay the spawning of sea bass, *Dicentrarchus labarax*, several weeks by holding them at simulated winter conditions of low water temperatures (Zanuy et al. 1986, Carrilo et al. 1991). Phase-shifting simply refers to exposing broodfish to altered photothermal regimes designed to lengthen rather than compress the annual reproductive period of fish. For example, by exposing fish to a prolonged summer of elevated water temperatures and long daylengths, the reproductive cycle could be extended beyond a natural 12 month period by a predicted length of time equivalent to that of the natural reproductive period plus the length of the extended artificial summer. By maintaining different groups of broodfish on dissimilar photothermal regimes, aquaculturists can produce eggs and fingerlings on predictable schedules year-round. Because exposure of striped bass to sustained high temperature (summer conditions) caused high rates of mortality in this study, it may be necessary to expose fish to lower water temperatures, but maintain long daylengths to prohibit the initiation of vitellogenesis and mortality.

The role of salt as a therapeutic to promote osmotic balance and reduce stress in striped bass is well documented (Rees and Harrell 1990). Smith and Jenkins (1984) reported a 65% decrease in post-spawning mortality of hybrid striped bass broodstock at high salinity, and recommended post-spawning salinities of 18 ppt to help control stress-induced hemorrhaging and scale loss. In this study, the efficacy of salt on survival and reproductive success of striped bass was not evaluated, but a salt solution (5 ppt) was maintained in all tanks for three days after handling and at each monthly sampling

period. Despite this precaution, significant mortality of broodfish held on the 9- and 12-month cycles occurred in the summer period after spawning. Higher salt concentrations sustained for a much longer period may have averted some of the losses.

Salinity may be an important cue triggering the timing of ovulation and spawning, in anadromous fish such as striped bass. Henderson-Arzapalo and Colura (1987) maintained striped bass in laboratory tanks on relatively high salinity (15 ppt) until near spawning in March and April when salinity was decreased to 2 ppt. Despite this manipulation, injection of HCG still was required to induce ovulation. Salinity appeared to be unrelated to maturation and spawning in this study, because spawning fish were maintained in freshwater or at relatively low salinities (5 ppt). At the projected spawning time, females and males were transferred and spawned in freshwater tanks. Manipulation of salinities or other biotic and environmental factors such as supplying bottom substrate or the presence of the opposite sex was unnecessary to induce maturation and spawning if exogenous hormones are employed.

Human chorionic gonadotropin (HCG) has been widely used to induce final maturation and spawning in wild, adult striped bass (Stevens 1966, Rees and Harrell 1990). Henderson-Arzapalo and Colura (1987) reported that HCG injection was necessary to trigger ovulation and spawning in striped bass held in laboratory tanks on a natural 12-month photothermal regime. Smith and Jenkins (1984) reported spawning 65% of the F_1 hybrid striped bass injected with HCG, but no spawning among three pairs of fish which were not injected. Smith and Jenkins (1986) achieved 70% spawning success in striped bass injected with HCG. HCG injection was necessary to induce ovulation in 87% of the female striped bass used in this study. However, continued use of HCG, a large foreign protein, may induce an immune response in domesticated broodfish (personal communication, C. Sullivan North Carolina State University). A

promising alternative to HCG is the use of time-release implants of gonadotropin releasing hormone (GnRH) that automatically administer controlled dosages on a sustained basis (Crim et al. 1983, Crim and Glebe 1984).

When the results for the tank- and strip-spawned fish were compared to examine the effect of natural and artificial methods of spawning on reproductive success in striped bass, no differences in spawning success (number of females spawning, fecundity, and fertility) were detected. Any potential stress associated with handling and egg sampling of strip-spawned fish after hormone injection was negligable. Latent time (period from injection to ovulation) was slightly shorter (35.4 h) for strip-spawned than tank-spawned (36.8 h) females, even though the tank-spawned fish were not handled after injection. The overall latent period in this study was substantially less than that of 51 h reported by Henderson-Arzapalo and Colura (1987) who injected HCG at the same dosage, but lower water temperatures (17°C) may have delayed ovulation in their study.

Blood was collected from 50% of the broodstock to measure hormone levels through the reproductive cycle and to compare the effect of bleeding on reproductive success. No significant differences in physiological measurements (ovary, egg, and testis size), spawning success (number of females spawned, fecundity, fertility), or mortality were detected between bled and non-bled fish. Handling stress (netting, anesthetization) probably had a much greater impact on fish health than the serial collection of 4 ml (< 0.2% body weight) of blood per fish on a monthly basis. Blood samples of volumes equivalent to 1% body weight, can be removed weekly without affecting fish health (Klontz and Smith 1968).

The ability to mature and to spawn striped bass out-of-season has several important advantages. First, photothermal control of the reproductive cycle will permit year-round spawning to meet the increasing demand for eggs and fingerling fish,

especially in intensive culture systems that require a continuous supply of seed fish to maximize production and profitability. Second, manipulation of striped bass by photothermal regimes may expedite the production of hybrids, such as the original cross (female striped bass x male white bass), a preferred fish for rearing in indoor tanks (Smith and Jenkins 1986). Finally, broodstock domestication and reliable control of spawning will reduce the current exploitation on wild, adult stocks, and facilitate genetic selection and improvement.

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CHAPTER 3:

PLASMA HORMONE AND VITELLOGENIN LEVELS IN STRIPED BASS, *Morone saxatilis*, EXPOSED TO 6-, 9-, AND 12-MONTH PHOTOTHERMAL REGIMES.

ABSTRACT

Striped bass, *Morone saxatilis*, broodstock (n=40) were induced to mature and to spawn when exposed to compressed 6- and 9-month cycles or to a 12-month reference cycle. Fish were sampled monthly to measure egg diameter or to detect spermiation. Monthly blood samples were obtained for 20 fish (equal numbers of each sex) held in each cycle. Vitellogenin (Vg), estradiol (E_2), and testosterone (T) were monitored in females' plasma. Maximum Vg levels were similar in females held on the 6-, 9-, and 12-month cycles, averaging 0.78, 1.05, and 0.80 mg/ml, respectively. Maximum E_2 levels were similar in females held on the 6-, 9-, and 12-month cycles, averaging 0.70, 1.40, and 1.35 ng/ml, respectively. Maximum T levels were similar in females held on the 6-, 9-, and 12-month cycles, averaging 0.40, 0.79, and 1.18 ng/ml, respectively. Maximum levels of Vg, E_2, and T were shifted to correspond with spawning (January, day 219; March, day 282; and June, day 373) for females held on the 6-, 9-, and 12-month cycles, respectively. Egg diameter was correlated with Vg ($r = 0.83$), E_2 ($r = 0.76$), and T ($r = 0.76$) levels. T and 11-keto testosterone (11KT) levels were measured in the plasma of male striped bass. Maximum T levels of males held on the 9-month (0.60 ng/ml) cycle were lower than those in the 6-month (1.08 ng/m) and 12-month (1.24 ng/ml) cycles. Maximum 11KT levels were similar (1.36, 0.82, and 1.58 ng/ml) among males held on the 6-, 9-, and 12-month cycles. Maximum levels of T and 11KT occurred during

spermiation in January (day 219), March (day 282), and June (day 373) for males held on the 6-, 9-, and 12-month cycles, respectively. Compression of the annual cycle accelerated the rate of change (timing), but not the absolute concentrations of plasma Vg and hormones. Vg and hormone profiles of male and female striped bass were advanced to reflect the maturational status of individuals held in the 6-, 9-, and 12-month photothermal regimes.

INTRODUCTION

Environmental factors, primarily water temperature and photoperiod, play an important role in fish reproduction (Billiard et al. 1978). Photothermal conditions influence gonadal steroids linking the environment and reproductive events. The extent to which water temperature and photoperiod, acting singly or in combination, control maturation and spawning is species-specific. Salmonids and certain cold water marine species are more responsive reproductively to photoperiod than to temperature (Duston and Bromage 1986, Zohar 1990), whereas cyprinids and other warm water species may be more responsive to temperature cues (Stacey 1984). Although the synergistic or antagonistic effects of these two variables on gonadal development have not been fully documented, it is probable that they co-modulate reproductive events in certain species. For example, photoperiod may initiate vitellogenesis, whereas water temperature may subsequently regulate vitellogenesis in grey mullet, *Mugil cephalus* (Kuo et al. 1974).

Induced maturation and out-of-season spawning by photothermal manipulation has been successfully employed in a variety of species including red drum, *Scianeops ocellatus* (Arnold 1988), sole, *Solea solea* (Devauchelle et al. 1987), and coho salmon, *Onchorynchus kisutch* (MacQuarrie et al. 1979). Captive populations of striped bass and their hybrids have been induced to mature and to spawn off-season in response to controlled photothermal regimes (Smith and Jenkins 1984, Smith and Jenkins 1986, Henderson-Arzapalo and Colura 1987).

Environmental cues influence vitellogenin and gonadal hormone concentrations, which follow distinct seasonal patterns (Zohar 1990). The female teleost reproductive cycle can be divided into four sequential periods; recrudescence, vitellogenesis, final maturation, and ovulation. Estrogens induce the production of vitellogenin (Bailey

1957), a calcium-binding lipophosphoprotein, and the major precursor of egg yolk (Wallace 1978). Vitellogenin is incorporated by the oocytes and restructured to form yolk proteins, phosvitin and lipovitellin (Tata and Smith 1979). Vitellogenin is specific to female fish and is detectable seven months before spawning in striped bass (Sullivan et al. 1991). Estrogen (E_2) concentrations are reported to increase with vitellogenesis in rainbow trout, *Onchorynchus mykiss* (van Bohemen and Lambert 1981, Bromage et al. 1982), Indian catfish, *Heteropnesustes fossils* (Lamba et al. 1983), plaice, *Pleuronectes platessa* (Wingfield and Grimm 1977), and during winter and spring in striped bass (Sullivan et al. 1991). E_2 induces vitellogenesis in rainbow trout (van Bohemen and Lambert 1981), winter flounder, *Pleuronectes americanus* (Emmerson and Peterson 1976), ayu, *Plecoglossus altivelis* (Aida et al. 1973), and striped bass (Sullivan et al. 1991). After vitellogenesis and throughout final maturation and ovulation, E_2 decreases to its lowest seasonal levels in rainbow trout (Scott et al. 1984), plaice (Wingfield and Grimm 1977), northern pike, *Esox lucius* (Simontocchi et al. 1978), and striped bass (Berlinsky and Specker 1991, Sullivan et al. 1991).

In females, testosterone (T) is a precursor to E_2 (Kagawa et al. 1982), and which therefore reflects its level. T remains low throughout vitellogenesis and then increases until ovulation in Indian catfish (Lamba et al. 1983). T peaks prior to ovulation and then decreases throughout spawning in pike (Simontocchi et al. 1978), winter flounder (Campbell et al. 1976), white sucker, *Catostomus commersoni* (Scott et al. 1983), and rainbow trout (Scott et al. 1980a). T reaches maximum levels just prior to the spring spawning season in female striped bass (Berlinsky and Specker 1991, Sullivan et al. 1991).

In male rainbow trout, T increases throughout spermatogenesis, peaks at the beginning of spermiation, and then decreases as spermiation continues (Scott and Baynes

1983). 11-keto testosterone (11KT) levels follow T levels in rainbow trout (Scott and Baynes 1983) and in the white sucker (Scott et al. 1983). In salmonids, 11KT may control spermiation (Fostier et al. 1982), but in goldfish, high T levels correspond to peak milt volumes (Kobayashi et al. 1986). Maximum T concentrations occur just prior to spawning in spring for male striped bass (personal communication, C. Sullivan, North Carolina State University).

Despite the increasing prominence of striped bass and *Morone* hybrids in aquaculture and advances in environmental regulation of maturation and spawning, the influence of photothermal cycles on gonadal steroids has received little attention. Berlinsky and Specker (1991) examined serum levels of E_2 and T in wild female striped bass collected in coastal waters, but provided little or no information on males or data on females throughout a complete reproductive cycle. Sullivan et al. (1991) serially measured plasma E_2, T, 11KT, and Vg concentrations in captive broodfish, males and females, held in tanks under natural photothermal conditions over a 15-month period. The purpose of the present study was to characterize the absolute levels, succession, and timing (in relation to egg size and spawning), of vitellogenin and reproduction hormones in striped bass exposed to 6-, 9-, and 12-month photothermal regimes and to examine their relationship to maturational status.

METHODS

Adult striped bass (n=20), (Chesapeake Bay stock) used in this experiment were obtained in 1984 as fingerlings from the National Fisheries Research Center at Leetown, West Virginia. They were reared in raceways at the Aquaculture Research Facility at Virginia Polytechnic Institute and State University. At age V, the experimental fish averaged 2.5 kg in weight and 54.5 cm in length (Table 1). Sex was determined by ultrasonic imaging and validated by presence of sperm or ova in individually tagged fish.

The fish were stocked into 1.9 m circular, fiberglass tanks (2200 L) in five environmental chambers (4 males, 4 females in each chamber) at the beginning of May 1990. The same two males and two females were bled monthly in each chamber. Early mortalities were replaced to maintain similar densities and sex ratios in each chamber (Appendix A). The environmental conditioning chambers (2.6 m x 2.6 m) were constructed with 10 cm thick styrofoam walls. Water quality was maintained using a recirculation system (Appendix B). Water flowed from the fish tank into a sump for solids removal, was then pumped up into a trickling filter for nitrification and aeration, and flowed back into the fish tank. The sump, used to remove solids, consisted of a 220 L plastic drum containing a multitube clarifier suspended near the bottom and a submersible pump (80 L/min) suspended on top of the sump. The trickling filter (60.9 x 60.9 x 121.9 cm tall) and the multitube clarifier located in the sump consisted of corrugated plastic (BioDeck, The Munter Corp. St.Petersburg, FL) biofilter material.

Three different photothermal cycles were used in the experiment. The compressed 6-month (Figure 1) and 9-month (Figure 2) cycles were duplicated in two environmental chambers. The 12-month (Figure 3) reference cycle was not

Table 1. Identification tag number, sex, cycle, environmental chamber, initial length and weight data of the broodstock.

Tag #	Sex	Cycle	Chamber #	Length (cm)	Weight (kg)
102	M	6	2	56.0	2.7
105	M	9	3	55.5	2.7
107	M	9	3	52.5	2.4
112	M	6	4	51.0	2.1
114	M	9	1	55.0	2.6
120	M	12	7	57.0	2.6
121	M	6	2	56.5	2.5
124	M	6	4	54.0	2.2
130	M	9	1	54.0	2.4
131	M	12	7	51.0	2.0
152	F	12	7	54.5	2.4
153	F	6	2	50.0	2.0
158	F	9	1	57.0	2.6
159	F	9	3	54.0	2.3
167	F	9	1	56.5	2.9
170	F	6	4	56.5	2.9
171	F	6	4	51.0	2.1
175	F	9	3	53.0	2.4
180	F	12	7	56.5	2.9
182	F	6	2	54.0	2.5

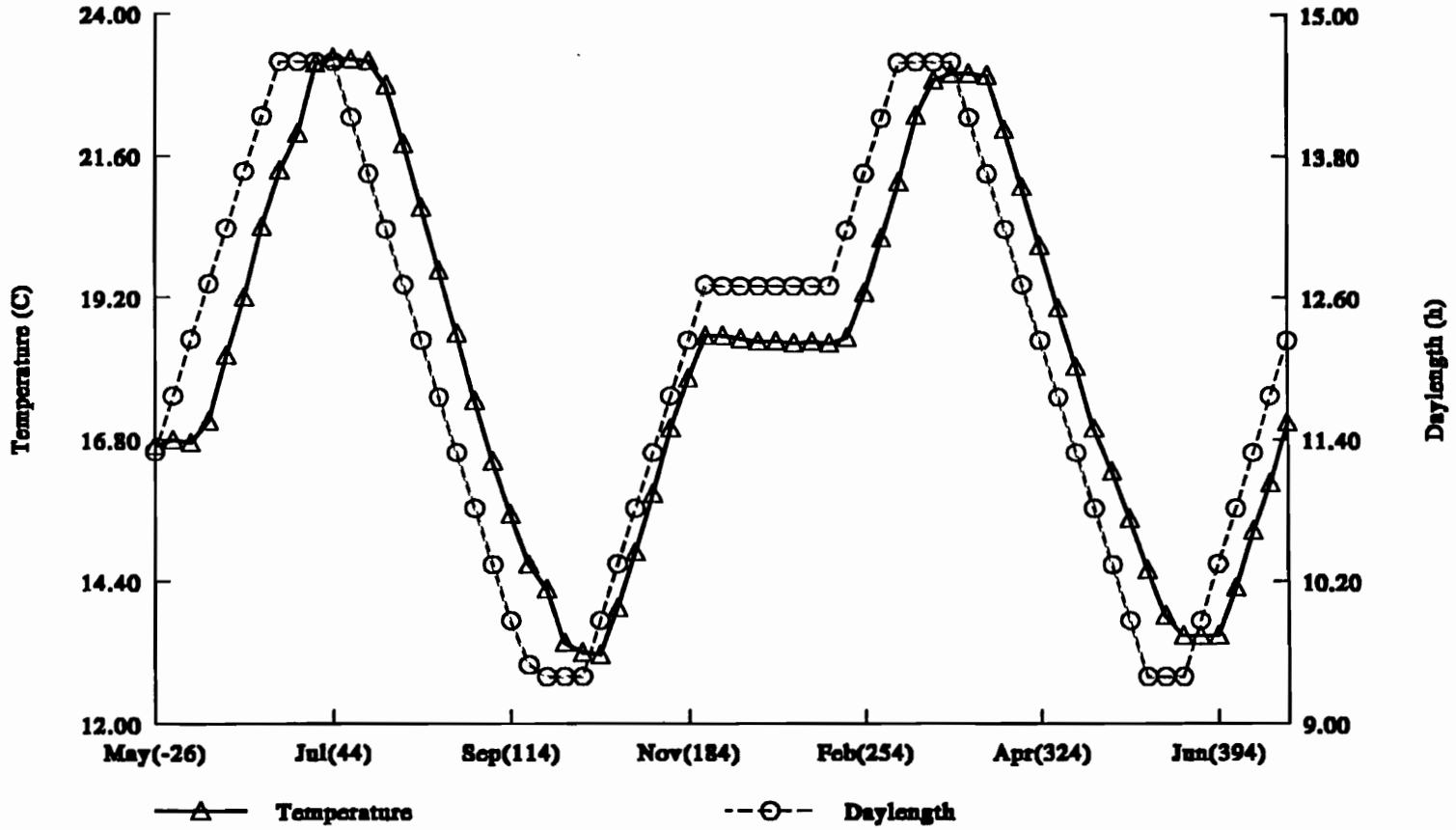

Figure 1. Six-month compressed temperature-photoperiod cycle from May 1990 through July 1991. Months are shown with (experimental days).

