CHANGES IN STEROID HORMONES DURING OOCYTE DEVELOPMENT IN THE STRIPED BASS, *Morone saxatilis*

By

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Abstract

Oocyte development in teleosts has been shown to occur in distinct phases each under separate endocrine control. Estradiol-17β (E_2) of ovarian origin stimulates the synthesis of vitellogenin, the major yolk precursor protein in all nonmammalian vertebrates. The role of other maturation inducing steroids (MIS) in promoting the breakdown of the germinal vesicle and preparing the oocyte for subsequent fertilization has been described for some teleost species. As the synthesis patterns of E_2 and nature of the MIS differ among species, it is of interest to document the circulating levels of reproductive hormones in this commercially important species. Serum levels of E_2, testosterone (T) and 17α-20β-dihydroxyprogesterone (DHP) were measured by radioimmunoassay (RIA) in striped bass, Morone saxatilis, at various times during the annual reproductive cycle. Samples were collected in coastal waters (Rhode Island, spring and fall; Long Island Sound, February), spawning rivers (Hudson River, May, November; Chesapeake Bay, December, March) and a striped bass hatchery (Verplank New York, May). Steroid levels, ovarian lipid content and gonadosomatic indices (GSIs) were correlated with stage of oocyte development as determined by histological analyses.

Levels of all reproductive parameters were low in fish classified immature or in the yolk vesicle stage. Significant increases ($p < 0.05$) in E_2, T, ovarian lipids and GSIs were measured in fish in the vitellogenic stage of development during several sampling periods. Maximum levels of all reproductive parameters were found in prespawning fish sampled in the Hudson River. Mean levels of E_2, T, ovarian lipids and GSIs for these fish were 2037 ± 465 pg/ml, 2981 ± 272 pg/ml, 24 ± 1

mg/g, and 5.6 ± 0.3 %, respectively. Elevated E_2 levels were not always present in fish with vitellogenic stage oocytes sampled several months prior to spawning. Significantly elevated DHP levels (1872 ± 352 pg/ml) were measured in human chorionic gonadotropin-injected fish sampled immediately after artificial spawning. Similar levels were found in two fish captured during the spawning season. This evidence suggests that DHP may serve as the maturation inducing steroid in this species.

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Preface

An understanding of the reproductive events which occur in an organism's life cycle is essential in order to manage wild populations and for efficient propagation in captivity. Efforts to restore declining populations of striped bass, Morone saxatilis, are currently underway by limiting commercial fishing and releasing hatchery reared stocks. In addition, aquaculture facilities are currently marketing striped bass hatched and reared in captivity for consumption. These efforts have been hampered by a lack of information concerning the age at which females become sexually mature and endocrine events which control the reproductive cycle. Investigations of the reproductive events occurring in other teleosts species have provided a framework which has been useful in striped bass hatchery and fishery management.

In mature females of these species, oocyte development occurs in specific stages which are controlled by different steroid hormones. Specifically, estradiol of ovarian origin stimulates hepatic synthesis of vitellogenin, the major yolk precursor protein. Following the vitellogenic phase of development, a maturation inducing steroid (MIS) prepares the oocyte for fertilization by breaking down the germinal vesicle and resuming the first meiotic division. The measurement of these hormones and histological examination of oocyte development have been utilized to determine maturity status, proximity to spawning and incidence of reproductive failure.

The objectives of this study were to correlate changes in circulating levels of steroid hormones with developmental events occurring in the oocytes throughout the reproductive cycle. This allows determination of criteria for assessing the maturity status of

individuals and the construction of a maturity schedule for native populations. The information generated in this study can abet the efforts of hatchery personnel in controlling reproduction in captive striped bass.

This dissertation has been written in manuscript form and will be submitted for publication to Fish Physiology and Biochemistry

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Introducion

The striped bass, Morone saxatilis, is an anadromous teleost indigenous to the Atlantic coast of North America. Spawning occurs in tidal rivers during the spring months primarily during May (Merriman 1941). Following spawning, striped bass undertake a feeding migration to coastal waters (McLaren et al. 1981). The major spawning grounds on the Atlantic coast are tributaries of the Chesapeake Bay, the Hudson River and the Roanoke River in North Carolina (Raney 1952).

