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Comparison of estradiol, testosterone, vitellogenin and cathepsin profiles among young adult channel catfish (*Ictalurus punctatus*) females from four selectively bred strains

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Abstract

Potential variation in reproductive performance as young broodfish among four selectively bred strains of channel catfish was evaluated by comparing profiles of plasma estradiol, testosterone, and vitellogenin, and activities of cathepsin D, L, and B in follicle-enclosed oocytes during vitellogenesis in 2- to 3-yr-old females. No differences among the four strains were detected for any of the parameters measured. Plasma steroid concentrations demonstrated temporal differences, with testosterone levels peaking in January and estradiol levels peaking in April. Monthly changes in circulating vitellogenin concentrations coincided with oocyte growth. Average oocyte size in May, the typical time of spawning, was smaller (1624 μm) than that considered normal for mature oocytes (approx. 3000 μm) in this species. Furthermore, only 9% of the females in this study spawned, and no significant correlations could be determined between the reproductive indices and spawning success. Activities of cathepsin D, L, and B were also similar between the four strains of catfish. Cathepsin L activity was highest in March, and cathepsin D activity was highest in March and May. Cathepsin B activity was generally lower than the activities of either cathepsin L or D, with the highest levels occurring in November, December, and March. This is the first report of cathepsin activities and their relationships to other reproductive parameters in channel catfish.
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1. Introduction

Efficient use of broodstock in commercial catfish fingerling production systems and breeding programs requires optimization of spawning success in young breeding populations. One possible reason for lack of

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spawning by some females is the age at the onset of puberty. Domesticated channel catfish (*Ictalurus punctatus*) females can become reproductively mature at 2 years of age; however, most producers use fish that are 3 years of age or older because older fish are more reliable spawners.

The time when fish reach sexual maturity and then spawn successfully is an important consideration in the operation of a commercial catfish farming enterprise. During the past few decades, several strategies have been developed to control the timing of sexual maturation in cultured catfish. These strategies include hormonal and environmental manipulations and genetic selection to accelerate maturation (Clemens and Sneed, 1962; Bates and Tiersch, 1998; Ponthier et al., 1998; Kestemont et al., 1999). Reports of the relationships among gonadal development, hormonal cycle, and spawning success in channel catfish are limited. However we know that timely and appropriate changes in titers of plasma sex steroids are necessary for successful reproduction to occur in all vertebrates (Ponthier et al., 1998).

Changes in the reproductive physiology of catfish species as they mature are associated with changes in levels of plasma steroids during the reproductive cycle (Singh and Singh, 1987). Causal relationships of steroidal hormones and production of essential components for oogenesis have been shown in catfish. One of these components is vitellogenin, the precursor to yolk proteins and the primary protein contributing to oocyte growth (Specker and Sullivan, 1994). Bradley and Grizzle (1989) demonstrated that vitellogenin was inducible as a 150 kDa plasma protein in response to estradiol administration in channel catfish. This is consistent with previous work showing that estradiol treatment induces the production of vitellogenin in the liver of Indian catfish (*Heteropneustes fossilis*; Sundararaj and Nath, 1981).

After vitellogenin is synthesized in the liver, it is released into the bloodstream. Vitellogenin is then accumulated in the developing oocytes via receptor mediated endocytosis, enzymatically processed into yolk proteins and stored in yolk granules or globules throughout the ooplasm (Mommsen and Walsh, 1988). The proteolysis of vitellogenin is predominantly by cathepsins (Hiramatsu et al., 2002a,c). Although the activities of cathepsins have been documented in some fish species, references to cathepsin activity in catfish species, including channel catfish, are lacking. A better understanding of cathepsin activities and their relationships to oocyte development may allow researchers to better understand factors influencing spawning success of young adult catfish.

Presently, there are few selective breeding programs, commercial or academic, for improving channel catfish traits, and because of the proprietary nature of commercial strains, little is known regarding putative differences in spawning success. The objective of this study was to evaluate potential differences in steroidogenic capacity, specifically plasma levels of estradiol and testosterone, vitellogenesis, specifically plasma vitellogenin levels, and the activity profiles of cathepsins L, D, and B in oocytes during vitellogenic growth in 2- to 3-yr-old channel catfish from four selectively bred channel catfish strains.

