Fluctuation of gonadosomatic index during oocyte development in the narrow-clawed crayfish *Astacus leptodactylus* (Eschscholtz, 1823) in Aras Dam Lake, Iran

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Abstract

This study was carried out with the aim of examining the seasonal reproductive cycle of the female crayfish *Astacus leptodactylus* from Aras Dam Lake, Western-Azerbaijan, Iran. Gonadosomatic index (GSI), and oocyte size were measured in females sampled seasonally in June, August, November(2011), January(2012). Development of the oocytes was categorized according to the diameter and the presence/absence of yolk granules. The ovary development was accompanied by increasing levels gonadosomatic index and egg diameter. Ovarian development histologicaly related to the seasonal GSI . This index was low in June (0.61±0.05) when oocytes started developing and reached the highest value in November (13.53±0.25), when vitellogenic oocytes were abundant in the mature ovary. Our results highlight the relationship between the ovary development and the GSI and egg diameter in the crayfish *A.leptodactylus* during the reproductive cycle and held important implications for the management of aquatic species. Thus, investigation of female *A. leptodactylus* reproduction is highly significant for fisheries managers as well as environmentalists concerned with perpetuating crayfish stocks.

Keywords: *Astacus leptodactylus*, ovarian development, gonadisomatic index, egg diameter, histological analysis, ELISA

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Introduction

Astacus leptodactylus is a widespread species distributed throughout Europe, eastern Russia, and the Middle East (Souty-Grosset et al. 2006; Ghiasvand et al., 2012). Due to their large size and commercial interest, *A. leptodactylus* have been stocked in a large number of water bodies, particularly during the last century (Skurdal and Taugbol, 2002). In Iran, the first crayfish introduction took place in 1988 and up to now crayfish introductions have been carried out successfully in 18 water bodies, although some difficulties may be encountered that can jeopardize the effectiveness of such introductions (Karimpour et al., 2011). Among others, Ghoolami (2002) reported the adverse effect of pestides (herbicides and insecticides) on crayfish stocks in Iran. As well, the effects of experimentally taint with *Aeromonas hydrophila* has been reported in *Astacus astacus* in Iran (SamCookiyaei et al., 2012). Motamedi Sedeh et al. (2012) also reported the Iranian White Spot Virus isolated on Crayfish *A. leptodactylus*. We know that certain chemicals can affect normal endocrine function and that certain endocrine-disrupting chemicals can substantially reduce some animal populations (Taylor and Harrison, 1999; Shanle and Xu, 2011).

The gonad index can serve as an indicator of the condition of the gonads. Thus, an understanding of the reproductive cycle of female *A. leptodactylus* will help interpret responses to seasonal variations and would provide a useful management tool.

Although the consumption of crayfish is not common in the Iranian population, *A. leptodactylus* is actively exported mainly toward Turkey and Europe, where crayfish are perceived as luxury food and therefore highly sought. Crayfish farming is virtually absent in Iran, and all production comes from the wild (Karimpour et al., 2011), although some studies have been done to investigate the effects of different energy sources on growth performances of crayfish in Iran (Valipour et al., 2011; Ghiasvand et al., 2012). A good understanding of reproduction is important in the fishery management of a species in nature since reproduction is an important characteristic expressing fitness and adaptation of a species to its habitat. The investigation of crayfish reproduction is highly significant for fisheries managers as well as environmentalists concerned with perpetuating crayfish stocks.

In this study, the fluctuation of GSI and oocyte size in the hemolymph of the female crayfish were measured throughout the reproductive cycle and the relationship between the GSI and oocyte development was examined.

Materials and methods

A total of eighty five female crayfish were captured during the four seasonal sampling from local fishermen from Aras Dam Lake, Western-Azerbaijan, Iran. Sampling was performed seasonally in June 2011 (22 immature females), August 2011 (20 maturing females), November 2011 (23

ripe females) and January 2012 (20 spent females), to cover the ovarian reproductive cycle. In this matter, another study on *A. astacus* was done six times during the ovarian reproductive cycle, as well (Lucic et al., 2006) which it was considered as a guideline in this study. Samples were captured by opera house traps. In the sampling, some opera house traps, 200mm diameters silken ropes, 8mm diameter nylon ropes, GPS, foldable plastic baskets, grab sampler and electro shocker were used. The specimens were transported by air in a Styrofoam box with some meshed ice to the fisheries laboratory, laboratory complex of Islamic Azad University (Tehran, Iran).

