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Life Cycle of the Fairy Shrimp, *Phallocryptus spinosa* Milne Edwards, 1840 (Crustacea: Anostraca) at Different Temperatures

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ABSTRACT

This study was conducted to evaluate a temperature regime ranged from 15 to 30°C on survival, growth, life span and reproductive traits (reproductive period, clutch number, offspring number) of *Phallocryptus spinosa* from Iran. Newly hatched nauplii (N= 200 and 3 replicates per each treatment) were allocated into containers and maintained at various temperature regimes (at 15, 20, 25 and 30˚C). During trial, the larvae were fed with unicellular algae *Dunaliella tertiolecta* and Lansy PZ (lipid-enriched yeast). Survival and growth rate of *P. spinosa* with an interval of 3 days to 18 days were examined. After attaining adulthood, 16 pairs of adult *P. spinosa* were transferred from all culture vessels into separate containers supplied with 300 ml of brackish water (5 ppt) and similar temperatures to compare their life span and reproductive traits. Based on results obtained, maximum growth at shortest period (13.2±2.0 mm on day 12) was observed at 30°C; whereas, all *P. spinosa* died before reaching sexual maturity by day 15. Maximum survival (86%) was observed at 15°C; however, minimum growth also was obtained at the same temperature with none of *P. spinosa* reaching sexual adulthood. On the 18th day, *P. spinosa* reached sexual adulthood only at 20 and 25°C. Furthermore, a significant difference was observed in most of the reproductive traits especially in total egg production per female at 20°C. Thus, it seems that 20°C is the suitable temperature to rear this fairy shrimp. Future studies should be focused on the feasibility of mass production of this species as a valuable live feed in the aquaculture industry.

Keywords: Fairy shrimp, Growth, Survival, Life span, Reproductive characteristics.

INTRODUCTION

Anostracans, commonly known as fairy shrimps are typical inhabitants of vernal pools, aquatic environments characterized by strongly variable abiotic conditions (Beladjal *et al*., 2003a). Like other crustacean species that inhabit vernal pools, the fairy shrimps are adapted to the unpredictable nature of the ponds by developing to maturity quickly and reproducing before the ponds dry. Females produce encysted embryos that remain quiescent until the pools are refilled; the nauplii are born when exposed to the appropriate environmental cues (Brendonck *et al*., 1990; Peck, 2004; Pociecha, 2007). Although much information is available on the ecology of anostracans (Lake 1969; Sluzhevskaya 1975; Sluzhevskaya-Drobysheva, 1982; Anderson and Hsu, 1990; Saiah and Perrin, 1990; Maeda-Martinez *et al*., 1995), relatively little is known about how populations differ in life history traits (Beladjal *et al*., 2007; Hawes *et al*., 2008). A number of studies have been carried out on growth, survival (Peck, 2004, 2005), respiration (Pociecha, 2007)

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reproductive and life span characteristics of anostraca populations from different parts of the world cultured under standardized laboratory methods (Lake, 1969; Daborn, 1977; Holtz *et al*., 2001; Beladjal *et al*., 2003b; Atashbar *et al*., 2012). However, more research is required to understand dynamics and life history characteristics in other species.

Phallocryptus spinosa (Milne Edwards, 1840) was collected in a pool close to the town of Tabriz, formerly indicated by Brehm (1954) as Estakr Chah, and presently named Eal Goli (Mura and Azari Takami 2000). These pools are scattered at the periphery of the lake in both West and East Azerbaijan. The size of the pools varies from a few square meters to maximum 2000 m^2 surface area and their depth is always less than 1 m. Therefore these pools are considered as temporary small water catchments that are dried during early summer and filled up again during winter rains. Water salinity in the pools begins from 0-5 ppt and gradually rises to saturation level. *P. spinosa* in these pools grows to maturity at very low salinities and starts reproduction before the pools dry up.

To the best of our knowledge, there are no available data in the literature on the growth and survival characteristics of Iranian *P. spinosa* in relation to temperature. The aim of our experiment was therefore to test the impact of various temperature regimes on the growth, survival and reproductive characteristics of fairy shrimp, *P. spinosa*.

MATERIALS AND METHODS

Source of Animals and Cysts

The soil containing cysts of *P. spinosa* was collected from Hasar pool (37° 49 'N 45° 50' E), located at north western region of Iran (West Azerbaijan) when the habitat was completely dry. The maximum surface and depth of this pool are about 120 m^2 and 40 cm, respectively. The soil sample was transferred into a 300 L tank filled with Tap water and subsequently washed over 500 and 150 µm sieves. The dormant cysts were dried at 37°C in an incubator and then kept in a refrigerator at 4°C. Dehydrated cysts were hatched using tap water at 25°C under constant fluorescent light. Salinity condition was prepared by dissolving Urmia Lake salt in dechlorinated tap water.

Culture Conditions

Two hundred newly born nauplii (Instar 1) were transferred to each of the 2-liter cylindrical bowls containing 1-liter aerated saline water (5 ppt) in four temperature treatments (15, 20, 25 and 30°C) with three replicates for each treatment. The animals in the culture bowls were kept under mild aeration and fed with unicellular algae (*Dunaliella tertiolecta*) at a concentration of 10,000 cells ml^{-1} daily (Mura *et al*., 1998) and Lansy PZ. At sexual maturity, 16 couples of pair-mated shrimps from each treatment were transferred in to smaller plastic bottle containing 300 ml aerated saline water (5 ppt) and were fed at a daily rate of a few drops of algal suspension containing approximately 5×10^5 cells ml^{-1}. The rest of the males were maintained in stock culture. Isolated cultures of couples continued as long as the females were alive if the male died earlier, the female received another male from the stock culture. The culture vessels were checked every three days for the production of cysts, which were counted and recorded separately (Beladjal *et al*., 2003a, b, Atashbar *et al*., 2012).