2. Material and methods

2.1. Animals and sampling

One hundred forty-four, two-year old female channel catfish broodfish from four different selectively bred commercial strains, the NWAC103 strain (A) and three proprietary strains (B, C, and D) were acquired in May 2003. Primary selection in each of these four strains was for growth traits. These fish were weighed (mean ± SE: 1.2 ± 0.2 kg), tagged with a passive integrated transponder (PIT) for individual identification, and stocked into each of four 0.04 ha earthen ponds at the Thad Cochran National Warmwater Aquaculture Center, Stoneville, MS at a rate of 9 fish per strain (36 broodfish per pond). Throughout the study, fish were fed a 36% crude protein diet at a rate of up to 17 kg ha^{-1} day^{-1}. Each month, approximately 20 fish were seined from each pond and 3 female catfish per strain per pond were removed and placed into four respective concrete raceways (192 ft^3) containing well water. Prior to sampling, the fish were rapidly anesthetized in a 250 mg l^{-1} bath of tricaine methanesulfonate (MS-222), identified by using a PIT tag reader model Power Tracker II (AVID manufacturer, Norco, CA), and weighed. Non-surgical collection of oocytes was not successful until the October sampling, at which time and each month thereafter, samples of blood and ovarian tissue were obtained from three fish per strain per pond. After blood and ovarian samples were obtained, fish were returned to their respective pond. To reduce the potentially adverse effects of sampling stress, individual fish sampled for a given month were not sampled again the following month. Sample collection was conducted on the following dates: 8 October, 18 November, 8 December, 12 January, 13 February, 11 March, 15 April, and 13 May.

As part of an effort to determine which individual females spawned and from which strain, apparently healthy 3-yr-old male channel catfish were moved from a pond with a mixed-sex population to each experimental pond (7 males per pond) with spawning cans on May

13. Every two to three days during the spawning season, the cans were checked for spawns. Egg masses were removed from the ponds and reared in a hatchery under standard conditions until hatching. Whole fry from each spawn and blood samples from each female broodfish were obtained and submitted to the USDA-ARS Catfish Genetics Research Unit, Stoneville, Mississippi for genotyping. The process used 40 ng of genomic DNA to amplify five microsatellite loci (IpCG0001, IpCG0018, IpCG0035, IpCG0070, IpCG0189; Waldbieser et al., 2001) and reaction products were resolved by capillary electrophoresis on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA; Waldbieser et al., 2003). Parental genotypes were deduced assuming Mendelian inheritance of microsatellite alleles from full-sib fry.

2.2. Estradiol (E_2) and testosterone (T) profiles

A 1-ml blood sample was obtained from the caudal vasculature of each fish using a syringe fitted with an 18-gauge needle. Each sample was placed into a 1.5-ml heparinized tube and stored on ice until centrifuged (3000 rpm for 10 min). The separated plasma was collected and then stored at −80 °C. Steroid analyses were conducted using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Oxford Biomedical Research, Oxford, MI). Steroids were extracted from plasma samples with 1 ml of ethyl ether and then incubated with 50 μl of E_2 or T enzyme conjugate in antibody coated plates for 1h at room temperature. The bound enzyme conjugate was detected by addition of 150 μl of substrate (3,3′,5,5′tetramethylbenzidinne), which bound to the enzyme conjugate and generated color after 30 min. The intensity of color development was inversely proportional to the amount of E_2 or T in the sample. Sample absorbances were read against standards at both 650 nm and 490 nm with a Model 550 microplate reader (Bio-Rad, Hercules, CA,).

