Variation of some biochemical parameters in female yellowfin seabream, Acanthopagrus latus (Houttuyn) during reproductive cycle

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Variation of some biochemical parameters in female yellowfin seabream, *Acanthopagrus latus* (Houttuyn) during reproductive cycle

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Abstract. In the present study changes of some blood parameters of wild female yellowfin seabream (*Acanthopagrus latus*) caught from Persian Gulf were assayed during reproductive cycle. Altogether, 120 female *A. latus* (15 each month) were captured monthly from October 2010 to May 2011 from the Mussa Creek in the north-west of Persian Gulf. Blood samples were collected from caudal vein; plasma was separated and kept at –80 °C till analysis. Total protein, glucose, cholesterol, triglyceride, electrolytes, calcium, sodium, chloride, magnesium, potassium plus hepatic enzymes, Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST), were assayed in plasma sample. Total protein and calcium increased parallel to ovarian development and decreased after spawning time. Cholesterol and triglyceride had a peak during vitellogenesis and decreased after spawning but glucose had a peak during spawning time. Most of the electrolytes (sodium, magnesium and potassium) did not show any significant changes during the reproductive cycle in *A. Latus*. AST reached a peak during final maturation of ovaries but ALT did not show any significant difference during different sampling times. Our findings showed that biochemical parameters could be used as indicators of physiological status during different maturation stage in this species.

Key words: fish, electrolyte, enzyme, metabolite, Persian Gulf, plasma, reproduction

Introduction

Yellowfin seabream, *Acanthopagrus latus* (Houttuyn, 1782), is a protandrous hermaphroditic sparid (porgies) from the order Perciformes (perch-likes) and class Actinopterygii (ray-finned fishes) (Abol-Munafi & Umeda 1994) with a wide distribution from southern Japan, China, Taiwan, southeastern Asia, the Persian Gulf, Australia and in the Indian Ocean to the southeastern Africa (Hayashi 1993). This euryhaline species is able to live in freshwater, brackish and seawater so has a high potential for aquaculture in the Indo-Pacific region because of its high market value and easy adaptation to captivity (Hesp et al. 2004, Sà et al. 2006). In addition, *A. latus* is observed in the shallow water of the estuarine zone that endures dual fluctuations in temperature and salinity (Savari et al. 2010). Yellowfin seabream is one of the most commercially important marine fish in Iran, and recently is cultured successfully in the coastal area of Iran, especially in the Khuzestan province.

Reproduction development process of yellowfin seabream fish consists of four main phases: (1) the initial growth phase: oogonial cells at this stage proliferate during mitotic and become nuclear oocytes (2) secondary growth phase characterized by the developing yolk in oocyte (3) oocyte maturation/ ovulation phase, when eggs reach the maximum size and are released from follicle and (4) the phase of oocyte atresia, when eggs are ripe reabsorbed if ovulation does not occur (Abou-Seedo et al. 2003, Karimi et al. 2014).

In fish, reproduction tends to be an important factor that affects the various internal parameters. Therefore, it is necessary to pay attention to hematological and biochemical parameters during the reproduction phase to evaluate physiological status of fish (Svobodová et al. 2001). The biochemical assessment of blood parameters is important in evaluating the health status of fish (Coles 1986). Interpretation of these parameters should be done carefully as they are affected by both

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internal and external factors such as species and strain, temperature, age, stress, photoperiod, nutritional state, reproductive stage, and the methodology used to determine these parameters (Hofer et al. 2000, Cnaani et al. 2004, Silverira-Coffigny et al. 2005, Asadi et al. 2006).

Rhythmicity in physiological and behavioral activities is a rife phenomenon in the living world, and a fundamental trait of organisms from bacteria to humans. Biological rhythms have a crucial role in the temporal organization of fish which allows the adjustment of life processes to daily, lunar and annual cycles (Gerkema 1992). It is accepted that the photoperiod, feeding and maturity stage are the most important factors affecting the physiological and behavioral rhythms in fish. Undulations in circulating metabolites, hormones, feeding and locomotor activity have been extensively described in fish (Ali 1992, Reebs 2002). Success in both the natural and artificial reproduction of fish is related to factors seriously affecting internal environment of the organism (Svoboda et al. 2000). The purpose of the present study was to evaluate variations in blood biochemical parameters in wild caught female yellowfin seabream in the different phases of the reproductive cycle.

