

## Some Biological Characteristics of Tadpole Shrimp, *Triops cancriformis*, from Seasonal Pools of West Azarbaijan (Iran)

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### ABSTRACT

The tadpole shrimp of genus *Triops* is a well-known living fossil whose fundamental morphology has been unchanged for 220 million years. We collected specimens of *Triops cancriformis* in temporary water bodies near the southern part of Urmia Lake (in the Fall of 2005). Some biological characteristics of this *Triops* were investigated. The feeding regime of *T. cancriformis* was found to be related to the fauna and flora of the temporary pools. Invertebrates and animal detritus were found to constitute major part of the feeding regime. The existence of *Triops* cysts and particles in the gut also showed certain degree of cannibalism. Morphological and histological investigations showed that the population of *T. cancriformis* was female and there was only one male among 400 samples collected. Observation of sperm among follicle ducts of a few samples indicated some degree of hermaphroditism, but the animal seemed to reproduce mainly through parthenogenesis. Fecundity, varying from 100 to 2500 cysts, was with a few exceptions related to the body size. The average cyst diameter was  $400 \pm 85 \mu\text{m}$ .

**Keywords:** Crustacean, Feeding Regime, Notostraca, Reproduction, Shrimp, Tadpole *Triops cancriformis*.

### INTRODUCTION

Notostracans are freshwater crustaceans adapted to temporary water bodies (Su and Mulla, 2001). They are commonly called tadpole shrimps because of their superficial resemblance to frog larvae. They are recognizable by their large, horseshoe-shaped dorsal carapace (Martin and Boyce, 2005). They are benthic branchiopods which swim and feed ventral surface down, burrowing into the bottom sediments in search of detritus and a variety of small organisms (Scholnick and Snyder, 1996). Their feeding depends on their habitats. Being facultative detritus feeders or scavengers or predators, they eat algae, bacteria, protozoa, rotifers, earthworms, insects, fairy shrimps, frog eggs and also tadpoles (Cvetkovic-Milicic and

Petrov, 1999). Tadpole shrimps are characterized by their wide fluctuations in population density. When their density becomes very high, they can barely find enough food, and they become very active in searching for food. Food shortage inhibits their development and reduces fecundity, while mortality increases as a result of cannibalistic behavior (Scholnick and Snyder, 1996).

The tadpole shrimp genus *Triops* is a well-known living fossil whose fundamental morphology has remained unchanged for 220 million years. While the order has wide geographical distribution, many species have a restricted local distribution (Suno-Uchi *et al.*, 1997).

The ability of *Triops* to develop and grow quickly is crucial to its success (Davis and Madison, 1999). Fecundity is related to oxy-

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gen tension and temperature and so small changes in temperature or oxygen dramatically change the total number of eggs laid in one season (Scholnick, 1995).

The females possess two ovisacs (brood pouches) on the 11<sup>th</sup> appendages. The two ovisacs hold an almost equal number of eggs. Eggs are laid randomly and not in clusters, although all the eggs in both brood pouches are released simultaneously (Seaman *et al.*, 1991). The fecundity of *T. cancriformis* is high; individuals may lay more than 1000 eggs (Fry and Mulla, 1996). Their eggs lie dormant in the soil and can survive as such for many years. They are resistant to severe drought and extreme temperatures (Takahashi, 1997). It has been suggested that the distribution of many branchiopods results from the transfer of resting eggs, either by birds, people or cattle and sheep. Most eggs hatch within a few days of being submerged, but some remain unhatched in the soil. This is an effective way for the shrimps to maintain their populations under unpredictable fluctuations in environmental conditions (Takahashi, 1997).

15 species of *Triops* have been reported in the world. *Triops cancriformis* inhabits temporary pools and rice fields in Eurasia and Africa (Cesari *et al.*, 2004; Cvčkovc-Milicic and Petrov, 1999). *T. cancriformis* distribution in Europe has been reported by Zaffagnini and Trentini (1980). While the order has a wide geographical distribution, many of the species have a restricted local distribution (Takahashi, 1997).

