Health and Reproduction of White Perch *Morone americana* from the Hackensack Meadowlands, NJ

Cathy Czerwinski
Department of Biological Sciences
Rutgers University
Newark, NJ 07102

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INTRODUCTION

White perch *Morone americana* are common fish from the Chesapeake Bay to the Hudson River, NY (Thoits, 1958; Bath and O'Connor, 1982). They are typically found in large numbers (Kasul et al. 1992) and have been steadily gaining support as a model to study reproduction in temperate basses (Jackson and Sullivan, 1995). The white perch is a multiple clutch, group-synchronous spawner with gonadal development similar to the congeneric striped bass *M. saxatilis* (Jackson and Sullivan, 1995).

The Hackensack River (Ha), NJ is located in a heavily populated and industrialized region and most of the discharge into the river stems from industrial and commercial sources. Contaminants at this site include metals (As, Cu, Hg, Ni, Pb, and Zn), PAHs, PCBs, chlordane, and thermal discharge (Ayers et al. 2000). In addition to point sources are chemical spills, landfills, and hazardous waste disposal sites. In a major comprehensive survey, Ayers et al. (2000) found that although water quality has improved since the 1970s, fish and invertebrate communities are still greatly impaired in urban areas along the river.

Fish health and reproduction are dependent upon external parameters such as water temperature, photoperiod, and contaminants (Stott et al. 1981; Wannemacher et al. 1992; Kime 1998; Gushiken et al. 2002; Watanuki et al. 2003). Fish reproduction is an ecologically relevant indicator of environmental degradation and endocrine disruption. Gonadotrophin releasing hormone (GnRH) is released from the hypothalamus in response to favorable environmental cues such as photoperiod and water temperature. GnRH acts on the pituitary to release gonadotrophin (GtH). In female teleosts GtH stimulates testosterone (T) production by thecal (outer follicle cells). T is converted to 17β-estradiol (E2) in the granulosa cells (inner follicle cells). E_2 then is transported to the liver where it binds and induces hepatic formation of vitellogenin (Vg), the egg yolk precursor protein. Vg is transported to the ovary by way of blood and is incorporated into the growing oocyte. Ovarian Vg uptake is the main source of oocyte growth and increase in ovarian weight (Nagahama 1983; Nagahama et al. 1993; Kime 1998). The actual physiological role of T is currently unknown (Kime 1998). Due to the numerous organs and processes involved with reproduction, this is a complex function. Reproduction involves the formation of gametes, actual release/spawning of eggs, fertilization,

embryonic development, and hatching and larval development (Heath 1995). Exposure of fish to adverse environmental conditions, including exposure to contaminants, can induce changes at any point along the hypothalamic-pituitary-gonadal (-liver) axis or hypothalamic-pituitary-interrenal axis, and thereby have the potential to impact reproductive and immune processes. A wide range of contaminants from industry and agriculture have been found to alter the functioning of the endocrine system ("endocrine disruption"), causing feminization of males, masculinization of females, and altering fertility, fecundity, gonadal size, etc. (Kime, 1998). White perch from areas of Lake Ontario have been shown to have indications of endocrine disruption, in that in some cases, >50% of the males had intersex gonads (Kavanagh et al, 2004).

It is of great interest to see if contaminants have any impact on the reproductive cycle, endocrines, or general health of white perch from the Hackensack Meadowlands. Parameters examined include gonad stage, organosomatic indices (ratio of liver or gonad weight to body weight), and the stress hormone cortisol, in addition to a health assessment during which fish weight and total length were recorded, along with the presence of histopathologies. Data collections were attempted monthly for one year.

METHODS

Sample Collection

As part of the MERI fisheries survey, white perch were collected by trap net set 24 hours prior to collection or otter trawl. Monthly sampling for an entire year was attempted beginning July 2001. No fish were obtained in January or in February. Fish were placed in MS-222 and weight and total length were recorded along with presence/absence of parasites, lesions, or abnormalities. Blood was removed from the caudal sinus with heparinized syringes, transferred to heparinized vacutainers, and kept on ice until return to the lab, at which point plasma was spun off at 10,000g for 15 minutes at 4°C. Scales were removed from the area of the appressed pectoral fin for ageing. Livers, spleens, and gonads were excised and livers and gonads weighed prior to fixation in 10% phosphate buffered formalin. Gonosomatic index (GSI) and hepatosomatic indices (HSI) were calculated by dividing the organ weight over body

weight and multiplying by 100. GSI and HSI are missing for part of Ha July 2001 due to scale malfunction.

HISTOLOGY

Tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E). The slides were viewed to confirm sex, obtain gonad stage of development, look for evidence of intersex, and note the presence/absence of abnormalities such as parasites and hyperplasia in the gonads, liver, and spleen. Stages of female gonadal development were based in those previously described by Jackson and Sullivan (1995) and Blazer (2002) and are summarized in Table 1 and Figures 1 and 2.

During staging of ovaries, 100 oocytes were viewed microscopically and characterized based on morphology. As white perch have asynchronous ovaries, the numbers of oocytes at each stage were counted, and the percent of each stage calculated. Stage 0 oocytes are immature, previtellogenic oocytes undergoing primary growth. They are basophilic with a large central nucleus and nucleoli. Oocytes at stage 1, the cortical alveoli stage, contain remnants of lipid droplets at the periphery of the oocyte. Stage 2 oocytes coincide with early stages of vitellogenesis. Yolk globules appear at the periphery of the oocyte indicating that vitellogenin is being incorporated into the oocyte. Stage 3 oocytes are in late stages of vitellogenesis. These oocytes have a well-developed follicle layer and chorion, with large yolk globules and lipid droplets in the ooplasm which appear as vacuoles when stained with hematoxylin and eosin (H&E). Stage 4 oocytes are mature and undergoing lipid droplet coalescence. The germinal vesicle (nucleus) migrates to the periphery indicating the resumption of meiosis. Post-ovulatory follicles, stage 5, are follicle cells that remain in the ovary after spawning has occurred. Atretic follicles are those that are being resorbed. They can be characterized by the presence of phagocytic follicle cells within the ooplasm and collapsing of the follicles.

Staging of the testes occurred by looking at the presence and relative quantity of spermatocytes, spermatids, and spermatozoa. The presence or absence of spermatogonia, spermatocytes, and spermatozoa in testes indicates the stage of development (Groman 1982; Blazer 2002). Testes characterized as stage 1 are pre-spermatogenic; they are immature and undeveloped, or regressed. They contain spermatogonia and possibly

residual sperm within lobules. Stage 2 testes are early spermatogenic and predominantly contain spermatocytes and spermatids. Mid spermatogenic testes, stage 3, contain approximately equal amounts of spermatocytes, spermatids, and spermatozoa. Stage 4, late spermatogenic testes contain spermatozoa almost entirely. Spawning/Post spawning testes, stage 5, are those of males that have spawned, or are in the process of spawning. Lobules will be empty or contain a reduced number of sperm.

While staging the gonads, pigmented cell foci (PCF) resembling macrophage aggregates (MA) were observed (Figure 3). Gonad slides were stained with Perl's stain for iron (Luna 1992) to make viewing of these foci more easily visible. With Perl's stain MA appear black (melanin), blue (hemosiderin), or yellow-brown (ceroid/lipofuscin), and PCF appear blue-green or yellow-brown (Figure 3A). Image-Pro Express (Media Cybernetics) imaging software was used to quantify and measure area of foci in gonads and spleens in ten random fields of view at 20X. Aggregates less than 50 μm^2, or the approximate size of three aggregated macrophages (Fournie et al. 2001), were ignored. Statistical analyses were performed to determine differences in quantity and size of these phagocytic foci between populations, gender, size, on a seasonal basis, as well as to compare gonadal foci with splenic MA.

Cortisol

Plasma cortisol was quantified using a competitive enzyme-linked immunosorbent assay (ELISA). Ninety-six well plates were coated with 50 µL of anti-cortisol monoclonal antibody (α-cortisol mAb, EastCoastBio, ME) at 1 µg per well, then incubated at 37°C for 1 hr. Plates were washed 3X with Dulbecco's Phosphate buffered saline (DPBS, Sigma) using a plate washer (Molecular Devices) and blocked for 1 hr with 300 µL of blocking buffer (0.5 g bovine serum albumin (BSA) in 100 mL DPBS). Plasma samples were added to washed plates, and 1:10,000 dilution of cortisol-HRPO conjugate added. Plates were incubated overnight at 37°C, and washed 5X with DPBS. One hundred µL developing solution (1%ABTS [diammonium salt] in citrate buffer with 30% hydrogen peroxide) was added to each well, and plates read at 405 nm after 40 min. Samples were analyzed in quadruplicate.

STATISTICS

Statistical analyses were performed using JMP-In analytical software (SAS Institutes) and results considered significant when $P \leq 0.05$. Differences in results between sexes were analyzed using t-tests, and site and monthly differences were analyzed using ANOVA. When significant differences were seen, Tukey-Kramer tests were run in order to discern which months or sites were different. Effects of age and size were analyzed using linear regressions. Multiple regressions of the various parameters of the fish with muscle concentration of various contaminants in individual fish (analyzed in the MERI fisheries study by MERI and P. Weis) were also run.