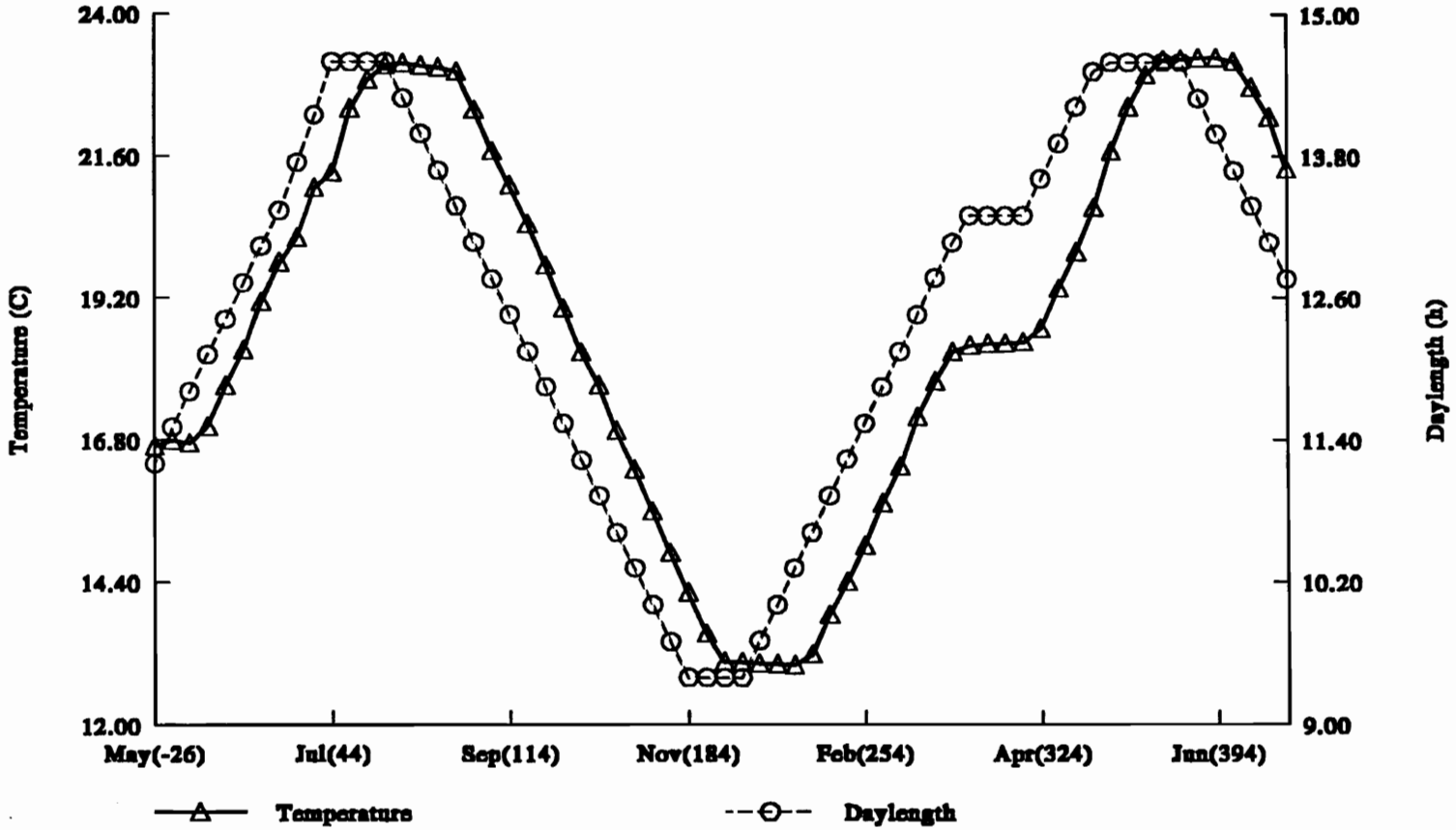

Figure 2. Nine-month compressed temperature-photoperiod cycle from May 1990 through July 1991. Months are shown with (experimental days).

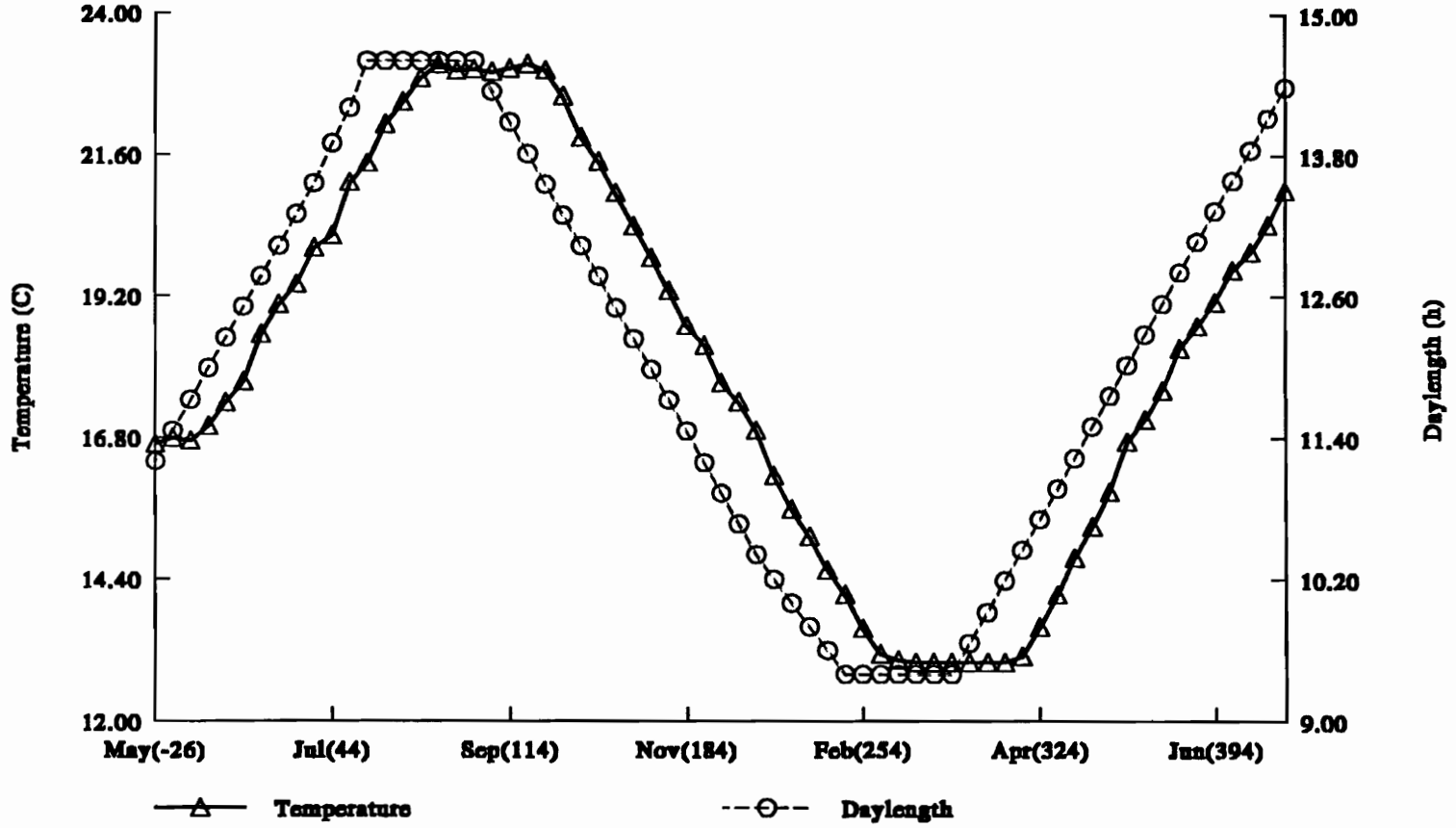

Figure 3. Twelve-month 'natural' temperature-photoperiod cycle from May 1990 through July 1991. Months are shown with (experimental days).

replicated. The artificial photothermal cycle was established based on the 1990 Farmers Almanac adjusted for the mid-Atlantic latitude and compressed from a 12-month natural cycle to 6 and 9 month intervals. Maximum and minimum temperature limits were established at 13.5 and 23.5° C to correspond to winter and summer conditions (Bain and Bain 1982).

Water temperature and photoperiod regimes were simultaneously controlled in each environmental chamber by a micro-computer network (Fly et al. 1991). Water temperatures were computer-adjusted by the addition of cold or hot water to the tank to maintain the desired temperatures. Photoperiod was supplied by three 60-watt incandescent lights suspended 2.5 m above the fish tank in each environmental chamber. Rheostatic-controlled (G.C. Controls, Smithville Flats, NY) crepuscular periods (1 hr in duration) simulated natural sunrise and sunset.

The environmental control system consisted of a Jameco/286 or DTK 386 personal computer linked with Campbell (CR10) data loggers (Campbell Scientific Co. Logan, UT) regulating light and temperature in tanks in the five environmental chambers. The host computer routinely queried each data logger to retrieve data, control water temperature, and alter photoperiod and temperature as programmed.

All fish were fed to satiation twice per day (morning and afternoon), using 1.2 cm pelleted "Bass Grower" feed (Biosponge Aquaculture Products Co. Sheridan, WY). This feed was comprised of a minimum of 44% crude protein, 8% crude fat, and a maximum of 3% crude fiber and 13% moisture (Appendix C). Dry weight of feed consumed by all fish in each environmental chambers at each feeding was recorded.

Water temperature and dissolved oxygen concentrations were measured on a daily basis using a YSI model 58 meter. Water quality parameters (pH, total ammonia nitrogen, and nitrite) were measured periodically using standard procedures (Hach

Chemical Co. Loveland, Co); throughout the study pH ranged from 8.8 to 8.0, TAN ranged from 1.0 to 0 mg/l, and nitrite ranged from 2.0 to 0 mg/l. Water was added to the system only to maintain water temperature control or to flush suspended solids from the sump tank.

Blood samples (4 ml) were collected from twenty broodfish each month with a 5 ml syringe and 3.8 cm (21-gauge) needle rinsed in heparin (10 mg/l) and NaCl (0.9%) solution. Blood was transferred into 5 ml culture tubes containing 50 ul of heparin (10 mg/l) and 24 ul/ml blood aprotinin solution. Samples were stored on ice until centrifuged (IECCentra-4B Centrifuge) at 3,000 rpm for 15 minutes. The plasma was extracted using heparinized Pasteur pipets, stored in 400 ul microcentrifuge tubes, and frozen at -40°C until analyzed. Small samples from fish were pooled and frozen for use as a group sample.

Plasma steroid and vitellogenin (Vg) analyses were done in collaboration with Dr. Craig Sullivan, North Carolina State University. Vitellogenin, estradiol (E_2), and testosterone (T) were measured in the plasma from females, and T and 11-keto testosterone (11KT) were measured in the male plasma. Vg was assayed using a single radial immunodiffusion assay as described by Mancini et al. (1965). Striped bass Vg was purified and the assay was developed and validated for striped bass as described by Sullivan et al. (1991). E_2, T and 11KT were measured using radio immunoassay techniques. Assay techniques were utilized as described by Berlinsky and Specker (1991) and developed for E_2 by Sower and Schreck(1982). T and 11KT methods were developed by Hourigan et al. (1991). With respect to recovery, parallelism, and extraction efficiency, radioimmunoassays were fully validated for use with striped bass plasma (Sullivan, unpublished data).

After blood sampling, abdominal pressure was applied to all fish in an attempt to express sperm from males. Females were catheterized using surgical tubing (1.2mm ID) attached to a 5 ml syringe. The tube was inserted into the urogenital pore and a slight vacuum was applied to collect the eggs. All eggs collected were placed in Cortland's solution to fix egg size prior to examination (Hoar 1969). Egg diameter was measured using a dissecting microscope equipped with an ocular micrometer. Mean diameter of the largest 20 eggs collected was determined.

Analysis of variance (ANOVA) procedures were used to compare monthly egg diameter and plasma hormone and vitellogenin levels among cycles. Peak egg diameter and hormone and vitellogenin levels of fish held on all three cycles were compared using ANOVA. Multiple analysis of variance (MANOVA) procedures were used to correlate egg diameter with Vg, E_2, and T.

RESULTS

Egg diameters were at a minimum during recrudescence in June 1990, but gradually increased in fish held in all cycles until spawning (Figure 4). Monthly egg diameters differed ($P < 0.05$) between fish held in different treatments during July, October through December 1990, and March through July 1991 (Table 2). Peak egg diameters differed between fish held in dissimilar treatments ($P < 0.05$). Maximum egg diameters for fish held in the 6-month, 9-month, and 12-month cycles occurred on days 224, 298, and 381, respectively (Table 3). Peak egg diameters corresponded to spawning for fish in all cycles. Maximum egg diameters occurred within a month of the predicted median spawning times, days 200, 290, and 380 for the 6, 9, and 12 month cycles, respectively. Peaks egg diameters of fish held in the 6-month cycle (721 um) were significantly ($P < 0.05$) lower than those in the 9-month (946 um) and 12-month (1,073 um) cycles.

Fewer (50%) females spawned in the 6-month cycle than the 9-month (100%), and 12-month cycles (100%). Spawning dates differed between cycles, occurring on days, 239, 306, 389 for the 6-month, 9-month, and 12-month cycles, respectively ($P < 0.05$). Median spawning dates occurred 39, 16, and 9 days after the projected spawning date for fish held on the 6-, 9-, and 12-month cycles (Table 3).

Mean plasma vitellogenin (Vg) levels in female striped bass were at a minimum until August, 1990, but increased in the 6 and 9 month cycles to a maximum of 0.717 mg/ml in January 1991 (day 219) and 0.99 mg/ml in February 1991 (day 255), respectively. Vg levels of fish held in the 12-month cycle increased gradually reaching a peak value of 0.647 mg/ml in July, 1991 (Figure 5). Vg levels were minimal during summer, increased in fall, and peaked prior to spawning in all cycles. During February

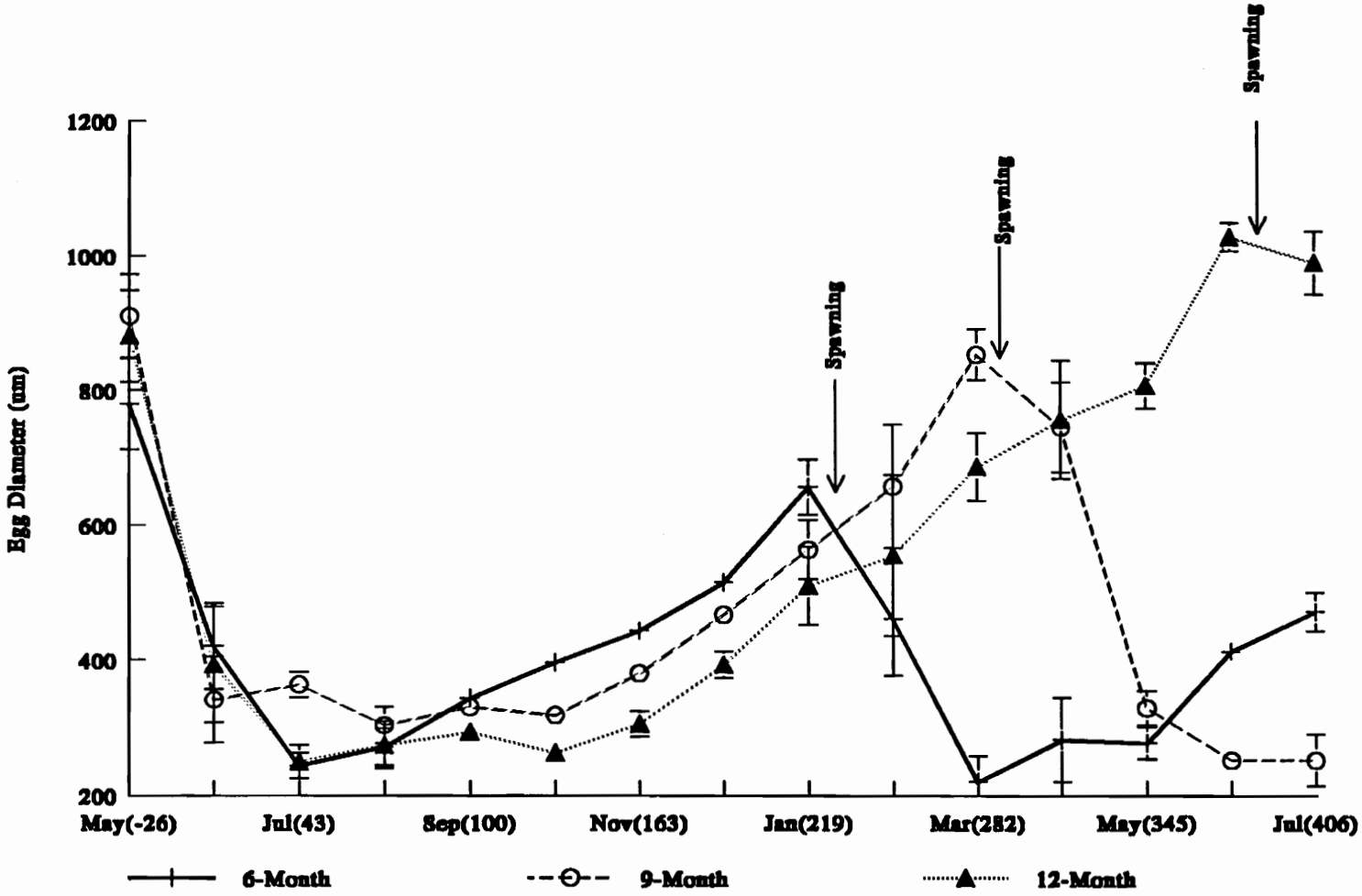

Figure 4. Mean monthly egg diameter (standard error) for all females held on the 6-, 9-, and 12-month cycles. Month shown with (experimental days).