*T. cancriformis* populations reproduce gonochorically, hermaphroditically or even parthenogenetically (Zaffagnini and Trentini, 1980; Cesari *et al.*, 2004). They comprise bisexual populations, with an equal male: female sex ratio or with a female bias, and unisexual populations, either hermaphroditic or parthenogenetic (Mantovani *et al.*, 2004). The more northerly European populations are parthenogenetic, while incidents of gonochorism begin appearing towards the southern latitudes, with the northern African populations presenting both sexes and amphigonic reproduction (Zaffagnini and Tren-

tini, 1980; Scanabissi *et al.*, 2005).

Although some scattered networks on this organism- under its general name "*Apus*"- have been traced to fish culture ponds in Iran (Kheirandish, 1975; Kohneshahri and Takami, 1974), no specific work on its identification and biological characteristics, particularly feeding and reproduction, have been conducted. In this work, therefore, efforts are concentrated towards precise identification, and study of the feeding and reproductive biology of this organisms in seasonal ponds of West Azarbaijan Province.

## MATERIALS AND METHODS

### Animals

The first adult specimens of *T. cancriformis* were collected in the Fall of 2003 from vernal pools of West Azarbaijan Province in Iran (Figure 1A) (Khodabandeh *et al.*, 2008). For biological studies 400 specimens (length = 2.5 cm) were collected during the Spring and Fall of 2005. 95 specimens were fixed for 24 hours in Bouin's fixative and alcohol 70% separately for feeding regime and reproduction studies.

### Feeding Regime

Study of the feeding regime was accomplished by investigating the gut contents of 50 specimens under the microscope, using Rose Bengal staining and histological methods. The *Triops*, gut was separated by scalpel under a stereo microscope, and its contents were preserved in alcohol 70% and then studied under a light microscope. Rose Bengal (0.5 g in 500 cc distilled water) was added to the contents of the gut to distinguish the vegetal or animal origins of the food.

For classic histological studies, the animals were fixed for 24 hours in Bouin's fixative. The specimens were then fully dehydrated in a graded ethanol series and embedded in paraffin. Sections (5µm) were cut on a mi-

crotome, collected on albumine-glycerine slides and stained with Haematoxylin, Eosin and Methyl green (Martoja and Martoja-Pierson, 1967; Khodabandeh *et al.*, 2005a and b).

### Reproduction

Morphological observation and a histological method were used for sex differentiation and identification of reproductive system structure. Fecundity was determined by counting the cysts in the ovisacs and gonads of 45 specimens. Biometry of the cysts was accomplished by light microscope with a micrometer. The diameters of 12 cysts from each specimen were measured, and the average, maximum and minimum diameter was calculated.