Total body length of seasonal samples was measured to the nearest 0.1 mm with a digital caliper, from the rostral apex to the posterior median edge of the telson, and ranged between 53.4 and 148.1 mm. The carapace length ranged between 35.0 and 62.3 mm and the wet weight was measured to the nearest 0.1 g and ranged between 42.9 and 92.4 g.

Soon after weighting crayfish and hemolymph collection, the crayfish were dissected and the ovaries were removed and weighted using a digital balance. Ovaries were treated for histological analysis. The ovarian performance was measured by calculation of the GSI as follows (Ferrè et al., 2012): Gonadosomatic Index (GSI): GSI = [wet weight of ovary (g)/ total body weight (g)] × 100.

Macroscopic description of ovarian maturation was used for help with staging of ovarian maturation, the maturation performance was measured by the consideration of the following parameters (Beatty et al., 2005);

I. Developing-1, Very small dark orange ovaries. A middle slot at the end of the ovary: II. Developing-2, Bright orange ovaries slightly thickened: III. Developing-3, Bright orange ovaries growing thickened: IV. Developing-4, slightly swollen Y-shaped ovaries, with pale milk oocytes: V. Mature-1, Y-shaped ovaries developing to the front of rostrum, containing mainly black oocytes: VI. Mature-2, Y-shaped ovaries containing mainly green oocytes: VII. Mature-3, Y-shaped ovaries containing mainly yellow oocytes. VIII. Mature-4, Y-shaped ovaries containing yellowish orange oocytes: IX. spent, tiny yellowish ovaries.

Ovarian samples were collected and fixed in Bouin's solution (Coccia et al., 2010). Samples were dehydrated in a graded ethanol series, then were transparented using xylene/or toluene solution and finally embedded in paraffin wax at 60°C. Slices (5-6 µm) were stained with Hematoxyline-Eosine and analyzed at light microscopy. From each slide, the horizontal and vertical diameter of nucleated oocytes was measured (Coccia et al., 2010). Only oocytes sectioned through the nucleus were measured. After measurement, based on a modified version of the method described by (Nakata and Goshima, 2004), nucleated oocytes were histologically staged into one of the following categories: stage I, Oogonia or non-vitellogenic oocytes with no yolk present in the cytoplasm; stage II, Previtellogenic oocytes with little yolk granules surrounding

the nucleus; stage III, Vitellogenic oocytes, yolk granules dispersed in the cytoplasm; stage IV, Full vitellogenic oocytes with the cytoplasm filled with yolk granules.

In addition, maturation of the ovaries was histologically graded according to a revised version of the protocol explained by (Beatty et al., 2005). Also the egg diameter measured for each female, were caculated as the meen length by using a profile projector (Nakata and Goshima, 2004). Therefore, ovarian performance was identified as follows;

I. Developing-1 (June, 22 crayfish), Previtellogenic and early vitellogenic oocytes dominate. Few vitellogenic oocytes. Oogonia developing in the germaria along with post-spawning follicles, maximum oocyte diameter 295 μm (Fig. 2A). II. Developing-2 (August, 7 crayfish), Mixed sizes of oocytes present along with each other, maximum oocyte diameter 570 μm (Fig. 2B). III. Developing-3 (August, 8 crayfish), Vitellogenic oocytes dominate. Few previtellogenic oocytes present, maximum oocyte diameter 750 μm (Fig. 2C). IV. Developing-4 (August, 5 crayfish), Full vitellogenic oocytes dominate, maximum oocyte diameter 950 μm (Fig. 2D). V-VIII. Mature 1-IV. (November, 8 crayfish), Full vitellogenic oocytes maximum oocyte diameter 1250 μm. Histologically, ovaries analyzed from females collected in November were mature, containing full vitellogenic oocytes prepared for ovulation. Therefore ovarian histology was impossible for November sampled ovaries and therefore morphologic identification of gonads plays an important role in their matter. IX. spent (January, 20 crayfish), Unspawned oocytes (mainly vitellogenic oocytes) present along with follicles maximum oocyte diameter 1250 μm (Fig. 2E).

Values were expressed as mean ± standard error (SE). Data were analyzed by one-way analysis of variance (ANOVA) and any significant difference was determined at the 0.05 level by Duncan's multiple range test. The analyses were carried out with the Statistica version 7.0 statistical package (Statsoft Inc, Tulsa, OK, USA).

Results

The *A.leptodactylus* female GSI ranged from 0.26-13.53% throughout the year (Fig. 1). The GSI value was low in June (0.61%) (Table 1), the ovary was orange in color and the maximum average diameter of oocytes in the ovary was 295 µm. The GSI value increased in August (1.35 %), the ovary became bright orange and the maximum average diameter of oocytes was 950 µm. The value of GSI recorded in November (13.53%) was significantly higher than the other months ($p<.05$), the ovarian color turned from black to green, to orange and finally to yellow as the oocyte maturation progressed. The maximum average oocyte diameter was 1250 µm. The smallest GSI value occurred in January (0.26%), after spawning (Table 1). The ovary was tiny and yellowish with few unspawned oocytes. The

maximum average diameter of oocytes was 225 µm.