Table 2. Mean monthly egg diameters (standard error) for female striped bass held in the 6-month (n=8), 9-month (n=8), and 12-month (n=4) cycles. Different letters represent significantly different in-row values.

Month	Day in Cycle	Cycles			P value
		6-Month Egg Diameter (um)	9-Month Egg Diameter (um)	12-Month Egg Diameter (um)	
May	-26	780 (67)	911 (62)	881 (88)	0.37
June	8	421 (64)	342 (64)	394 (86)	0.69
July	43	244 (19)^{a}	364 (19)^{b}	250 (25)^{a}	< 0.01
August	71	272 (27)	304 (27)	275 (35)	0.67
September	100	343 (12)	331 (13)	294 (16)	0.08
October	129	397 (11)^{a}	319 (12)^{b}	263 (15)^{c}	< 0.01
November	163	444 (13)^{a}	381 (14)^{b}	306 (19)^{c}	< 0.01
December	191	516 (14)^{a}	468 (16)^{b}	394 (20)^{c}	< 0.01
January	219	658 (41)	565 (44)	511 (58)	0.12
February	255	462 (85)	659 (91)	556 (120)	0.32
March	282	221 (38)^{a}	855 (38)^{b}	688 (50)^{c}	< 0.01
April	318	283 (62)^{a}	747 (67)^{b}	758 (88)^{b}	< 0.01
May	345	278 (24)	330 (26)	809 (34)^{a}	< 0.01
June	373	414 (15)^{a}	253 (17)^{b}	1031 (21)^{c}	< 0.01
July	406	473 (29)^{a}	253 (38)^{b}	993 (59)^{c}	< 0.01

Table 3. Elapsed experimental days (standard error) from the initiation of the study (Day 1) to when maximum egg diameters and maximum concentrations of Vg, E_2 and T, at spawning occurred in females held on the 6 (n=4), 9 (n=4), and 12-month (n=2), cycles.

Reproductive	Cycles			P-value
Parameter	6-Month	9-Month	12-Month	
Egg Diameter	225 (7)	299 (8)	381 (10)	<0.01
Spawning	239 (9)	306 (6)	389 (7)	<0.01
Vitellogenin (Vg)	205 (13)	284 (13)	390 (19)	<0.01
Estradiol (E_2)	205 (9)	282 (9)	373 (13)	<0.01
Testosterone (T)	212 (8)	291 (8)	373 (11)	<0.01

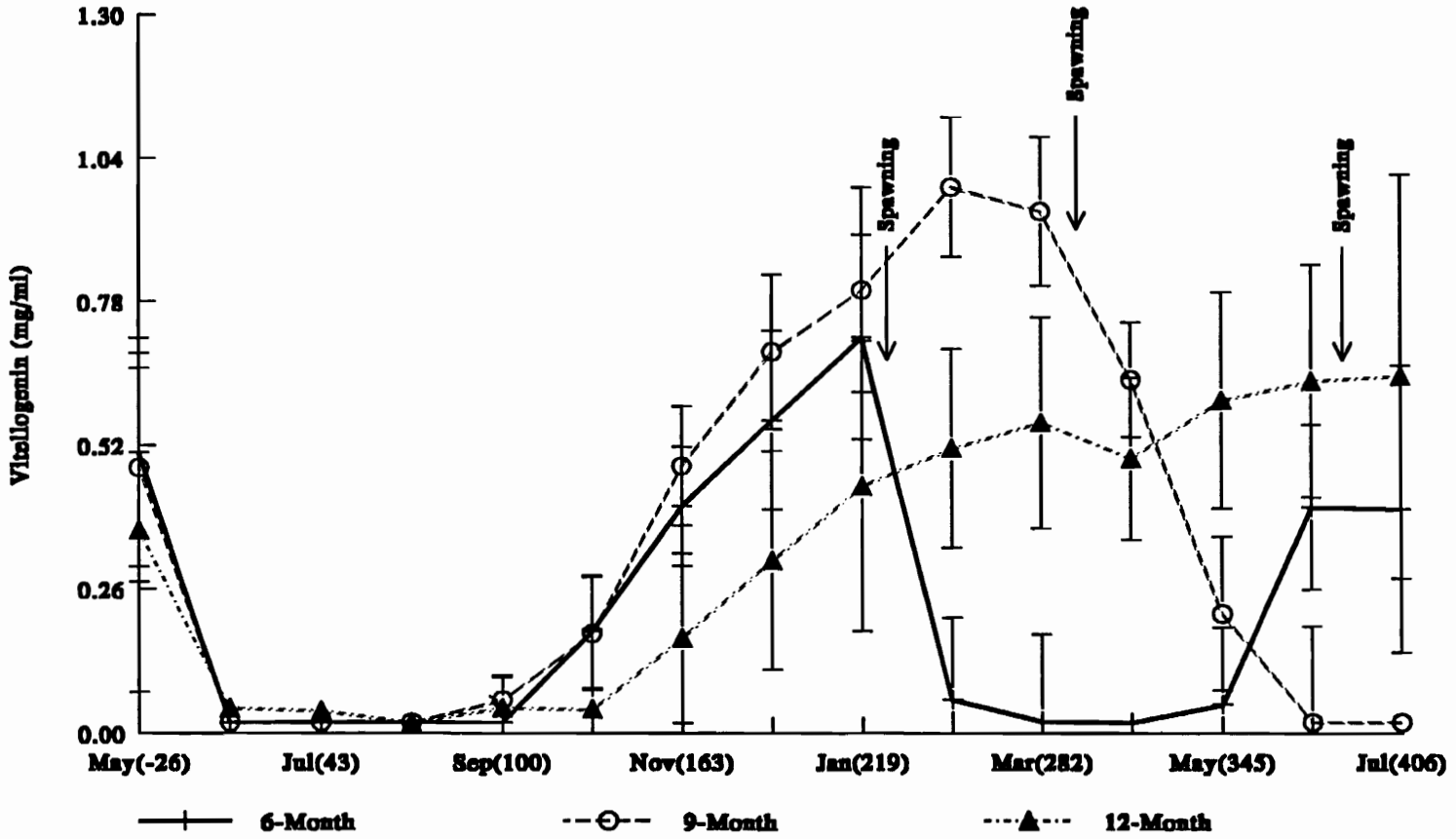

Figure 5. Mean monthly plasma Vg levels (standard error) for all females held on the 6-, 9-, and 12-month cycles. Month and (experimental day) shown.

(day 255) and March 1991 (day 282), Vg levels in the 9-month females were higher ($P < 0.05$) than those females held on the 6- and 12-month cycles. In April 1991 (day 318), Vg levels in females held on the 9- and 12-month females were higher ($P < 0.05$) than in the females held on the 6-month cycle (Table 4).

Maximum plasma Vg values for females held on the 6-, 9-, and 12-month cycles occurred at different ($P < 0.05$) times, days 205, 284, and 390, respectively (Table 3). Peak Vg levels occurred 34, 22, and 1 days before spawning for fish held on the 6, 9, and 12-month cycles, respectively. No significant ($P > 0.05$) differences were detected in the maximum plasma Vg levels between fish held on the 6- (0.78 mg/ml), 9- (1.053 mg/ml), and 12-month (0.799 mg/ml) cycles (Table 5).

Estradiol (E_2) for females in all cycles remained at minimal levels through summer months, and increased to detectable levels in September (day 100), October (day 129), and November (day 163) for fish held in the 6-, 9-, and12-month cycles, respectively, and then peaked prior to spawning (Figure 6). E_2 levels for fish held in the 9-month cycle and those held on the 12-month cycle were significantly greater than the others in March (day 282) and June (day 373) 1991, respectively (Table 6). Peak E_2 levels occurred after 205, 282, and 373 days for the females held on the 6-, 9-, and 12-month cycles respectively (Figure 6). Maximum E_2 levels occurred on different ($P < 0.05$) dates (Table 3) and days before spawning (34, 24, and 16) for fish held on the 6-, 9-, and 12-month cycles respectively. Maximum E_2 levels for the 6- (0.696 ng/ml), 9- (1.395 ng/ml), and 12-month (1.346 ng/ml) cycles were similar (Table 5).

Female testosterone (T) levels were low (< 0.2 ng/ml) except for short (1 month), sharp increases that occurred in females held on all cycles near spawning (Figure 7). Maximum T values for females held on the 9-month and 12-month cycles were greater than those held on other cycles during March (day 282) and May (day 345), respectively

Table 4. Mean monthly vitellogenin level (standard error) for female striped bass held in the 6-month (n=4), 9-month (n=4), and 12-month (n=2) cycles. Different letters represent significantly different in-row values.

Month	Day in Cycle	Cycles			P value
		6-Month Vitellogenin (mg/ml)	9-Month Vitellogenin (mg/ml)	12-Month Vitellogenin (mg/ml)	
May	-26	0.507 (0.207)	0.480 (0.207)	0.367 (0.292)	0.92
June	8	0.020 (0)	0.020 (0)	0.046 (0.021)	0.27
July	43	0.020 (0)	0.020 (0)	0.040 (0.015)	0.13
August	71	0.020 (0)	0.020 (0)	0.020 (0)	1.00
September	100	0.020 (0)	0.060 (0.041)	0.046 (0.058)	0.59
October	129	0.183 (0.102)	0.179 (0.102)	0.043 (0.144)	0.71
November	163	0.410 (0.108)	0.483 (0.108)	0.171 (0.153)	0.31
December	191	0.566 (0.162)	0.690 (0.140)	0.312 (0.198)	0.36
January	219	0.717 (0.186)	0.803 (0.186)	0.447 (0.263)	0.55
February	255	0.061 (0.147)[a]	0.990 (0.127)[b]	0.515 (0.180)[c]	<0.01
March	282	0.022 (0.156)[a]	0.946 (0.135)[b]	0.562 (0.191)[c]	0.01
April	318	0.020 (0)[a]	0.640 (0.104)[b]	0.497 (0.147)[b]	0.01
May	345	0.052 (0.139)	0.216 (0.139)	0.603 (0.196)	0.14
June	373	0.409 (0.150)	0.020 (0)	0.638 (0.211)	0.13
July	406	0.406 (0.260)	0.020 (0)	0.647 (0.367)	0.50

Table 5. Peak blood plasma concentrations (standard error) of Vg, E_2, and T for females held on the 6 (n=4), 9 (n=4), and 12 (n=2) month cycles.

Blood	Cycles			P-value
Parameter	6-Month	9-Month	12-Month	
Vg (mg/ml)	0.780 (0.164)	1.053 (0.164)	0.799 (0.232)	>0.05
E_2 (ng/ml)	0.696 (0.203)	1.395 (0.203)	1.346 (0.288)	>0.05
T (ng/ml)	0.399 (0.282)	0.792 (0.282)	1.184 (0.399)	>0.05

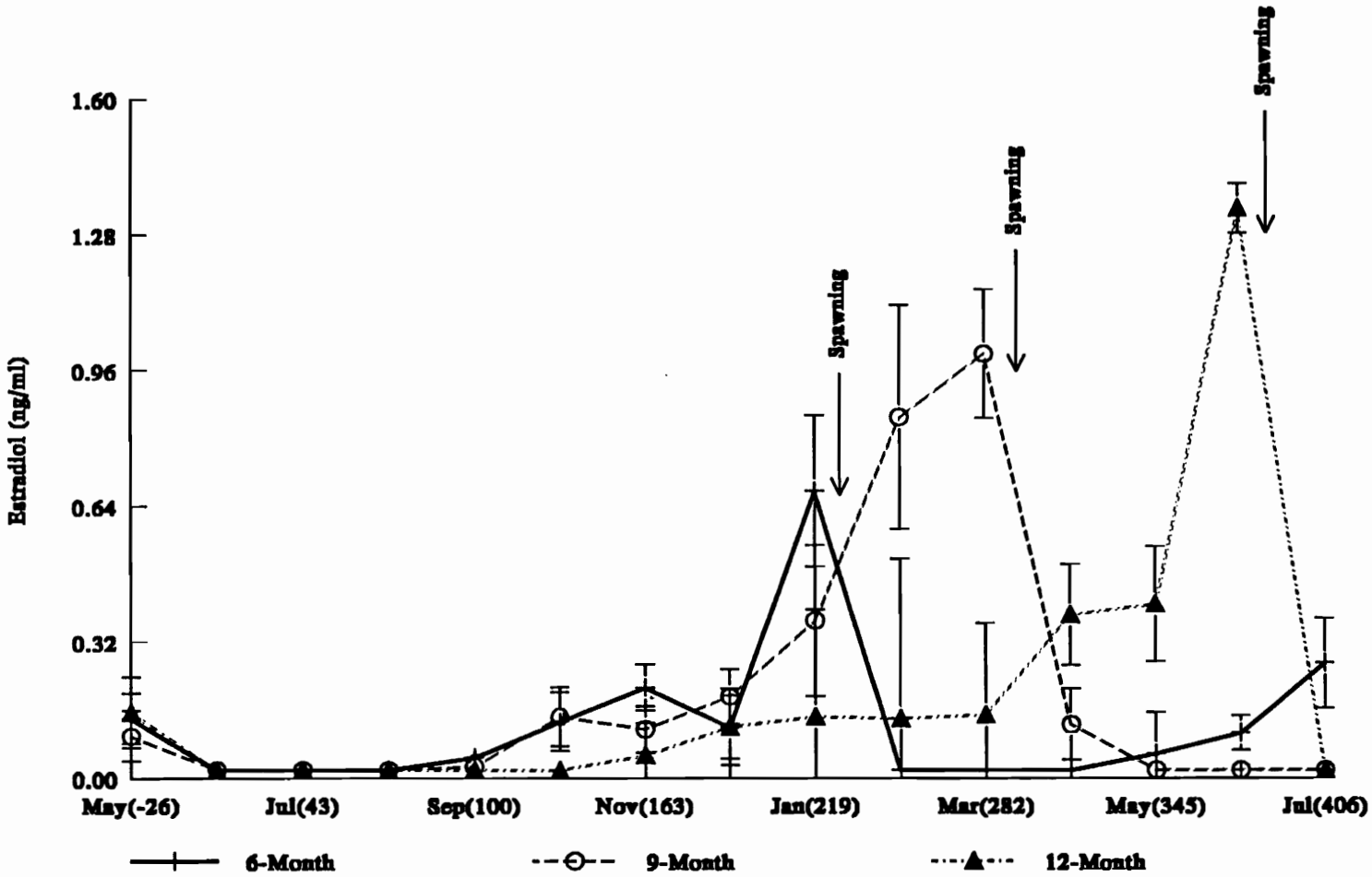

Figure 6. Mean montly plasma E_2 levels (standard error) for all females held on the 6-, 9-, and 12-month cycles. Month with (experimental day) shown.

Table 6. Mean monthly estradiol levels (standard error) for female striped bass held in the 6-month (n=4), 9-month (n=4), and 12-month (n=2) cycles. Different letters represent significantly different in-row values.