RESULTS & DISCUSSION

EXTERNAL PARAMETERS

The age of white perch from the Hackensack River ranged from 1 to 6 years, with a mean of 3 years. An ANOVA showed no significant difference in age between males and females (P=0.34). They averaged 232±3 mm in length with a range from 124 mm to 278 mm. Weights ranged from 104-380 g, with a mean weight of 205±7 g.

Linear regressions of data from female white perch from the Hackensack River showed significant positive correlations of fish age on length (r^2=0.28, P<0.05) and weight (r^2=0.36, P<0.01). However, there were no significant correlations between age and GSI or HSI (P>0.05). Regressions showed that there was a positive, though weak, correlation between age and GSI (r^2=0.17, P<0.01). Among the males, linear regressions revealed significant positive correlations between age and HSI (r^2=0.14, P<0.05). Though not significant, there was a positive correlation between age and GSI (r^2=0.11, P=0.07).

Ectoparasites seen on white perch included *Lernaea sp.* (Copepoda). Approximately 8% of fish were affected, and there was no significant association of gender on infestation (df=1, χ^2=2.43, P=0.12) (Figure 5). However, there was a significant effect of sampling location on percent of fish infested (df=11, χ^2=58.34, P<0.001). Eight out of eight fish from TN1 were infested, one out of three was infested from GN2, and three out of 16 fish were infested from TN2. These two nets were in more saline areas of the river, suggesting that salinity plays a role in parasite incidence.

Other ectoparasites seen were leeches and an isopod (unidentified spp.). There was a significant association between sampling location and parasite abundance (df=11, χ^2=25.77, P=0.007). Infested fish were seen at site S2, TN2, TN4, TN5, and T5 (Figure 5). Thirteen percent of all fish sampled had leeches or isopods on their body surface.

ORGANOSOMATIC INDICES

Hepatosomatic index (HSI) varied seasonally (Figure 6). In females, HSI was at basal levels in July (1.65± 0.17) and gradually rose through December (3.11± 0.19). HSI remained at this maximum through March, after which there was a gradual decrease to basal levels in June (1.88± 0.25). HSI was negatively correlated with length (r2=0.13, P=0.002).

In males, HSI followed a similar trend as that seen in females; however, they appeared to have a biphasic pattern of increase (Figure 6). Basal levels were seen in July (1.33± 0.28), and HSI rose through October (1.95± 0.3) and held steady through November. There was another gradual increase through March (3.28± 0.2), during which time maximum HSI was recorded. HSI then decreased through June (1.67± 0.19). There was no correlation between HSI and length (r2=0.057, P=0.06). Maximum HSI in males and females was significantly greater than levels seen in summer and autumn (P<0.0001).

Seasonal variations were also seen in gonadosomatic index (GSI) (Figure 6). In females, GSI was at basal levels in July (1.25± 0.89), rose through November (3.18± 0.75), and decreased in December (1.21± 0.6). Another increase occurred through the maximum in April (10.66± 1.15), after which there was a gradual decrease through June. Maximum GSI in April was significantly greater than values for autumn and winter (P<0.0001). There was no significant correlation between HSI and GSI (r^2=0.16, P=0.19), and a weak correlation of GSI with length (r2=0.07, P=0.024).

In males, GSI was at basal levels in August (0.22± 0.72) and rose through November (3.01± 1.01) (Figure 6). There was a decrease seen in December (0.52± 0.62) and an increase through May (8.05± 0.51). After this maximum, GSI decreased through June (5.52± 0.49). The maximum GSI was significantly greater than all other months except April. Though not significant, there is a weak correlation between HSI and GSI

(r^2=0.22, p=0.08), and significant positive correlation between GSI and length (r2=0.11, p=0.009).

Increases in HSI are associated with increased protein synthesis, including synthesis of vitellogenin (Vg), used in yolk biosynthesis. However, HSI may also reflect synthesis of metallothioneins, detoxification enzymes, or other proteins (Heath 1995), or even hepatic accumulation of metals as previously described in white perch (Frazier 1984; Bunton et al. 1987). As Vg is a hepatically synthesized protein, increased HSI corresponds with increased circulating Vg. Incorporation of Vg into oocytes results in increased GSI. It is therefore expected that there will be a correlation between GSI and HSI. Though not significant, there was a strong positive trend. There are no known data on seasonal changes in HSI for white perch to compare these results to.

GSI is a measure of reproductive readiness. During gamete production and maturation there is typically an increase in GSI through the spawning season, with a subsequent decrease to basal levels post-spawn (Kime 1998). This reflects what was seen in this study. Maximum GSI in April-May is thought to correspond to spawning.

Studies by Jackson and Sullivan (Jackson and Sullivan 1995) showed a biphasic increase in GSI of male and female white perch with maximum occurring in April and a subsequent decline in May. In this study, GSI increased from basal levels in November with a second major increase to maximum GSI in April. This biphasic pattern of increase has also been seen in male white perch (Jackson and Sullivan 1995). Ha males exhibited the first increase in GSI in September and October, with the second increase in April.

Gonad Stages

In developing ovaries there was an inverse relationship between the percentage of oocytes at stage 0 and those at stages 1, 2, and 3 (Figure 7). During the expected breeding season in spring, ovaries contained oocytes at all stages of development. Stage 0 oocytes occurred in greatest quantities August through September, and the percentage of oocytes at this stage decreased through May. The number of oocytes at stage 1 increased from basal levels in August and September to maximum number of stage 1 oocytes in May. The same pattern was seen for oocytes at stage 2. Stage 3 oocytes began to appear in November, and were at their highest concentration through April. A

high percentage of oocytes at this stage remained through June. Stage 4 oocytes were not seen until June. Stage 5, post-ovulatory follicles were at their maximum concentration in June 2001 and again in the summer of 2002 after the spawning period was thought to occur. As would be expected, the number of atretic follicles increased April through June, with a maximum occurring in May.

Spearman correlations were performed to determine correlations between oocyte stages of development with other stages, and with GSI and HSI. The numbers of oocytes at stage 0 were significantly negatively correlated with the number of oocytes at all other stages ($P<0.05$). Also, there were significant negative correlations between the number of oocytes at stage 0 and GSI and HSI ($P<0.05$). Stage 1 oocytes were significantly positively correlated with oocytes at stages 2-4, and both GSI and HSI ($P<0.05$). There were positive correlations between the number of oocytes at stage 2 and stages 1, 3, and 4, and GSI and HSI. Oocytes at stage 3 were positively correlated to oocytes at stage 1, 2, 4, and 5, and GSI and HSI. Stage 4 oocytes were positively correlated with those at stages 1-6, and GSI. Stage 5 oocytes were positively correlated to those at stages 3 and 4, length, body weight, and GSI. Oocytes at stage 6 were positively correlated with those at stage 4 and 5 ($P \leq 0.05$).

All males captured July through September were sexually immature (Stage 1) (Figure 8). Testes contained only spermatogonia. In October, approximately 60% of males were sexually immature, with the remaining males undergoing sexual maturation as seen by development of spermatocytes and spermatids (stages 2 and 3). Testicular development continued through April, during which time Stage 4 males (late spermatogenic stage, testes contained spermatozoa) were seen. The differences in testicular development corresponds significantly with month ($DF=27$, $\chi^2=50.25$, $P=0.0043$). Sexually immature males were most abundant in July and August of 2001, and June and July 2002. The greatest numbers of sexually mature fish were seen in the spring, with spawned males present in April and May. Spearman correlations revealed significant positive correlations between stage and GSI and HSI ($P<0.05$). While there were no cases of intersex gonads as seen by Kavanagh et al (2004) in white perch from the Great Lakes, an occasional male had small, circular basophilic structures within the gonad (Figure 9). Their appearance is similar to immature, previtellogenic oocytes. The

actual significance of these structures cannot be elucidated, though if they are indeed immature oocytes, this rare condition could be an indication of endocrine disruption.

Vitellogenic oocytes are those from stage 2 through stage 5. Interestingly, HSI increases concomitant with development of oocytes at stages 2 and 3, and decreases prior to spawning. This suggests that HSI reflects the initial incorporation of Vg into the developing oocytes. Also, negative correlations of stage 0 oocytes with other stages, GSI, and HSI reflect developmental changes. With the transition of stage 0 oocytes to more advanced stages, we would expect to see increased HSI due to an increase in Vg production. In addition to this, we would expect increases in GSI to reflect incorporation of Vg into the developing oocytes.

The presence of mature oocytes and post-ovulatory follicles from April through June indicates spawning within this time frame. Previous studies revealed spawning of white perch April through May in laboratory-reared white perch (Jackson and Sullivan 1995), May through June in Maine (Thoits 1958), and April through June in the Hudson River, NY (Klauda et al. 1988). The presence of mature and post-ovulatory follicles over a three month period, as well as various oocyte stages present together within ovaries indicates multi-clutch, protracted spawning. Protracted spawning of white perch has been described previously (Stanley and Danie 1983; Jackson and Sullivan 1995). Although no post-spawn males were seen in spring, based on the presence of advanced reproductive stages at this time and on milt production, it appears that spawning occurred during this time. No evidence of intersex was seen in the fish examined in this study.