2.3. Vitellogenin (Vtg) determination

Preparation of vitellogenin antigen and monoclonal antibody production was performed following the procedure described by Goodwin et al. (1992). Semipurified Vtg was prepared by two precipitations with *N-N*-dimethyl formamide of serum obtained from male channel catfish that had been implanted with a silastic estrogen implant. Serum samples were loaded into microtiter plates containing double dilution samples of channel catfish serum that had been previously diluted 1:100 in PBS-BSA. The Vtg concentrations used to create the standard curve were 200, 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0.39 ng ml^{-1}. The absorbance of the reaction mixture in each well was measured at 405 nm using a Multiscan EX plate reader (Thermo Electron Corporation, Woburn, MA).

2.4. Oocyte maturation

To follow oocyte maturation visually, samples of follicle-enclosed oocytes were collected by cannulation of the oviduct, as described by Markman and Doroshov (1983), and placed into a 10% formalin solution until they were analyzed no more than 1 month later. When removed from the formalin solution, oocytes were placed into Stockard's and Serra's solutions to clear the eggs for visualization of the germinal vesicle, following the procedure described by Stoeckel (2000). The germinal vesicles of the oocytes were observed using a compound microscope with transmitted light. The diameters of oocytes in the samples were measured using an ocular micrometer. An oocyte was classified as mature when the germinal vesicle was clearly differentiated and had moved from the middle to the periphery of the oocyte (Nagahama, 1983).

2.5. Enzymatic activity of cathepsins

The enzymatic activities of cathepsins B, L, and D from samples of ovarian tissue collected during vitellogenic growth were assayed. Follicle-enclosed oocytes were obtained by cannulation of the oviduct, placed in liquid nitrogen, and then transported on dry ice to the laboratory. All samples were stored at −80 °C until analysis.

2.5.1. Cathepsin D

Proteolytic activity of cathepsin D was measured by spectrophotometric methods using bovine hemoglobin (5% w/v) as a substrate according to the method described by Takahashi and Tang (1981). Briefly, hemoglobin was hydrolyzed by cathepsin D to liberate peptides that are soluble in a dilute solution of trichloroacetic acid (TCA). After incubation at 37 °C for 20 min, 2 ml of 10% TCA was added to precipitate protein, followed by filtration, and measurement of absorbance at 280 nm (Spectronic 2000, Bausch and Lomb, Hilliard, Ohio). Controls were carried out with incubation mixtures to which TCA was added immediately after the addition of enzyme. One unit of enzyme activity was defined as the net extinction value of 1.0 of the filtrate in excess of the control. All determinations were performed within the linear range of the assay, up to a net absorbance of 0.3.

2.5.2. Cathepsin B

The enzymatic activity of cathepsin B was assayed using Z–Arg–Arg–NNap (Sigma Chemical Company, St Louis, MO) as a substrate according to the method described by Barrett and Kirschke (1981). Briefly, samples were pre-incubated for 5 min at 40 °C with buffer/activator followed by addition of the substrate (Z–Arg–Arg–NNap) to start the reaction, liberating 2-naphthylamine. Substrate hydrolysis was assayed colorimetrically by coupling it with a diazonium salt, Fast Garnet (Sigma). The colored product was maintained in solution by using a nonionic detergent, Brij 35 (4% solution; Sigma) and read at 520 nm on the spectrophotometer. The production of 2-naphthylamine was linear as determined by the preparation of a standard curve with 0.1, 0.2, 0.3 and 0.4 mL of 0.2 mM 2-naphthylamine (Sigma). Enzyme activity per milligram of protein was calculated by the ΔA_{520} knowing that 100 nmol of 2-naphthylamine (0.5 mL of 0.2 mM solution) corresponds to 10 milliunits (10×10^{-3} μmol min^{-1}).

2.5.3. Cathepsin L

The enzymatic activity of cathepsin L was assayed by adding azocasein (2% w/v) containing 6 M urea to the sample for 30 min at 37 °C as described by Barrett and Kirschke (1981). The azo-coupling groups confer an intense yellow color on the protein. Proteolytic degradation of azocasein by cathepsin L yields peptides soluble in dilute TCA, which are quantified spectrophotometrically by the change in absorbance at 366 nm. The ΔA_{366} due to enzyme activity is not linear with enzyme concentration, and requires a calibration curve be utilized in which the inhibition of proteinase activity against Z–Phe–Arg–NMec (Sigma) is measured in the presence of E-64 (L-*trans*-epoxysuccinylleucylamido(4-guanidino)butane; Sigma) (Barrett and Kirschke, 1981). Specific activity of cathepsin L was obtained according to the relationship of μmol of products formed per mg of protein.