Month	Day in Cycle	Cycles			P value
		6-Month Estradiol (ng/ml)	9-Month Estradiol (ng/ml)	12-Month Estradiol (ng/ml)	
May	-26	0.140 (0.059)	0.099 (0.059)	0.155 (0.083)	0.83
June	8	0.020 (0)	0.020 (0)	0.020 (0)	1.00
July	43	0.020 (0)	0.020 (0)	0.020 (0)	0.13
August	71	0.020 (0)	0.020 (0)	0.020 (0)	0.78
September	100	0.049 (0.017)	0.028 (0.017)	0.020 (0)	0.33
October	129	0.133 (0.069)	0.145 (0.069)	0.020 (0)	0.51
November	163	0.213 (0.054)	0.115 (0.054)	0.054 (0.076)	0.26
December	191	0.120 (0.074)	0.192 (0.064)	0.121 (0.090)	0.72
January	219	0.677 (0.178)	0.371 (0.178)	0.145 (0.252)	0.26
February	255	0.020 (0)	0.851 (0.265)	0.141 (0.375)	0.16
March	282	0.020 (0)[a]	1.001 (0.153)[b]	0.149 (0.216)[a]	0.01
April	318	0.020 (0)	0.127 (0.084)	0.385 (0.119)	0.10
May	345	0.058 (0.096)	0.020 (0)	0.410 (0.136)	0.10
June	373	0.107 (0.041)[a]	0.020 (0)[b]	1.346 (0.058)[c]	<0.01
July	406	0.271 (0.106)	0.020 (0)	0.020 (0)	0.28

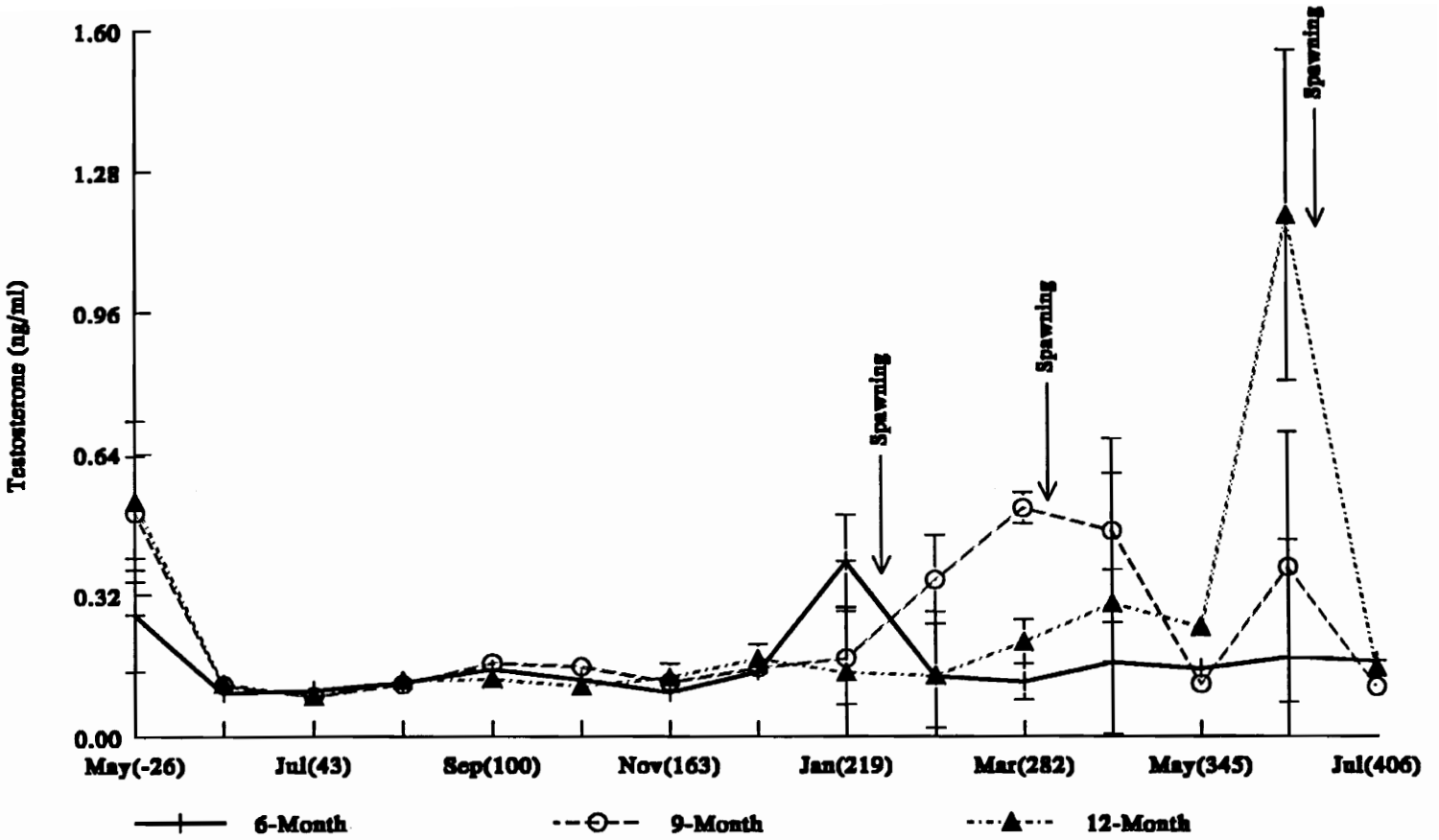

Figure 7. Mean monthly plasma T levels (standard error) for all females held on the 6-, 9-, and 12-month cycles. Month with (experimental day) shown.

(Table 7). Maximum T levels occurred on different ($P<0.05$) dates (212, 291, and 373) and days prior to spawning (27, 15, and 16) for females held on the 6-, 9-, and 12-month cycles (Table 3). Peak T levels (Table 5) of fish held on the 6- (0.399 ng/ml), 9- (0.792 ng/ml), and 12-month (1.184 ng/ml) cycles were similar ($P > 0.05$).

Within each cycle, egg diameters and Vg, E_2, and T levels peaked synchronously, within the level of temporal resolution availiable (Table 2). Maximum concentrations for Vg, E_2, and T in females held on the 6-month cycle occurred within a seven day period, 205-212, in January. Females held on the 9-month cycle were less synchronous, reaching maximum of Vg, E_2, and T concentrations on days 282-291, in March. Maximum levels of E_2, T, and Vg for females held on the 12-month cycle occurred on days 373, 373, and 390, in June. Despite the fact that the maximum Vg, E_2, and T levels of females held on the 6 month cycle that reached spawning condition (egg diameter > 700 um) were nearly double those females that did not reach spawning condition (< 700um), no statistical significant differences were detected (Table 8).

Vitellogenin, E_2, and T concentrations of females held in all cycles were correlated ($P < 0.05$) with egg diameter throughout the experimental period. Of the steroids, Vg had the highest degree of correlation ($r = 0.83$) compared to E_2 ($r = 0.76$), and T ($r = 0.76$).

All (100%) of males held in the 6-month, 9-month, and 12-month cycles spermiated. Spermiation for males held on the 6-, 9-, and 12-month cycles occurred on days 219-282, 282-345, and 345-406, respectively (Table 9). These dates were within a month of those estimated (200, 290, and 380).

Testosterone (T) in the plasma of males was relatively low (< 0.2 ng/ml) until increasing temperature and photoperiod in the spring initiated 2 to 3 fold increases in males held on all cycles (Figure 8). T levels were highest in males held on the 6-month

Table 7. Mean monthly testosterone levels (standard error) for female striped bass held in the 6-month (n=4), 9-month (n=4), and 12-month (n=2) cycles. Different letters represent significantly different in-row values.

Month	Day in Cycle	Cycles			P value
		6-Month	9-Month	12-Month	
		Testosterone (ng/ml)	Testosterone (ng/ml)	Testosterone (ng/ml)	
May	-26	0.275(0.129)	0.506 (0.129)	0.532 (0.182)	0.40
June	8	0.099 (0.015)	0.116 (0.015)	0.120 (0.021)	0.66
July	43	0.104 (0.013)	0.091 (0.013)	0.091 (0.018)	0.76
August	71	0.122 (0.009)	0.118 (0.009)	0.127 (0.019)	0.89
September	100	0.152 (0.017)	0.166 (0.017)	0.130 (0.024)	0.52
October	129	0.129 (0.015)	0.157 (0.015)	0.114 (0.021)	0.25
November	163	0.100 (0.022)	0.123 (0.022)	0.134 (0.031)	0.63
December	191	0.147 (0.026)	0.154 (0.026)	0.176 (0.032)	0.78
January	219	0.397 (0.106)	0.177 (0.106)	0.144 (0.149)	0.30
February	255	0.137 (0.117)	0.355 (0.117)	0.138 (0.144)	0.34
March	282	0.124 (0.041)^{a}	0.518 (0.041)^{b}	0.214 (0.050)^{a}	<0.01
April	318	0.168 (0.209)	0.466 (0.209)	0.301 (0.295)	0.62
May	345	0.153 (0.018)^{a}	0.119 (0.018)^{a}	0.247 (0.025)^{b}	0.01
June	373	0.180 (0.266)	0.384 (0.307)	1.184 (0.376)	0.17
July	406	0.171 (0.014)	0.113 (0.019)	0.156 (0.019)	0.14

Table 8. Maximum concentrations (standard error) of Vg, E_2, T of females held on a 6-month cycle. Comparison of females with egg diameters > 700 um (n=2) and < 700 um (n=2).

Blood	Female Egg Diameter		P value
Parameter	> 700 um	< 700 um	
Vg (mg/ml)	0.977 (0.223)	0.584 (0.223)	>0.05
E_2 (ng/ml)	1.025 (0.267)	0.367 (0.267)	>0.05
T (ng/ml)	0.595 (0.190)	0.203 (0.190)	>0.05

Table 9. Elapsed experimental days (standard error) from the initiation of the study (Day=1) when maximum concentrations of T and 11KT, spermiation range, and spawning occurred for males held on the 6 (n=4), 9 (n=4), and 12-month (n=2), cycles.

Reproductive Parameter	Cycles			P-value
	6-Month (days)	9-Month (days)	12-Month (days)	
Spermiation	219-282	282-345	345-406	
Spawning	239 (9)	306 (6)	389 (7)	<0.05
T	246 (12)	291 (12)	376 (17)	<0.05
11-kt	246 (9)	291 (9)	390 (13)	<0.05

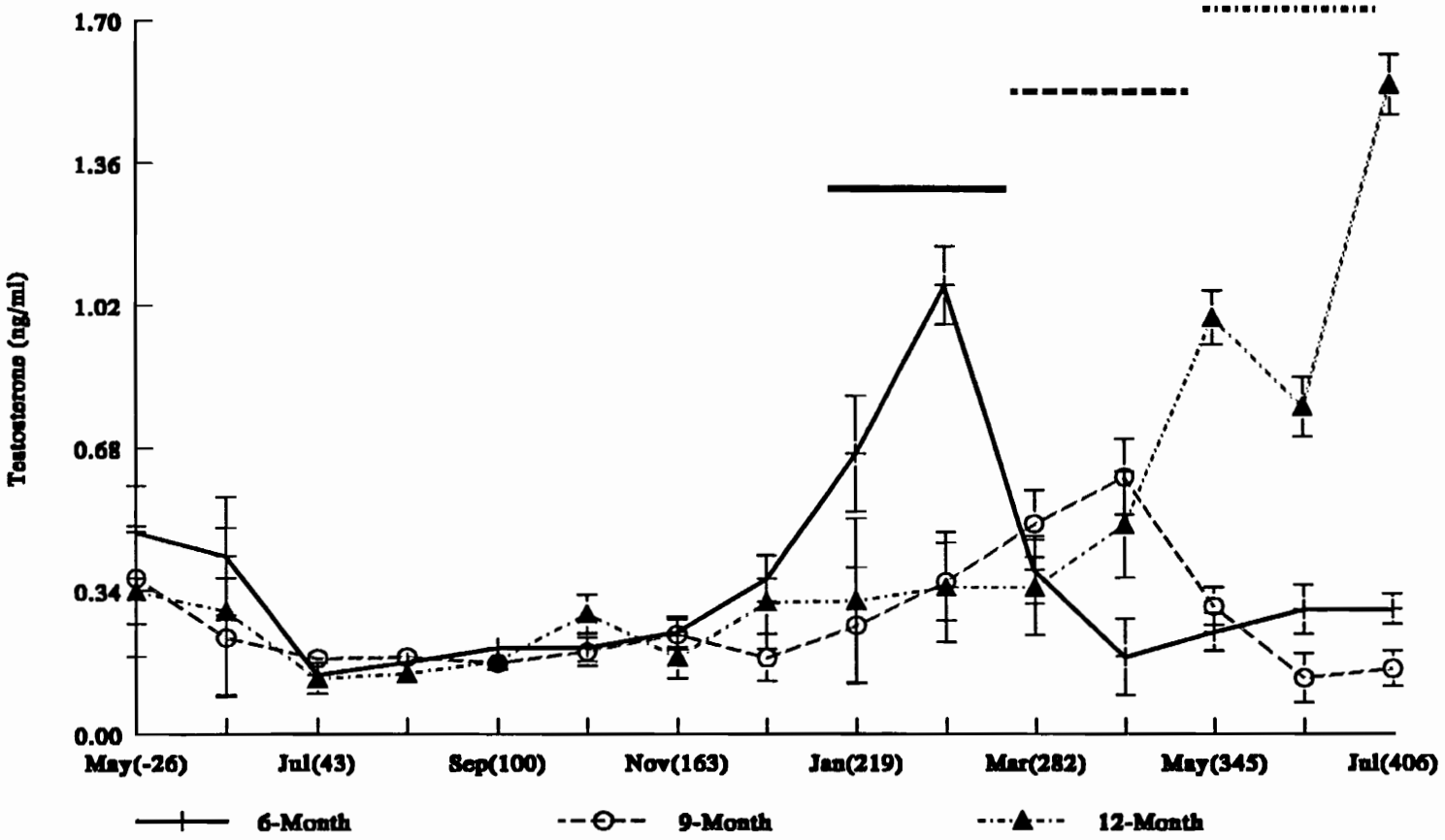

Figure 8. Mean monthly plasma T levels (standard error) for males held on the 6-, 9-, and 12-month cycles. Month with (experimental days) shown. Upper lines represent periods of spermiation.

cycle during August (day 73) and September (day 100) 1990 and January (day 219) through February (day 255) 1991 (Table 10). They were highest in males held on the 12-month cycle during June (day 373) and July (day 406) 1991. Maximum T levels occurred on different ($P < 0.05$) days (246, 291, and 376) (Table 9). Maximum concentrations occurred at 15, 7, and 13 days prior to spawning for fish held on the 6-, 9-, and 12-month cycles, respectively. Peak T levels of fish held on the 6- (1.079 ng/ml) and 12-month (1.236 ng/ml) cycles were higher ($P < 0.05$) than for those held on the 9-month (0.592 ng/ml) cycle (Table 11).

11-keto testosterone (11KT) remained low (<0.2 ng/ml) from June (day 8) through December (day 191) 1990 for males in all cycles (Figure 9). Plasma 11KT levels then increased in January (day 219), March (day 282), and June (day 373) for the 6-, 9-, and 12-month cycles, respectively. In each cycle, 11KT remained low until temperature and photoperiod increased, and peaked after initiation of spermiation for males in all cycles. 11KT was highest in males held on the 6-month cycle during February (day 255) 1991 and lowest during April (day 318) 1991 (Table 12). Males held on the 12-month cycle had the highest 11KT concentrations from May (day 345) through July (day 406) 1991. Maximum 11KT levels occurred at different ($P < 0.05$) times (Table 9), on days 246, 291, 390 for males held on the 6-, 9-, and 12-month cycles, respectively (Figure 9). Maximum 11KT levels occurred 11, 15, and 1, days after spawning for males held on the 6-, 9-, and 12-month cycles, respectively. Maximum 11KT levels for fish held on the 6- (1.363 ng/ml), 9- (0.821 ng/ml), and 12-month (1.375 ng/ml) cycles were similar (Table 11).

Maximum concentrations of steroids in males in all cycles corresponded to the period of spermiation (Table 9). Spermiation was initiated about one month prior to and concluded one month after maximum concentrations occurred. T and 11-kt peak

Table 10. Mean monthly testosterone levels (standard error) for male striped bass held in the 6-month (n=4), 9-month (n=4), and 12-month (n=2) cycles. Different letters represent significantly different in-row values.

Month	Day in Cycle	Cycles			P value
		6-Month Testosterone (ng/ml)	9-Month Testosterone (ng/ml)	12-Month Testosterone (ng/ml)	
May	-26	0.480 (0.110)	0.371 (0.110)	0.339 (0.156)	0.71
June	8	0.423 (0.141)	0.229 (0.141)	0.291 (0.199)	0.63
July	43	0.141 (0.025)	0.180 (0.025)	0.132 (0.036)	0.47
August	71	0.170 (0.017)	0.182 (0.017)	0.143 (0.024)	0.42
September	100	0.205 (0.017)	0.168 (0.017)	0.130 (0.023)	0.31
October	129	0.205 (0.033)	0.195 (0.033)	0.285 (0.046)	0.31
November	163	0.242 (0.036)	0.236 (0.036)	0.182 (0.051)	0.63
December	191	0.369 (0.056)	0.181 (0.056)	0.313 (0.112)	0.14
January	219	0.668 (0.138)	0.258 (0.138)	0.317 (0.195)	0.16
February	255	1.070 (0.093)^{a}	0.362 (0.093)^{b}	0.349 (0.131)^{b}	<0.01
March	282	0.390 (0.080)	0.500 (0.080)	0.348 (0.114)	0.50
April	318	0.183 (0.090)^{a}	0.612 (0.090)^{b}	0.498 (0.127)^{b}	0.03
May	345	0.242 (0.045)^{a}	0.303 (0.045)^{a}	0.993 (0.064)^{b}	<0.01
June	373	0.296 (0.058)^{a}	0.133 (0.058)^{a}	0.780 (0.071)^{b}	<0.01
July	406	0.297 (0.036)^{a}	0.155 (0.042)^{a}	1.552 (0.072)^{b}	<0.01

Table 11. Mean maximum concentrations (standard error) of T and 11KT in males held on 6 (n=4), 9 (n=4), and 12 (n=2) month cycles. Different letters represent significantly different ($P < 0.05$) in row values.