Until recently, the striped bass fishery was of great economic importance. However, declines in population numbers and high tissue concentrations of polychlorinated biphenyls necessitated imposing severe restrictions on commercial and recreational fishing (Horn and Skinner 1985). In order to better understand the reasons for this population decline and to preserve existing stocks, extensive research has been conducted on the life history of this species and striped bass hatcheries have been established in several states (Parsons 1974, Rogers and Westin, 1975).

Striped bass, like many teleosts from temperate waters, have an annual reproductive cycle with group synchronous ovarian development (Specker et al. 1987). Several investigations have focused on the dynamics of oocyte growth and factors influencing fecundity (Merriman 1941; Jackson and Tiller 1952; Lewis 1962). In these studies discrepancies exist in the degree of oocyte development observed in fish sampled from different locations. Although the migration patterns are not completely understood, striped bass return to their natal waters to spawn and the time of spawning is related to latitude (Raney, 1952, Nichols and Miller 1967). For instance, in the St. John's River

of Florida spawning may be initiated as early as February but not until July in the St. Lawrence River (Setzler et al. 1980). It is possible that the differences observed in oocyte development reflect the sampling of different populations of fish and their proximity to the spawning grounds.

The endocrine control of reproductive events in striped bass has received little attention. Investigations conducted with other teleost species have shown that oocyte development occurs in distinct phases which are under different endocrine control (Trant and Thomas 1988). Initially, oocytes undergo primary growth which is not under the influence of hormones from the anterior pituitary (Barr 1963). Pituitary dependent, secondary growth is initiated with the synthesis of endogenous yolk or yolk vesicles (Wallace and Selman 1981). Yolk vesicles give rise to cortical alveoli, which have been shown to play an important role in the fertilization process (Yamamoto 1956, Selman and Wallace 1986). The endocrine control of yolk vesicle synthesis is not well understood and the period of vitellogenesis which follows yolk vesicle synthesis has received considerably more research effort.

Vitellogenin is a phospholipoprotein synthesized by the liver which serves as the major precursor for yolk proteins in teleosts and other oviparous vertebrates (Wallace 1978). The sequestering of vitellogenin by the oocytes greatly increases their size and leads to an increase in gonad weight (Dodd 1972). Estradiol-17β (E_2) has been shown to stimulate vitellogenin synthesis and release by the liver in several species of teleosts and amphibians (Ng and Idler 1983). E_2 and testosterone (T) generally increase upon ovarian recrudescence and remain elevated throughout the period of vitellogenin uptake (Pankhurst

and Conroy 1988).

Vitellogenesis is followed by the period of final oocyte maturation, during which time the germinal vesicle breaks down and meiosis resumes (Nagahama 1987). In many species of teleosts, this process is controlled by the maturation inducing steroid (MIS) 17α-20β-dihydroxyprogesterone (DHP)(Young et al. 1983; Nagahama and Adachi 1985; Greeley et al. 1986). Recently, another progestin 17α-20β-21α-trihydroxyprogesterone (THP) has been found to act as the MIS in two species of teleosts, the Atlantic croaker, Micropagonias undulatus and the sea trout, Cynoscion nebulosis (Trant et al. 1986; Trant and Thomas 1986).

The objective of the present study was to monitor seasonal changes of steroid hormones and reproductive events in immature and mature, wild, female striped bass. Specifically, levels of E_2, T and DHP were measured in serum and these levels were related to developmental changes in oocytes and ovarian lipid content.

Materials and Methods

Striped bass were obtained by several methods of capture between November 1984 and May 1988. Sample dates, locations and methods of capture are shown in Table 1.

Freshly caught fish were stunned by a blow to the head and blood was collected by caudal puncture using nonheparinized syringes. Serum was separated by centrifugation and stored at -20 C until it was used for hormone analysis. Body and gonad weights, fish fork length and macroscopic condition of the ovary were recorded for each fish. Gonadosomatic indices were calculated as gonad weight/body weight X 100. Striped bass sampled at the Verplank Hatchery (Verplank, N.Y.)

were injected with human chorionic gonadotropin (HCG) at a dose of 150 I.U./kg body weight upon capture. Beginning 20 h after hormone injection, ova were collected from the urogenital pore using a 3.0 mm O.D. glass catheter. Oocytes were classified according to the criteria established by Stevens (1966), and fully developed oocytes were expelled by exerting mechanical pressure on the abdomen of the animal for artificial fertilization. Following expulsion of the eggs, blood samples were collected as described above.