### RESULTS

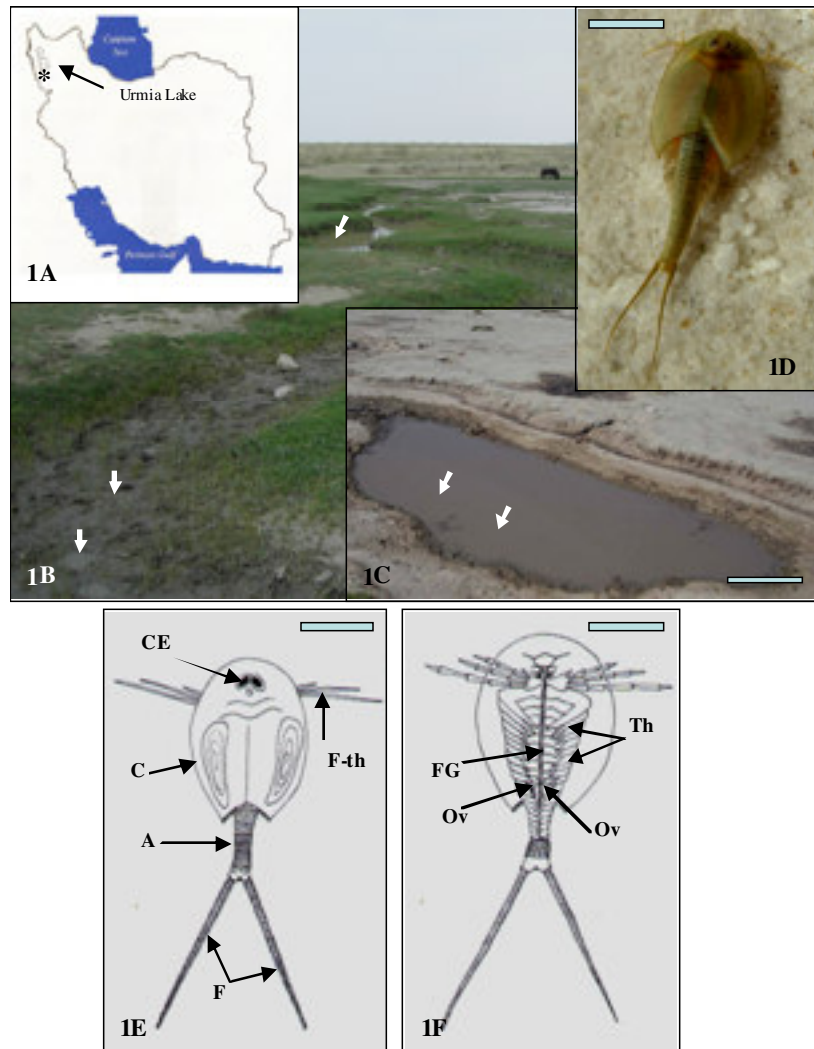
*Triops cancriformis* is reported from throughout the southern Palaearctic region, and from vernal pools of West Azarbaijan Province in Iran (Khodabandeh *et al.*, 2008). Morphologically the material is a mixture of specimens bearing the carapace cainal spine arrangements of both *T. c. cancriformis* and *T. c. simplex* subspecies, casting doubts as to the validity of the subspecific designations. Specimens appeared with the filling of ditches and vernal pools by rain and irrigation. They were also found in almost drained out ditches (Figure 1B). During the course of sampling the pond had conductivity values ranging between 450-860  $\mu\text{S cm}^{-1}$  (22°C) and a pH ranging from 7.5 and 8.5. The highest population of *T. cancriformis* occurred in ponds that had little aquatic vegetation but supported other invertebrates (cladocerans, hemipterans, coleopterans) and had relatively high conductivity (860  $\mu\text{S cm}^{-1}$ ) (Figure 1C).

Morphological investigation shows that the body (total length= 2-5 cm) consists of a head and a trunk mostly covered by a large (length =1-2 cm) dorsal carapace (Figures

1D-F). The anterior dorsal portion of the carapace is equipped with a relatively unique optical arrangement (Figure 1E). The trunk consists of a thorax bearing appendages and an abdomen without them. The oral region of the cephalon possesses specific appendages including antennules, antennae, maxillae and mandibles that are covered by a labrum and used for food handling. The anterior portion of the thorax consists of 11 segments, each bearing a pair of appendages (thoracopods) (Figure 1F). The 11<sup>th</sup> appendages of females form ovisacs (brood pouches) (Figure 1F). The many appendages posterior to the 11<sup>th</sup> move the spent feeding and respiratory current away from the body. The proximal portion of the abdomen possesses 65 pairs of fine hair-like appendages that beat in a rhythmic fashion to assist in movement and food channeling. The distal portion of the abdomen terminates in a prominent telson and subsequently branches into two large caudal furca (Figures 1D-F).

Stereo microscopic studies revealed that the gut of *T. cancriformis* was filled with microscopic vegetal, animal, and unknown particles (Figures 2A-H). Invertebrates and animal detritus were found to constitute a major part of the *T. cancriformis* feeding regime. The existence of *Triops* cysts and *Triops* particles in the guts also showed some degree of cannibalism (Figures 2H and 2D). Application of Rose Bengal showed that the vegetal detritus did not have any color and animal detritus were pink color (not shown). Histological studies showed that in the stained sections the vegetal detritus was stained green by Methyl Green and animal detritus was stained red by Fuchsin (Figures 3A-C). The gut of *Triops* in the pool with flora and without flora was more green and red, respectively (Figure 3A).