Fertilization Success

Although reproductive parameters on adults have been examined and determined, fish reproduction also consists of successful fertilization, embryogenesis, hatching, and larval development (Heath 1995). During the springs of 2002 and 2003, collection of gametes from male and female white perch was attempted with the hope of fertilizing eggs and monitoring embryonic development. Although we were able to collect milt from many males, no ripe eggs could be stripped from females, though many females were collected. Thus, we were unable find females that were ready to spawn, and therefore could not perform this aspect of the study. We attempted capture of white perch

using trawl in areas of the Hackensack River with low salinity as this is where white perch are thought to spawn (Thoits 1958; Bath and O'Connor 1982; Stanley and Danie 1983). However, a recent study by McGrath and Austin (McGrath and Austin 2004) indicates that white perch may actually exhibit site fidelity, and are not apt to move with changes in tide or temperature. Success may be achieved in the future if collections take place in areas with a history of successful large catches of larval and juvenile white perch, as opposed to traveling to lower salinities.

HISTOPATHOLOGY

GRANULOMAS

Granulomas form as a chronic inflammatory response characterized by proliferation of reticulo-endothelial cells and phagocytes which may be surrounded by a fibrous capsule with a necrotic center (Wolke 1975; Post 1987). They form in response to bacterial and fungal infections, as well as parasites. In the white perch sampled for this study, granulomas were seen in various viscera (Figure 10). Metacercaria, larval stages of trematode worms, were seen within these granulomas. These parasites are thought to be in the family Echinostoma, as characterized by an oral sucker surrounded by spines (Figure 10A-C).

Metacercaria are larval digenetic trematodes and as such, their presence within white perch indicates their role as a second intermediate host with their first intermediate host most likely a mollusk. In addition to the presence of metacercaria in the spleens, livers, and gonads, they have also been noted in the heart, gills, intestine, and even muscle, though their presence in these tissues has not been quantified. Currently, granulomas in the liver, spleen, and gonad are being quantified and measured with Image Pro Express image analysis software. Statistical analyses will be performed to determine differences in infection between sexes and sites.

PIGMENTED CELL FOCI

There were no significant associations between the area of the spleen occupied by macrophage aggregates (MAs) and fish age, sex, length, weight, or month. There was no effect of age, weight, or length on MA area, though they were larger in males than in

females ($P<0.01$). There was a significant difference in splenic MA area monthly ($P<0.01$) (Figure 11), with MA area in March significantly less than those in June and May. There was no significant difference of area in April. Although there were no significant differences between the number of MAs monthly, there was a strong trend indicative of potential seasonal effects ($P=0.08$). There were no correlations between the numbers of splenic MAs and age, weight, length, or sex.

Seasonal differences in foci may be correlated with the normal, annual reproductive cycle. In both sexes, gonadal foci and splenic MAs were most abundant in the post spawning period in spring. ANOVAs were performed to see if the number or area of foci was associated with gonadal stage of development. Among females, there was no significant correlation of foci with developmental stage. As some researchers noted similar appearance of gonadal foci with atretic follicles (Johnson et al. 1988), a pairwise correlation was employed to determine if there was a correlation between follicular atresia and the number and/or size of gonadal foci. No significant correlation was seen.

Among males there was a significant correlation between stage 1 testes and the number of pigmented cell foci in the testes ($P<0.01$), but not the other stages. Stage 1 testes are in an immature or regressed state. Interestingly, Grier and Taylor (Grier and Taylor 1998) saw MA in regressed testes of the common snook (*Centropomus undecimalis*). Though typically seen in the interstitium, occasionally they were seen in lobules. As seen in this study, the number of MAs in snook was low at the beginning and middle of the reproductive season, with an increase during late maturation and regression. Although there is still speculation as to actual composition of gonadal PCF, images of these foci ingesting sperm have been obtained (Grier and Taylor 1998).

Correlations were also performed to determine correlations between splenic MAs and gonadal foci. There was a significant, positive correlation between the number of gonad foci and spleen MA ($r^2=0.32$, $P<0.01$), and although the relationship between their areas was not significant, a weak trend was detected ($r^2=0.05$, $P=0.07$).

A significant negative correlation was seen in the number of splenic MA and concentration of organochlorine pesticides ($r^2=0.27$, $P=0.05$), and between Cu concentration and area of gonadal PCF ($r^2=0.14$, $P=0.02$). There was also a trend of

increasing number of gonadal PCF with increasing organochlorine pesticide concentrations (r^2=0.23, P=0.08).

Macrophage aggregates (MAs) are types of pigmented cell foci that develop as a nonspecific, generalized stress response that typically form in liver, kidney, and spleen of teleosts (Montero et al. 1999; Blazer and Dethloff 2000; Fournie et al. 2001). Stressful situations such as tissue breakdown and age, size, exposure to pathogens, nutritional deficiencies, or diets rich in metals, and stocking density have been shown to affect MA presence and size (Agius 1979; Agius and Roberts 1981; Agius and Agbede 1984; Vogelbein et al. 1987; Montero et al. 1999; Manera et al. 2000; Fournie et al. 2001). MAs have also been shown to change in size and number based on water quality (Blazer et al. 1994; Moles and Norcross 1998; Facey et al. 1999; Fournie et al. 2001). Though there have been observations of pigmented cell foci in gonads of various teleosts (Smith 1966; Ellis et al. 1976; Groman 1982; Ravaglia and Maggese 1995), the actual composition of these structures was not elucidated.

It is possible that the foci are present within testes to assist Sertoli cells in the removal of unreleased gametes. Various researchers have described seasonal differences in quantity of hepatic MAs (Stentiford et al. 2003) and sex differences in macrophage activity (Broeg et al. 1999). However, it is also possible that these foci are composed entirely of Sertoli cells.

Although we cannot assume that pigmented cell foci are related to splenic MAs, and hence composed of macrophages, it is possible that these aggregates are actually composed of follicle cells in ovaries, and Sertoli cells in testes. If this is the case, it would be inappropriate to call them macrophage aggregates (Blazer 2002).

Hepatic Altered Foci

Incidence of hepatic altered foci is significantly correlated with sex. Males had significantly higher occurrence of hepatic altered foci than females (DF=1, χ^2=4.39, P=0.04). There was no correlation between numbers of fish with altered foci and age or fish size. However, there were significant differences based on month (DF=9, χ^2=30.17, P<0.0001) and sampling location (DF=11, χ^2=22.68, P=0.02). Fish with hepatic altered foci were caught in September (25%), March (19%), June (15%), and July (30%).

Affected fish were taken from S2, TN3, T4, and T5. Of all fish sampled, approximately 9% had vacuolated foci in their livers. There was no significant correlation of hepatic altered foci with metals or the organic contaminants tested, however.

Altered foci are regions of liver cells that stain differently from the surrounding stroma. Differences in cells that may be seen include vacuolated/clear cell foci, eosinophilic foci, and basophilic foci (Hinton et al. 1992; Couillard et al. 1997; Couillard et al. 1999). In white perch, the altered foci were irregularly contoured, colorless regions that did not result in compression of surrounding, normal tissue (Figure 12). This type of lesion is considered preneoplastic, and is considered to be a result of exposure to environmental carcinogens (Hinton et al. 1992). Clear cell foci have been described in carp *Cyprinus carpio* from West Point Lake in Georgia and Alabama, winter flounder *Pleuronectes americanus* from various estuaries in northeast US, white sucker *Catostomus commersoni* from Lake Ontario, brown bullhead *Ictalurus nebulosus* from Lake Erie, and English sole *Parophrys vetulus* from Puget Sound (Hinton et al. 1992). There was a significant association between PAH concentrations and frequency of flounder and sole with this lesion (Schiewe et al. 1991; Chang et al. 1998). No correlations were seen with PCBs or metals (Couillard et al. 1997; Chang et al. 1998).

Vacuolated foci may appear during gonadal maturation due to vitellogenesis and transfer of lipids from liver to gonads, or exposure to carcinogens (Couillard et al. 1997). Interestingly, in the current study, male white perch had greater incidence of foci than females, and this lesion was mostly seen in the pre- and post-spawning season. However, there were no correlations between the presence of altered foci and GSI. Any relationship between sex and presence of lesions may be due to species-specific responses. There were no sex related occurrences in hepatic lesions in various fish species from the Pacific Coast, USA, though female European flounder *Platichthys flesus* had higher incidence than males (Au 2004).

Cortisol

Cortisol levels averaged 526.42±53.19 µg/mL. Although males had greater levels of plasma cortisol than females (603.01±87.13 and 492.28±86.2 µg/mL, respectively) the difference was not significant ($p=0.37$). There was no significant correlation of cortisol

levels with metals concentrations or with PCBs, organochlorine compounds, or DDT and related compounds (DDX). However, there were significant negative correlations of cortisol with dissolved oxygen (DO) (r2=0.17, P=0.02) and GSI (r2=0.16, P=0.02). Plasma cortisol concentrations varied significantly from month to month (P<0.001). Highest concentrations were detected October through December, and were significantly greater than concentrations seen in all other months except September (Figure 13A). There were no significant differences in cortisol concentrations based on sampling site or metals concentrations (Figure 13B).