Ovarian tissue for histological analysis was removed from the midlength of the ovary, fixed in Bouin's solution, dehydrated in an ethanol series and cleared in methyl salicylate prior to paraffin infiltration and embedment. The tissue was sectioned at 10 μm and stained with hematoxylin and eosin. In order to detect the presence of carbohydrates, specifically acid mucopolysaccharides, tissues were stained with alcian blue and metanil yellow according to the procedures of Humason (1979).

Sections of ovarian tissue were viewed and photographed with a Nikon S-U microscope equipped with a M-35 camera. Micrograph slides were projected onto a Scriptel glass digitizing tablet and the diameters of at least 50 oocytes were calculated using a sigma scan digitizing program. Oocytes were selected randomly, but only oocytes which had been sectioned through the nucleus were measured. This ensured both primary and secondary oocytes, if present, were measured. Oocytes were classified according to developmental stage using the criteria established by Wallace and Selman (1981), Specker et al. (1987) and Tam et al. (1987). Briefly, primary growth oocytes were small (150 μm) and contained ooplasm which stained uniformly with

hematoxyln. Oocytes in the early yolk vesicle (cortical alveoli) stage were characterized by the presence of opaque spheres in the periphery of the ooplasm. These spheres increased in number and filled the ooplasm as the yolk vesicle stage progressed. Oocytes in the most advanced stages of yolk vesicle synthesis attained diameters of approximately 300 μm. Oocytes in the early vitellogenic stage attained diameters of approximately 350 μm and were characterized by the presence of yolk globules in addition to yolk vesicles.

Ovarian lipid content

Ovarian lipid levels were determined for fish in the immature, yolk vesicle and vitellogenic stages of development. Fragments of ovarian tissue weighing 1.0 g were removed from the midlength of the ovary. The fragment was then minced with scissors and homogenized with a Polytron tissue homogenizer (force 6) following the addition of 5 ml of tap water. Lipids were extracted by adding 10.0 ml ethyl ether (Fisher, reagent grade) and vortexing at high speed for 20 seconds. The ether carrying the ovarian lipids was decanted into 16x125 mm glass tubes which had been previously weighed. The ether was evaporated with air and the procedure following homogenization was repeated twice. Lipid levels were calculated as the final weight of the glass tubes minus the initial weight. Values were expressed as mg/g of ovary.

Radioimmunoassay

E_2, T and DHP were measured in sera by RIA following ether extraction. Steroids were extracted from serum (200 μl) twice with ethyl ether (Fisher, reagent grade), dried under a stream of nitrogen and resuspended in 200 μl phosphate buffer containing 10% bovine serum albumin (Sigma Chemical Co., St louis) and 0.1% sodium azide (assay

buffer). Extraction efficiency as determined by recovery of tritiated steroids extracted from plasma was at least 90% for all steroids.

Antisera to E2 and T were purchased from Endocrine Sciences (Tarzana, California) and the antisera to DHP was a gift from Dr. Y. Nagahama. Radiolabelled [2,4,6,7 H^3 (N)] E2 and [1,2,6,7, H^3 (N)] T were purchased from New England Nuclear (Boston, Mass.) and radiolabelled 17α-hydroxyprogesterone was purchased from Amersham International (Arlington Height, Ill.). DHP was prepared from 17α-hydroxyprogesterone by enzymatic conversion as described by Scott et al. (1982) and separated from the parent compound by thin layer chromatography (Vanderkraak et al. 1984).

E_2, T and DHP were evaluated by validated RIA using the methods of Kagawa et al. (1981). In all assays standard concentrations of steroids ranged from 3-1280 pg/tube and were reconstituted in 200 µl of assay buffer. Standards and samples were incubated with 3600-4000 counts per minute (100 µl) of tritiated steroid and 200 µl of dilute antibody (approximate dilution 1:62,000) for 3 h at room temperature or at 4°C overnight. No differences were detected between assays when incubation characteristics were varied in this manner. Bound steroids were separated from free steroids by the addition of 500 µl dextran-coated charcoal (2.5% Norit containing 0.25% charcoal, Sigma Chemical Co., St Louis, Mo.). Assay tubes were centrifuged at 2000 x g for 10 minutes. Five hundred microliters of the resulting supernatant was suspended in 5.0 ml scintillation fluid (Budget Solve, Research Products International, Mount Prospect, Ill.). All samples were assayed in duplicate and counted on a Beckman LS-1801 scintillation counter.

Parallelism

Parallelism was determined by measurements of aliquots of different volumes of the same plasma pools. A linear response which was parallel to the standard curves was obtained over a range of 50-200 µl.