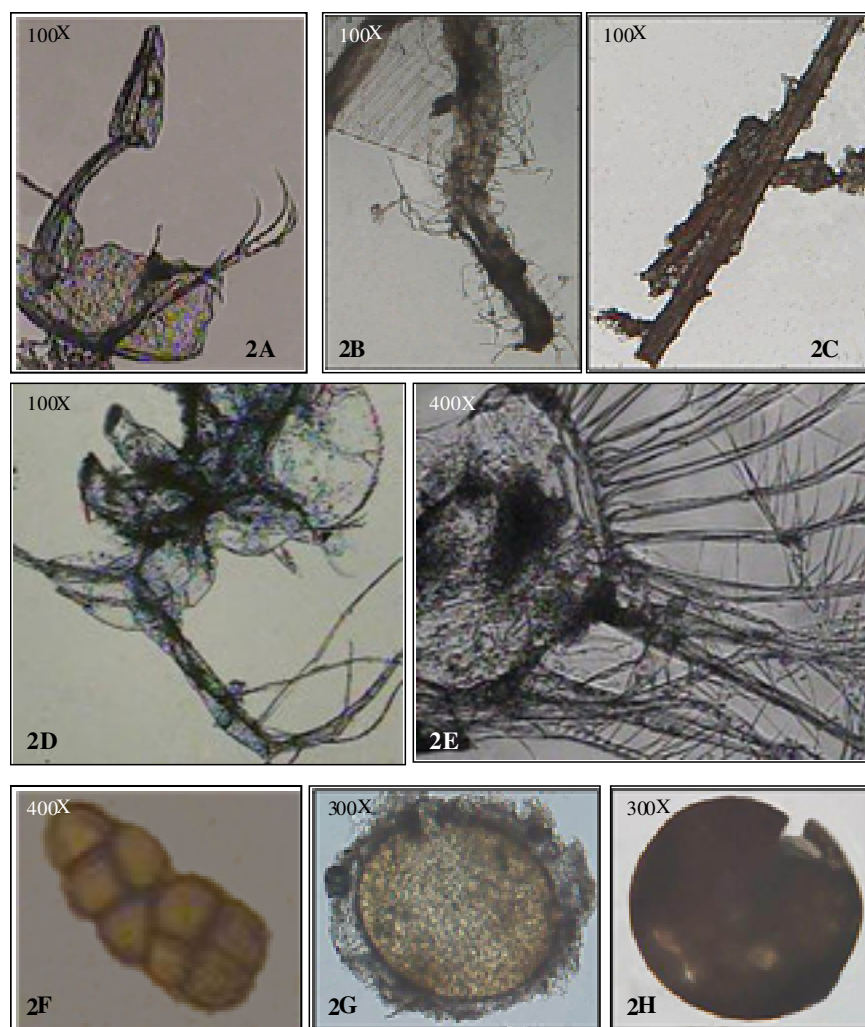
Morphological sex determination showed that most of the population of *Triops* were female and there was only one male among 400 collected samples. The paired gonads extend almost the entire length of the trunk on either side of the gut in females (Figure 3E). These gonads were filled by numerous oocytes and cysts that occupied the body



**Figure 1.** Map of Iran Star showing collection location: (1A) Sampling ditches and vernal pools; (1B and 1C) *Triops cancriformis*; (1D) Dorsal; (1E) Ventral, (1F) View of *Triops cancriformis*. Abbreviations: A= Abdomen; C= Carapace; CE= Compound eyes; F= Furca; FG= Food groove; F-th= First thoracopod; Ov= Ovisac, Th= Thoracopods. Scale bars: 1C (15 cm); 1D, 1E and 1F (1 cm). Arrows (samples).

cavity together with the gut (Figure 3E). Each gonad consists of a gemarium at the tip of several follicles whose ducts join the longitudinal oviduct and, at the end, leading to the ovisac (Figure 3D). Each follicle consists of an oocyte and three nurse cells (Figures 3D and 3F). The follicle duct wall is a single layer of closely packed cells lying on the basal lamina (Figures 3F and 3G). Folli-

cle duct cells on average are 32  $\mu\text{m}$  high and 10  $\mu\text{m}$  wide (Figure 3H). They possess rather short, scattered microvilli. The elliptical nucleus was nearly central. The cytoplasm of the follicle duct cells seem to secrete eggshell material (Figure 3H). At the end of vitellogenesis the oocytes descend to the follicle duct, then the ripe oocytes pass along the follicle ducts and enter the longi-