Material and Methods

Sampling

Altogether, 120 female yellowfin seabream were caught monthly (15 each month) from the Mussa Creek, Persian Gulf, Iran, from October 2010 to May 2011. Sampling was done carefully with hook to minimize damages. The fish were anesthetized with 2-phenoxy ethanol (0.2 %) and then blood samples were collected from the caudal vein with a heparinized syringe immediately after capture. Samples were kept on ice, transferred to laboratory (Martemyanov 2001) and then centrifuged at 9000 G for 10 min to separate the plasma. Plasma was kept in –80 ºC for further analysis. After sampling, the fish was killed by a sharp blow to the head. Biometry was done and identification of gonadal stage by visual examination of ovary was done as described by Shinkafi et al. (2011) (Table 1).

Table 1. Occurrence of different maturation stage in gonads of female *A. latus* during the reproductive cycle.

Maturation stages	Month of occurrence in this study
Immature stage (I)	October to December
Developing stage (II)	January
Mature stage (III)	February
Ripe stage/final maturation(IV)	March
The spawning stage (V)	April
Resting (VI)	May

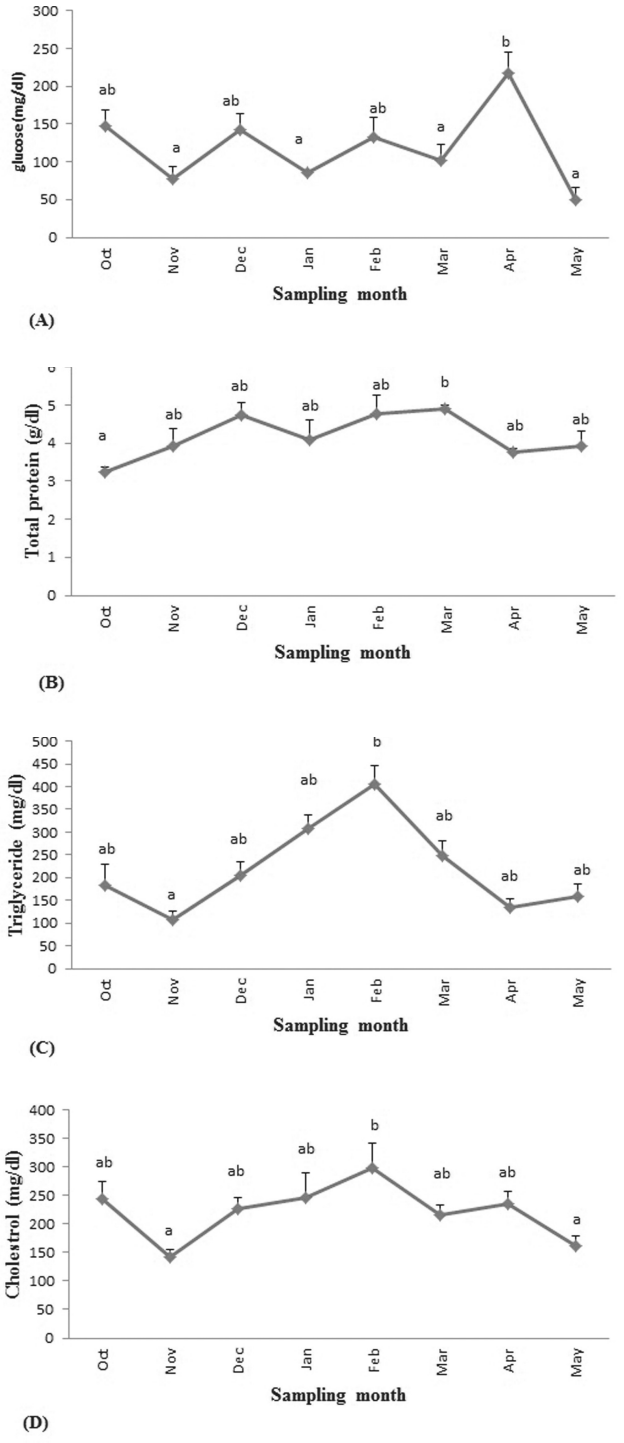

Fig. 1. Monthly variation of metabolites in female yellowfin seabream (*A. latus*) during reproductive cycle. (A) glucose (B) total protein (C) triglyceride (D) cholestrol. Data are expressed as the mean ± SE (n = 15). Different letters denotes significant variation ($P < 0.05$).