Blood	Cycles			P-value
Parameter	6-Month	9-Month	12-Month	
T (ng/ml)	1.079 (0.103)	0.592 (0.103)[a]	1.236 (0.146)	0.013
11-kt (ng/ml)	1.363 (0.298)	0.821 (0.298)	1.575 (0.421)	>0.05

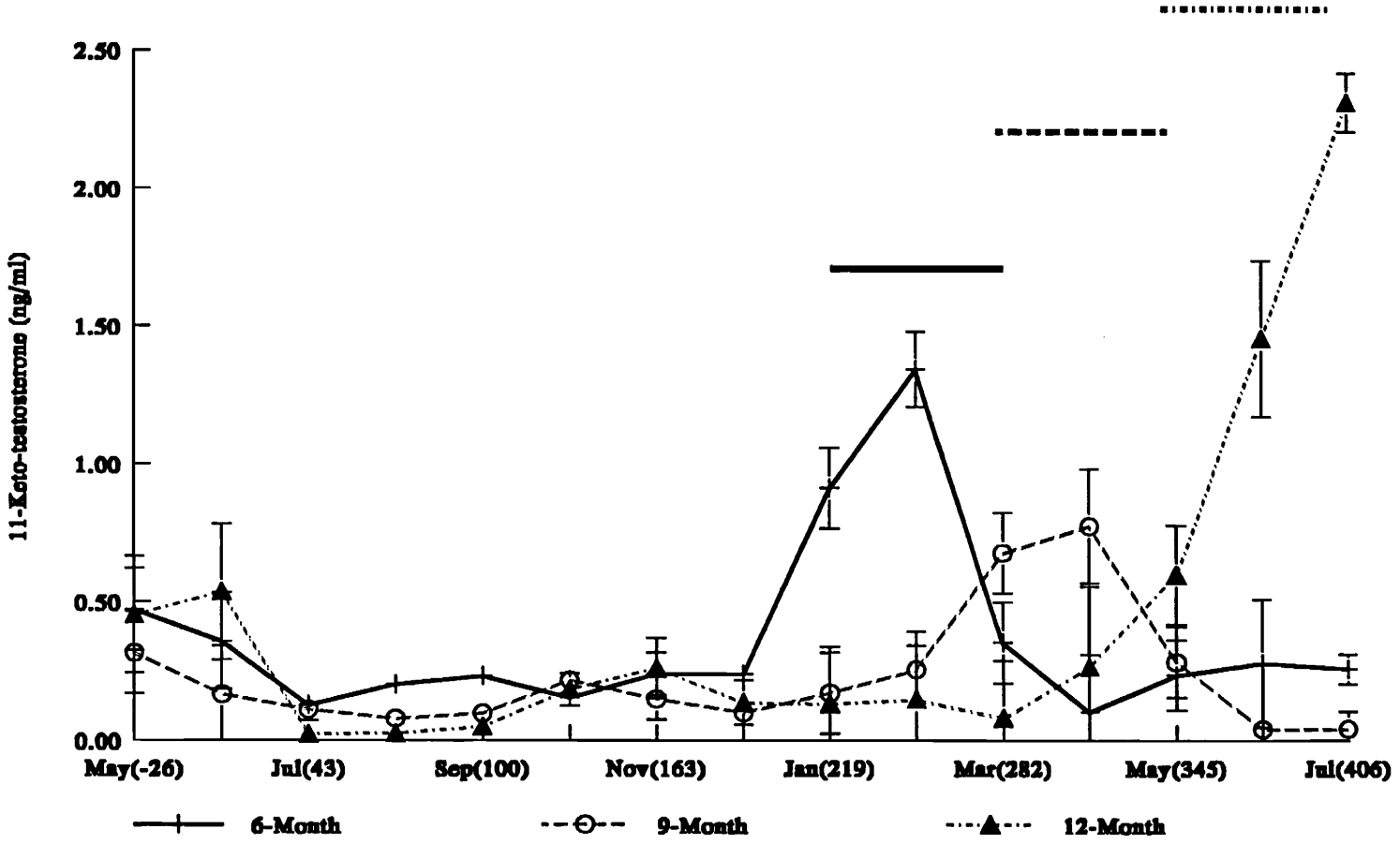

Figure 9. Mean monthly plasma 11KT levels (standard error) for males held on the 6-, 9-, and 12-month cycles. Month with (experimental days) shown. Upper lines represent periods of spermiation.

Table 12. Mean monthly 11-keto testosterone levels (standard error) for male striped bass held in the 6-month (n=4), 9-month (n=4), and 12-month (n=2) cycles. Different letters represent significantly different in-row values.

Month	Day in Cycle	Cycles			P value
		6-Month	9-Month	12-Month	
		11KT (ng/ml)	11KT (ng/ml)	11KT (ng/ml)	
May	-26	0.473 (0.149)	0.317 (0.149)	0.455 (0.211)	0.74
June	8	0.359 (0.174)	0.167 (0.174)	0.537 (0.246)	0.49
July	43	0.128 (0.034)	0.112 (0.034)	0.024 (0.048)	0.27
August	71	0.202 (0.032)^{a}	0.080 (0.032)^{b}	0.026 (0.045)^{b}	0.02
September	100	0.233 (0.030)^{a}	0.098 (0.030)^{b}	0.051 (0.042)^{b}	0.02
October	129	0.154 (0.041)	0.218 (0.041)	0.184 (0.058)	0.56
November	163	0.239 (0.076)	0.150 (0.076)	0.260 (0.108)	0.63
December	191	0.239 (0.040)	0.101 (0.040)	0.137 (0.080)	0.12
January	219	0.914 (0.146)^{a}	0.171 (0.146)^{b}	0.132 (0.206)^{b}	0.02
February	255	1.345 (0.137)^{a}	0.256 (0.137)^{b}	0.149 (0.193)^{b}	<0.01
March	282	0.353 (0.146)	0.678 (0.146)	0.081 (0.206)	0.11
April	318	0.103 (0.206)	0.776 (0.206)	0.266 (0.291)	0.13
May	345	0.236 (0.127)	0.283 (0.127)	0.599 (0.188)	0.30
June	373	0.279 (0.232)^{a}	0.042 (0.232)^{a}	1.457 (0.284)^{b}	0.03
July	406	0.259 (0.054)^{a}	0.043 (0.062)^{a}	2.318 (0.107)^{b}	<0.01

occurred simultaneously (days 246 and 291, Table 9) for males held on the 6- and 9-month cycles, respectively, but were less synchronous for males held on the 12-month cycle.

DISCUSSION

Maturation and spawning of striped bass were advanced by five months and three months in fish exposed to compressed 6- and 9- month photothermal cycles, respectively, as compared to those held on a 12-month reference cycle. The experimental variations in photoperiod and water temperature elicited corresponding changes in plasma steroid levels. Photothermal manipulation advanced the annual successional pattern of plasma Vg and gonadal hormones in female striped bass, but did not alter the sequence or absolute levels of Vg and gonadal hormones present.

Plasma Vg and hormone profiles reflected the maturational status of striped bass regardless of the photothermal cycle on which they were held. Estradiol (E_2) became detectable in fish held on all cycles during their respective fall seasons at days 129, 177, and 198 before spawning for fish held on the 6-, 9-, and 12-month cycles. E_2 has been reported to induce vitellogenesis in striped bass (Sullivan et al. 1991) and plasma Vg levels became detectable when E_2 was first detected. Vg activity is initiated during periods of decreasing daylength and temperatures (near autumnal equinox) and reaches maximum levels in striped bass during the spring spawning season (Sullivan et al. 1991).

Vg and E_2 attained maximum levels about one month prior to spawning in female striped bass held on the 6- and 9-month cycles and within two weeks of spawning for females held on the 12-month cycle. Similarly, peak E_2 levels have been reported to occur about two months prior to spawning in rainbow trout (Scott et al. 1984), Indian catfish (Lamba et al. 1983), and plaice (Wingfield and Grimm 1977). Bromage et al. (1982) found that maximum E_2 levels occurred in rainbow trout on the same date of the year for fish held on 6-, 9-, and 12-month cycles, which corresponded to two weeks, two months and four months prior to spawning, respectively. Berlinsky and Specker (1991)

reported the highest E_2 levels in "prespawning" striped bass. Sullivan et al. (1991) suggested that Vg levels in striped bass escalate most rapidly near the winter solstice and crest at the vernal equinox, just (weeks) prior to spawning.

Maximum levels of T in females held on the 6- and 9-month cycles occurred seven days after peak E_2 levels (about two weeks before spawning) and then decreased rapidly. T and E_2 levels peaked simultaneously in females held on the 12-month cycle. A similar succession of peak E_2 and then T levels has been reported for winter flounder (Campbell et al. 1976), Indian catfish (Lamba et al. 1983), and white sucker (Scott et al. 1983). T, a precursor to E_2, attains maximum levels after E_2 due to a decrease in aromatization of T to E_2 (van Bohemen and Lambert 1981).

Compression of the annual cycle did not affect the magnitude of Vg and steroid levels in female striped bass when compared to those exposed to the reference 12-month cycle. Maximum levels of Vg, E_2, and T were similar among females held on all cycles. Maximum E_2 levels in the present study (1.0 ng/ml) were lower than the 2.2 ng/ml reported for wild, Atlantic Coast stocks (Berlinsky and Specker 1991) and the 2.0 ng/ml for Chesapeake Bay stocks held in laboratory tanks (Sullivan et al. 1991). Bromage et al. (1982) reported lower peak E_2 values for rainbow trout held on a compressed 6-month cycle than on 9- and 12-month cycles, and they attributed these differences to short photoperiods early in the year. Peak Vg levels found in this study (0.5 to 1.0 mg/ml) were identical to those reported for striped bass by Sullivan et al. (1991).

Maximum T values in female striped bass reported in this study (1.0 - 1.5 ng/ml) were comparable to those for tank reared striped bass (0.4 - 1.4 ng/ml) reported by Sullivan et al. (1991), but lower than the 3.0 ng/ml measured in wild striped bass by Berlinsky and Specker (1991).

Blood parameters (Vg, E_2, and T) measured in females were highly correlated with egg diameters. Similar high correlations of E_2 and the gonadosomatic index have been determined for striped mullet, where r = 0.95 (Dindo and MacGregor 1981) and Atlantic salmon, where r = 0.70, (Idler et al. 1981). Of the blood parameters measured, Vg exhibited the highest correlation with egg diameter (r = 0.83). Because vitellogenesis is directly related to yolk formation in developing oocytes (Zohar 1990), a close Vg - egg size relationship was expected.

The number of females successfully spawned was lower in the 6-month cycle (50%) compared to the 9- and 12-month females, each (100%). Non-spawning females exhibited lower hormone and Vg levels than spawning fish, averaging two times lower in non-spawning females. Large individual variances and small sample size (n=4) did not allow for detection of statistical significance.

As in females, photothermal manipulation of male striped bass advanced the annual successional pattern of plasma Vg and gonadal hormones and affected the absolute levels of androgens present, but did not alter the sequence of steroids through the reproductive cycle. Male androgen levels remained at low levels in all cycles until early spring when their concentrations increased sharply. 11KT increased from basal level about one month after T levels increased. T is a precursor of 11KT, and 11KT is considered to be more important in late maturation and the initiation of spermiation (Scott et al. 1983).

Maximum concentrations of T and 11KT occurred during the middle of spermiation for striped bass in all cycles and then decreased rapidly through the rest of the spawning season. Maximum T and 11KT levels preceded spawning in white suckers (Scott et al. 1983). Maximum T levels coincided with initiation of spermiation in the

Indian catfish (Lamba et al. 1983). Maximum 11KT levels peaked at the initiation of spermiation in rainbow trout (Scott et al. 1980B).

T levels for fish held on the 6- and 12-month cycles escalated more sharply in the spring and were significantly higher than those for fish held in the 9-month cycle. However, because blood sampling was conducted only at monthly intervals, a short, sharp spike in T concentrations in the males held on the 9-month cycle may have occurred but not sampled. Maximum T and 11KT levels measured in male striped bass in this experiment (1.08 and 1.57 ng/ml, respectively) were lower than peak T concentrations of 10 ng/ml reported for striped mullet (Dindo and MacGregor 1981) or the 1.5 ng/ml measured for rainbow trout and peak 11KT concentrations of 2.5 ng/ml for rainbow trout (Scott et al. 1980B). Maximum T levels measured in striped bass males in this study were lower than peak T levels reported by Sullivan et al. (1991), 2.0 ng/ml for striped bass held in laboratory tanks under natural photoperiod and temperature. 11KT concentrations in striped bass have not been reported in the literature.

Vg and hormone profiles of male and female striped bass were advanced to reflect the compressed 6- and 9-month photothermal regimes on which the fish were held. Compression of the annual cycle accelerated the rate of change (timing), but not the absolute concentrations of plasma Vg and hormones. Maximum concentrations of Vg, E_2, and T generally corresponded to those reported for striped bass near spawning (Sullivan et al. 1991, Berlinsky and Specker 1991). Plasma concentrations of Vg, E_2, and T, and their interrelationships, were highly correlated with egg diameter, and can be used to estimate the reproductive status of striped bass throughout the year.

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APPENDIX A

Table 1. Raw data for reproductive parameters for fish number 102.

Fish ID: 102	Sex: Male	Cycle: 6-Month	Chamber: 2	Initial length: 56.0
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: Yes	Ending length: 62.8
Spawn Date: N/A	# of Males: N/A	Fert %: N/A	Type: N/A	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation	Testosterone (ng/ml)	11 keto-testosterone (ng/ml)
3/1/90	-	-	Y	-	-
5/3/90	11.6	-	Y	0.475	0.546
6/5/90	7.8	-	N	0.379	0.466
7/10/90	4.3	-	N	0.149	0.156
8/7/90	6.3	-	N	0.197	0.213
9/5/90	4.0	-	N	0.219	0.256
10/4/90	6.6	-	N	0.152	0.067
11/7/90	7.0	-	N	0.243	0.171
12/5/90	6.4	10.5	N	0.268	0.155
1/2/91	17.3	23.9	Y	0.743	0.900
2/7/91	11.3	19.0	Y	1.110	0.987
3/6/91	8.0	12.1	Y	0.345	0.260
4/11/91	3.9	6.7	N	0.201	0.118
5/8/91	6.4	7.4	N	0.254	0.235
6/5/91	-	-	N	-	-
7/8/91	5.1	9.0	N	0.247	0.224
8/12/91	7.0	11.9	N	-	-
9/6/91	11.8	18.1	N	-	-

Table 2. Raw data for reproductive parameters for fish number 103.

Fish ID: 103	Sex: Male	Cycle: 9-Month	Chamber: 1	Initial length: 57.9
Stock Date: 4/11/91	Mort: N/A	Cause: N/A	Bled: Yes	Ending length: 59.6
Spawn Date: N/A	# of Males: N/A	Fert %: N/A	Type: N/A	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation	Testosterone (ng/ml)	11 keto-testosterone (ng/ml)
4/11/91	23.8	30.0	Y	0.873	0.708
5/8/91	14.8	21.7	Y	0.302	0.020
6/5/91	7.1	11.7	N	0.122	0.002
7/8/91	8.1	9.3	N	0.196	0.002
8/12/91	9.3	10.7	N	-	-
9/6/91	8.9	11.9	N	-	-

Table 3. Raw data for reproductive parameters for fish number 104.

Fish ID: 104	Sex: Male	Cycle: 9-Month	Chamber: 3	Initial length: 55.5
Stock Date: 5/3/90	Mort: 6/22/91	Cause: Post-spawn	Bled: No	Ending length: 61.2
Spawn Date: 4/17/91	# of Males: 1	Fert %: 47.8	Type: Strip	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation
3/1/90	-	-	Y
5/3/90	13.7	-	N
6/5/90	8.6	-	N
7/10/90	6.0	-	N
8/7/90	5.1	-	N
9/5/90	5.7	-	N
10/4/90	6.1	-	N
11/7/90	7.0	-	N
12/5/90	6.8	13.4	N
1/2/91	9.5	13.7	N
2/7/91	14.4	18.1	N
3/6/91	24.1	32.1	Y
4/11/91	20.1	29.0	Y
5/8/91	16.8	26.4	N
6/5/91	6.8	10.0	N

Table 4. Raw data for reproductive parameters for fish number 105.

Fish ID: 105	Sex: Male	Cycle: 9-Month	Chamber: 3	Initial length: 55.5
Stock Date: 5/3/90	Mort: 5/14/91	Cause: Post-spawn	Bled: Yes	Ending length: 60.3
Spawn Date: 3/26-27/91	# of Males: 1, 2	Fert %: 71.1, 49.0	Type: Strip, Tank	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation	Testosterone (ng/ml)	11 keto-testosterone (ng/ml)
3/1/90	-	-	Y	-	-
5/3/90	12.6	-	Y	0.844	0.546
6/5/90	9.7	-	Y	0.344	0.205
7/10/90	2.0	-	N	0.114	0.002
8/7/90	6.1	-	N	0.197	0.044
9/5/90	6.9	-	N	0.225	0.163
10/4/90	5.8	-	N	0.198	0.292
11/7/90	4.4	-	N	0.257	0.396
12/5/90	9.4	10.5	N	0.179	0.209
1/2/91	5.4	9.8	N	0.265	0.141
2/7/91	11.6	11.4	N	0.677	0.564
3/6/91	38.8	38.9	Y	0.663	1.288
4/11/91	11.9	22.9	Y	0.728	1.666
5/8/91	14.0	16.7	Y	0.473	0.853

Table 5. Raw data for reproductive parameters for fish number 106.

Fish ID: 106	Sex: Male	Cycle: 6-Month	Chamber: 4	Initial length: 56.5
Stock Date: 5/3/90	Mort: 5/17/90	Cause: Jumper	Bled: No	Ending length: 56.8
Spawn Date: N/A	# of Males: N/A	Fert %: N/A	Type: N/A	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation
3/1/90	-	-	Y
5/3/90	10.8	-	N

Table 6. Raw data for reproductive parameters for fish number 107.

Fish ID: 107	Sex: Male	Cycle: 9-Month	Chamber: 3	Initial length: 52.5
Stock Date: 5/3/90	Mort:	Cause:	Bled: Yes	Ending length: 62.7
Spawn Date: 3/27/91	# of Males: 2	Fert %: 49.0	Type: Tank	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation	Testosterone (ng/ml)	11 keto-testosterone (ng/ml)
3/1/90	-	-	Y	-	-
5/3/90	12.1	-	Y	0.269	0.574
6/5/90	3.8	-	N	0.190	0.228
7/10/90	1.1	-	N	0.151	0.121
8/7/90	3.0	-	N	0.228	0.018
9/5/90	5.0	-	N	0.134	0.093
10/4/90	4.9	-	N	0.145	0.168
11/7/90	6.6	-	N	0.183	0.013
12/5/90	5.8	10.0	N	0.154	0.144
1/2/91	6.2	10.7	N	0.246	0.097
2/7/91	9.5	13.1	N	0.253	0.154
3/6/91	14.1	19.3	Y	0.418	0.408
4/11/91	8.3	12.6	Y	0.278	0.233
5/8/91	8.1	9.5	N	0.208	0.106
6/5/91	7.4	8.3	N	0.142	0.110
7/8/91	7.0	10.0	N	0.127	0.128
8/12/91	6.0	10.0	N	-	-
9/6/91	6.7	9.0	N	-	-

Table 7. Raw data for reproductive parameters for fish number 108.