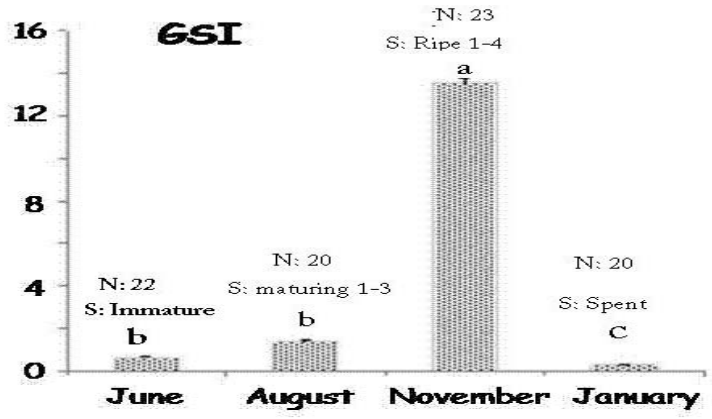

Figure 1: Gonadosomatic index in the female of *A.leptodactylus*. Bars represent mature size females (15 individual per sampling month; June, August, November 2011 and January 2012). Values are given as mean ± SE. Values with different alphabetic superscripts are significantly different (*p<.05*). S= Stage of maturation, N= number of specimens.

Table 1: Seasonal values of gonadosomatic indices and hemolymph levels of steroids in the female *A.leptodactylus*.

Months	Number of specimens	Stage of maturity	Gonadosomatic Index (GSI)
June	22	Immature	0.61 ± 0.05^{b}
August	20	Maturing 1-3	1.35 ± 0.05^{b}
November	23	Ripe1-4	13.53 ± 0.25^{c}
January	20	Spent	0.26 ± 0.01^{a}

Values are mean ± S.E. (within a column values with common alphabetic superscripts are not significantly different; *p>.05*).

Females collected in June had developing ovaries with mixed oocyte population represented by mainly previtellogenic and vitellogenic oocytes. Oogonia were developing in the germaria along with post-spawning follicles (Fig. 2A). In August vitellogenic and

full vitellogenic oocytes dominated the developing ovaries, although few previtellogenic oocytes were still present (Fig. 2B, C, D). Histologically, ovaries analyzed from females collected in November were mature, containing full vitellogenic oocytes prepared for ovulation. Females collected in January had spent ovaries containing few unspawned oocytes (Fig. 2E).