Recovery

Authentic steroids in three different concentrations were added to serum pools of known steroid concentrations. Recoveries greater than 90% of expected concentrations were obtained for the three steroids assayed.

Sensitivity

The sensitivities of the standard curves, taken as the smallest dose of standard which could be distinguished reliably from the buffer blank with 95% confidence limits, were 15, 30 and 30 pg/ml for E_2, T, and DHP, respectively.

Precision

The precision of the assays was determined by calculating the interassay and intrassay coefficients of variation. The interassay coefficient of variations for E_2 (n=6), T (n=8) and DHP (n=6) were 7, 12 and 13 % respectively. The intraassay coefficients of variations (n= 10 for all) were 3, 9 and 11% respectively.

Specificity

The cross-reactivities of the E_2 and T antibodies were specified by the manufacturer. The antibody for E_2 cross-reacted 1.3% with estrone and 0.6% with estriol. Cross-reactivity of this antibody was less than 0.1% with all other steroids tested. Testosterone antibody cross-reacted 20% with dihydrotestosterone, 3.6% with

delta-1-dihydrotestosterone and 52% with delta-1-testosterone. These cross-reactivity estimates were calculated at a level of 50% bound testosterone. The antibody to DHP cross-reacted 2.5% with 17α-20β-dihydroxy-5β-pregnane-3-one. Cross-reactivity was less than 1% for all other steroids tested (Young et al. 1983).

Statistics

The results are expressed as mean ± SE, with the number of samples (n) in parentheses. Differences between means were tested by analysis of variance followed by Tukey's HSD multiple comparison test (Sokal and Rolf 1981). Significance was accepted for all tests at $p < 0.05$.

Results

Reproductive characteristics of the fish sampled throughout the year are summarized by reproductive stages. Figure 1 shows photomicrographs of each developmental stage. Figures 2-4 illustrate the differences in reproductive parameters between immature and mature fish. Changes in E_2 (Fig. 5), T (Fig. 6), DHP (Fig. 7) ovarian lipids (Fig. 8) and gonadosomatic indices (Fig. 9) are shown first for immature fish and then for fish in progressively more advanced stages. A summary of the characteristics of the fish sampled at each location are shown in Table 2. Figure 10 illustrates the proportion of fish in each developmental stage used in this study.

Oocyte Stages

Figures 1 illustrates various stages of oocyte development. Primary growth oocytes had a maximum diameter of 150 μm with uniformly staining ooplasm (Fig 1a.). Fish with only primary growth oocytes were classified as immature while those with both primary and secondary growth were classified as mature. As fish initiated secondary growth

and entered the yolk vesicle stage, the ooplasm no longer stained uniformly due to the synthesis of yolk vesicles (Fig.1b,c). Oocytes that stained positively with alcian blue had diameters that were greater than approximately 300 µl.

Oocytes which had entered the vitellogenic stage of development were also characterized by the presence of yolk globules. These appeared as spheres located initially around the periphery of the ooplasm which stained with metanil yellow. As the vitellogenic phase progressed (oocyte diameters 500 µm) these yolk globules were found throughout the ooplasm, increased in size and finally fused into large polygonal masses (Fig.1d). The highly developed oocytes sampled from fish during the spawning season (oocyte diameters 0.8-1.0 mm) were difficult to section in some cases, as the ooplasm became brittle and porous during fixation. The resulting loss of histological details did not permit distinction between oocytes in the final maturation stage and those in the terminal stages of vitellogenesis. Fish which had recently spawned could be identified by the presence of atretic oocytes (Fig. 1e). These oocytes were of ovulatory size (1.0 mm), but no nuclei were present in serial sections, indicating degradation had begun. These follicles could be distinguished from those in the final maturation stage of development which had undergone germinal vesicle breakdown, as the latter still retained yolk globules and remainders of nuclear material.

Immature

Fish classified as immature and in the yolk vesicle stage were observed throughout the year except on the spawning grounds during the spawning season. Levels of E_2 and T were low (< 100 pg) throughout

the year. Immature fish sampled in Maryland during December had elevated levels of E_2 (46 ± 5 pg/ml, n = 11) relative to fish sampled in R.I waters during the spring (26 ± 3 pg/ml, n = 51) or fall (20 ± 2 pg/ml, n = 5). No differences were found in T or ovarian lipid levels between sampling dates or locations. Mean T and ovarian lipid levels were 68 ± 11 pg/ml (n = 29) and 1.0 ± 0.6 mg/g (n = 17), respectively. Immature fish sampled from Maryland during December had lower GSIs (0.2 ± 0.0%, n = 12) than fish sampled at other times of the year (0.3 ± 0.2 %, n = 107).