When combining sexes, there was no significant correlation of cortisol levels with metal concentrations. Interestingly, in females, there were negative correlations of cortisol with Hg (r^2=0.21, P=0.005). A trend of decreasing cortisol corresponding to elevated muscle DDX was seen (r^2=0.46, P=0.09). Sampling location had a significant effect on cortisol levels (P=0.006). Fish from TN6 had significantly more cortisol than all other sites except TN5, TN1, and GN1. In females there were significant differences in plasma cortisol concentration by month sampled (P=0.001), with greatest concentrations seen in October through December. The concentration of plasma cortisol was negatively correlated with water temperature (r^2=0.22, P=0.02).

Males exhibited some positive correlations between plasma cortisol and metals. Significant correlations were seen of cortisol with As (r^2=0.12, P=0.03), Cr (r^2=0.24, P=0.001), and Pb (r^2=0.31, P<0.001). Similar to the females, cortisol concentration was negatively correlated with Hg (r^2=0.26, P<0.01). Significant differences in cortisol levels were also seen based on sampling location (p=0.05), with fish from TN6 having greatest cortisol concentrations, as well as month (P<0.0001). Cortisol levels were greatest October through December. There was also a significant negative correlation between water temperature and cortisol concentration (r^2=0.37, P<0.01).

Cortisol is the main corticosteroid in fish and is released following exposure to stress. It affects metabolism of carbohydrates, protein, and lipids, as well as immune function and osmoregulation (Mommsen et al. 1999). Although stresses such as density, competition (McCormick 1998; Kubilay and Ulukoy 2002), parasites and disease (Stolze and Buchmann 2001; Bilodeau et al. 2003), and toxicants like Cd and atrazine (Wu et al. 2002; Lacroix and Hontela 2004; Waring and Moore 2004) have been shown to increase

plasma cortisol, exposure of fish to β-napthoflavone did not result in cortisol increase (Mommsen et al. 1999). It would seem that fish obtained from sites with greatest contaminant concentrations would have greatest concentrations of plasma cortisol. However, this was not the case when looking at combined data. However, when analyzing data for males and females separately, significant differences in cortisol by site were seen (Figures 13B). Initially, it was thought that this could be due to increased contaminant levels at specific sites. However, after reviewing concentrations of metals by site (Figure 14) no significant correlations were seen. A trend was noticed, though, when looking at graphs of cortisol levels by site: similar concentrations were seen in all samples obtained by the same method of sampling. Being caught in a net is a stressful situation. Cortisol results for fish obtained by similar methods were pooled and an ANOVA showed that although there were differences in cortisol by sampling method, the results were not significant. These differences may reflect undetected stressors such as predators, diseases, population density, or contaminants not analyzed.

GENERAL CONCLUSIONS

The overall health and reproduction of the white perch from the Hackensack appear to be fairly typical and normal. No striking indications of reproductive impairment, major disease, or endocrine disruption were seen. These "negative" findings of the absence of major problems are probably related to the apparently successful reproduction of this species and its high numbers in the Hackensack estuary, as seen in the MERI fisheries study.

CONTINUING STUDIES

Future studies will consist of analyzing reproductive hormones using plasma of sampled fish. Jackson and Sullivan (Jackson and Sullivan 1995) showed an increase in blood plasma E_2 and T concomitant with the onset of vitellogenesis in November of their sampling period with a rise to maximal levels during the spawning season. Hormone levels reflected the biphasic pattern of the reproductive endocrine cycle of white perch (Jackson and Sullivan, 1995). We expect to find similar results. Testosterone and 17β-estradiol (E_2) will be quantified using dissociation enhanced lanthanide

fluoroimmunoassay (DELFIA, Perkin-Elmer). Hormones will be extracted from plasma by ether. Twenty five µL of plasma will be washed with 1.5 mL anhydrous diethyl ether (Sigma), voretexed, immersed in liquid nitrogen, and the unfrozen ether containing hormones will be decanted. The ether will be allowed to evaporate off, and the hormones will remain in microcentrifuge tubes. Purified hormone samples will be analyzed for testosterone in both sexes and E_2 in females. This assay is currently under development, and results will be verified using radioimmunoassay. Upon completion of this assay, results will be submitted to MERI.

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Table 1. Characterization of gonadal development in white perch A. Ovarian Stages B. Testes stages

A.

Oocyte Stage	Histological Features
0	Immature, previtellogenic oocytes; perinucleolar stage; large central nucleus with nucleoli present
1	Cortical alveoli/lipidic stage; unstained remnants of lipid droplets throughout
2	Early vitellogenic stage; yolk globules present in periphery indicating initiation of vitellogenesis
3	Late vitellogenic; well developed follicle layer and chorion; larger yolk globules and lipid droplets
4	Final maturation; undergoing lipid droplet coalescence; germinal vesicle migrating to periphery
5	Post-ovulatory follicles; follicle cells remaining post-spawn
Atresia	Follicles collapse inward; phagocytic follicle cells present within

B.

Testis Stage	Histological Features
1	Pre-spermatogenic; immature and undeveloped, or regressed; contain spermatogonia and possibly residual sperm within lobules
2	Early spermatogenic; predominantly contain spermatocytes and spermatids
3	Mid spermatogenic; contain approximately equal amounts of spermatocytes, spermatids, and spermatozoa
4	Late spermatogenic; almost entirely spermatozoa
5	Spawning/Post spawning; have spawned, or are in the process of spawning; lobules empty or with reduced number of sperm

Figure Legends

Figure 1. Female gonad stages. **A.** Stages 0-3 **B.** Stage 4 **C.** Stage 5 **D.** Atresia

Figure 2. Male gonad stages A. Stage 1 B. Stage 2 C. Stage 3 D. Stage 4 E. Stage 5

Figure 3. Appearance of pigmented cell foci.

Figure 4. Size (Lt) and weight (Wt) of white perch on a seasonal basis.

Figure 5. Incidence of parasitism and hepatic lesions in white perch from different collection sites.

Figure 6. Seasonal changes in HSI and GSI in males and females.

Figure 7. Seasonal changes in ovarian developmental stage.

Figure 8. Seasonal changes in testicular development.

Figure 9. Basophilic structures in testes, indicated by arrow.

Figure 10. Parasite-induced granulomas. Spines around oral sucker (os) indicated with arrow. The asterisk is located at the acetabular (blind) sucker.

Figure 11. Changes in Pigmented Cell Foci (PCF) **A.** abundance and **B.** size

Figure 12. Hepatic altered foci.

Figure 13. Average plasma cortisol concentration **A.** by month, and **B.** by site.

Figure 14. Concentrations of muscle metal concentration of white perch from different sampling sites.

Figure 15. Concentration of plasma cortisol by sampling method.

Figure 1. Female stages. **A.** Stages 0-3. Numbers on oocytes reflect stage **B.** Stage 4 **C.** Stage 5 **D.** Atresia