Fish ID: 108	Sex: Male	Cycle: 9-Month	Chamber: 1	Initial length: 59.0
Stock Date: 8/7/90	Mort: N/A	Cause: N/A	Bled: Yes	Ending length: 63.9
Spawn Date: 3/17/91	# of Males: 2	Fert %: 73.1	Type: Tank	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation	Testosterone (ng/ml)	11 keto-testosterone (ng/ml)
8/7/90	5.3	-	N	0.140	0.113
9/5/90	3.3	-	N	0.185	0.075
10/4/90	5.1	-	N	0.252	0.226
11/7/90	6.8	-	N	0.312	0.093
12/5/90	6.9	10.5	N	0.216	0.052
1/2/91	7.0	12.9	N	0.261	0.369
2/7/91	14.6	20.0	N	0.278	0.194
3/6/91	24.1	27.9	Y	0.570	0.653
4/11/91	15.0	18.3	Y	0.567	0.498
5/8/91	8.8	12.9	N	0.230	0.151
6/5/91	4.5	8.6	N	0.135	0.017
7/8/91	3.6	7.4	N	0.143	0.002
8/12/91	4.3	7.4	N	-	-
9/6/91	6.1	9.5	N	-	-

Table 8. Raw data for reproductive parameters for fish number 109.

Fish ID: 109	Sex: Male	Cycle: 6-Month	Chamber: 4	Initial length: 53.0
Stock Date: 6/5/90	Mort: 4/28/91	Cause: Unknown	Bled: No	Ending length: 57.3
Spawn Date: 1/7/91	# of Males: 2	Fert %: 5.5	Type: Tank	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation
6/5/90	8.0	-	Y
7/10/90	4.6	-	Y
8/7/90	-	-	N
9/5/90	5.3	-	N
10/4/90	9.8	-	N
11/7/90	8.3	-	N
12/5/90	12.0	17.3	N
1/2/91	21.8	30.7	Y
2/7/91	15.5	19.3	Y
3/6/91	9.3	12.6	N
4/11/91	6.4	7.4	N

Table 9. Raw data for reproductive parameters for fish number 111.

Fish ID: 111	Sex: Male	Cycle: 9-Month	Chamber: 1	Initial length: 56.0
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: No	Ending length: 61.8
Spawn Date: 3/17/91 4/9/91	# of Males: 2,2	Fert %: 73.1, 10.8	Type: Tank, tank	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation
3/1/90	10.8	-	Y
5/3/90	12.0	-	Y
6/5/90	7.0	-	N
7/10/90	3.7	-	N
8/7/90	5.5	-	N
9/5/90	4.5	-	N
10/4/90	4.0	-	N
11/7/90	6.0	-	N
12/5/90	8.8	16.3	N
1/2/91	6.9	13.9	N
2/7/91	-	19.3	N
3/6/91	25.1	29.5	Y
4/11/91	17.9	23.1	Y
5/8/91	8.9	12.4	N
6/5/91	-	10.5	N
7/8/91	6.3	7.9	N
8/12/91	6.3	8.8	N
9/6/91	4.9	8.1	N

Table 10. Raw data for reproductive parameters for fish number 112.

Fish ID: 112	Sex: Male	Cycle: 6-Month	Chamber: 4	Initial length: 51.0
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: Yes	Ending length: 56.1
Spawn Date: 1/24/91	# of Males: 2	Fert %: , 36.5	Type: Tank	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation	Testosterone (ng/ml)	11 keto-testosterone (ng/ml)
3/1/90	10.8	-	Y	-	-
5/3/90	10.6	-	Y	0.574	0.428
6/5/90	4.8	-	Y	0.991	0.703
7/10/90	3.4	-	N	0.159	0.157
8/7/90	5.4	-	N	0.142	0.271
9/5/90	4.5	-	N	0.224	0.255
10/4/90	6.6	-	N	0.225	0.105
11/7/90	9.1	-	N	0.331	0.278
12/5/90	6.8	18.0	N	0.283	0.226
1/2/91	14.9	22.7	Y	0.232	0.402
2/7/91	18.5	22.6	Y	1.129	1.833
3/6/91	18.5	27.1	Y	0.492	0.433
4/11/91	6.4	9.8	N	0.195	0.079
5/8/91	7.4	11.2	N	0.197	0.252
6/5/91	9.5	12.4	N	0.196	0.379
7/8/91	10.1	14.3	N	0.398	0.442
8/12/91	11.1	14.8	N	-	-
9/6/91	11.5	15.5	N	-	-

Table 11. Raw data for reproductive parameters for fish number 113.

Fish ID: 113	Sex: Male	Cycle: 6-Month	Chamber: 2	Initial length: 54.0
Stock Date: 9/5/90	Mort: N/A	Cause: N/A	Bled: No	Ending length: 61.3
Spawn Date: 2/3/91	# of Males: 2	Fert %: 64.7	Type: Tank	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation
9/5/90	4.7	-	N
10/4/90	5.9	-	N
11/7/90	6.2	-	N
12/5/90	5.4	9.0	N
1/2/91	13.9	20.7	Y
2/7/91	9.1	13.8	Y
3/6/91	5.7	6.0	N
4/11/91	4.5	5.7	N
5/8/91	7.1	11.4	N
6/5/91	6.4	7.1	N
7/8/91	7.1	8.3	N
8/12/91	12.1	-	N
9/6/91	13.3	17.4	N

Table 12. Raw data for reproductive parameters for fish number 114.

Fish ID: 114	Sex: Male	Cycle: 9-Month	Chamber: 1	Initial length: 55.0
Stock Date: 5/3/90	Mort: 8/8/90	Cause: Handling	Bled: Yes	Ending length: 56.3
Spawn Date: N/A	# of Males: N/A	Fert %: N/A	Type: N/A	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation	Testosterone (ng/ml)	11 keto-testosterone (ng/ml)
3/1/90	12.7	-	Y	-	-
5/3/90	7.4	-	N	0.231	0.146
6/5/90	6.6	-	N	0.131	0.002
7/10/90	5.4	-	N	0.182	0.104
8/7/90	5.9	-	N	0.142	0.037

Table 13. Raw data for reproductive parameters for fish number 117.

Fish ID: 117	Sex: Male	Cycle: 9-Month	Chamber: 1	Initial length: 52.5
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: No	Ending length: 58.7
Spawn Date: 4/9/91	# of Males: 2	Fert %: 10.8	Type: Tank	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation
3/1/90	9.8	-	Y
5/3/90	10.9	-	Y
6/5/90	6.4	-	N
7/10/90	5.8	-	N
8/7/90	6.2	-	N
9/5/90	6.1	-	N
10/4/90	6.0	-	N
11/7/90	8.4	-	N
12/5/90	9.5	10.7	N
1/2/91	9.5	13.7	N
2/7/91	10.2	13.1	N
3/6/91	22.4	30.2	Y
4/11/91	22.1	32.1	Y
5/8/91	18.0	26.2	Y
6/5/91	7.1	11.4	N
7/8/91	6.0	12.1	N
8/12/91	7.5	12.4	N
9/6/91	8.2	11.0	N

Table 14. Raw data for reproductive parameters for fish number 119.

Fish ID: 119	Sex: Male	Cycle: 6-Month	Chamber: 2	Initial length: 50.0
Stock Date: 5/3/90	Mort: 8/8/90	Cause: Handling	Bled: No	Ending length: 52.7
Spawn Date: N/A	# of Males: N/A	Fert %: N/A	Type: N/A	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation
3/1/90	18.6	-	Y
5/3/90	9.8	-	Y
6/5/90	5.4	-	Y
7/10/90	4.0	-	N
8/7/90	7.0	-	N

Table 15. Raw data for reproductive parameters for fish number 120.

Fish ID: 120	Sex: Male	Cycle: 12-Month	Chamber: 7	Initial length: 57.0
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: Yes	Ending length: 60.6
Spawn Date:6/6-21/91	# of Males: 1,1	Fert %: 30.8, 40.9	Type: Strip, Tank	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation	Testosterone (ng/ml)	11 keto-testosterone (ng/ml)
3/1/90	-	-	N	-	-
5/3/90	12.2	-	Y	0.407	0.875
6/5/90	13.9	-	Y	0.469	1.074
7/10/90	3.9	-	N	0.112	0.034
8/7/90	6.0	-	N	0.117	0.002
9/5/90	7.4	-	N	0.167	0.002
10/4/90	6.1	-	N	0.187	0.202
11/7/90	4.0	-	N	0.135	0.301
12/5/90	5.8	6.8	N	-	-
1/2/91	6.2	7.3	N	0.152	0.168
2/7/91	7.3	9.0	N	0.165	0.191
3/6/91	6.6	8.3	N	0.190	0.033
4/11/91	8.9	11.2	N	0.381	0.346
5/8/91	14.3	20.5	N	1.067	0.659
6/5/91	18.8	26.4	Y	0.646	2.082
7/8/91	18.7	21.7	Y	1.552	2.318
8/12/91	8.7	10.7	N	-	-
9/6/91	6.1	9.3	N	-	-

Table 16. Raw data for reproductive parameters for fish number 121.

Fish ID: 121	Sex: Male	Cycle: 6-Month	Chamber: 2	Initial length: 56.5
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: Yes	Ending length: 64.2
Spawn Date: 2/3/91	# of Males: 2	Fert %: 64.7	Type: Tank	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation	Testosterone (ng/ml)	11 keto-testosterone (ng/ml)
3/1/90	-	-	Y	-	-
5/3/90	10.9	-	Y	0.508	0.539
6/5/90	5.0	-	N	0.195	0.157
7/10/90	4.8	-	N	0.165	0.146
8/7/90	5.0	-	N	0.177	0.203
9/5/90	6.1	-	N	0.194	0.287
10/4/90	4.1	-	N	0.247	0.315
11/7/90	7.0	-	N	0.263	0.436
12/5/90	6.3	8.0	N	0.322	0.270
1/2/91	16.9	23.9	Y	0.520	0.914
2/7/91	12.3	15.7	Y	0.897	1.193
3/6/91	11.0	15.7	Y	0.528	0.483
4/11/91	5.7	6.7	N	0.186	0.162
5/8/91	7.3	9.8	N	0.236	0.266
6/5/91	7.9	9.0	N	0.346	0.265
7/8/91	5.4	8.6	N	0202	0.164
8/12/91	7.7	10.0	N	-	-
9/6/91	12.3	15.0	N	-	-

Table 17. Raw data for reproductive parameters for fish number 122.

Fish ID: 122	Sex: Male	Cycle: 6-Month	Chamber: 4	Initial length: 56.5
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: No	Ending length: 63.0
Spawn Date: 10/8/91	# of Males: 2	Fert %: 15.3	Type: Tank	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation
3/1/90	-	-	Y
5/3/90	8.8	-	Y
6/5/90	6.6	-	N
7/10/90	3.7	-	N
8/7/90	4.0	-	N
9/5/90	5.0	-	N
10/4/90	6.9	-	N
11/7/90	6.3	-	N
12/5/90	8.8	12.0	N
1/2/91	8.5	13.2	Y
2/7/91	9.6	14.0	N
3/6/91	5.0	8.3	N
4/11/91	5.6	8.1	N
5/8/91	6.2	8.3	N
6/5/91	8.0	11.0	N
7/8/91	8.1	12.6	N
8/12/91	11.8	16.9	N
9/6/91	14.6	15.5	Y

Table 18. Raw data for reproductive parameters for fish number 124.

Fish ID: 124	Sex: Male	Cycle: 6-Month	Chamber: 4	Initial length: 54.0
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: Yes	Ending length: 60.8
Spawn Date: 1/7-24/91 9/16/91.	# of Males: 2,2,1,2	Fert %: 5.5, 36.5, 81.8, 15.3	Type: Tank, tank, strip, tank	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation	Testosterone (ng/ml)	11 keto-testosterone (ng/ml)
3/1/90	12.0	-	Y	-	-
5/3/90	9.7	-	Y	0.361	0.380
6/5/90	4.8	-	N	0.125	0.111
7/10/90	2.7	-	N	0.090	0.051
8/7/90	-	-	N	0.162	0.121
9/5/90	4.1	-	N	0.182	0.135
10/4/90	9.4	-	N	0.196	0.128
11/7/90	7.2	-	N	0.130	0.071
12/5/90	9.3	13.7	N	0.601	0.305
1/2/91	10.5	11.5	Y	1.178	1.440
2/7/91	7.0	8.1	Y	1.142	1.365
3/6/91	4.4	11.4	N	0.194	0.235
4/11/91	4.9	5.7	N	0.150	0.052
5/8/91	8.5	9.8	N	0.279	0.190
6/5/91	11.9	12.1	N	0.345	0.192
7/8/91	7.3	11.4	N	0.339	0.206
8/12/91	14.4	17.4	N	-	-
9/6/91	17.3	27.1	Y	-	-

Table 19. Raw data for reproductive parameters for fish number 126.

Fish ID: 126	Sex: Male	Cycle: 6-Month	Chamber: 2	Initial length: 53.5
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: No	Ending length: 61.8
Spawn Date: N/A	# of Males: N/A	Fert %: N/A	Type: N/A	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation
3/1/90	10.3	-	Y
5/3/90	10.6	-	N
6/5/90	6.0	-	N
7/10/90	4.1	-	N
8/7/90	5.0	-	N
9/5/90	5.6	-	N
10/4/90	5.6	-	N
11/7/90	6.6	-	N
12/5/90	6.4	8.5	N
1/2/91	11.4	15.1	N
2/7/91	9.9	11.7	N
3/6/91	6.1	7.4	N
4/11/91	6.0	8.1	N
5/8/91	7.6	10.5	N
6/5/91	9.0	10.5	N
7/8/91	6.2	10.7	N
8/12/91	8.3	10.2	N
9/6/91	9.0	11.7	N

Table 20. Raw data for reproductive parameters for fish number 127.

Fish ID: 127	Sex: Male	Cycle: 12-Month	Chamber: 7	Initial length: 53.5
Stock Date: 5/3/90	Mort: 8/31/91	Cause: Post-spawn	Bled: No	Ending length: 59.2
Spawn Date: 7/6/91	# of Males: 2	Fert %: 32.1	Type: Tank	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation
3/1/90	14.7	-	Y
5/3/90	12.3	-	Y
6/5/90	7.9	-	N
7/10/90	6.6	-	N
8/7/90	5.7	-	N
9/5/90	6.3	-	N
10/4/90	6.7	-	N
11/7/90	4.4	-	N
12/5/90	5.6	8.8	N
1/2/91	8.3	9.8	N
2/7/91	9.3	15.7	N
3/6/91	10.1	11.7	N
4/11/91	14.9	19.0	N
5/8/91	18.8	26.7	Y
6/5/91	22.0	27.6	Y
7/8/91	16.2	25.7	Y
8/12/91	7.4	10.7	N

Table 21. Raw data for reproductive parameters for fish number 128.

Fish ID: 128	Sex: Male	Cycle: 9-Month	Chamber: 3	Initial length: 55.0
Stock Date: 5/3/90	Mort: 6/19/91	Cause: Post-spawn	Bled: No	Ending length: 59.6
Spawn Date: N/A	# of Males: N/A	Fert %: N/A	Type: N/A	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation
3/1/90	10.1	-	N
5/3/90	11.3	-	N
6/5/90	7.2	-	N
7/10/90	7.6	-	N
8/7/90	4.8	-	N
9/5/90	5.2	-	N
10/4/90	5.9	-	N
11/7/90	3.8	-	N
12/5/90	4.0	8.0	N
1/2/91	6.8	11.5	N
2/7/91	8.5	14.3	N
3/6/91	16.3	23.6	Y
4/11/91	13.1	17.9	Y
5/8/91	7.1	11.9	Y
6/5/91	7.4	7.1	N

Fish ID: 129	Sex: Male	Cycle: 12-Month	Chamber: 7	Initial length: 55.5
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: No	Ending length: 61.1
Spawn Date: 7/6/91	# of Males: 2	Fert %: 32.1	Type: Tank	

Sampling	Average Gonadal	Maximum Gonadal	Spermiation

Date	Diameter (mm)	Diameter (mm)	
3/1/90	14.0	-	Y
5/3/90	9.3	-	Y
6/5/90	6.6	-	N
7/10/90	4.7	-	N
8/7/90	2.6	-	N
9/5/90	2.0	-	N
10/4/90	4.2	-	N
11/7/90	3.8	-	N
12/5/90	5.4	6.3	N
1/2/91	6.7	8.3	N
2/7/91	6.8	13.6	N
3/6/91	7.1	10.5	N
4/11/91	10.5	15.0	N
5/8/91	15.7	23.1	Y
6/5/91	18.1	29.0	Y
7/8/91	11.5	15.0	Y
8/12/91	6.9	10.2	N
9/6/91	6.3	11.7	N

Table 23. Raw data for reproductive parameters for fish number 130.

Fish ID: 130	Sex: Male	Cycle: 9-Month	Chamber: 1	Initial length: 54.0
Stock Date: 5/3/90	Mort: 3/9/91	Cause: Unknown	Bled: Yes	Ending length: 57.6
Spawn Date: N/A	# of Males: N/A	Fert %: N/A	Type: N/A	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Spermiation	Testosterone (ng/ml)	11 keto-testosterone (ng/ml)
3/1/90	11.8	-	Y	-	-
5/3/90	9.3	-	Y	0.138	0.002
6/5/90	6.8	-	N	0.249	0.236
7/10/90	5.2	-	N	0.271	0.222
8/7/90	5.2	-	N	0.205	0.187
9/5/90	4.3	-	N	0.127	0.059
10/4/90	4.3	-	N	0.184	0.185
11/7/90	7.1	-	N	0.191	0.097
12/5/90	7.4	10.0	N	0.176	0.002
1/2/91	6.1	11.5	N	0.260	0.076
2/7/91	8.8	10.2	N	0.241	0.112
3/6/91	10.6	13.6	N	0.349	0.364

Table 25. Raw data for reproductive parameters for fish number 152.