2.6. Statistical analysis

Data were analyzed using Proc mixed, SAS 9.1 (SAS Institute, 2002) and evaluated using a two-way ANOVA, where month and strain were the main effects. The dependent variable was the concentration for each parameter, and ponds were the replicates ($n=4$). Least-squares difference was used to evaluate differences at significance levels of 0.05. When the effect of strain was insignificant ($P>0.05$), data were pooled across strains and analyzed by month.

3. Results

The average weight of the female broodfish prior to the final sampling was 1.5±0.2 kg (mean±SE). No significant differences ($P>0.05$) in E_2 or T concentrations were observed among four strains; however, temporal differences ($P<0.05$) were detected (Table 1; Fig. 1A). Estradiol concentrations began to increase in November and fluctuated between the monthly samples until April, at which time E_2 concentrations in the plasma were highest. Testosterone levels in the plasma remained low in monthly samples taken from October through December, and peaked in January. Circulating levels of testosterone declined after January, in contrast to the elevated E_2 concentrations closer to the spawning season. Mean plasma concentrations of T were less than those observed for E_2 for most monthly samples, except January, when the mean concentration of circulating T was greater.

Strain also had no effect ($P>0.05$) on oocyte diameters or circulating Vtg concentrations (Table 1; Fig. 1B). The greatest increases ($P<0.05$) in oocyte size were observed

Table 1
Differences of least squares means for reproductive parameters in 2- to 3-yr-old female channel catfish corresponding to temporal presentations in Fig. 1

Month	E_2 (ng ml^{-1})	T (ng ml^{-1})	Vtg (mg ml^{-1})	Oocyte size (μm)	Cathepsin B (U mg^{-1})	Cathepsin D (U mg^{-1})	Cathepsin L (U mg^{-1})
Oct	0.04^{a}	0.01^{a}	4.3^{a}	756^{a}	2.35^{a}	5.41^{a}	6.58^{a}
Nov	0.15^{cd}	0.06^{ab}	4.2^{a}	1206^{b}	10.32^{c}	7.58^{ab}	17.00^{bc}
Dec	0.13^{bc}	0.05^{ab}	5.2^{ab}	1096^{b}	11.19^{c}	7.26^{ab}	16.32^{b}
Jan	0.09^{b}	0.22^{c}	7.4^{b}	1096^{b}	5.44^{b}	13.65^{b}	21.68^{c}
Feb	0.17^{d}	0.10^{a}	15.5^{c}	1333^{d}	0.66^{a}	5.43^{a}	27.43^{d}
Mar	0.08^{ab}	0.05^{ab}	12.8^{c}	1169^{bc}	9.15^{c}	48.62^{d}	35.25^{e}
Apr	0.26^{e}	0.11^{b}	21.4^{d}	1231^{cd}	5.75^{b}	29.73^{c}	18.90^{bc}
May	0.16^{cd}	0.11^{b}	31.2^{e}	1624^{e}	7.24^{b}	43.69^{d}	19.28^{bc}
PSE	0.02	0.03	1.1	51	0.76	2.76	1.93

Means within columns having different superscripts are significantly different ($P<0.05$). PSE = pooled standard error.