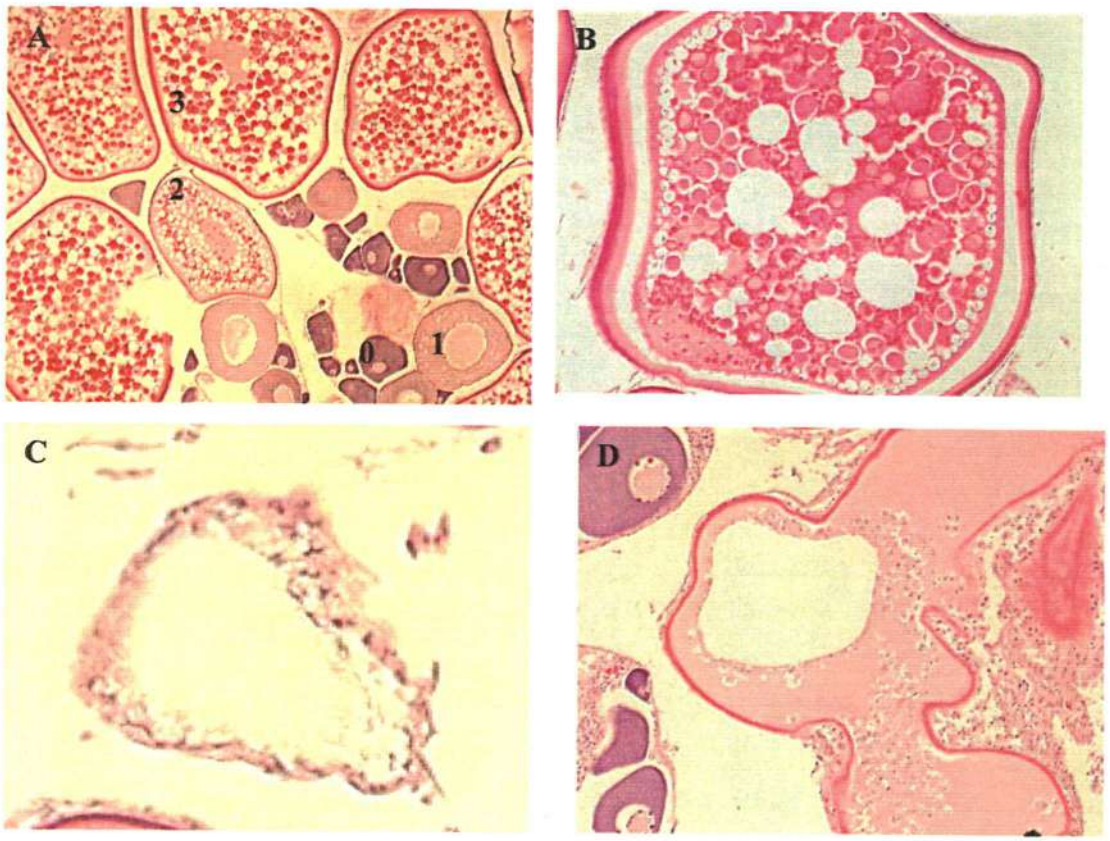


Figure 2. Male stages A. Stage 1 B. Stage 2 C. Stage 3 D. Stage 4 E. Stage 5

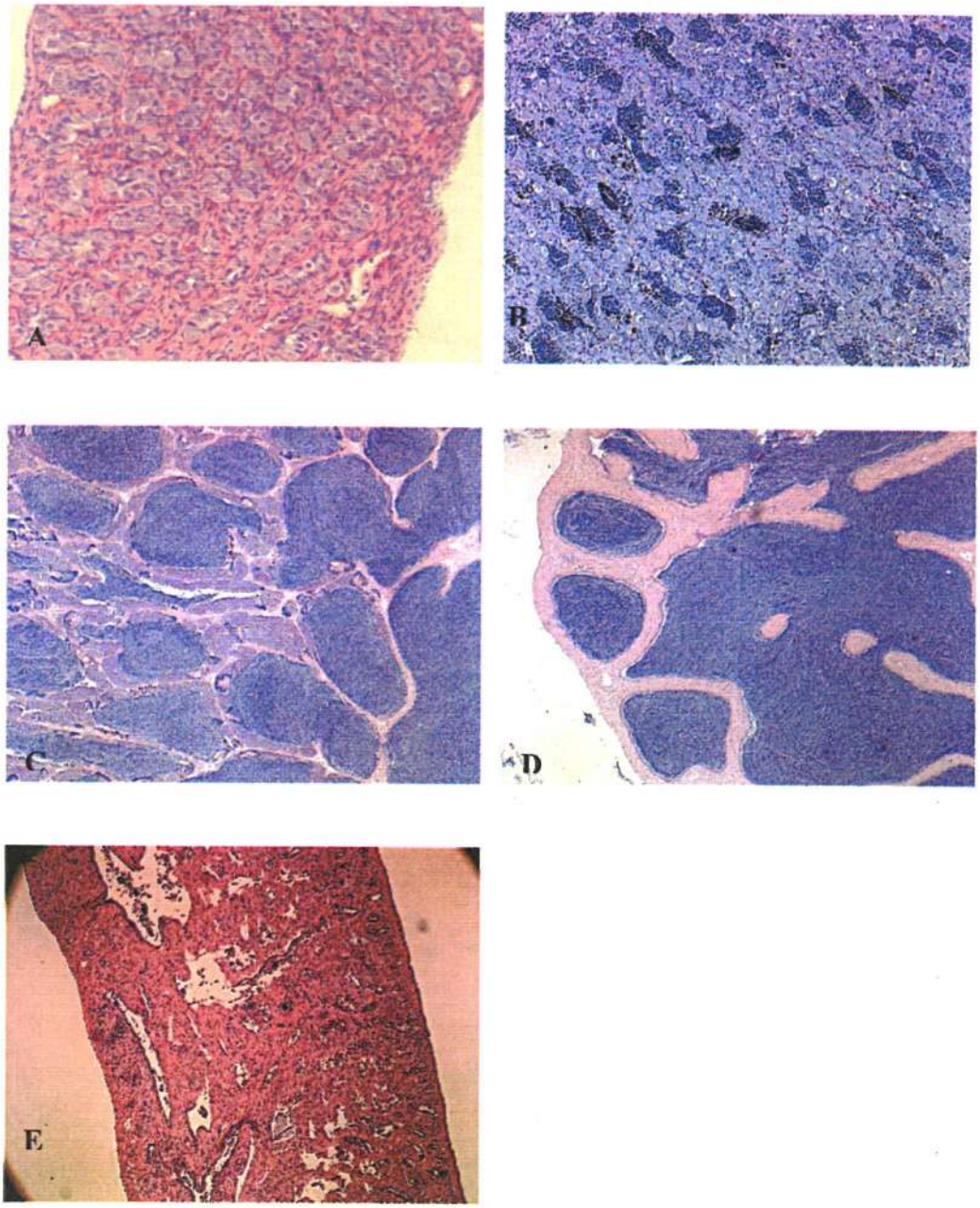
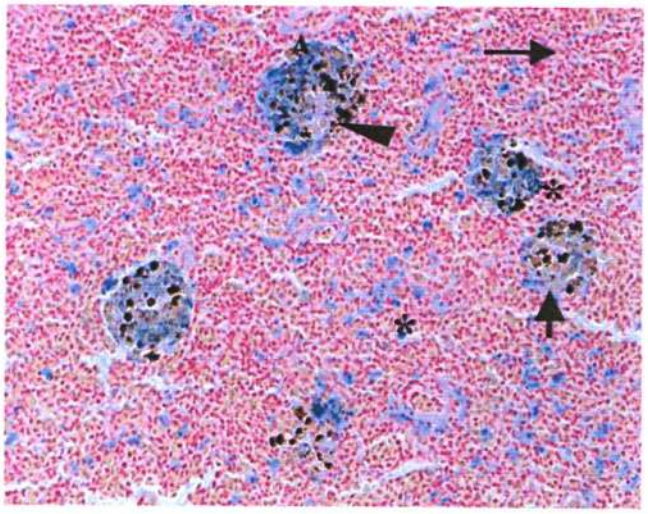
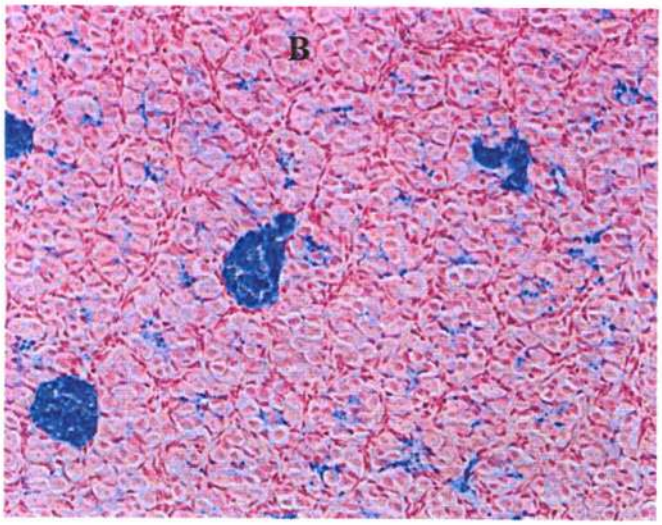


Figure 3. Appearance of pigmented cell foci. A. Splenic MAs with hemosiderin (asterisk), melanin (arrow head), and lipofuscin/ceroid (arrow) B. PCF in testis C. PCF in ovary





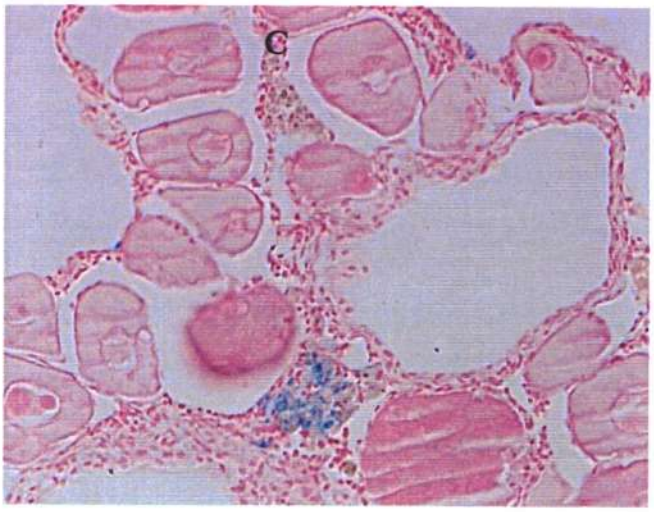


Figure 4. Size (Lt) and weight (Wt) of white perch on a seasonal basis.