Fish ID: 152	Sex: Female	Cycle: 12-Month	Chamber: 7	Initial length: 54.5
Stock Date: 5/3/90	Mort: 7/21/91	Cause: Post-spawn	Bled: Yes	Ending length: 64.6
Spawn Date: 7/6/91	Latent Time: 31	Fert %: 32.1	Eggs/kg: 18,568	Type: Tank

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)	Testosterone (ng/ml)	Estradiol (ng/ml)	Vitellogenin (mg/ml)
5/3/90	20.0	-	975	0.6380	0.0852	0.404
6/5/90	15.6	-	-	0.1484	0.0020	0.002
7/10/90	11.8	-	275	0.1026	0.0052	0.002
8/7/90	9.0	-	225	0.1274	0.0020	0.002
9/5/90	7.2	-	275	0.1676	0.0104	0.002
10/4/90	9.1	10.4	225	0.1414	0.0118	0.002
11/7/90	10.3	12.4	300	0.1662	0.0934	0.240
12/5/90	12.7	16.0	400	0.1868	0.1856	0.459
1/2/91	13.8	18.2	515	0.1694	0.2126	0.775
2/7/91	17.0	20.5	625	0.1814	0.2468	0.936
3/6/91	17.9	25.6	700	0.2568	0.2538	0.955
4/11/91	19.6	26.0	605	0.3936	0.5226	0.786
5/8/91	19.4	27.6	795	0.1934	0.0532	0.905
6/5/91	23.3	30.0	935	2.0056	1.3096	0.915
7/8/91	15.2	21.4	1100	0.1824	0.0020	1.237

Table 26. Raw data for reproductive parameters for fish number 153.

Fish ID: 153	Sex: Female	Cycle: 6-Month	Chamber: 2	Initial length: 50.0
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: Yes	Ending length: 57.7
Spawn Date: N/A	Latent Time: N/A	Fert %: N/A	Eggs/kg: N/A	Type:N/A

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)	Testosterone (ng/ml)	Estradiol (ng/ml)	Vitellogenin (mg/ml)
5/3/90	19.9	-	-	0.3172	0.1906	1.025
6/5/90	16.4	-	400	0.1194	0.0020	0.051
7/10/90	14.2	-	275	0.1226	0.0020	0.002
8/7/90	9.2	-	275	0.1334	0.0278	0.002
9/5/90	9.7	-	375	0.1524	0.0848	0.002
10/4/90	13.9	17.3	400	0.1008	0.1172	0.171
11/7/90	17.7	21.3	475	0.0962	0.2712	0.537
12/5/90	17.0	20.0	600	0.1682	0.2448	0.811
1/2/91	21.8	30.0	775	0.8588	1.2684	0.898
2/7/91	14.8	18.8	250	0.1302	0.0082	0.108
3/6/91	19.2	27.6	225	0.1026	0.0072	0.067
4/11/91	12.9	21.0	300	0.1314	0.0272	0.002
5/8/91	8.7	13.3	250	0.1836	0.0906	0.002
6/5/91	14.2	21.2	350	0.2008	0.0456	0.332
7/8/91	16.7	21.2	398	0.1800	0.1214	0.007
8/12/91	13.5	17.6	585	-	-	-
9/6/91	11.5	15.7	305	-	-	-

Table 27. Raw data for reproductive parameters for fish number 155.

Fish ID: 155	Sex: Female	Cycle: 9-Month	Chamber: 3	Initial length: 57.6
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: No	Ending length: 65.4
Spawn Date: N/A	Latent Time: N/A	Fert %: N/A	Eggs/kg: N/A	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)
6/5/90	14.4	-	Plugged Vent
7/10/90	11.9	-	-
8/7/90	11.0	-	-
9/5/90	11.8	-	-
10/4/90	9.2	17.1	-
11/7/90	15.6	19.6	-
12/5/90	16.1	21.6	-
1/2/91	18.2	24.2	-
2/7/91	29.3	43.4	-
3/6/91	25.2	39.0	-
4/11/91	34.2	39.3	-
5/8/91	28.6	32.4	-
6/5/91	-	-	-
7/8/91	-	-	-
8/12/91	-	-	-
9/6/91	-	-	-

Table 28. Raw data for reproductive parameters for fish number 156.

Fish ID: 156	Sex: Female	Cycle: 12-Month	Chamber: 7	Initial length: 50.5
Stock Date: 5/3/90	Mort: 10/7/90	Cause: Handling	Bled: No	Ending length: 52.0
Spawn Date: N/A	Latent Time: N/A	Fert %: N/A	Eggs/kg: N/A	Type:

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)
3/1/90	23.2	-	1000
5/3/90	21.0	-	1000
6/5/90	21.6	-	675
7/10/90	14.8	-	275
8/7/90	11.8	-	325
9/5/90	12.2	-	325
10/4/90	10.2	13.9	250

Table 29. Raw data for reproductive parameters for fish number 157.

Fish ID: 157	Sex: Female	Cycle: 6-Month	Chamber: 4	Initial length: 57.5
Stock Date: 9/5/90	Mort: N/A	Cause: N/A	Bled: No	Ending length: 62.3
Spawn Date: 1/7/91	Latent Time: 34	Fert %: 5.5	Eggs/kg: 66,993	Type: Tank

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)
9/5/90	9.7	-	350
10/4/90	18.6	26.4	425
11/7/90	13.9	20.4	375
12/5/90	12.4	15.6	515
1/2/91	19.5	30.5	800
2/7/91	15.1	20.0	212
3/6/91	17.0	23.1	212
4/11/91	14.8	18.3	280
5/8/91	12.4	17.1	330
6/5/91	15.2	20.2	450
7/8/91	14.9	20.2	465
8/12/91	19.3	25.7	630
9/6/91	29.0	35.7	885

Table 30. Raw data for reproductive parameters for fish number 158.

Fish ID: 158	Sex: Female	Cycle: 9-Month	Chamber: 1	Initial length: 57cm
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: Yes	Ending length: 66.5cm
Spawn Date: 3/17/91	Latent Time: 38	Fert %: 73.1	Eggs/kg: 78,554	Type: Tank

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)	Testosterone (ng/ml)	Estradiol (ng/ml)	Vitellogenin (mg/ml)
3/1/90	25.8	-	-	-	-	-
5/3/90	21.8	-	1090	0.8690	0.2298	0.2700
6/5/90	18.6	-	-	0.1206	0.0020	0.0020
7/10/90	18.3	-	420	0.1260	0.0020	0.0020
8/7/90	20.0	-	325	0.1276	0.0054	0.0020
9/5/90	13.8	-	375	0.1360	0.0062	0.0020
10/4/90	10.1	-	325	0.1278	0.0148	0.0020
11/7/90	12.6	18.7	375	0.1608	0.1194	0.3800
12/5/90	13.3	20.4	515	0.1144	0.0606	0.7790
1/2/91	17.3	23.1	565	0.1260	0.213	0.7930
2/7/91	22.4	34.6	725	0.1706	0.3568	1.0520
3/6/91	29.6	37.6	950	0.4636	1.3218	1.0910
4/11/91	15.4	21.2	350	0.1522	0.0356	0.7310
5/8/91	16.1	20.7	325	0.0900	0.0020	0.1710
6/5/91	13.2	17.4	240	0.8594	0.0020	0.0020
7/8/91	13.2	19.8	275	0.1193	0.0020	0.0020
8/12/91	10.4	12.4	255	-	-	-
9/6/91	14.5	16.7	240	-	-	-

Table 31. Raw data for reproductive parameters for fish number 159.

Fish ID: 159	Sex: Female	Cycle: 9-Month	Chamber: 3	Initial length: 54cm
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: Yes	Ending length: 62.8cm
Spawn Date: 3/27/91	Latent Time: 39	Fert %: 49.0	Eggs/kg: 120,185	Type: Tank

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)	Testosterone (ng/ml)	Estradiol (ng/ml)	Vitellogenin (mg/ml)
3/1/90	22.4	-	-	-	-	-
5/3/90	25.8	-	800	0.3830	0.0841	0.572
6/5/90	16.3	-	-	0.0763	0.0020	0.002
7/10/90	10.3	-	350	0.0527	0.0020	0.002
8/7/90	9.7	-	225	0.1001	0.0020	0.002
9/5/90	11.5	-	300	0.1295	0.0161	0.002
10/4/90	10.0	15.1	275	0.1542	0.1544	0.002
11/7/90	13.6	17.1	375	0.0662	0.1318	0.121
12/5/90	13.2	18.0	475	0.1059	0.0688	0.556
1/2/91	14.4	16.7	565	0.1391	0.3257	0.556
2/7/91	21.0	25.6	725	0.1972	0.4481	0.940
3/6/91	22.9	38.0	825	0.5123	1.1719	0.943
4/11/91	13.2	20.2	510	0.1739	0.0020	0.858
5/8/91	16.3	21.7	300	0.1227	0.0020	0.693
6/5/91	14.1	22.6	240	0.1588	0.0020	0.010
7/8/91	9.9	12.6	225	0.1061	0.0020	0.002
8/12/91	10.0	12.6	255	-	-	-
9/6/91	10.1	14.5	350	-	-	-

Table 32. Raw data for reproductive parameters for fish number 160.

Fish ID: 160	Sex: Female	Cycle: 6-Month	Chamber: 4	Initial length: 58.5cm
Stock Date: 3/20/91	Mort: N/A	Cause: N/A	Bled: Yes	Ending length: 71.1cm
Spawn Date: 9/16/91	Latent Time: 37	Fert %: 81.8	Eggs/kg: 61,110	Type: Strip

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)	Testosterone (ng/ml)	Estradiol (ng/ml)	Vitellogenin (mg/ml)
4/11/91	17.1	26.2	265	0.219	0.002	0.067
5/8/91	14.8	20.5	250	0.131	0.072	0.208
6/5/91	14.0	20.7	500	0.219	0.273	0.866
7/8/91	16.8	23.1	565	0.194	0.645	0.841
8/12/91	23.0	38.8	885	-	-	-
9/6/91	29.2	45.7	1070	-	-	-

Table 33. Raw data for reproductive parameters for fish number 161.

Fish ID: 161	Sex: Female	Cycle: 9-Month	Chamber: 1	Initial length: 52.5
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: No	Ending length: 56.5
Spawn Date: N/A	Latent Time: N/A	Fert %: N/A	Eggs/kg: N/A	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)
3/1/90	20.9	-	
5/3/90	23.0		950
6/5/90	19.1		350
7/10/90	11.7		280
8/7/90	9.5		250
9/5/90	10.1		325
10/4/90	10.4		325
11/7/90	11.9	17.3	400
12/5/90	14.8	16.9	475
1/2/91	13.8	20.4	565
2/7/91	16.5	21.2	700
3/6/91	26.0	31.2	900
4/11/91	18.1	23.3	610
5/8/91	13.1	15.2	250
6/5/91	9.2	10.2	255
7/8/91	13.8	19.5	255
8/12/91	8.3	13.1	240
9/6/91	10.2	14.1	255

Table 34. Raw data for reproductive parameters for fish number 163.

Fish ID: 163	Sex: Female	Cycle: 12-Month	Chamber: 7	Initial length: 57.0
Stock Date: 8/7/90	Mort: 7/4/91	Cause: Post-spawn	Bled: No	Ending length: 65.2
Spawn Date: 6/21/91	Latent Time: 34.5	Fert %: 24.8	Eggs/kg: 37,886	Type: Strip

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)
8/7/90	9.9	-	325
9/5/90	10.1	-	350
10/4/90	11.7	13.8	350
11/7/90	12.0	15.1	325
12/5/90	13.9	15.1	400
1/2/91	15.1	16.2	515
2/7/91	17.1	22.4	475
3/6/91	19.3	22.4	550
4/11/91	20.1	28.6	725
5/8/91	21.4	29.8	750
6/5/91	23.7	33.8	1075

Table 35. Raw data for reproductive parameters for fish number 165.

Fish ID: 165	Sex: Female	Cycle: 6-Month	Chamber: 2	Initial length: 53.5
Stock Date: 8/7/90	Mort: N/A	Cause: N/A	Bled: No	Ending length: 61.5
Spawn Date: 10/8/91	Latent Time: 38	Fert %: 15.3	Eggs/kg: 43,063	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)
8/7/90	12.6		425
9/5/90	13.1		325
10/4/90	14.8	18.9	350
11/7/90	15.2	18.0	400
12/5/90	14.5	18.2	450
1/2/91	22.7	24.4	700
2/7/91	17.6	23.4	600
3/6/91	13.7	17.6	225
4/11/91	9.9	13.6	280
5/8/91	11.0	16.9	250
6/5/91	13.3	20.5	400
7/8/91	16.0	21.7	435
8/12/91	18.7	25.0	635
9/6/91	21.4	29.3	725

Table 36. Raw data for reproductive parameters for fish number 166.

Fish ID: 166	Sex: Female	Cycle: 6-Month	Chamber: 4	Initial length: 58.0
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: No	Ending length: 63.4
Spawn Date: N/A	Latent Time: N/A	Fert %: N/A	Eggs/kg: N/A	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)
3/1/90	25.9	-	-
5/3/90	25.3	-	900
6/5/90	18.8	-	425
7/10/90	11.6	-	275
8/7/90	-	-	225
9/5/90	10.9	-	325
10/4/90	12.9	18.4	375
11/7/90	13.8	18.0	425
12/5/90	12.5	17.4	450
1/2/91	11.7	17.7	350
2/7/91	12.1	14.5	212
3/6/91	9.4	13.3	225
4/11/91	9.5	16.0	280
5/8/91	13.3	16.9	265
6/5/91	14.3	20.7	400
7/8/91	13.5	21.0	395
8/12/91	15.8	21.9	585
9/6/91	32.1	36.0	845

Table 37. Raw data for reproductive parameters for fish number 167.

Fish ID: 167	Sex: Female	Cycle: 9-Month	Chamber: 1	Initial length: 56.5
Stock Date: 5/3/90	Mort: 7/17/91	Cause: Post-spawn	Bled: Yes	Ending length: 65.8
Spawn Date: 3/16/91	Latent Time: 31	Fert %: 48.4	Eggs/kg: 113,533	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)	Testosterone (ng/ml)	Estradiol (ng/ml)	Vitellogenin (mg/ml)
3/1/90	21.1	-	1100	-	-	-
5/3/90	26.0	-	1010	0.6221	0.0002	0.024
6/5/90	20.7	-	350	0.0865	0.0002	0.002
7/10/90	14.9	-	350	0.1126	0.0002	0.002
8/7/90	12.6	-	425	0.1122	0.0002	0.002
9/5/90	11.1	-	375	0.1229	0.0238	0.002
10/4/90	14.6	-	325	0.2015	0.0655	0.024
11/7/90	16.1	22.2	375	0.1741	0.3648	0.557
12/5/90	14.3	20.0	515	0.0981	0.1209	0.794
1/2/91	17.9	24.4	650	0.2349	0.3368	0.703
2/7/91	21.5	33.2	750	0.2255	0.5540	0.968
3/6/91	25.4	43.7	950	0.7765	1.9541	0.881
4/11/91	15.1	23.8	660	0.4503	0.3766	1.091
5/8/91	15.1	20.0	375	0.1245	0.0002	0.365
6/5/91	8.6	13.1	255	0.1209	0.0002	0.002
7/8/91	8.7	17.1	255	0.1343	0.0002	0.002

Table 38. Raw data for reproductive parameters for fish number 168.

Fish ID: 168	Sex: Female	Cycle: 6-Month	Chamber: 2	Initial length: 52.5
Stock Date: 5/3/90	Mort: 8/7/90	Cause: Jumper	Bled: No	Ending length: 54.6
Spawn Date: N/A	Latent Time: N/A	Fert %: N/A	Eggs/kg: N/A	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)
3/1/90	24.7	-	1020
5/3/90	25.8	-	975
6/5/90	15.1	-	-
7/10/90	12.8	-	-
8/7/90	10.1	-	-

Table 39. Raw data for reproductive parameters for fish number 170.

Fish ID: 170	Sex: Female	Cycle: 6-Month	Chamber: 4	Initial length: 56.5cm
Stock Date: 5/3/90	Mort: 1/24/91	Cause: Spawning	Bled: Yes	Ending length: 61.1cm
Spawn Date: 1/23/9`	Latent Time: 42	Fert %: 36.5	Eggs/kg: 27,556	Type: Tank

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)	Testosterone (ng/ml)	Estradiol (ng/ml)	Vitellogenin (mg/ml)
3/1/90	24.3	-	920	-	-	-
5/3/90	27.7	-	925	0.472	0.312	0.693
6/5/90	17.9	-	650	0.094	0.002	0.002
7/10/90	12.4	-	250	0.115	0.002	0.002
8/7/90	-	-	250	0.145	0.012	0.002
9/5/90	12.3	-	325	0.178	0.010	0.002
10/4/90	18.2	26.2	425	0.163	0.332	0.413
11/7/90	17.7	26.9	475	0.152	0.414	0.522
12/5/90	15.2	24.5	600	-	-	-
1/2/91	20.5	24.8	725	0.331	0.785	1.055

Table 39. Raw data for reproductive parameters for fish number 170.