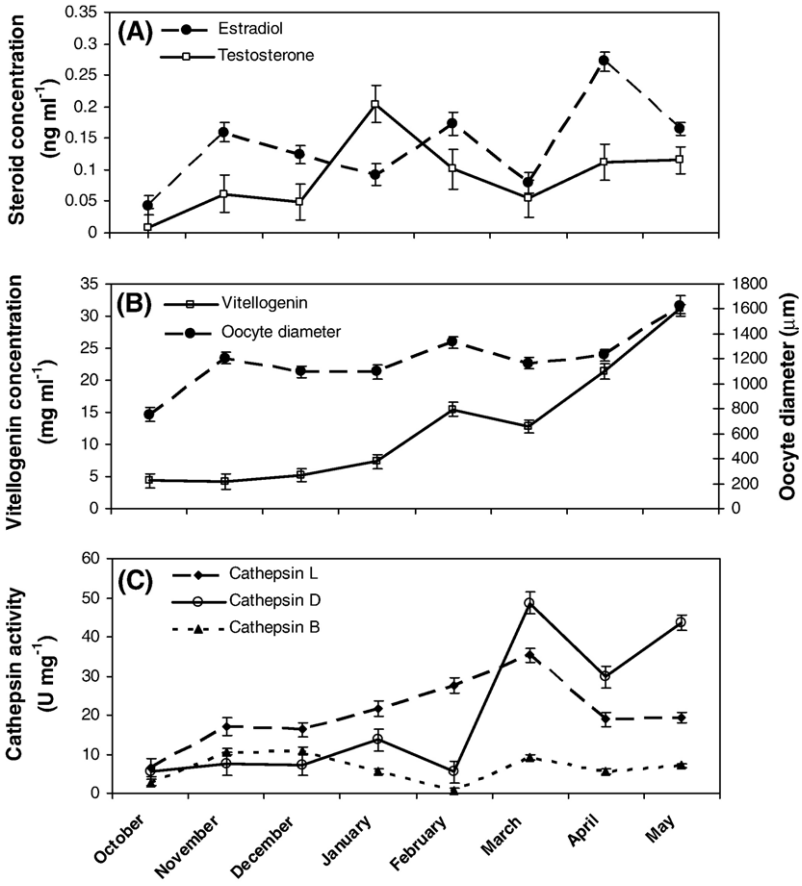

Fig. 1. Monthly, mean (±SEM), plasma concentrations of estradiol and testosterone (A); oocyte diameter and plasma vitellogenin concentration (B); and activities of cathepsins L, D, and B (C) in 2- to 3-yr-old female channel catfish from four select strains. There were no effects of strain or significant interactions between strain and month for any of the parameters ($P>0.05$). The effect of month was significant ($P<0.05$) for all the parameters and statistical separation of the means is presented in Table 1.

between October and November, and between April and May. The average May oocyte diameter (1624 μm) is suggestive of mid-vitellogenic oocytes, and not oocytes about to enter final oocyte maturation. Furthermore, germinal vesicle migration was not observed in any of the oocyte samples. Changes in oocyte diameter were positively correlated ($P<0.05$; $r=0.91$) with plasma Vtg concentrations from January to May. There was a large increase in plasma Vtg concentrations between January (7.4 ng ml^{-1}) and May (31.2 ng ml^{-1}).

Activities of the three cathepsins were also unaffected ($P>0.05$) by strain, but did demonstrated significant ($P<0.05$) temporal changes (Table 1; Fig. 1C). No significant correlations were observed between any of the three cathepsins and circulating steroid concentrations. Only cathepsin D activity showed some correlation ($R=0.69$; $P=0.056$) with plasma Vtg concentrations over time.

Cathepsin L activity was elevated in all samples compared to those taken in October when the oocytes were very small (756 μm). Between October and March there was a large increase in cathepsin L activity. Then from March to April, activity decreased by approximately 50% on average. Cathepsin D activity was highest in March, April, and May, peaking in March and May. Cathepsin B activity was generally lower than the activities of either cathepsin L or D, with the highest levels occurring in November, December, and March.

Spawning occurred between May 26th and June 10th; however, the incidence of spawning was low and only 9% of the fish (6/64) spawned. Genotype analysis identified the six spawning females as three from strain B, two from strain A, one from strain C, and none from strain D. No significant correlations ($P>0.05$) could be determined between the reproductive parameters measured and spawning success.