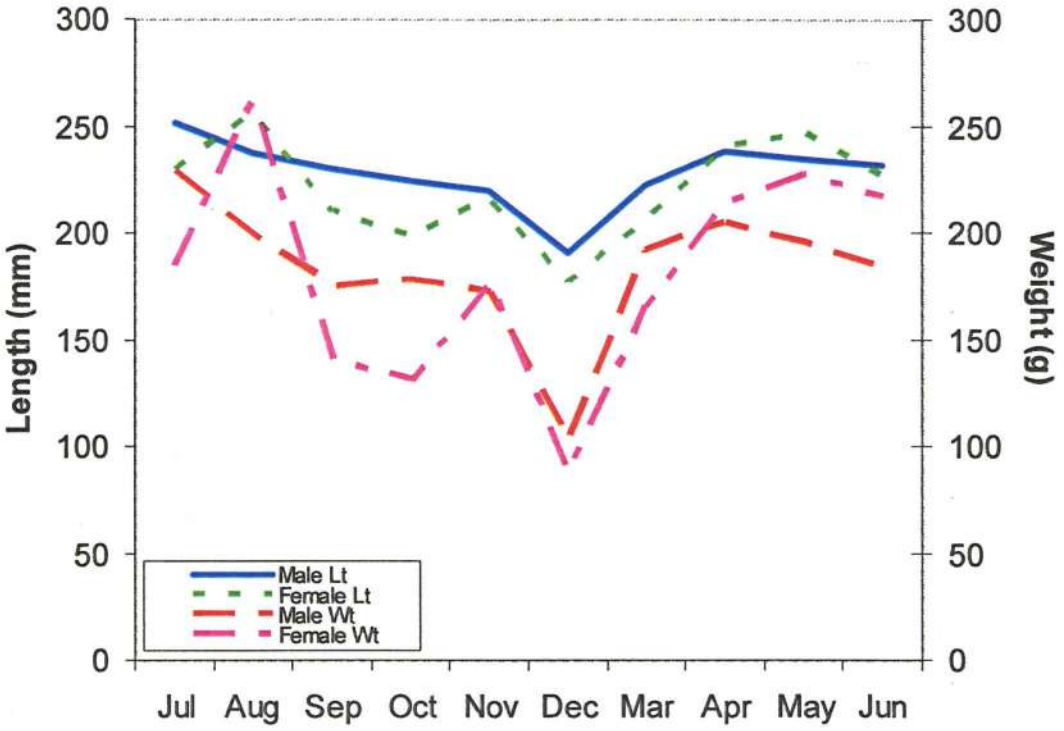


Figure 5. Incidence of parasitism and hepatic lesions in white perch from different collection sites.

	GN1	GN2	S2	TN1	TN2	TN3	TN4	TN5	TN6	T4	T5	T7
Lernaea	8%	33%	0%	100%	19%	0%	0%	0%	0%	0%	0%	0%
Endoparasites	0%	0%	25%	0%	11%	0%	19%	44%	0%	0%	11%	0%
Altered Foci	0%	0%	25%	0%	0%	10%	0%	0%	0%	17%	44%	0%

Figure 6. Seasonal changes in HSI and GSI in males and females.

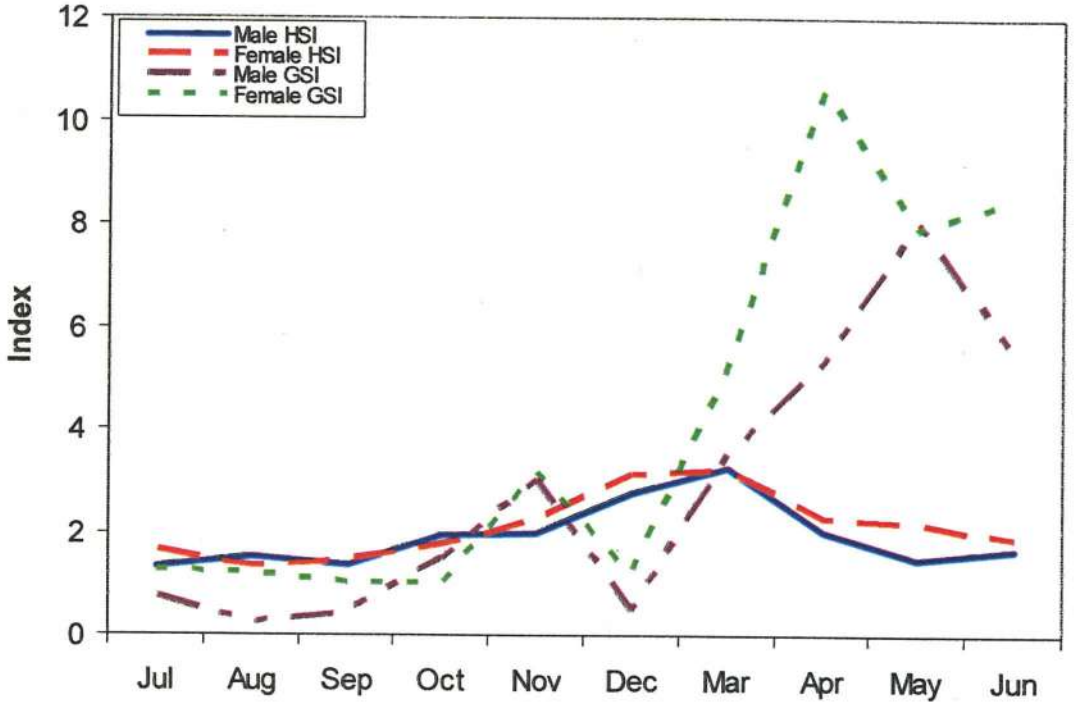


Figure 7. Seasonal changes in ovarian developmental stage.

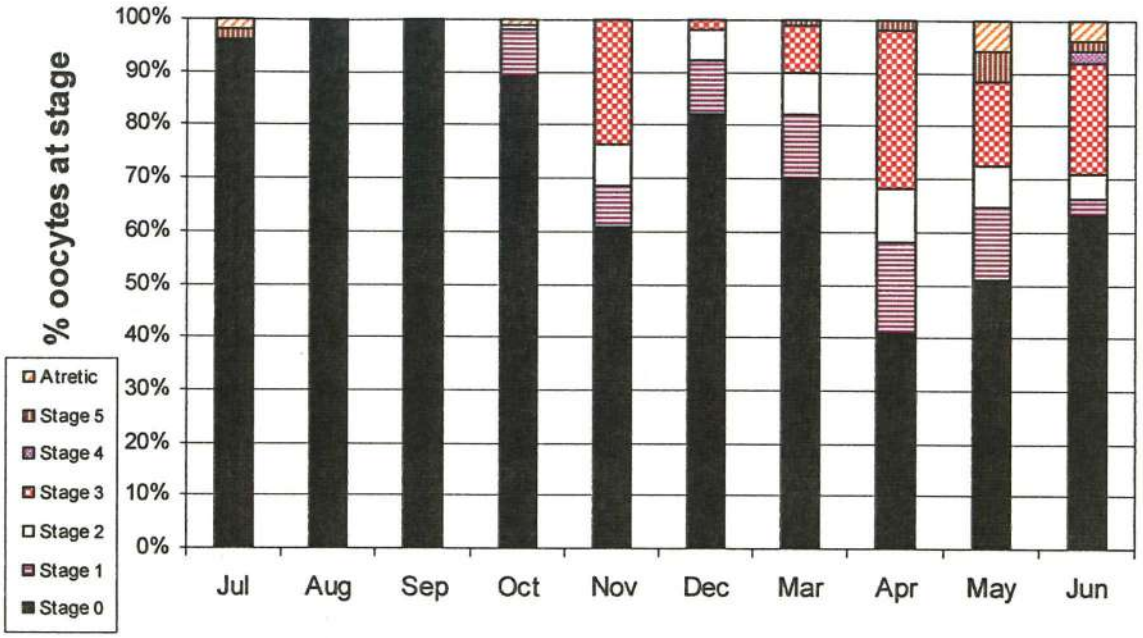


Figure 8. Seasonal changes in testicular development.

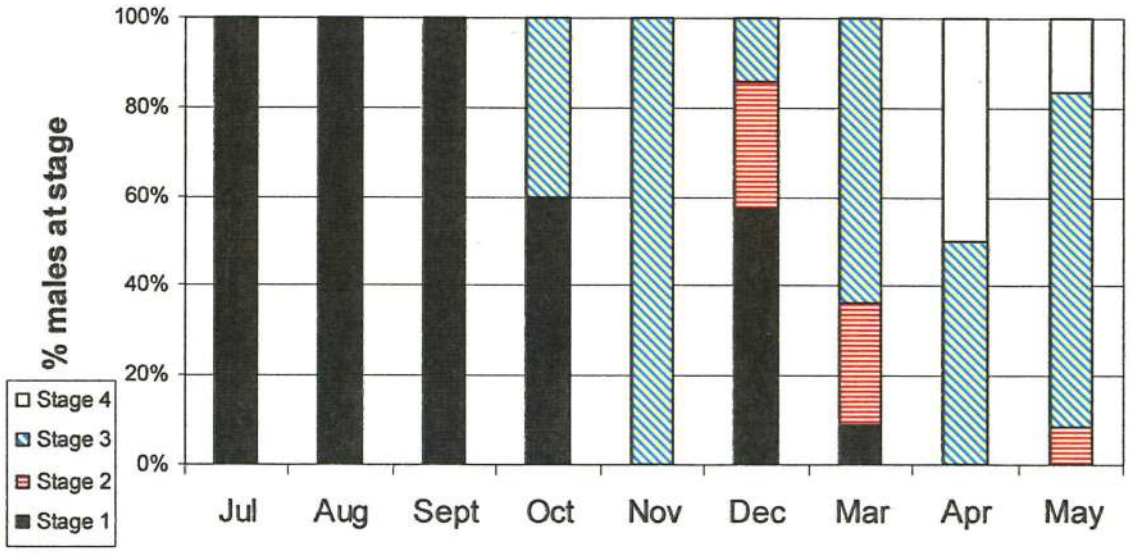


Figure 9. Basophilic structures in testes, indicated by arrow.

