The Effect of Age/Size on Plasma Sex Steroids and Aromatase activity of Asian Seabass (*Lates calcarifer*) when transferred to ideal hatchery conditions.

Saman Athauda\textsuperscript{a,b,*}, Trevor Anderson\textsuperscript{a}

\textsuperscript{a}School of Marine Biology & Aquaculture, James Cook University, Townsville, QLD 4811, Australia

\textsuperscript{b}Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.

Corresponding Author email: sbathauda@pdn.ac.lk

ABSTRACT

Asian Seabass, *Lates calcarifer* precocious sex change is problematic for efficient hatchery operation. Seabass grown in freshwater farms were transferred to brackish water (32 g/L) at 28 °C and fed to satiety until acclimated. Blood, brain and gonad were collected and analysed. Brain aromatase activity appeared to respond to age/size rather than environmental conditions while gonadal aromatase was detectable only in the 700 – 1000 g. Plasma T was increased in response to the environmental change in fish groups of 300-500 g and 700-1000 g while the 50-100 g and 2.5-4 kg had no significant increase while Plasma E\textsubscript{2} increased significantly in all groups of fish and 11-KT was detected in the 700 - 1000 g and 2.5 - 4 kg fish having no significant difference. Data indicate that the hormonal conditions are pre-requisite for inducing sex change in *L. calcarifer* are present in this species from 435 ±27 g.

Keywords: Asian Seabass; *Lates calcarifer*; Sex steroids; Aromatase activity; Sex change

1. INTRODUCTION

The sex inversion of fish is a complex and labile mechanism under the control of genetic, physiological and environmental factors (Baroiller and Guiguen, 1999; Delvin and Nagahama, 2002). Asian Seabass, *Lates calcarifer* (Bloch, 1790), also known as barramundi is a protandrous hermaphroditic species (Moore, 1979) reaching first maturity as a male and changing to female within two years of age in farmed conditions (Athauda et al, 2012; Athauda and Anderson, 2012) or in six to eight years (Guiguen *et al.*, 1994; Allan and Stickney, 2000) in the wild.

Asian Seabass is an important species for both aquaculture and fisheries throughout its natural distribution area from Australia to the Arabian Sea. This is a commercially cultured species in Australia and Southeast Asia and recently expanded to North America and Europe (Katersky and Carter, 2007). Obtaining seeds for culture operations from wild broodstock is expensive, highly seasonal, and unreliable and conflicts with wild stock resources management (Barlow *et al.*, 1996). Hence, brackish water hatcheries are used to obtain juveniles for rearing in aquaculture. However, the early sex change producing precocious females in far higher numbers than observed in the wild creates a significant management problem for hatchery managers who have difficulty in maintaining sufficient male brood stock for commercial rearing purposes. Consequently, hatchery seed production is limited by the inability of culturists to maintain functional males in the breeding population.

In northern Australia, male Asian Seabass in Van Diemen Gulf and Southeast Gulf of Carpentaria mature between 3 and 5 years age in wild. In contrast, sexually precocious maturity was reported in northeast Gulf of Carpentaria fish, which mature in their first or second year (Davis, 1985, Garrett, 1986). However, gonad development was retarded in landlocked freshwater Asian Seabass from the same region (Davis, 1985). Although the catadromous migratory pattern has been reported in previous Seabass research (Garrett, 1986) the environmental cues, chemical phenomena that led to its migration behavior towards brackish water are yet to be explored.

It was previously found that temperature (Athauda et al, 2012) and temperature and salinity (Athauda and Anderson, 2012) have an effect on sex change in L calcarifer. Haddy and Pankhurst (1998) also suggested that salinity may play a role in regulating reproductive activity in fish generally. There is relatively little published data or information on the effects of salinity on the timing of reproductive development (Bromage et al., 2001) or reproductive physiology (Haddy and Pankhurst, 2000) in fish. Previous studies with the protandrous species Amphiprion melanopus (Godwin and Thomas, 1993) and Acanthopagrus schlegeli (Wu et al., 2009) showed that sex steroids have significant role in sex change and gonadal development in teleost fish and oestradiol, 17β (E₂) when administered to L. calcarifer can stimulate sex change (Anderson and Forrester, 2001).