4. Discussion

Using the information provided by Grizzle (1985) and Silverstein and Small (2004), the development of channel catfish eggs can be related to the results obtained in the present study. Vacuolated oocytes with a size that ranged from 240 to 650 μm appeared in October. Grizzle (1985) stated that during vitellogenesis, oocytes increase in size from approximately 650 μm to 3000 μm. At approximately 3000 μm, oocytes have completed maturation in preparation for ovulation. Average oocyte sizes throughout our study (756–1624 μm) suggest that the majority of oocytes sampled were early to mid-vitellogenic. The results of the present research suggest that on the last day of oocyte evaluation (13 May), the oocytes were either still vitellogenic, with many not likely to reach maturity, or already undergoing absorption. As a result, the May sample might have represented fish undergoing diverse reproductive processes including vitellogenesis, early stages of post-vitellogenic maturation, and atresia. Furthermore, at the time of the May sampling, the germinal vesicle was visible in the central position and had not yet moved to the periphery, a condition that signals the onset of the resumption of meiosis. These observations correspond to the low incidence of spawning observed.

Plasma concentrations of sex steroids are useful indicators of steroidogenic activity during oocyte development and maturation. Timely and appropriate changes in circulating steroid titers are considered critical for successful reproduction. Monthly levels of reproductive steroids in the plasma of 2- to 3-yr-old female channel catfish in the present study varied during vitellogenesis. As maturation proceeded, coordinated changes were apparent for many of the reproductive indices measured. The increase in E_2 that occurred in November is likely responsible for the induction of vitellogenin production, which was increased in the plasma from December through May. MacKenzie et al. (1989) reported relatively unchanged E_2 concentrations from September to January in 3-yr-old channel catfish, and observed maximum concentrations in February, similar to those observed in the present study with younger fish. The levels of E_2 determined in the present study are lower than those reported by Bradley and Grizzle (1989). This difference may be attributed to the young age of channel catfish evaluated. It appears that many of these fish were immature, which is further supported by the low incidence of spawning. Several researchers (Massoud et al., 1983; Pacoli et al., 1990; Ponthier et al., 1998; Kumar et al., 2000) have evaluated reproductive parameters in channel catfish that were 3 years old or older, but data for channel catfish less than 3-years-old are considerably less common. A study by Davis et al. (1986), reported less marked seasonal changes in plasma E_2 levels of 2-yr-old channel catfish relative to 3-yr-old channel catfish. Similarly, E_2 concentrations of young adult, 3-yr-old, striped bass are reportedly lower than those of 4-yr-old individuals (Holland et al., 2000).

Plasma testosterone concentrations increased in January about the same time that Vtg levels began to increase, and were generally higher after this increase in Vtg. Testosterone is the primary precursor for estradiol in most teleost fishes and production of this androgen from vitellogenic ovaries is thought to be related to its use in the aromatization to estradiol (Kagawa et al., 1983). The pattern observed for T is consistent with this action.

Plasma Vtg concentrations observed in the present study are in agreement with what has been reported in the literature for channel catfish (Pacoli et al., 1990). Increasing levels of Vtg from January to May can be attributed to vitellogenin growth of the oocytes, and were highly correlated to oocyte size. Pacoli et al. (1990) reported that prior to spawning levels of plasma Vtg in channel catfish were 30.2 mg ml^{-1} in May. Similar levels (31.2 mg ml^{-1}) were measured in the plasma of fish sampled in May of the present study.

The initial increase in cathepsin L activity in November followed by monthly increases until March may be associated with the incorporation of protein into the oocytes during the early and mid-vitellogenic growth phases. Carnevali et al. (1999a) reported that higher cathepsin L activity in seabream (*Dicentrarchus labraz*) occurred during mid-vitellogenesis, thus supporting the results of the current study. Kestemont et al. (1999) reported that cathepsin L in perch was absent in freshly fertilized eggs, but increased at the onset of yolk vesicle proteolysis. Cathepsin L activity decreased after ovulation and began to reappear after fertilization of the eggs. Similarly, Hiramatsu et al. (2002b) concluded that cathepsin L was involved in secondary proteolysis of the yolk proteins during final maturation of oocytes in white perch. On average, oocytes sampled in the present study did not appear to have reached the stage of final oocyte maturation. More research is needed to determine if cathepsin L is involved in the secondary proteolysis of yolk proteins at this stage in channel catfish.