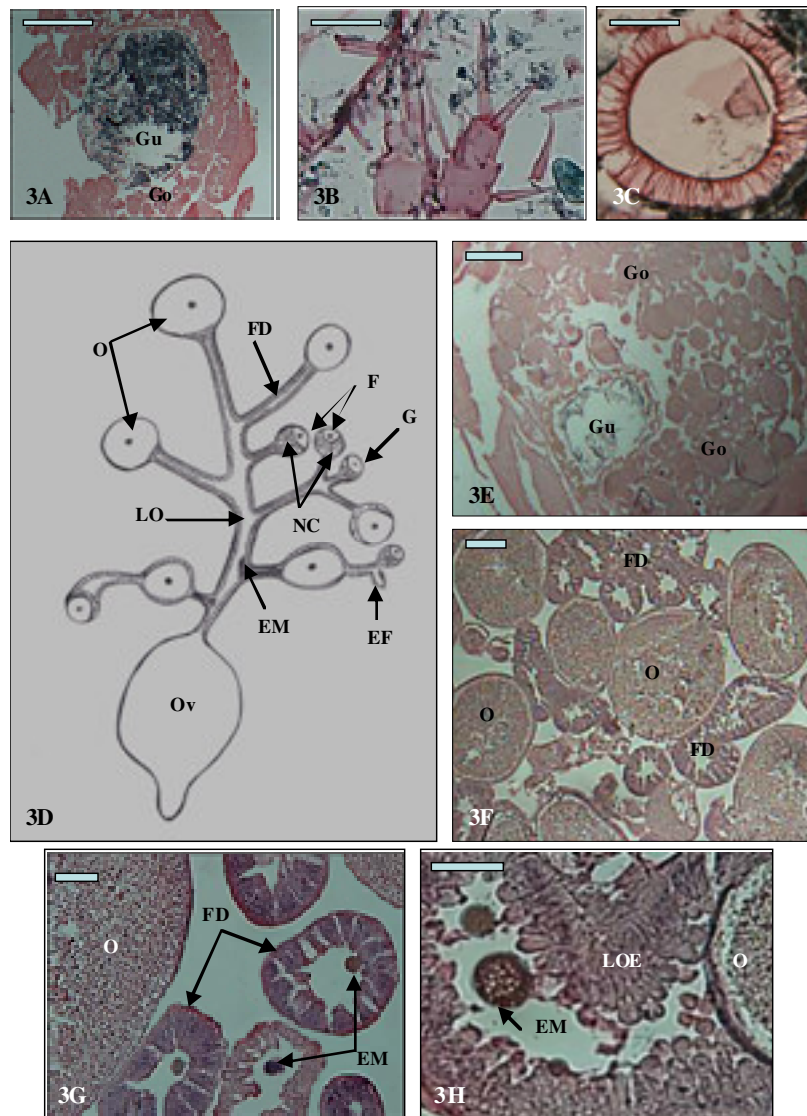


**Figure 2.** (2A-C) Some vegetal particles, (2D and 2E) Animal particles and cysts from *Triops cancriformis* gut.

tudinal oviduct to reach the ovisac (Figure 3D). The number of follicles and oocytes depended on the size and habitat of *Triops*, and were different even in two gonads of the same *Triops*. Some samples did not have any cysts in their ovisacs, but their gonads were full of cysts. In their ovisacs were brownish, spherical and separated cysts. In their gonads some cysts were milk-white in color and others were brown. The cysts were

present in a tubular and complicated reproductive system on either side of the gut, which at the end were carried in two ovisacs (Figure 3D). Fecundity, that with a few exceptions was found to be related to the body size, varied from 100 to 2500; the average cyst diameter was  $400 \pm 85 \mu\text{m}$ .