Protandrous sex inversion in Asian Seabass could be associated with a shift in gonadal steroidogenesis from androgens to estrogens (Guiguen and Jalabert, 1995). Thus, increases of aromatase activity which is the critical enzyme for above phenomena might further stimulate E₂ production and ovarian development, and finally cause sex inversion into females. Results of recent research (Guiguen et al., 2010) also showed that E₂ has a stimulatory effect on the aromatase activity in Asian Seabass.

Since we have previously described an effect of temperature and salinity on sex change in L. calcarifer (Athauda et al, 2012; Athauda and Anderson, 2012), this study sought to determine the age/size at which a change to “ideal” conditions might induce changes of the serum steroids E₂, testosterone (T) and 11 keto-testosterone (11-KT) concentrations and gonad and brain aromatase activity in cultured Asian Seabass.

2. MATERIALS AND METHODS

2.1. Experimental Fish

Groups of Asian Seabass (Lates calcarifer) at different sizes (50 - 100 g, 300 - 500 g, 700 - 1000 g and 2.5 - 4 kg) were obtained from a commercial farm (GFB Fisheries, Kelso) in north Queensland (19°S, 147°E) where they had been maintained in freshwater (0 g/L) under natural conditions of temperature (28 °C) and photoperiod (12: 12 Light: Dark). Fish were randomly taken from the cages in which they were cultured at the farm in and immediately transported in oxygenated tanks (80 L) to the Marine and Aquaculture facility (MARFU) at James Cook University, Townsville, Australia, where they were used for the experiment. Fish were fed with commercially formulated barramundi pellet (50% protein, 18 MJ/Kg, Ridley Aquafeeds, Brisbane, Australia) to satiety twice daily (0930 and 1430) when held on the farm and in the same manner in the Aquaculture facility at James Cook University. Fish were acclimatized to the Aquarium conditions (28 C and 14:10 L: D), which are the usual conditions during the breeding season.
2.2. Experimental Design and Sampling Procedures

Eight groups of Asian Seabass (n = 8/group, two groups per size class) according to their body weight were allocated to 3000 L tanks contained in a single enclosed room. A group (n=8) of fish of each size class were sampled under the initial freshwater conditions prior to commencing the exposure of the remaining fish to higher salinity for the experimental period. Gradual changes of water salinity from freshwater (0 g/L) to brackish water (32 g/L) were undertaken over a one week period for each of the size classes fish and subsequently maintained throughout the experimental period of nine weeks at 28 ºC and at 32 g/L, respectively. One third of the tank water was changed everyday throughout the experimental period in addition to removing uneaten feed, faeces or any foreign material twice a day by siphoning. Both saline and freshwater was supplied from the main supply of the Aquarium system to adjust required salinity levels for each experimental tank and siphoned water was discharged to waste water system.

Temperature and salinity was measured twice daily and adjustments were made if required to maintain experimental conditions. During this period water quality parameters (pH, Ammonia, Nitrate and Nitrite levels) were measured using standard test kits (Aquarium Pharmaceuticals, Inc. Chalfont, PA, USA) once a week throughout the experimental period and were maintained at constant and equal levels in all the units. Dissolved oxygen (DO) was measured everyday by DO meter and maintained above 5 mg/L. A UV sterilizer (QL 160, Rainbow Aq Products, Elmonte, CA 91734) was used to sterilize some proportion of the water as a preventive method of disease control in addition to the biological filtration.

Fish were anaesthetized to collect the blood samples for steroid hormone assays at the beginning (n = 6 or 8) and the end (n = 7 or 8) of the 09 weeks experimental period. A 2.5 mL blood sample was extracted from the caudal vein using a 5 mL syringe and an 18-gauge hypodermic needle. The blood samples were immediately transferred to 2.5 mL fluoride-oxalate tubes (Sarstedt, Technology Park, SA 5095, Australia), mixed gently and stored on ice until transported to the laboratory. Blood samples were centrifuged at 14000 g for 10 min at 4 ºC (Eppendorf centrifuge 5415C, Hamburg, West Germany) and the plasma stored at -80 ºC until the assay for E2 (Oestradiol-17 β), T (Testosterone) or 11-KT (11 Keto Testosterone).

Upon collection of the blood samples, the fish were sacrificed, brain and gonads were removed aseptically, transferred to labelled vials, and placed in liquid N2 at -80 ºC for aromatase assay.