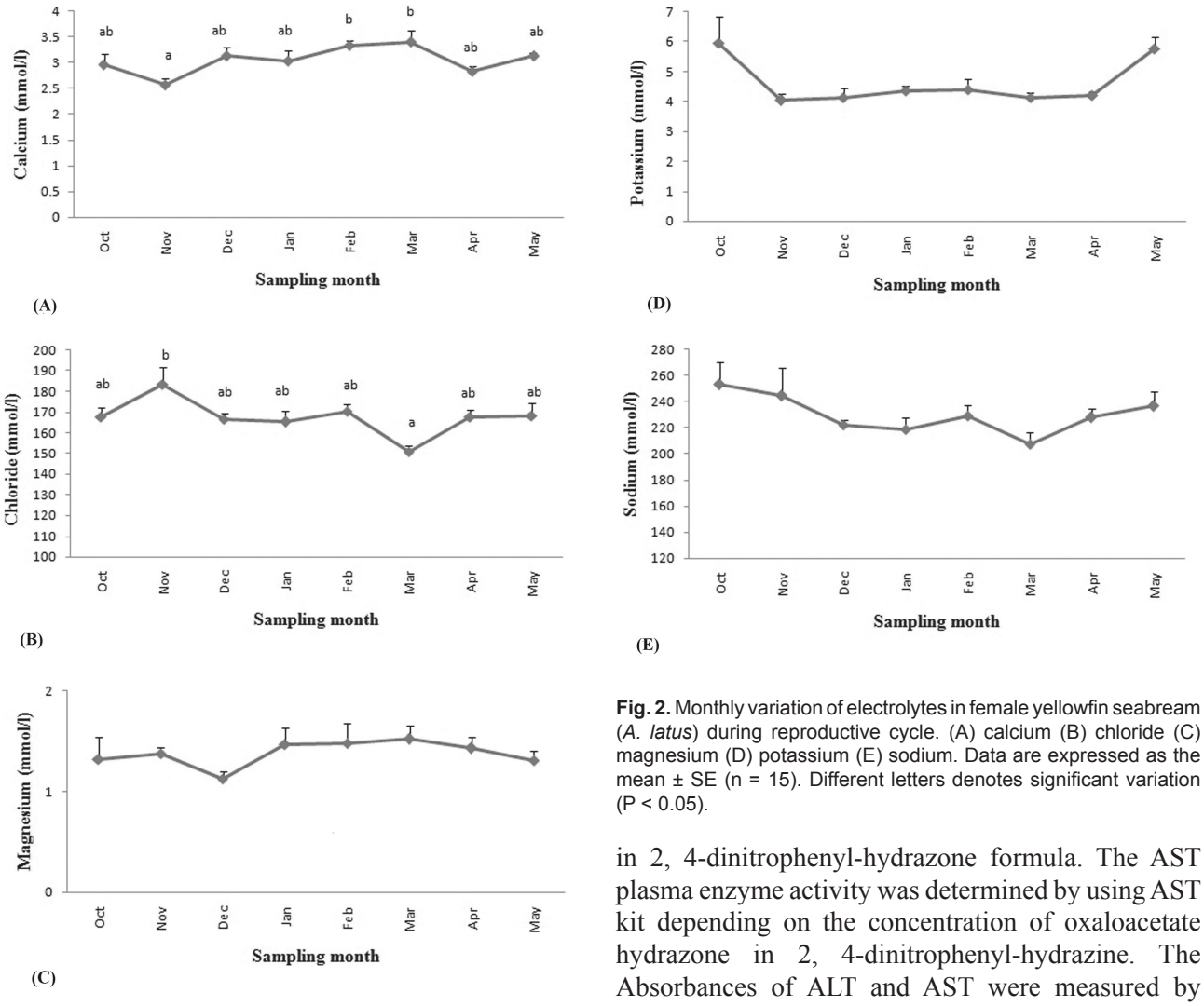

Fig. 2. Monthly variation of electrolytes in female yellowfin seabream (*A. latus*) during reproductive cycle. (A) calcium (B) chloride (C) magnesium (D) potassium (E) sodium. Data are expressed as the mean ± SE (n = 15). Different letters denotes significant variation ($P < 0.05$).

Plasma biochemical analyses

Plasma total protein concentration was determined on the basis of Lowrey method and using bovine serum albumin as a standard protein. Plasma glucose was colorimetrically measured using a commercial kit (Ziestchem, Iran). Cholesterol and triglycerides (TG) were measured by colour reaction using the auto analyzer Technicon RA-1000 (612 and 600 nm was used for triacylglycerol and cholesterol, respectively). Electrolytes including calcium, sodium, chloride, magnesium and potassium were assayed plasma sodium and potassium were determined with flame photometer. Plasma calcium (Ca^{+2}), magnesium (Mg^{+2}) and chloride (Cl^{-}) values were measured by commercial kits (Ziestchem, Iran).

Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) were determined with Pars-Azmoon commercial reagent kits (Tehran, Iran). The ALT plasma enzyme activity was calculated depending on the concentration of pyruvate hydrazone in 