Fish ID: 170	Sex: Female	Cycle: 6-Month	Chamber: 4	Initial length: 56.5cm
Stock Date: 5/3/90	Mort: 1/24/91	Cause: Spawning	Bled: Yes	Ending length: 61.1cm
Spawn Date: 1/23/9`	Latent Time: 42	Fert %: 36.5	Eggs/kg: 27,556	Type: Tank

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)	Testosterone (ng/ml)	Estradiol (ng/ml)	Vitellogenin (mg/ml)
3/1/90	24.3	-	920	-	-	-
5/3/90	27.7	-	925	0.472	0.312	0.693
6/5/90	17.9	-	650	0.094	0.002	0.002
7/10/90	12.4	-	250	0.115	0.002	0.002
8/7/90	-	-	250	0.145	0.012	0.002
9/5/90	12.3	-	325	0.178	0.010	0.002
10/4/90	18.2	26.2	425	0.163	0.332	0.413
11/7/90	17.7	26.9	475	0.152	0.414	0.522
12/5/90	15.2	24.5	600	-	-	-
1/2/91	20.5	24.8	725	0.331	0.785	1.055

Table 40. Raw data for reproductive parameters for fish number 171.

Fish ID: 171	Sex: Female	Cycle: 6-Month	Chamber: 4	Initial length: 51cm
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: Yes	Ending length: 67.3cm
Spawn Date: N/A	Latent Time: N/A	Fert %: N/A	Eggs/kg: N/A	Type: N/A

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)	Testosterone (ng/ml)	Estradiol (ng/ml)	Vitellogenin (mg/ml)
3/1/90	21.0	-	1020	-	-	-
5/3/90	20.1	-	800	0.100	0.002	0.185
6/5/90	13.7	-	-	0.071	0.002	0.002
7/10/90	9.4	-	200	0.095	0.002	0.002
8/7/90	7.6	-	225	0.118	0.002	0.002
9/5/90	10.7	-	325	0.148	0.093	0.002
10/4/90	13.4	16.3	425	0.100	0.031	0.093
11/7/90	15.3	21.9	425	0.062	0.090	0.303
12/5/90	13.6	17.8	475	0.121	0.080	0.837
1/2/91	18.2	26.0	725	0.256	0.657	0.889
2/7/91	16.1	22.1	425	0.142	0.002	0.076
3/6/91	10.9	14.5	212	0.117	0.002	0.002
4/11/91	8.8	13.1	280	0.182	0.002	0.002
5/8/91	12.0	14.1	290	0.156	0.069	0.002
6/5/91	11.8	17.9	415	0.139	0.078	0.437
7/8/91	14.4	20.5	510	0.131	0.290	0.776
8/12/91	18.9	29.8	700	-	-	-
9/6/91	23.8	31.7	885	-	-	-

Table 41. Raw data for reproductive parameters for fish number 172.

Fish ID: 172	Sex: Female	Cycle: 9-Month	Chamber: 3	Initial length: 50.0
Stock Date: 5/3/90	Mort: 5/10/90	Cause: Jumper	Bled: No	Ending length: 50.5
Spawn Date: N/A	Latent Time: N/A	Fert %: N/A	Eggs/kg: N/A	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)
3/1/90	21.1	-	990
5/3/90	16.8	-	800

Table 42. Raw data for reproductive parameters for fish number 173.

Fish ID: 173	Sex: Female	Cycle: 9-Month	Chamber: 3	Initial length: 55.5
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: No	Ending length: 61.3
Spawn Date: 3/26/91	Latent Time: 35.5	Fert %: 71.1	Eggs/kg: 76,974	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)
3/1/90	20.1	-	-
5/3/90	23.0	-	600
6/5/90	17.0	-	250
7/10/90	14.0	-	400
8/7/90	9.2	-	225
9/5/90	8.8	-	300
10/4/90	9.2	13.6	325
11/7/90	12.0	15.6	350
12/5/90	13.7	18.2	425
1/2/91	13.8	20	590
2/7/91	19.8	29.3	675
3/6/91	30.4	39.3	900
4/11/91	18.3	27.1	980
5/8/91	14.9	26.9	320
6/5/91	16.1	21.7	250
7/8/91	13.0	16.7	255
8/12/91	13.7	18.3	255
9/6/91	11.5	19.3	255

Table 43. Raw data for reproductive parameters for fish number 174.

Fish ID: 174	Sex: Female	Cycle: 6-Month	Chamber: 2	Initial length: 55.5
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: No	Ending length: 63.0
Spawn Date: 2/3/91	Latent Time: 35.5	Fert %: 64.7	Eggs/kg: 98,930	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)
3/1/90	22.8	-	860
5/3/90	18.4	-	425
6/5/90	14.0	-	325
7/10/90	10.0	-	225
8/7/90	10.7	-	250
9/5/90	9.5	-	400
10/4/90	15.8	20.7	400
11/7/90	14.9	20.2	525
12/5/90	11.0	12.7	525
1/2/91	19.5	27.1	675
2/7/91	20.4	26.8	990
3/6/91	20.1	28.3	225
4/11/91	9.4	11.9	280
5/8/91	13.7	17.1	250
6/5/91	14.8	21.0	400
7/8/91	18.5	25.5	605
8/12/91	22.5	32.1	750
9/6/91	28.7	38.1	885

Table 44. Raw data for reproductive parameters for fish number 175.

Fish ID: 175	Sex: Female	Cycle: 9-Month	Chamber: 3	Initial length: 53.0
Stock Date: 5/3/90	Mort: 5/31/91	Cause: Post-spawn	Bled: Yes	Ending length: 58.9
Spawn Date: 4/17/91	Latent Time: 40	Fert %: 47.8	Eggs/kg: 15,992	Type: Strip

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)	Testosterone (ng/ml)	Estradiol (ng/ml)	Vitellogenin (mg/ml)
3/1/90	-	-	960	-	-	-
5/3/90	21.2	-	1060	0.6581	0.0801	1.076
6/5/90	14.3	-	350	0.2174	0.0020	0.002
7/10/90	10.8	-	325	0.0740	0.0020	0.002
8/7/90	10.5	-	325	0.1189	0.0020	0.002
9/5/90	12.7	-	300	0.1940	0.0251	0.002
10/4/90	10.9	16.7	325	0.1734	0.0444	0.157
11/7/90	13.4	19.8	425	0.1678	0.0866	0.636
12/5/90	15.6	18.0	475	0.1607	0.3008	0.723
1/2/91	15.0	24.0	600	0.2152	0.3891	0.895
2/7/91	21.0	32.2	675	0.2749	0.6425	1.087
3/6/91	27.9	37.3	950	0.6469	1.1307	0.659
4/11/91	22.3	33.6	1030	1.4142	0.4713	0.605
5/8/91	-	-	280	0.1406	0.0020	0.002

Table 45. Raw data for reproductive parameters for fish number 177.

Fish ID: 177	Sex: Female	Cycle: 12-Month	Chamber: 7	Initial length: 57.5
Stock Date: 11/7/90	Mort: 6/12/91	Cause: Post-spawn	Bled: No	Ending length: 65.6
Spawn Date: 6/6/91	Latent Time: 36	Fert %: 30.8	Eggs/kg: 38,110	Type: Strip

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)
11/7/90	13.1	16.9	325
12/5/90	9.4	13.1	425
1/2/91	14.6	18.9	563
2/7/91	18.9	29.0	700
3/6/91	23.1	34.6	900
4/11/91	25.8	36.9	1050
5/8/91	26.8	34.3	975
6/5/91	26.8	34.1	1125

Table 46. Raw data for reproductive parameters for fish number 178.

Fish ID: 178	Sex: Female	Cycle: 6-Month	Chamber: 4	Initial length: 50.5
Stock Date: 5/3/90	Mort: 8/8/91	Cause: Handling	Bled: No	Ending length: 51.2
Spawn Date: N/A	Latent Time: N/A	Fert %: N/A	Eggs/kg: N/A	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)
3/1/90	20.1	-	800
5/3/90	17.4	-	575
6/5/90	15.3	-	-
7/10/90	7.7	-	250
8/7/90	-	-	-

Table 47. Raw data for reproductive parameters for fish number 179.

Fish ID: 179	Sex: Female	Cycle: 12-Month	Chamber: 7	Initial length: 54.5
Stock Date: 5/3/90	Mort: 7/23/90	Cause: Unknown	Bled: No	Ending length: 55.5
Spawn Date: N/A	Latent Time: N/A	Fert %: N/A	Eggs/kg: N/A	Type: N/A

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)
3/1/90	22.2	-	990
5/3/90	21.1	-	750
6/5/90	21.6	-	300
7/10/90	11.6	-	300

Table 48. Raw data for reproductive parameters for fish number 180.

Fish ID: 180	Sex: Female	Cycle: 12-Month	Chamber: 7	Initial length: 56.5
Stock Date: 5/3/90	Mort: 8/15/91	Cause: Post-spawn	Bled: Yes	Ending length: 63.6
Spawn Date: 6/21/91	Latent Time: 40	Fert %: 40.9	Eggs/kg: 87,273	Type: Tank

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)	Testosterone (ng/ml)	Estradiol (ng/ml)	Vitellogenin (mg/ml)
5/3/90	23.9	-	800	0.3289	0.2241	0.630
6/5/90	18.3	-	300	0.0910	0.0020	0.002
7/10/90	13.3	-	150	0.0799	0.0020	0.002
8/7/90	10.5	-	225	-	-	-
9/5/90	7.7	-	225	0.0922	0.0020	0.002
10/4/90	8.1	12.9	225	0.0858	0.0020	0.002
11/7/90	11.3	13.3	275	0.1011	0.0151	0.002
12/5/90	10.8	20.0	350	0.1644	0.0548	0.167
1/2/91	14.3	19.3	450	0.1175	0.0755	0.436
2/7/91	13.9	22.4	425	0.0938	0.0336	0.611
3/6/91	18.5	22.7	600	0.1677	0.0430	0.700
4/11/91	17.7	21.4	650	0.2073	0.2457	0.758
5/8/91	22.5	27.6	700	0.3007	0.7655	1.496
6/5/91	29.0	38.8	980	0.3610	1.3812	0.004
7/8/91	13.9	19.5	885	0.0572	0.0020	0.002
8/12/91	12.1	17.4	240	-	-	-

Table 49. Raw data for reproductive parameters for fish number 181.

Fish ID: 181	Sex: Female	Cycle: 9-Month	Chamber: 1	Initial length: 50.5
Stock Date: 5/3/90	Mort: 6/14/91	Cause: Post-spawn	Bled: No	Ending length: 55.8
Spawn Date: 4/9/91	Latent Time: 36.5	Fert %: 10.8	Eggs/kg: 57,462	

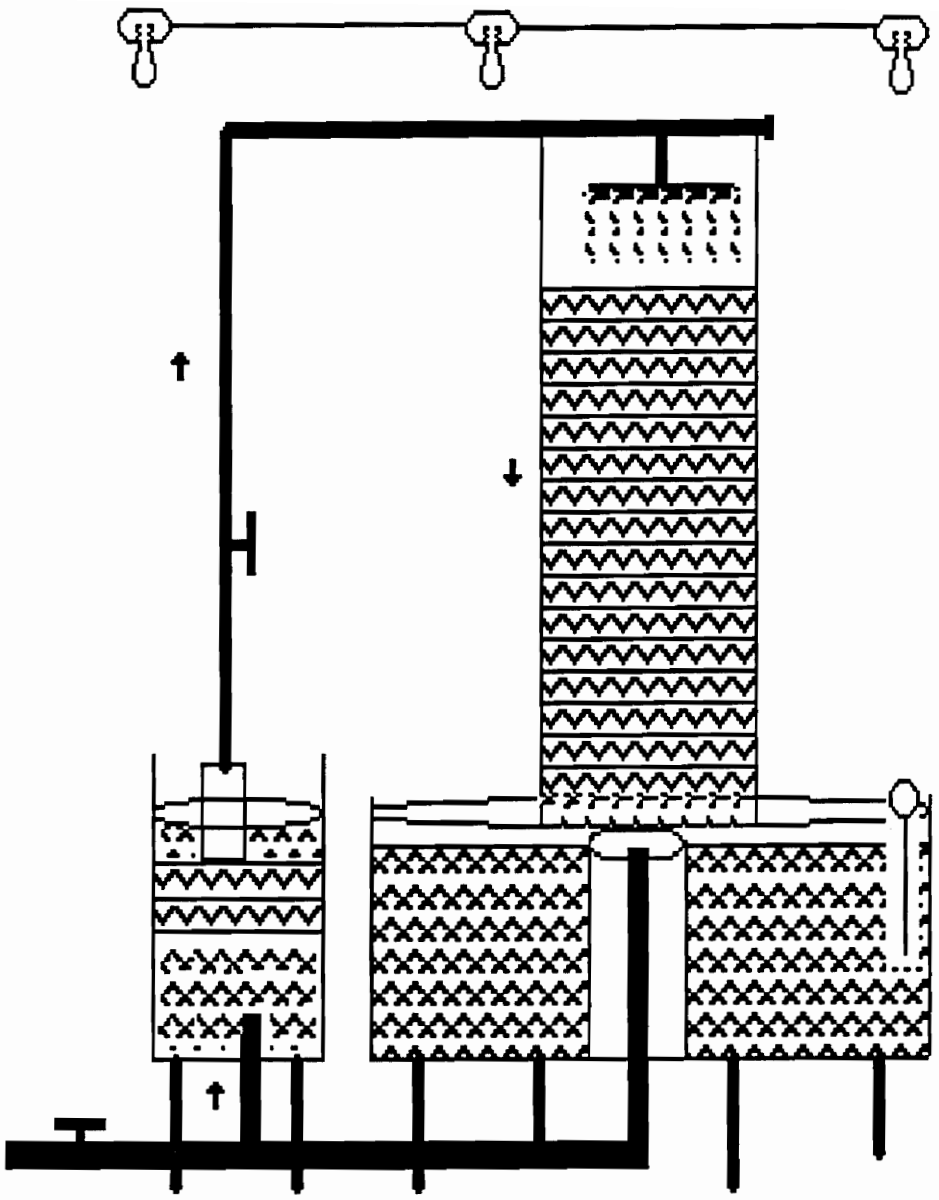
Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)
3/1/90	19.0	-	950
5/3/90	18.4	-	975
6/5/90	15.6	-	400
7/10/90	12.7	-	420
8/7/90	11.6	-	375
9/5/90	11.9	-	350
10/4/90	16.9	-	325
11/7/90	13.3	17.3	375
12/5/90	15.1	18.4	425
1/2/91	13.1	19.6	450
2/7/91	15.4	18.8	425
3/6/91	21.8	26.6	650
4/11/91	22.1	30.5	980
5/8/91	14.6	17.9	425
6/5/91	13.9	17.4	275

Table 50. Raw data for reproductive parameters for fish number 182.

Fish ID: 182	Sex: Female	Cycle: 6Month	Chamber: 2	Initial length: 54.9
Stock Date: 5/3/90	Mort: N/A	Cause: N/A	Bled: Yes	Ending length: 61.9
Spawn Date: N/A	Latent Time: N/A	Fert %: N/A	Eggs/kg: N/A	

Sampling Date	Average Gonadal Diameter (mm)	Maximum Gonadal Diameter (mm)	Egg Diameter (um)	Testosterone (ng/ml)	Estradiol (ng/ml)	Vitellogenin (mg/ml)
5/3/90	20.2	-	800	0.2114	0.0554	0.1240
6/5/90	14.1	-	350	0.1131	0.0020	0.0020
7/10/90	10.2	-	225	0.0833	0.0020	0.0020
8/7/90	11.8	-	225	0.0902	0.0020	0.0020
9/5/90	9.7	-	325	0.1307	0.0083	0.0020
10/4/90	11.2	19.1	375	0.1515	0.0498	0.0540
11/7/90	15.1	20.2	450	0.0876	0.0764	0.2780
12/5/90	13.9	16.7	515	0.1505	0.0360	0.0490
1/2/91	15.4	17.6	515	0.1412	0.0020	0.0260
2/7/91	9.3	11.2	225	0.1381	0.0020	0.0020
3/6/91	9.5	12.9	210	0.1523	0.0020	0.0020
4/11/91	8.8	14.3	280	0.1394	0.0020	0.0020
5/8/91	7.6	11.9	250	0.1418	0.0020	0.0020
6/5/91	11.1	11.1	350	0.1624	0.0318	0.0020
7/8/91	12.0	13.1	413	0.1802	0.0257	0.0020
8/12/91	11.5	13.8	470	-	-	-
9/6/91	8.9	13.8	350	-	-	-

APPENDIX B



Appendix B. Schematic of recirculating system inside each environmental chamber.

APPENDIX C

Table 1. Amino acid composition in Biosponge Bass Grower Feed.

Amino Acid	MMOL/10g Feed
Aspartic	2.48
Threonine	1.06
Serine	1.70
Glutamic	2.94
Proline	2.68
Glycine	2.60
Alanine	2.85
Valine	1.27
Isolucine	0.92
Leucine	2.30
Tyrosine	0.35
Phenylalanine	0.89
Lysine	1.46
Histidine	0.75
Arginine	0.87
Mehionine	0.10
Cystine	0.60

Table 2. Mineral analysis for Biosponge Bass Grower feed.

Mineral	ppm
Manganese	0.79
Copper	0.19
Iron	1.63
Aluminum	1.23
Boron	0.06
Phosphorus	186.70
Potassium	152.60
Calcium	288.30
Magnesium	29.19
Sodium	40.36
Sulfer	59.84
Zinc	3.69

VITA

William Granville Blythe was born March 30, 1967, in St. Louis, Missouri. He graduated from Parkway Central High School in Chesterfield, Missouri in 1985 and received a B.S. Degree in Fisheries at the University of Minnesota, St. Paul, Minnesota in 1989. In August 1989, he became a candidate for the Master of Science degree in Fisheries and Wildlife Sciences (Fisheries Science) at Virgina Polytechnic Institute and State University, Blacksburg, Virgina.