2.3. Sex Steroids Assay

Plasma levels E2, T and 11-KT were measured by radioimmunoassay following extraction with ethyl acetate using the reagents and protocols describe by Pankhurst and Conroy (1987). Briefly, 300 µL of plasma were extracted with 1.5 mL ethyl acetate, 200 µL extract was added to each assay tube for evaporation and resuspending in assay buffer, with the reagent and supernatant was mixed with ReadyGel scintillation fluid (Ecolite, USA) and 3H-labelled steroid in each samples were measured in duplicate using a liquid scintillation counter (Beckman, QuantaSmart-1.31, USA). Extraction efficiency (recovery level of 3H-labelled steroid from plasma) was determined and assay values were corrected accordingly for E2, T and 11-KT. Interassay variability measured using aliquots of a pooled internal standard was determined for E2, T and 11-KT. The detection limit of E2, T and 11-KT in plasma was 3 ng/tube, and all samples were measured in a single assay. E2, T and 11-KT concentrations were determined against a standard curve.

2.4. Measurements of Aromatase Activity

Aromatase activity in the gonad and brain was measured by tritiated water release assay (radiometric method), which had been previously validated for use of the Asian Seabass in our laboratory (Anderson & Forrester, 2001). Macerated gonad tissue was thoroughly homogenised at 24000 g in 10 volumes (w/v) of a cold solution containing 100 mM KCl, 10 mM K2HPO4, 1 mM ethylenediaminetetraacetic acid (EDTA) and 2
mM dithiothreitol (pH 7.4) using a Heidolph diax 600 homogeniser with 10G tools. The homogenate was then sonicated for 30 sec (Unisonics FX8, Sydney, Australia) and centrifuged at 1000g for 5 min at 4 °C. The non-lipid portion of the supernatant was recovered by piercing the tube immediately above the pellet with a heated 18 gauge needle. Aliquots of extract were stored at −80 °C prior to assay. Extract (150 µL) was incubated at 30 °C with 450 µL of solution containing 100mM KCl, 10mM K₂HPO₄, 1mM ethylenediaminetetraacetic acid (EDTA) and 2mM dithiothreitol, 5mM glucose-6-phosphate, 1mM β-nicotinamide adenine dinucleotide phosphate (NADP), 2U glucose-6-phosphate dehydrogenase plus 66.67 nM androst-4-ene-3, 17-dione (pH 7.4). At 10 and 30 minutes, a 200 µl aliquot of the reaction mixture was terminated by mixing with 100µL of 30% trichloroacetic acid containing 60mg/mL charcoal. After standing for 30 min, the mixture were then centrifuged at 10,000g for 5 min. and the supernatant (200 µL) was added to a 1.0 x 3.0 cm column packed with equal volumes of 50-100 and 100-200 mesh AG50W-X4 resin (Bio-Rad, Hercules, CA). Samples were then eluted with 2.3 mL of deionized water, with the final 1.5 mL collected and mixed with 15 mL Ready Gel (Beckman, USA). DPM (Disintegration per minute) was measured over 5 min with a liquid scintillation counter (Beckman, QuantaSmart-1.31, Hicksville, NY 11801, USA).

Aromatase activity was expressed using the production of ³H₂O from ³H-androstenedione (³H-A) according to the method previously used in our laboratory (Anderson & Forrester, 2001). The Bradford (1976) method was used for determining protein concentrations of the crude supernatant fraction by monitoring of absorbance of protein-dye complex. Aromatase activity was assayed in the gonad and brain tissues of all fish except for 50 g, which as the lowest size was not able to be sampled for aromatase activity in either tissue. Aromatase activity was expressed as the reaction velocity of the conversion of androstenedione to tritiated water (formation of ³H₂O during aromatization of ³H-A) using fmoles/mg protein/min as the units.

2.5. Data Analysis

Each tank was considered as one experimental unit and each fish as one replicate. Analysis of Variance (ANOVA); two-way comparison was used with Tukey’s HSD test to test for the difference between treatment for hormone and aromatase or steroids levels. Least Significant Difference (LSD) was used to compare the significant levels within and in between of treatments and their interactions. Significant effects were assumed at α = 0.05. All data were analyzed using SPSS version 12.0 and where appropriate, proportion data were normalized by transformation to ensure assumptions of the ANOVA were met.