In the follicle ducts of a few females several sperm cells and also degenerated spermatozoa were observed (Figures not shown).



**Figure 3.** Histological stained sections: (3A-C) Transversal sections of trunk of *Triops cancriformis*; (3B and 3C) Detritus into the gut; (3D) Schematic graph of a gonad and ovisac, (3E-H) Gonad sections. Abbreviations: EF= Empty follicles; EM=Eggshell materials; F= Follicles; FD Follicle ducts; G= Germarium Go= Gonad; Gu= Gut; LO=Longitudinal oviduct; LOE= Longitudinal oviduct epithelium; Oo= Oocyte, Ov= Ovisac. Scale bars: 3A (1mm); 3B and 3C (400  $\mu$ m); 3E (250  $\mu$ m); 3F and 3G (25  $\mu$ m), 3H (3  $\mu$ m).

## DISCUSSION

Adult *Triops* (length 2-5 cm) were used for biological studies. The gut of *T. cancriformis* was filled with small invertebrate and

microscopic vegetal and unknown particles. Their feeding regime was found to be related to the fauna and flora of the temporary pools. The vegetal or animal origin of the detritus depended on the abundance of floral and faunal forms in the pools. Invertebrate



and animal detritus were found to constitute a major part of *T. cancriformis* feeding regime. Some *Triops* particles in the guts also showed a certain degree of cannibalism.

Taylor *et al.* (1987) have suggested that *Triops* is omnivorous and feeds mainly on algae, bacteria, protozoa, rotifers and detritus. Tietze and Mulla (1989) have reported that *T. cancriformis* progress from juveniles feeding on phytoplankton to mature adults feeding on plant material and that only the largest individuals  $\geq 10$  mm are carnivorous; they have also suggested that *T. longicaudatus* was polyphagous and found to feed upon invertebrates and plant material in both daylight and darkness. They also noted that, in the laboratory, early instar mosquito larvae were consumed. In a more detailed study in the laboratory, this species was found to be a predator of mosquito larvae (*Culex quinquefasciatus*) and is currently being considered as a biological control against mosquitoes (Tietze and Mulla, 1989).

Our investigation showed that the population of *Triops* were almost exclusively female and there was only one male among 400 collected samples. Observation of sperm among the follicle ducts of a few females indicated some degree of hermaphroditism, but the demonstration of a high amount of degenerated spermatozoa in the gonads of hermaphroditic specimens is contrary to hermaphroditic reproduction and the animals seemed to reproduce mainly through parthenogenesis. The presence of male *T. cancriformis* had already been reported in Hungary (Abonyi, 1926), in Yugoslavia (Petrov and Cvetkovic, 1996 and 1999) and in Austria (Scanabissi *et al.*, 2005). Trentini and Scanabissi (1982) have suggested that the sex ratio of *T. cancriformis* in Italy is heavily biased in favor of the females, which display testicular lobes scattered along their follicle ducts. Such populations have been considered to be hermaphroditic by some authors, while other authors consider them to be parthenogenetic with rudimentary hermaphroditism. Whenever testicular lobes are present, they lie along some female gonadal

tubules and their number varies throughout the population. In addition, the activity of the testicular vesicles is obviously low when compared with those of the bisexual populations. They have never seen sperm cells in their specimens, which leads them to exclude any possibility of self-fertilization. Also, an ultrastructural analysis of the gonad of males of *T. cancriformis* in Austria has shown a truly functional structure, presenting morphofunctional characteristics typical of normal gametic development and maturation (Scanabissi *et al.*, 2005). In *T. cancriformis* Engelmann *et al.* (1997) found signs of sperm degeneration.

We observed that the female reproductive apparatus consisted of two gonads lying ventrolaterally in the haemocoel and filling the body cavity along with the gut. Each female gonad consists of a germarium at the tip of several follicles whose ducts join the longitudinal oviduct. These results are in agreement with the results of previous studies (Trentini and Scanabissi, 1978 and 1982).