2, 4-dinitrophenyl-hydrazone formula. The AST plasma enzyme activity was determined by using AST kit depending on the concentration of oxaloacetate hydrazone in 2, 4-dinitrophenyl-hydrazine. The Absorbances of ALT and AST were measured by spectrophotometer at 340 nm.

Statistical analyses

All data are expressed as mean ± standard error. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. The accepted statistical significance level was $P < 0.05$. The SPSS 13.0 (Chicago, USA) was used for analysis.

Results

Morphology of fish

In this study fish mean total length and weight were 26.65 ± 0.53 cm, 400.29 ± 25.99 g, respectively. There was no significant difference in total length and weight of female yellowfin seabream during the period of study ($P > 0.05$). The fish was at maturity stage in February and reached ripe stage in March (Table 1).

Metabolic changes

Monthly changes in blood metabolites are presented in Fig. 1. Glucose levels showed significant fluctuations

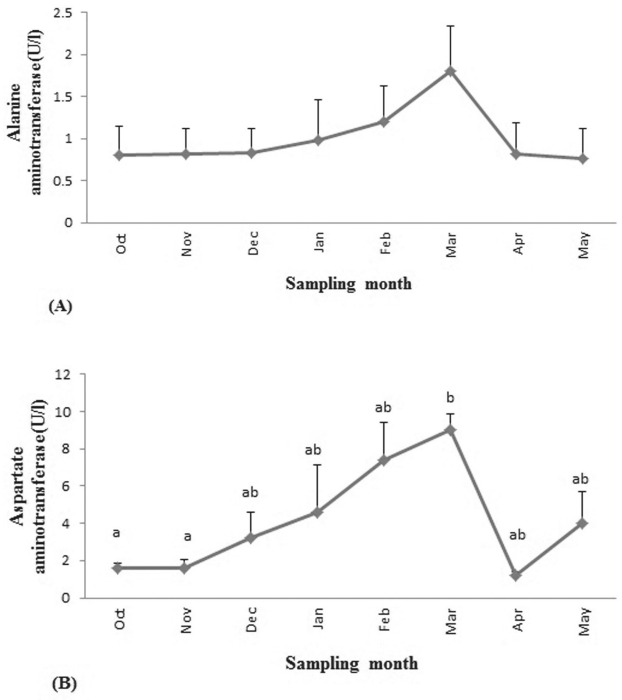

Fig. 3. Monthly variation of plasma enzymes activities in female yellowfin seabream (*A. latus*) during reproductive cycle. (A) ALT (B) AST. Data are expressed as the mean ± SE (n = 15). Different letters denotes significant variation (P < 0.05).