3. RESULTS

3.1. Growth

Initial and final average weights of fish are shown in Table 1. Average Daily Growth (ADG) decreased as the size of the fish increased with the highest growth rate in the smallest fish (50 - 100 g) and lowest growth rate in the largest fish (2.5 - 4 kg). Average size at the end of the experiment was significantly greater than the beginning weight in all fish groups except 2.5 – 4 kg, the largest fish group. The highest ADG (7.05% body weight/d) was recorded in smallest size group (50- 100 g) while the lowest (0.35% body weight/d) was in largest size group 2.5 – 4 kg (Table 1). Survival of experimental fish was 100% at the end of the experiment period.

3.2. Water Quality

Mean water quality parameters during the experiment period are shown below (Table 2). Ammonia and nitrite levels were measured routinely and were maintained at less than 1 mg/L and nitrate was maintained around 12 mg/L. Average temperature was measured daily and maintained at 28 °C, while pH was measured weekly and maintained at pH 8.0. Salinity was maintained according to the experimental
design and maintained at 32 g/L. All water exchanges required to maintain favorable conditions for cultured fish and adjustments of water quality parameters occurred at the same time each day.

3.3. Aromatase activity
3.3.1. Aromatase activity in brain

The aromatase activity in brain at the beginning and end of the experimental period is shown in Fig. 1. The level of aromatase activity in brain at the beginning of the experiment was significantly (p < 0.05) greater in the brain of 700–1000 g size fish group and 2.5–4 kg size fish group than in the brain of 300 g to 500 g animals, although this was not the case at the end of the experiment.

The level of aromatase activity in fish group 300–500 g was significantly increased at the end of the experiment and in the remaining two groups (700-1000 g and 2.5–4 kg) was lower, but not significantly, compared to their initial aromatase activity. Aromatase levels were not assayed in brain of smallest group (50–100 g) of fish.

3.3.2. Aromatase activity in gonad

The changes in aromatase activity in gonad during the nine week experimental period are shown in Fig. 2. Gonadal aromatase activity recorded at the end of the experiment in fish group 700–1000 g was significantly greater than the beginning of the experiment. Fish in the 2.5–4 kg group had a very small amount of gonadal aromatase activity, which is not significantly different to the low or undetectable values found in all other weight groups (50–100 g and 300–5000 g).

3.4. Plasma steroids
3.4.1. Testosterone (T)

Two-way ANOVA of the testosterone data showed significant effects between groups and between times, but no interaction of group x time. There was no significant difference in plasma T levels in fish at different weight groups at the beginning of the experiment, although the mean value for the largest group was approximately twice that of the other groups (Fig. 3). Initial T values were recorded as 0.127, 0.136, 0.163 and 0.295 ng/mL in the 50–100 g, 300–500 g, 700–1000 g and 2.5–4 kg groups, respectively.

Within each fish group, plasma T levels increased with the increase appearing to be dependent on fish size. After nine weeks of being held at higher salinity and temperature, T values were recorded as 0.239, 0.361, 0.356 and 0.346 ng/mL in the 50–100 g, 300–500 g, 700–1000 g and 2.5–4 kg groups respectively. The smallest (50–100 g) and the largest (2.5–4 kg) fish had the smallest relative increases, which were not significantly different (p < 0.05).

3.4.2. Estradiol 17-β (E₂)

There was no difference in plasma E₂ levels among beginning fish at different weight groups (Fig. 4). E₂ concentrations were slightly higher in the larger fish than in the smaller fish. The E₂ levels at the beginning were 0.002 and 0.003 ng/mL respectively for the 50–100 g and 300–500 g weight group fish while E₂ concentration was 0.034 ng/mL in the 700–1000 g and 2.5–4 Kg weight groups. At the end of the experiment period E₂ levels were significantly increased (p < 0.05) within each of the fish groups and between each fish size classes being 0.056, 0.106, 0.142 and 0.211 ng/mL at 50–100 g, 300–500 g, 700–1000 g and 2.5–4 kg, respectively (Fig. 4). However, Plasma E₂ levels of 50–100 g and 300–500 g and 300–500 g and 700–1000 g were not significantly different among each other. Plasma E₂ concentrations
in all fish smaller than 700 g were significantly \( p < 0.05 \) lower than those of the 2.5 – 4 kg plasma \( E_2 \) level (Fig. 4.) where the value was recorded as 0.211 ng/mL.

3.4.3. KetoTestosterone (11-KT)
There were no measurable amounts of 11-KT in the two smallest groups (50 - 100 g and 300 - 500 g) of fish (Fig. 5). Some amount of 11-KT was measured in plasma of 700 – 1000 g and 2.5 – 4 kg fish groups. However, there was no significant difference in 11-KT concentrations in these fish at the beginning and the end of the experiment (Fig. 5).