The ovarian follicles consisted of an oocyte and three nurse cells. These oocyte follicles were surrounded by a thin layer of follicle cells. The oocytes were initially smaller than nurse cells. The cytoplasm of oocytes was filled with differently sized yolk globules that were surrounded by membranes of the endoplasmic reticulum. The nurse cells had yolk globules too and they were characterized by slightly darker cytoplasm. This was also in agreement with the results of previous studies (Trentini and Scanabissi, 1978 and 1982; Engelmann *et al.*, 1997).

The follicle duct wall is a single layer of closely packed cells lying on the basal lamina. The oocyte has no coating at the beginning of its descent but pushes the shell material towards the end of the tubules into the oviduct where it accumulates. The egg covering begins to form as the egg passes through this material before entering the longitudinal oviduct or uterus. The egg coating is uniform and becomes the definitive eggshell only after a vacuolization takes place within it. It is already reported that, when the eggs are finally deposited in the



ovigerous pockets, they display the characteristic shell structure (Trentini and Scanabissi, 1978 and 1982; Tommasini et al., 1989).

In our study fecundity was, with a few exceptions, related to the body size and means that the larger specimens have more cysts; fecundity varied from 100 to 2500 cysts. Fry and Mulla (1996) reported that the fecundity of *T. cancriformis* was high: individuals may lay more than 1000 eggs. Scholnick (1995) found a positive linear relationship between the number of eggs and wet mass for *T. longicaudatus*; Seaman et al. (1991) have also reported a similar relationship between carapace length and fecundity for *T. granarius*. Su and Mulla (2001) have reported a positive correlation between any two parameters of growth (CL at Death), longevity and egg production. Longer surviving *T. newberryi* with a larger body size at death produced more eggs during their lifetime. This relationship is also true for other branchiopod species. We observed that the average cyst diameter in the ovisacs of *T. cancriformis* was not significantly different. Cyst diameter was not found to be related body size. Some of the cysts in the gonads were milky-white and the other were brown. Previous investigations have shown that *T. granarius* has two types of eggs, one drought resistant, the other not (Seaman et al., 1991). Trentini and Scanabissi (1982) have found that the cyst shell of *T. cancriformis* is subdivided into two layers: an outer uniform and compact one about 3 µm thick (cortex) and an inner alveolar layer which is about 25 µm thick. The chitinous embryonic cuticle lies deep to it.

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## مطالعه برخی خصوصیات زیستی میگوی بچه وزغی، *Triops cancriformis*، در آبگیرهای فصلی استان آذربایجان غربی

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### چکیده

میگوی بچه وزغی جنس *Triops* فسیل زنده شناخته شده‌ای است که در طول ۲۲۰ میلیون سال گذشته تغییرات اساسی ریخت شناسی انجام نداده است. در پاییز سال ۲۰۰۵، نمونه‌های از گونه اروپایی این جنس، یعنی *Triops cancriformis* در آبهای استخرهای فصلی واقع در بخش جنوبی دریاچه ارومیه جمع‌آوری کرده و برخی خصوصیات زیستی آنها مورد مطالعه قرار گرفت. رژیم تغذیه‌ای این سخت پوستان وابسته به مجموعه زیستی موجود در استخرهای فصلی بوده و بی‌مهرگان کوچک و پوده‌های جانوری بیشترین محتویات روده را تشکیل می‌دادند. وجود سیستم‌ها و خرده‌های تنه مربوط به هم‌نوعان در دستگاه گوارش نمونه‌های بررسی شده نشانگر حالت همجنس‌خواری در آنها بود. بررسیهای بافت‌شناسی و ریخت‌شناسی نشان داد که غالب افراد جمعیت ماده بوده و تنها یک مورد از ۴۰۰ نمونه گرفته شده نر می‌باشد. مشاهده اسپرم در لوله‌های فولیکولی برخی از نمونه‌ها نشانگر دوجنسی بودن آنها بود، اما به نظر می‌رسد که روش عمده تولید مثل آنها بکرزایی باشد. باروری بسته به سائز جانور، بین ۱۰۰ - ۲۵۰۰ سیستم متفاوت بود. متوسط قطر سیستم‌ها  $400 \pm 85$  میکرون اندازه‌گیری شد.