Yolk vesicle stage

The reproductive parameters measured for fish in the yolk vesicle stage were low throughout the year in all sampling locations. E_2, T and ovarian lipid content averaged 33 ± 6 pg/ml (n = 19), 81 ± 14 pg/ml (n = 30) and 1.5 ± 0.3 mg/g (n = 68), respectively. No differences were found between these values and those measured in immature fish at any sampling period. Mean GSI level was less than 1.0% for all sampling locations except Maryland, December had a level of 1.2 ± 0.4 % (n = 3). Significant differences were found between the GSIs measured in these fish and those sampled in Rhode Island waters during the spring months (0.6 ± 0.0, n = 26).

Vitellogenesis

Striped bass sampled on the spawning grounds during the spring months demonstrated the highest degree of reproductive development. E_2 levels of striped bass sampled in the Hudson River (May) and Maryland (March) averaged 2220 ± 318 pg/ml (n = 41) and 2037 ± 465 pg/ml (n = 6), respectively. Statistical differences were observed for E_2 between these periods and all other sampling periods (364 ± 83

pg/ml, n = 31). E_2 levels measured in fish sampled at all locations except Maryland, December, were different from those measured in immature and yolk vesicle stage fish. Testosterone production paralleled that of E_2 throughout the year. A mean level of 2981 ± 272 pg/ml (n = 26) was recorded for fish sampled on the Hudson River spawning grounds during May. This level differed from levels measured at all other locations (102 ± 14 pg/ml, n = 17). GSIs for vitellogenic fish were always greater than 1.0% and a maximum of 10.2% was recorded for one fish. Striped bass sampled on the Hudson River spawning grounds had GSIs which averaged 5.6 ± 0.3% (n = 41) and were significantly different from those sampled at other locations (3.0 ± 0.3%, n = 47)). GSIs from vitellogenic fish were different from fish in the immature and yolk vesicle stages throughout the year. Ovarian lipid levels for fish in the vitellogenic stage were different in all sampling periods from those in the immature and yolk vesicle stages. Ovarian lipid levels from fish sampled during the spring months in Rhode Island (22 mg/g ± 4, n =10) and from the Hudson River spawning grounds (24.0 ± 0.9 mg/g, n = 6) were different from those in Maryland in December (11.0 ± 0.5 mg/g, n = 5).

Final Maturation

Striped bass sampled at the Verplank Hatchery had E_2, T and DHP levels of 86 ± 11 pg/ml (n = 10), 61 ± 9.3 pg/ml (n = 10) and 1872 ± 352 pg/ml (n = 11), respectively. DHP levels were low or nondetectable in fish sampled in other developmental stages. Two fish sampled in the Hudson River during the spawning season 1986 had DHP levels which were not different from those sampled at the Verplank Hatchery. These fish were judged, a posteriori, to be in the final maturation stage.

Summary of Results

Striped bass which were immature had low E_2 and T levels (100 pg/ml), GSIs (1.0%) and ovarian lipid levels (2 mg/g) (see Figures 2, 3 and 4). No differences were found in reproductive parameters between immature fish and those in the yolk vesicle stage of development. Elevated E_2, T, ovarian lipid levels and GSIs were observed in fish in the vitellogenic stage. Maximum levels of all reproductive characteristics were found in prespawning fish sampled in the Hudson River. Mean levels of E_2, T, ovarian lipids and GSIs for these fish were 2037 ± 465 pg/ml, 2981 ± 272 pg/ml, 24 ± 1.0 mg/g, and 5.6 ± 0.3% respectively. Striped bass in the final maturation stage had elevated levels of DHP. The mean DHP level in HCG injected fish sampled at the Verplank hatchery was 1872 ± 352 pg/ml.

Discussion

This study describes the endocrine changes which occur during the reproductive stages of the female striped bass. Mature striped bass sampled in coastal waters, soon after returning from their spawning migration, were undergoing gonadal recrudescense. These fish were primarily in the yolk vesicle stage of development. Vitellogenesis is initiated during the late fall (November) and winter months (December-February). Upon returning to the spawning areas during the spring, oocytes had grown to near their maximum size. The transition into the final maturation stage of development occurs very close to the time of spawning. These aspects of reproductive development in wild populations of striped bass are described and compared to reported changes occurring in other teleost species.