in different months, with the highest level recorded during in April (217.80 ± 27.43 mg dl^{-1}) and the lowest level was seen in May (50.00 ± 15.95 mg dl^{-1}). Total protein showed significant changes during reproductive cycle. The lowest and highest levels were recorded in October (3.24 ± 0.14 g dl^{-1}) and March (4.90 ± 0.10 g dl^{-1}), respectively. Lowest blood cholesterol values were observed in November (143.00 ± 11.82 mg dl^{-1}), then an increasing pattern was observed until highest level recorded in February (297.50 ± 44.07), then it decreased till resting stage in May (Fig. 1).

Triglyceride concentration increased gradually. In November, the lowest level (106.20 ± 13.32 mg dl^{-1}) was recorded in immature female, then its value increased progressively during the gonadal development and reached the maximum value (404.80 ± 72.64 mg dl^{-1}) at the beginning of the spawning stage which was synchronous with the vitellogenesis stage (February) (Fig. 1).

Electrolytes changes

Blood calcium content increased significantly from November to March, with the highest values being observed during the final maturation stage, and then decreased significantly (Fig. 2A). Monthly changes in plasma chloride are presented in Fig. 2B. Chloride levels showed significant decrease in the ripe fish (March) comparing to fish in resting stage (November). Highest level was recorded during in November (183.40 ± 8.58 mEq l^{-1}). There were no significant fluctuations in magnesium, sodium and potassium values at different months (Fig. 2C, D, E).

Enzyme changes

Plasma ALT levels in the yellowfin seabream did not show significant variations among the different months (Fig. 3A). Changes in AST levels during the reproductive cycle are shown in Fig. 3B. AST values increased till March, decreased in April and increased again in May at the end of reproductive cycle (Fig. 3B).

Discussion

The study of blood biochemical parameters during reproductive period in fish has received great attention (Svobodová et al. 2001, De Pedro et al. 2005, Bayir et al. 2007, Bani & Vayghan 2011). The study of blood parameters is one of the noteworthy diagnostic tools that show the physiological status of animal (Svoboda et al. 2000).

Our results showed that blood glucose was changed significantly which is similar with results described by Svoboda et al. (2001), De Pedro et al. (2005) in tench (*Tinca tinca*), and Bayir et al. (2007) in siraz (*Capoeta capoeta umbla*). Decreased plasma glucose levels in the pre-spawning period in *A. latus* may be due to the higher energy demand needed for gonadal development (Bani & Vayghan 2011). Further, the use of lipid and protein levels as the main energy sources during the spawning season had been reported in migrating American shad (Leonard & McCormick 1999). During the spawning period glucose increased significantly that is in accordance with previous reports in *T. tinca* (Guijarro et al. 2003) and *Rutilus frisii kutum* (Firouzbakhsh et al. 2013). This increase of glucose during spawning could be proposed as an additional energy source for response to energy demands.

Blood protein content could be used for assessing the physiological status and condition of fish, and therefore is an important diagnostic tool (Svetina et al. 2002). A significant difference in total protein values in plasma of *A.latus* was found during the reproductive cycle. Total protein showed peak in March (Fig. 1B) and in accordance with Table 1 it is stage IV. Increased protein synthesis and its release into blood in pre-spawning could account for increased total protein levels as reported by Bani & Vayghan (2011). Similar

pattern is reported in other species (Svobodová et al. 2001, De Pedro et al. 2005). Decrease in total protein levels during reproductive cycle has been reported in rainbow trout (Miller et al. 1983), pike (Lenhardt 1992), and wels (Svobodová et al. 1997). This could be caused by using total protein as an energy source (Bani & Vayghan 2011).