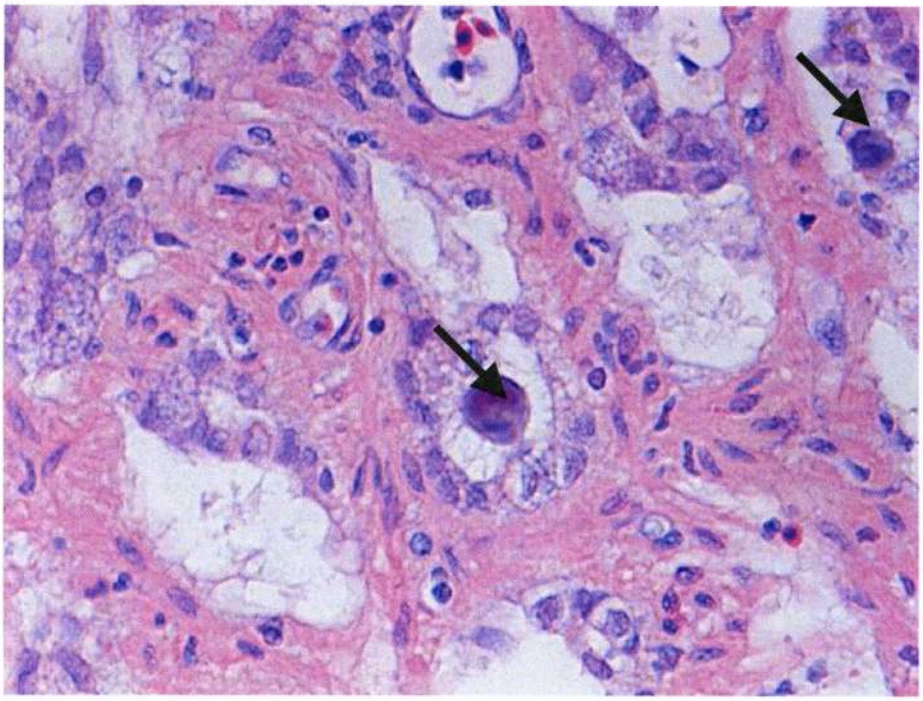
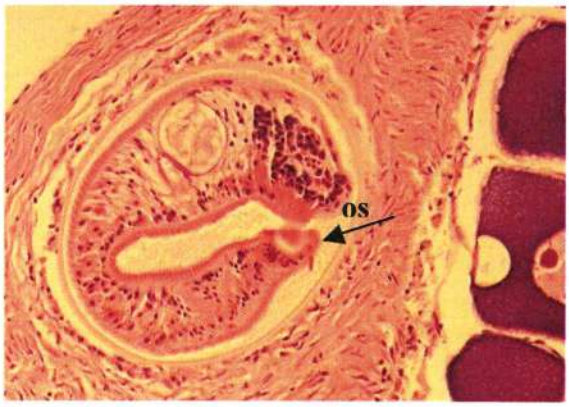
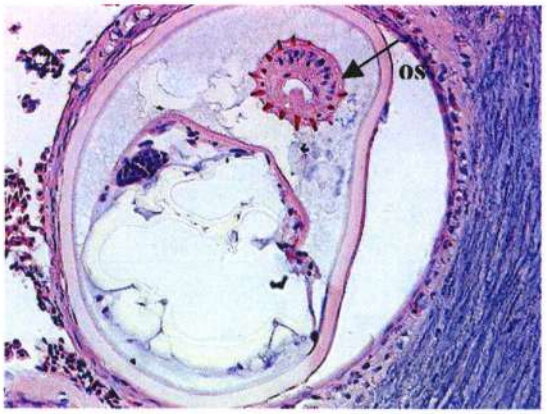


Figure 10. Parasite induced granulomas. Spines around oral sucker (os) indicated with arrow. The asterisk is located at the acetabular (blind) sucker.





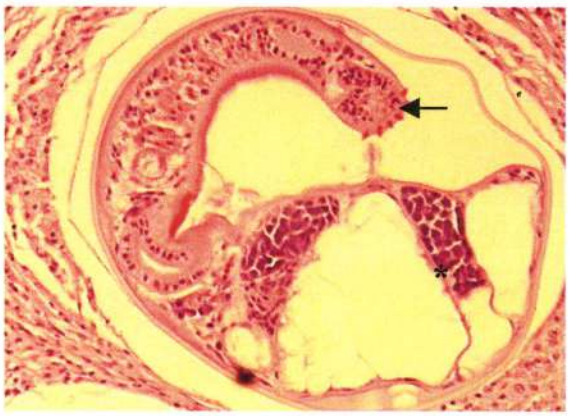
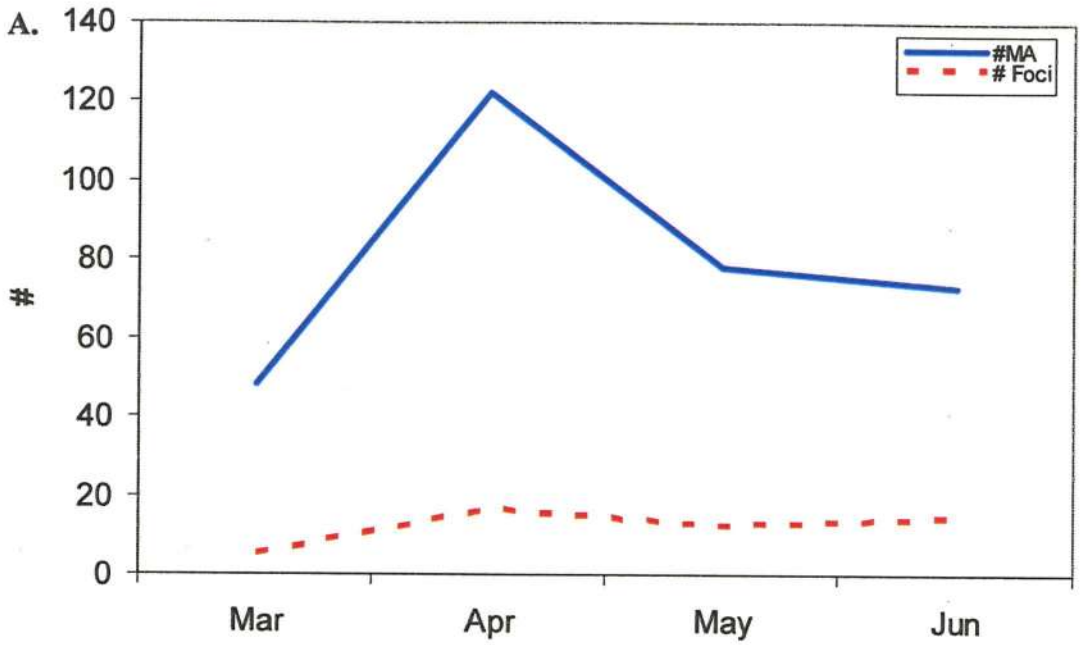


Figure 11. Seasonal changes in PCF **A.** abundance and **B.** size.



B.

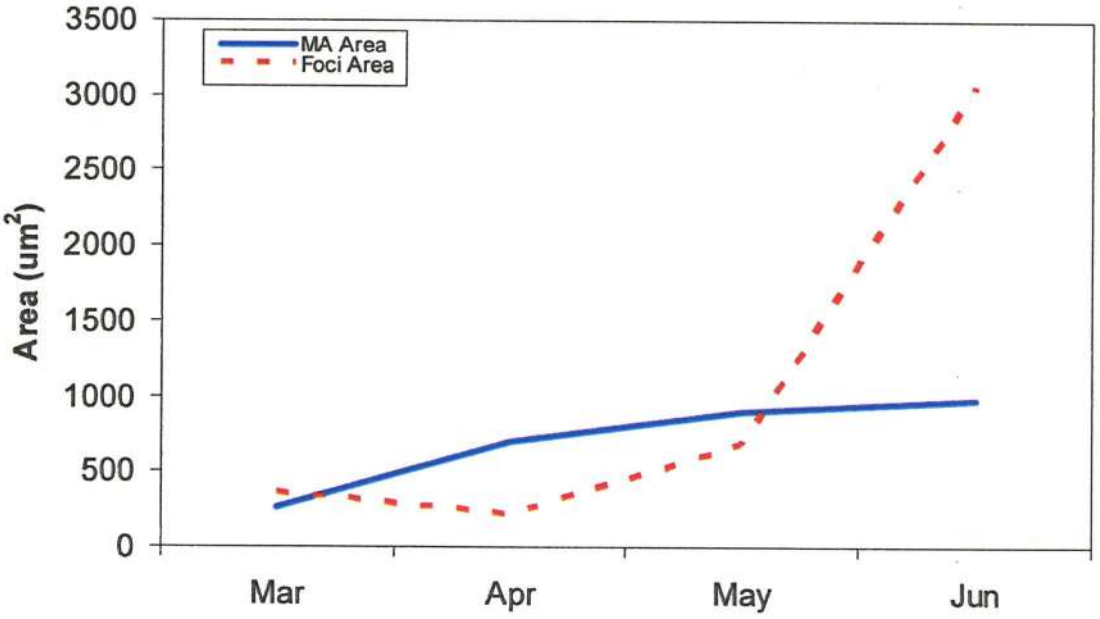


Figure 12. Hepatic altered foci. Normal hepatic tissue surrounds altered foci (asterisk).