4. DISCUSSION
The highest ADG was recorded in the smallest size fish group 50 – 100 g followed by 300 – 500 g and 700 – 1000 g size groups while the lowest was in the largest size fish (2.5 – 4 kg) group. This is commonly observed phenomena in fish and shows a standard growth vs size relationship (De Silva and Anderson, 1995). Numerous exogenous factors have been shown to affect the growth of fish. In this experiment, the environmental factors (salinity and temperature and photoperiod) are optimum for \( L. \) calcarifer growth and dietary factors (protein and energy) are also in sufficient levels. Growth rates observed in this study were similar to previously (Raso and Anderson, 2003) observed values for Asian Seabass under a healthy growing environment.

Aromatase activity level in brain increased significantly \( p < 0.05 \) after nine weeks in the 300 g – 500 g experimental fish, but other groups (700 – 1000 g and 2.5 – 4 kg) showed no significant change in brain aromatase activity. The significant change that occurred with the change in brain aromatase was the increase in size of the fish, with no changes occurring in the larger animals with the change to a higher temperature and salinity environment. The size of the 300 – 500 g group increased to be equivalent to the starting size of the 700 – 1000 g group and the aromatase activity in the brain increased to a level that was not significantly different to that at the beginning of the experiment in the 700 – 1000 g group. The level of brain aromatase activity was not different between groups in all fish of 636 ± 18 g or above. The brain is a site where environmental effects are largely integrated and temperature and salinity changes might be expected to exert an effect on the brain aromatase activity. However, the data presented here indicate that brain aromatase activity is a function of size/age of the fish rather than being influenced by external environmental parameters.

In the protandrous black porgy, aromatase inhibitor Fadrozole blocked the natural sex change and significantly suppressed the brain aromatase activity (Lee et al. 2001) although it would also have blocked gonadal aromatase activity. Aromatase activity in gonads was not detectable in most groups of Asian Seabass in this study with one clear exception of the 700 – 1000 g fish after nine weeks at 28 °C, in which the gonadal aromatase activity level increased significantly \( p < 0.05 \) over its initial level. Previous research (Kitano et al., 2000; Kwon et al., 2001) has shown that gonadal aromatase plays a decisive role in sexual differentiation. In the black porgy, the increase of gonadal aromatase activity was associated with both natural and controlled sex changes (Lee et al., 2000, 2001).

The role of T as one of the precursors for \( E_2 \) (Devlin and Nagahama, 2002) has been well documented. There were significantly \( p < 0.05 \) increased T levels in the fish groups 300-500 g and 700 – 1000 g while the smallest (50 - 100 g) fish and largest (2.5 – 4 kg) groups of fish were not significantly different to the initial level. Factors affecting the levels of T in circulation involve regulation of both synthesis of T and its conversion to other hormones. It is likely that production of T is not yet fully functionally in 50 – 100 g fish, which are immature. In contrast, 2.5 – 4 kg fish contained relatively high concentrations in the circulation at the beginning of the experiment but did not show a significant increase.
by the end of the experiment indicating that metabolism of T was approximating its synthesis in these fish as would be expected in mature fish.

The end T level of smaller fish (50 – 100 g) is greater, but not significantly, than the initial T level circulating in 300 – 500 g fish although the weight of the two groups (435 ± 27 g in the smaller group vs 463 ± 13 g in the larger of the two groups) were similar and indicated an effect of salinity also on T production in Asian Seabass as previously described by Ahauda and Anderson (2012). The concentration of T observed in fish over about 1000 g, which was the finishing size of the 300 – 500 g group, had a consistent mean between 350 and 400 pg/mL suggesting this may be the steady state level of T in Asian Seabass held in seawater conditions.

All fish in this study showed low levels of circulating E₂ at the beginning of the experiment. However, by the end of the 9 weeks experimental period, there was a significant increase in the circulating E₂ concentration in all the fish groups. The E₂ level showed a clear increase with increasing size, with the lowest value recorded in 50 – 100 g fish while the highest value was found in 2.5 – 4 kg. A lower level of E₂ in small fish (50 – 100 g) is likely to be due to the absence of functional gonadal tissue while in larger fish, the higher concentration of E₂ will reflect a greater amount of gonadal tissue.