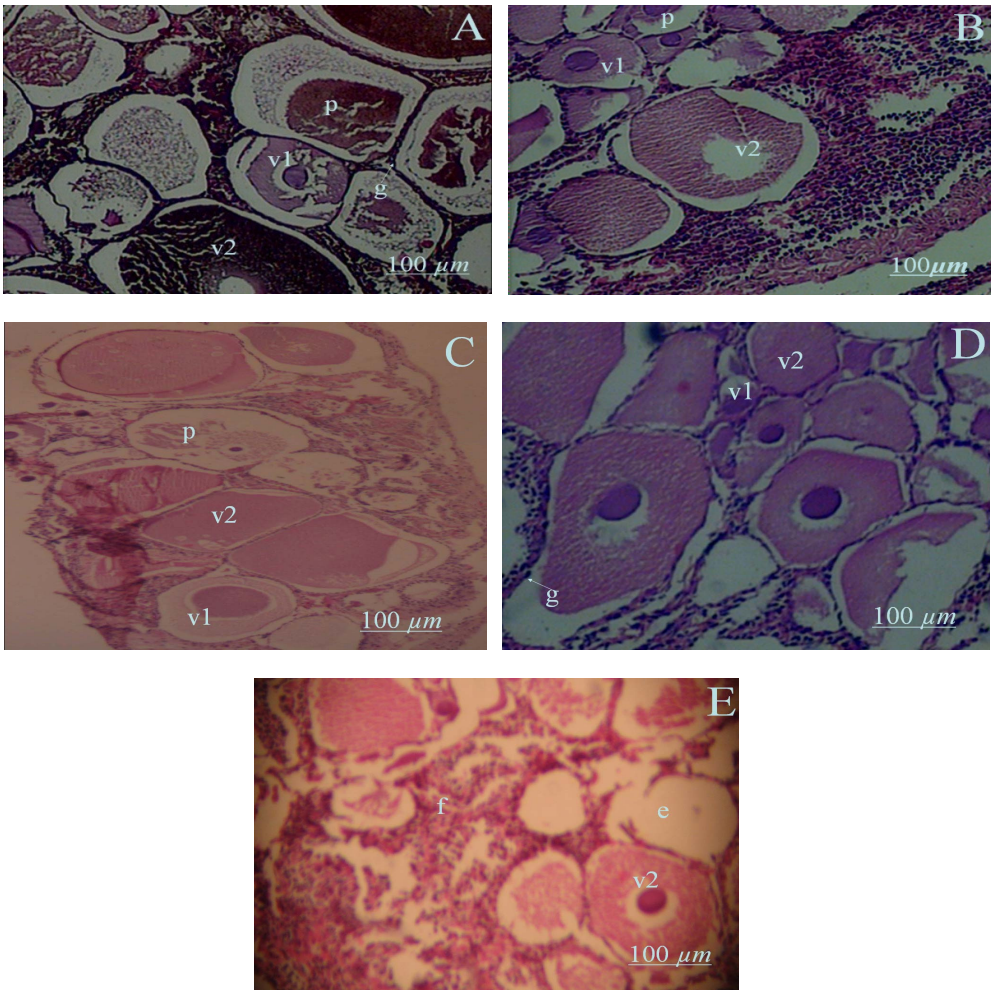

Figure 2: Maturation stages of the ovary of *A.leptodactylus* from Aras Dam reservoir. (A) June; (B, C, D) August; (E) January. Ovaries collected on June and august show mixed stages of previtellogenic and vitellogenic oocytes, but spent ovaries of January sampled females presented a few well developed oocyes. F-follicular epithelium; e-empty space; p-previtellogenic oocytes; v1- primary vitellogenic oocytes; v2-Secondary vitellogenic oocytes.

Discussion

In this study we report the ovarian and oocyte development and in the hemolymph throughout the reproductive cycle of the female of the narrow clawed *A. leptodactylus* from Iran.

The reproductive cycle described here resembled that one reported by Stucki (1999) for *A. leptodactylus* from Switzerland and by Hubenova et al. (2009) from South Bulgaria. In *A. leptodactylus* from Switzerland females with freshly spawned eggs were found in mid December, but the majority of them started spawning at the end of December. Similarly, we found that *A. leptodactylus* females captured in January from Aras Dam Lake had completed the spawning season, as shown by their ovaries, which appeared yellowish and were smaller than the ovaries of sexually immature females (data not shown).

In *A. leptodactylus* from South Bulgaria, oocytes start maturing in June and in August-September enlarge their size until they reach their final dimensions. Copulation takes place in November. Interestingly, these authors reported that females can reach their first sexual cycle with a body weight of about 10 g. Such earlier maturity is explained as the result of different environmental conditions, such as temperature and abundance of food. The ovary of mixed populations, formed by small yang females at their first year of sexual maturity along with sexually mature adult females of bigger size, is characterized by the presence of oocytes of variable diameter even though they are at the same developmental stage. This is also the case of *A. leptodactylus* from Turkey, reported by Unis and Erkan (2012), where the analyzed population comprised juveniles and mature females, with a wide range of body weight, from few grams to more than 100 g. Therefore, the discrepancy between the oocyte diameter reported by these authors and the results presented here may be explained with the fact that the population from Turkey was a mixed population, including not only sexually mature female but also juveniles at their first reproductive cycle. On the contrary, in our study, we chose to investigate the reproductive cycle of only adult sexually mature females.

On the other hand, Harlioglu and Turkgulu (2000) summon us about the lack of relation between female size and egg diameter in *A. leptodactylus,* although these authors reported a study on the significance of egg size in the freshwater crayfish, *Pacifastacus leniusculus*, where a positive linear relation was found between egg size and carapace size, with the largest females producing the largest eggs (Mason, 1978).

In addition, in a previous study on the Aras Dam Lake population of *A.leptodactylus* there were a positive correlation between female size and egg size (Hosseinpour and Karimpour, 2001). Caution is then invoked when producing regression models to estimate fecundity for *A. leptodactylus*, due to the fact that there is no relation between female size and egg size and the egg size of this species is very variable, even for the same size crayfish.

The data presented in this study show that GSI and oocyte size fluctuate in the female of *A. leptodactylus*, throughout the reproductive cycle in a way consistent with the physiological maturation of oocytes. Such results confirm the relationship of oocyte size and ovarian development in *A. leptodactylus* and reinforce previous observations in this field.

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