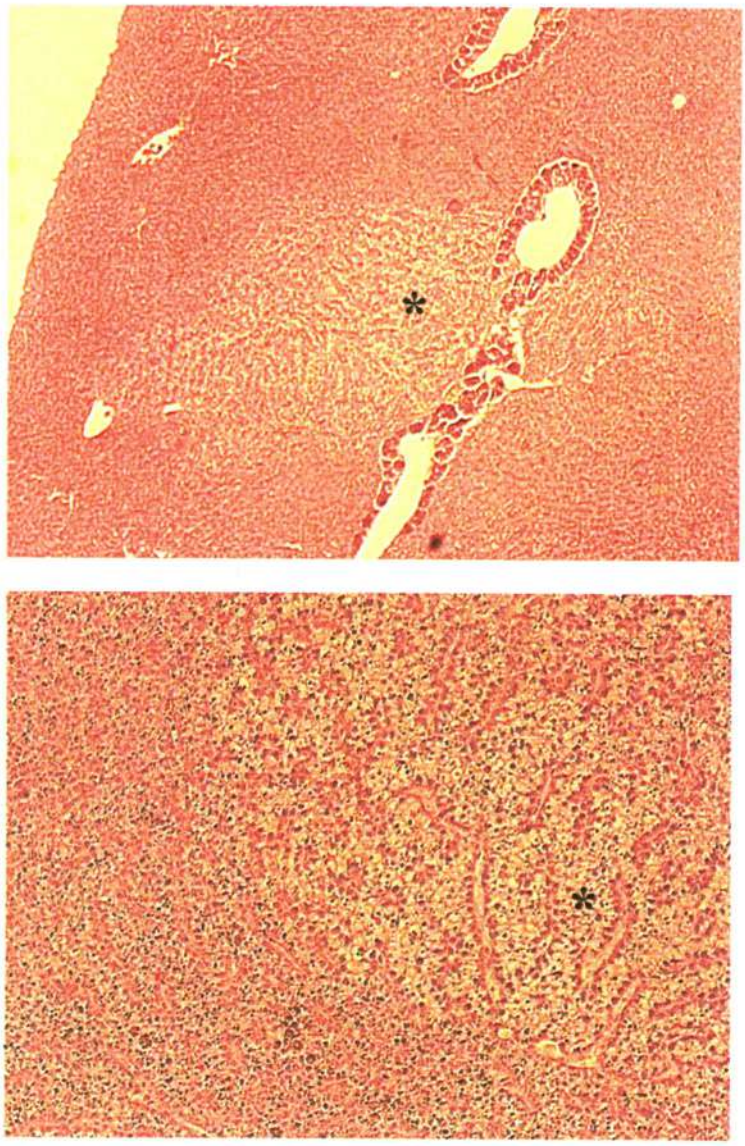
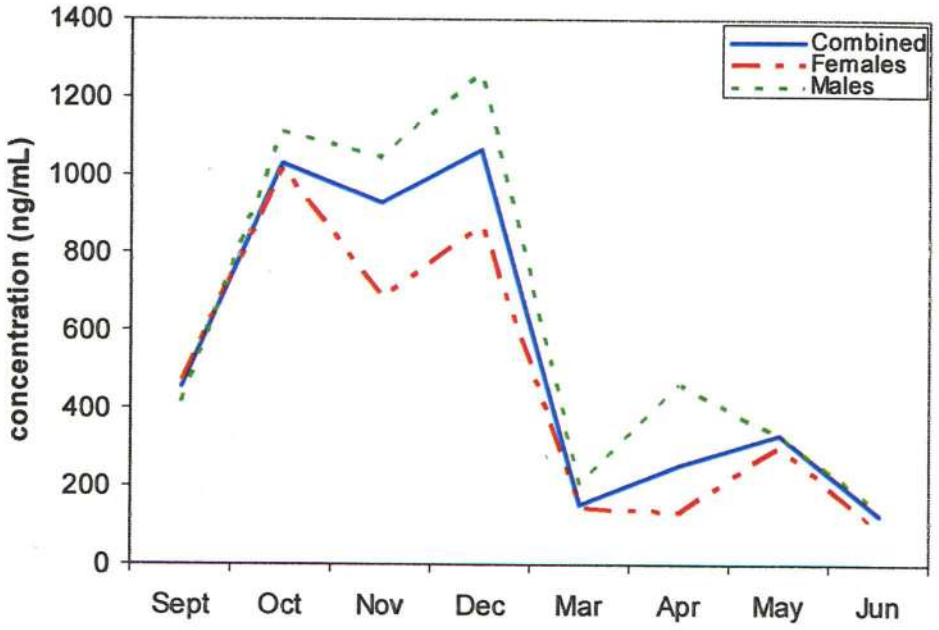


Figure 13. Average plasma cortisol concentration **A.** by month, and **B.** by site.

A.



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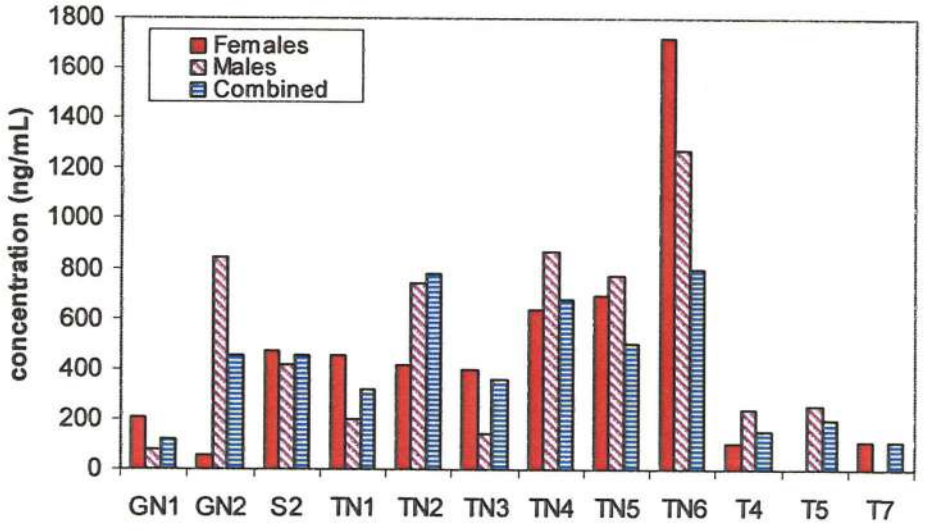

Figure 14. Concentrations of muscle metal (ug/g) of white perch from different sampling sites.

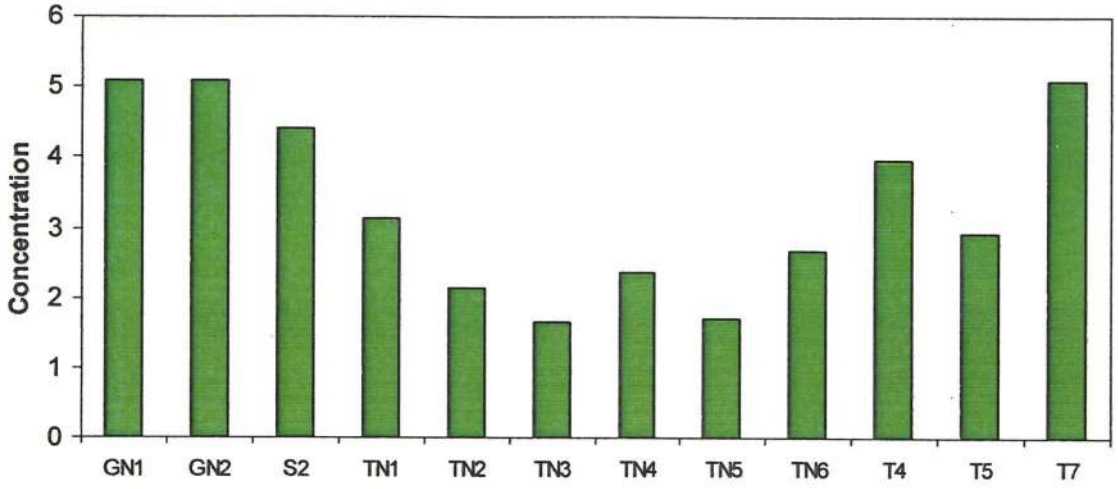

Figure 15. Concentration of plasma cortisol by sampling method.