Steroid levels, GSIs and ovarian lipid levels were low in immature

and yolk vesicle stage fish throughout the year. This is in agreement with findings in other teleost species (Wingfield and Crim 1977, Pankhurst and Conroy 1987). Several investigators have interpreted the appearance of yolk vesicles in the oocytes of striped bass to mean that these fish are mature and will spawn in the next spawning season (Merriman 1941; Lewis 1962). However, Chadwick (1965) suggested that yolk vesicle synthesis may be initiated more than one year prior to spawning. The present findings can be interpreted to support Chadwick's idea. In striped bass, the major part of oocyte growth occurs during the winter and early spring months (Scofield 1931; Lewis 1962). Fish with yolk vesicle stage oocytes as the most advanced clutch present were sampled in the Hudson River in April. The largest oocyte diameters observed in these fish were 250 μm, and it is unlikely development could have proceeded to the extent necessary for ovulation to occur within a 2 or 3 week period. In addition, the ovaries of fish sampled on the Hudson River spawning grounds during the spawning season, contain a population of yolk vesicle stage oocytes in addition to the clutch of oocytes about to be ovulated. Yolk vesicle synthesis can occur more than a year in advance of spawning in rainbow trout (Bromage and Cumaranatunga 1987), brook trout (Tam et al. 1987) and white sturgeon (Chapman et al. 1987).

Estradiol stimulates the synthesis of vitellogenin and elevated plasma levels of E_2 are generally detected during the vitellogenic stage in teleosts (Wiegand 1982); although secretory patterns and absolute concentrations of estradiol vary greatly between species (Pankhurst and Conroy 1988). Elevated ovarian lipid levels and high GSIs were found in all fish with vitellogenic stage oocytes throughout

the year. Elevated steroid levels were not always associated with vitellogenic stage oocytes, however. Conceivably, low E_2 levels may be sufficient to stimulate early hepatic synthesis of vitellogenin in this species. Elevated plasma levels of E_2 were also not observed in bluefish throughout the vitellogenic period (Macgregor et al. 1981) or in blue cod in the early stages of vitellogenesis (Pankhurst and Conroy 1987). Alternatively, E_2 levels may initially increase with the onset of vitellogenesis, drop to basal levels and then increase prior to spawning. The low E_2 levels observed during the winter and early spring months may represent sampling in the trough between the two hormone peaks. A bimodal E_2 secretion pattern such as this was observed in the reproductive cycle of the brown bullhead (Burke et al. 1984).

The most advanced stages of vitellogenesis were observed in fish sampled in the Hudson River during the spawning season. However, striped bass with well developed vitellogenic oocytes were also observed during the fall and spring months in coastal Rhode Island waters. The initiation and duration of vitellogenin synthesis may be influenced by specific environmental and physiological factors. Sampling different stocks of fish during their spawning migration may also have contributed to the variability in oocyte development observed.

Fish with very large (0.6 mm) vitellogenic oocytes were observed in R.I. coastal waters during June in 1986 and 1987. As spawning occurred during the month of May during those years, these fish either failed to spawn that season or were preparing to spawn in a location other than the major spawning areas. It has been suggested that

striped bass spawn in rivers along the Atlantic coast other than those associated with the major spawning areas (Raney, 1952). In addition, populations do spawn in river systems in Canada (Leim and Scott 1968).

Vitellogenesis is initiated earlier in large coastal migrant fish than in smaller fish. Striped bass sampled in R.I during November were of two distinct populations. Large fish (> 12 kg) were sampled from waters near Block Island. These fish, with only one exception, were in the vitellogenic stage of development. One large (16 kg) fish sampled at that time was in the advanced yolk vesicle stage of development. Small fish (< 5 kg) were sampled at this time of year along the coast at Pt. Judith, Rhode Island. These fish were immature or in the yolk vesicle stage of development. Greeley et al. (1987) also observed the influence of fish size on ovarian development in striped mullet. Fish > 32 cm were found to enter vitellogenesis two months earlier than smaller fish in this study.