Differences in concentration of plasma cholesterol were found in female *A. latus* before and after spawning ($P < 0.05$). Variation in plasma cholesterol concentrations in relation to the reproductive cycle of fish had been reported by Diwan & Krishnan (1986). Vitellogenesis in *A. latus* begins in February, synchronically with the maximum value of plasma cholesterol. Decrease in the cholesterol value after spawning has also been reported in *T. tinca* (Svobodová et al. 2001). TG concentration in plasma of *A. latus* females was also significantly higher during February (maturation stage) than during May (resting period). The increased levels of blood TG reflect the vitellogenesis process of females. Cholesterol and TG are two important compounds being used during ovary development and vitellogenesis as well as for biosynthesis of sex hormones, therefore their plasma levels fluctuate (Svobodová et al. 2001). Decrease in the plasma cholesterol content of females after spawning may be due to the entrance of cholesterol into membranes and endogenous structures of eggs (Svobodová et al. 2001).

As observed in the present study, plasma calcium concentration increased steadily with reproductive development in female. Srivastava & Srivastava (1994) found that Ca^{+2} and Mg^{+2} levels increased in the pre-spawning stage while during spawning and post spawning decreased consistently. Martemyanov (2001) indicated that the plasma Ca^{+2} and Mg^{+2} levels were maintained within definite ranges during the reproductive cycle in *Rutilus rutilus*. Calcium is necessary for the formation of vitellogenin molecules (Yeo & Mugiya 1997). In female tilapia (*Oreochromis mossambicus*) the plasma calcium levels decreased after ovariectomy and increased after treatment with E2. These results showed that the gonads play a basic role in the calcium regulatory system of females (Tsai & Wang 2000). Estrogen treatment was showed to induce an increase in circulating levels of vitellogenin in (Mosconi et al. 1998), which is accompanied by an increase in blood plasma calcium (Persson et al. 1997). Although in this study the plasma chloride levels showed some significant fluctuations. Findings of Smit et al. (1981) in rainbow trout (*Oncorhynchus mykiss*), Allanson et al. (1971) in an African cichlid (*Tilapia mossambica*), Cataldi et al. (1998) in the Adriatic sturgeon (*Acipenser naccarii*), Cameron (1976) in Arctic grayling (*Thymallus arcticus*) and Jeffries et al. (2012) in the Pacific salmon (*Oncorhynchus* spp.) suggested that changes in chloride concentration are related to environmental parameters more than the reproductive cycle. Svobodová et al. (2001) reported that the values of Na^{+} in blood ranged in 144 and 145 $mmol.l^{-1}$ in all phases of the reproductive cycle in *T. tinca*. Comparable Na^{+} concentration values, very stable either before, or after the reproductive cycle were also detected in brood common carp (Svoboda et al. 2000). In present survey there was no significant variation in plasma Mg^{+2} during the reproductive cycle. It is thought that the level of free, ionic Mg^{+2} is kept at a stable and low value because it is an important determining factor of cellular function by its control over catalytic reactions (Imanpoor & Abdollahi 2011).

The plasma ALT levels in the yellowfin seabream showed no significant change between the different reproductive stages. But, the AST levels increased parallel to ovarian development and decreased after spawning (Fig. 3B). ALT is a crucial enzyme in utilization of protein and carbohydrates and functions at the linkage between protein and carbohydrate metabolism by inter converting ketogluterate, oxalacetate, and pyruvate on one hand and alanine, aspartate, and glutamate on the other hand (Tripathi & Verma 2003) that suggest a role for this enzyme to supply increased energy demand during reproduction. A significant increase in AST level might be due to the spawning period (Lusková 1997a). Similarly to *A. latus*, in the course of natural reproduction of salmonid, Lusková (1997a, b) found a noticeable effect of the reproductive stage (spawning) on the activity of enzymes studied.

Our findings highlight the relation of blood biochemical parameters with the reproductive cycle of *A. latus* but the role of other endogenous and exogenous parameters should be considered to evaluate the observed changes.

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