The plasma E₂ level increased in all groups after the fish had been kept for 9 weeks under saline water conditions, compared with those of similar size that had been held on farm in freshwater. These data clearly indicate that salinity has a stimulatory effect on plasma E₂ level in L. calcarifer and that this effect occurs in all fish of this species over 463 ± 13 g.

E₂ was detected in fish as small as 463 ± 13 g and T was detected in animals as small as 50 g. Brain and adipose tissue have been shown to produce these steroids (Zhang et al., 2004) with aromatase being involved in the synthesis of E₂ from T. The absence of significant aromatase activity in the gonads in the same groups of fish that showed both brain aromatase activity and circulating concentrations of E₂ suggests the brain is an important initial site of E₂ synthesis. The increase in brain aromatase in response to the change of environment at sizes greater than 636 ± 18 g, but the increase in E₂ at smaller sizes in response to increasing salinity suggests that regulation of aromatase activity is not the sole process by which E₂ is regulated but it may effect the rate of the response to environmental changes.

In this study, 11-KT was detected only in the groups of larger fish (700 – 1000 g and 2.5 – 4 kg) at both the initial, freshwater, and after nine weeks of higher salinity conditions (Fig. 5). The presence of 11-KT is an indicator of maturity as a male in fish (Pankhurst, 1998). Thus, hormonal indicators of mature male L. calcarifer were present only in animals that had reached 636 ± 18 g while being held in freshwater conditions and a shift to 28 °C and 32 g/L salinity for nine weeks was insufficient to cause 11-KT production to cease. The presence of high concentrations of E₂ in these animals suggests that remaining for a longer period in these conditions will result in sex change to female. In the case of the smallest group, the absence of 11-KT in the circulation may reflect an absence of functional gonadal material.

That E₂ responds to increasing salinity and there is some brain aromatase activity present in fish of 463 ± 13 g indicates that the hormonal conditions that are pre-requisite for inducing sex change in L. calcarifer (Anderson and Forrester, 2001) are present from this size, much earlier than would be expected from observations of normal (wild) ontogeny of sex change was to occur.
Table

Table 1. Mean fish weight at the beginning and the end of experiment and average daily growth (ADG) over the experiment for each group of fish. Values are mean ± S.E. Values with different superscripts are significantly different (p < 0.05).

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial Wt (g)</th>
<th>Final Wt (g)</th>
<th>ADG (% body weight/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 – 100 g</td>
<td>81 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>435 ± 27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.05</td>
</tr>
<tr>
<td>300 – 500 g</td>
<td>463 ± 13&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1025 ± 35&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.96</td>
</tr>
<tr>
<td>700 – 1000 g</td>
<td>636 ± 18&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1225 ± 61&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.49</td>
</tr>
<tr>
<td>2.5 – 4.0 kg</td>
<td>4408 ± 445&lt;sup&gt;g&lt;/sup&gt;</td>
<td>5375 ± 530&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Table 2. Average water quality parameters in experimental fish tanks. Values are mean ± S.E.

<table>
<thead>
<tr>
<th>Salinity (mg/L)</th>
<th>pH</th>
<th>Ammonia (mg/L)</th>
<th>Nitrite (mg/L)</th>
<th>Nitrate (mg/L)</th>
<th>Temperature °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 ± 0.24</td>
<td>8.0 ± 0.02</td>
<td>0.64 ± 0.05</td>
<td>0.037±0.004</td>
<td>12.72 ± 0.55</td>
<td>28.3 ± 0.07</td>
</tr>
</tbody>
</table>

Figure

Figure 1. Aromatase activity in brain of different weight groups fish at the beginning and end of the experiment. Values are mean ± S.E. Values with different superscripts are significantly different (p < 0.05).
Figure 2. Aromatase activity in gonad of different weight groups fish at the beginning and end of the experiment. Values are mean ± S.E. Values with different superscripts are significantly different (p < 0.05).

Figure 3. Mean Testosterone levels in fish of different weight groups at the beginning and end of the experiment. Values are mean ± S.E. Values with different superscripts are significantly different (p < 0.05).
Figure 4. Mean Estradiol 17-β levels in fish at different weight groups at the beginning and end of the experiment. Values are mean ± S.E. Values with different superscripts are significantly different (p < 0.05).

Figure 5. Mean 11 Keto Testosterone levels in fish of different weight groups at the beginning and end of the experiment. Values are mean ± S.E. Values with different superscripts are significantly different (p < 0.05).

REFERENCES