The highest E_2 levels observed in striped bass were approximately 3 ng/ml and were observed in prespawning fish. This is at the lower end of the spectrum of values reported for other species, but is similar to plasma levels reported in the striped mullet (Dindo and Macgregor 1981) and the king mackeral (Macgregor et al. 1981). Estradiol levels reported for various species show a wide range with maximum values of less than 1 ng/ml found in the orange roughy, blue cod and bluefish (Pankhurst and Conroy 1988; Pankhurst and Conroy 1987; Macgregor et al. 1981) to over 50 ng/ml in some salmonid species such as brook trout and rainbow trout (Scott et al. 1980; Tam et al. 1986). This variation between species in steroid concentrations may be due in part to differences in sampling procedure. The stress of capture has

been shown to significantly decrease plasma levels of reproductive steroids in the brown trout, Salmo trutta, (Pickering et al. 1987) and dogfish, Scyciorhinus canicula (Jenkins and Dodd 1982). Furthermore, Safford and Thomas (1987) demonstrated that different methods of capture could influence gonadal steroid levels to different degrees. In the present study, no statistical differences were found between testosterone levels of male fish captured by haul seine or gill net during a period of active steroidogenesis (see appendix B5). A great deal of variation was observed in steriod levels of fish captured by both methods. This was also found to be true of estradiol and testosterone levels in female fish captured near the time of spawning.

Testosterone serves as a precursor for estradiol synthesis and may also have additional unrelated functions in teleosts (Scott et al. 1984). Testosterone and E_2 levels typically fall during the final maturation stage as the shift in steroidogenesis favors the production of DHP (Scott et al. 1984; Pankhurst and Conroy 1987). The shift is believed to result from a gonadotropin surge (Scott et al. 1983;). Striped bass also demonstrate this sequential pattern of steroid hormone synthesis. HCG-injected fish which had completed final maturation and spawned had higher DHP and lower E_2 and T levels than fish in the advanced stages of vitellogenesis. A similar hormone profile was observed in two fish sampled on the Hudson River spawning grounds during the spawning season. These fish were believed to be in the final maturation stage based on steroid hormone profiles. The drop in testosterone production does not appear to be a prerequisite for final maturation however, since Fitzpatrick et al. (1986) found that T and 11-keto-testosterone levels remained elevated throughout the final

maturation period in coho salmon.

Gonadotropin levels peak shortly before spawning in many teleost species (Peter 1981). Evidence suggests that the gonadotropin surge is required for the shift in steroidogenesis necessary for DHP production (Scott et al. 1982). DHP levels have been measured in captive striped bass during the final maturation period (Thomas 1988); however, it remains unclear specifically how far in advance of spawning DHP levels rise. As only 2 of 41 fish sampled during the spawning season showed elevated DHP levels, it appears the shift in steroidogenesis towards DHP production occurs very close to the time of actual spawning.

Histological observations of ovarian samples of fish captured on the spawning grounds were not sufficient to determine unequivocally the proximity to spawning or whether germinal vesicle breakdown had occurred. Oocytes from fresh ovarian tissue were collected from prespawning fish sampled on the Hudson River spawning grounds during May, 1987. These oocytes were classified according to the methods of Stevens (1966) in order to determine their relative proximaty to spawning. This staging system was established to predict the number of hours anticipated until ovulation in striped bass which had previously been injected with HCG. Although the samples analysed were obtained from fish which had not been injected with HCG, this staging system did suggest the relative degree of oocyte development. The most advanced stage observed at this time was equivalent to 10 hours before ovulation. No correlation was found, however, between steroid hormone levels and proximity to spawning as determined by this staging system. Without exogenous gonadotropin administration, spawning of captive striped bass is difficult in hatcheries (Stevens 1966). Stress of

capture has been shown to reduce gonadotropin to basal levels in white suckers (Stacey et al. 1984) and induce follicular atresia in pike (de Montalembert et al. 1971). The stress associated with capture near the time of spawning may prevent the gonadotropin surge necesssary for DHP production and successful ovulation.

The results of this study provide hormone profiles which accompany oocyte development during the reproductive cycle of the striped bass. Fish which were immature and in the yolk vesicle stage of development had low levels of steroid hormones and low GSIs. Estradiol levels were elevated in vitellogenic fish and reached peak levels during the prespawning period. During the periovulatory period E_2 levels declined as steroid pathways shifted toward DHP production. Further research is necessary in order to determine specifically when yolk vesicle synthesis is initiated and the interval between their synthesis and spawning. In addition, with more frequent sampling prior to spawning it may be possible to determine when the shift in steroidogenesis resulting in DHP production occurs.

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