In catfish, cathepsin D activity was observed to be highest in March and May. Patiño and Sullivan (2002) stated that cathepsin D in seabream is responsible for the production of yolk proteins from vitellogenin. Hiramatsu et al. (2002b) concluded that both cathepsin D and cathepsin L were responsible for the specific

conversion of vitellogenin into its constituent yolk proteins. Kwon et al. (2001) reported that maximum cathepsin D activity of rainbow trout (*Oncorhynchus mykiss*) occurred prior to or at the onset of vitellogenesis, and was abundantly expressed throughout vitellogenic growth and final maturation of the oocytes. Carnevali et al. (1999a) and Kestemont et al. (1999) concluded that cathepsin D in perch was highest in early vitellogenic oocytes when yolk protein was being deposited at a high rate. In channel catfish the enzymatic activity of cathepsin D was elevated at the mid- to late-vitellogenic phases, with the highest activity observed in May, near the spawning season. While these results may be indicative of Vtg proteolysis, it is possible that since the oocytes were not of mature size on average and few fish spawned, cathepsin D may have been playing a role in follicular atresia. Carnevali et al. (2006) reported that lysosomal enzymes are involved in oocyte re-absorption and that degeneration of immature follicles (follicular atresia) is under the control of cathepsins. Furthermore, there is evidence that cathepsin D is an important mediator of programmed cell death, and has been implicated in apoptosis induction and potentiation. (Kågedal et al., 2001).

Few studies have investigated cathepsin B activity during oogenesis in teleosts. Cathepsin B activity was the lowest among the cathepsins monitored in this study. In contrast to the observations for cathepsins D and L, the highest activities occurred in November, December and March. Kwon et al. (2001) observed high cathepsin B activity in rainbow trout throughout all stages of oogenesis. Like cathepsin D, Carnevali et al. (1999a) found that elevated cathepsin B activity occurred within early vitellogenic oocytes, results supported by those of the present study.

Carnevali et al. (1999b) reported maximum levels of activity of cathepsins B and D to be 8 and 31 U mg^{-1} in seabream, respectively, in early vitellogenic oocytes, while maximum cathepsin L activity (0.10 U mg^{-1}) occurred later in vitellogenesis. For channel catfish, cathepsin L activities were substantially higher than those reported for seabream. This difference could be species-specific. One possible explanation for the differences may reside in the fact that eggs of seabream float, whereas channel catfish eggs do not. Therefore, the differences in enzymatic activities could be in response to a need to maintain buoyancy throughout the hydration process in seabream. These investigators observed that the activity of cathepsin D was significantly higher in sinking eggs compared to that of floating eggs, where cathepsin L activity was predominant (Carnevali et al., 2001).

5. Conclusion

Channel catfish have been important commercial and sport fish for many years, with captive spawning occurring as early as the late 1890's (Dunham and Smitherman, 1984). Modern farm-raised channel catfish have ancestry from many river systems. Dunham and Smitherman (1984) documented 92 channel catfish stocks in the United States. A variety of breeding programs to improve channel catfish traits for commercial aquaculture has existed over the years, but few comparisons have been made between selectively bred strains for physiological or biochemical reproductive parameters possibly relating to spawning. The present study is the first to make such comparisons between four commercially reared strains, three of which were selectively bred in a commercial setting. Although no differences were observed among the strains for any of the traits measured in the 2- to 3-yr-old females used in this study, valuable data for young-adult channel catfish have been collected that may prove useful in future research to assess what controls spawning success in channel catfish. Furthermore, this is the first report of cathepsin activities and their relationships to other reproductive parameters in channel catfish. These data represent the foundation for future investigations into the roles of cathepsins in oocyte maturation and embryo development.

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