Determination of Survival and Growth

Growth rate and survival were determined on days 1, 3, 6, 9, 12, 15 and 18. For determining body length (from the tip of the head to the posterior margin of the telson) five animals from each replicate as long as they were immature (15 animals for each treatment) were measured under a stereomicroscope equipped with drawing

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tube (STEMI SV 11, ZEISS) and digitized using a digitizer (Summa Sketch TM III) connected to a computer (Beladjal *et al.,* 2003a, b, Atashbar *et al*., 2012).

Determination of Reproductive Characteristics

The reproductive characteristics (number of clutches, total number of offspring, clutch size, offspring per day during the reproductive period) and the life span characteristics (pre-reproductive period, reproductive period, post reproductive period and life span) were determined for populations of different temperature regimes according to Browne *et al.* (1984, 1988).

Data Analysis

Data were analyzed using one-way ANOVA. The Tukey's test was employed to detect significant differences among treatments at the 0.05 significance level. All data analysis was carried out using the SPSS version 11.5 package (Chicago, IL, USA).

RESULTS

Survival

The Survival data is shown in Table 1. The result indicated that survival declines in *P. spinosa* when temperature increases. Higher survival was observed for specimens reared at 15°C and minimum survival was observed at 30°C. All animals died by day 12 at 30°C, while survivorship was over 80% at 15°C (by day 15).

Growth

The growth data of *P. spinosa* is shown at Table 2. The animals' growth continued at all temperatures throughout their life time. Comparison of growth on day 12 revealed significantly higher size (13.2±2.0 mm) at 30°C than animals at 15°C (5.3 ± 0.7mm) and the growth rates at 20 and 25°C were not significantly different. The results indicated that growth increases in *P. spinosa* when temperature increases.

Reproductive Characteristics

The reproductive and life span characteristics of *P. spinosa* at four different temperatures are summarized in Table 3. Significantly longer life span was detected at 15 and 20˚C (about 52 and 48 days, respectively) compared to the fairy shrimps reared at 25 and 30˚C. The pre-reproductive period was significantly longer at 20˚C in comparison to those at higher temperature (25˚C). The animals became sexually mature at about 18 and 15 days at 20 and 25˚C, respectively. *P. spinosa* produced a significantly higher number of cysts at 20°C (173.8 cysts) compared with 25°C (113.7

Table 1. Survival rate (%) of *P. spinosa* at different temperatures (±SD).[a]

	Temperatures (°C)			
Time (Days)	15	20	25	30
1	100.0 ± 0.0 [a]	100.0 ± 0.0 [a]	100.0 ± 0.0 [a]	100.0 ± 0.0 [a]
3	99.0 ± 0.5 [a]	93.5 ± 5.8 [a]	98.5 ± 0.0 [a]	96.1 ± 1.2 [a]
6	92.3 ± 5.9 [b]	78.6 ± 7.6 [b]	88.0 ± 5.5 [b]	50.0 ± 5.0 [a]
9	88.0 ± 9.9 [c]	75.1 ± 1.0 [b]	75.1 ± 6.7 [b]	36.6 ± 2.8 [a]
12	86.1 ± 9.9 [c]	71.1 ± 0.2 [bc]	63.8 ± 12.5 [b]	21.8 ± 2.0 [a]
15	82.0 ± 8.8 [c]	67.5 ± 1.3 [bc]	59.8 ± 14.0 [b]	0.0 ± 0.0 [a]
18	78.8 ± 8.1 [c]	63.8 ± 0.7 [bc]	56.6 ± 14.6 [b]	0.0 ± 0.0 [a]

[a] Values in each row that share the same superscript are not significantly different (P< 0.05).

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Table 2 Growth rate (mm) of *P. spinosa* at different temperatures (±SD).[a]

	Temperatures (°C)			
Time (Days)	15	20	25	30
1	0.38 ± 0.0 [a]	0.41 ± 0.0 [a]	0.40± 0.0 [a]	0.43 ± 0.0 [a]
3	1.4 ± 0.3 [a]	2.1 ± 0.4 [b]	3.3 ± 0.2 [c]	3.1 ± 0.7 [c]
6	2.2 ± 0.1 [a]	4.5 ± 0.8 [b]	6.5 ± 0.9 [c]	7.3 ± 1.0 [d]
9	4.0 ± 1.2 [a]	6.7 ± 1.3 [b]	8.2 ± 0.6 [c]	11.3 ± 1.9 [d]
12	5.3 ± 0.7 [a]	9.9 ± 1.0 [b]	9.5 ± 0.8 [b]	13.2 ± 2.0 [c]
15	6.5 ± 0.8 [a]	10.4 ± 1.2 [b]	11.4 ± 0.9 [b]	-
18	7.4 ± 1.3 [a]	11.0 ± 0.8 [b]	12.9 ± 2.0 [c]	-

[a] Values in each row that share the same superscript are not significantly different (P< 0.05).

Table 3 Mean values of various reproductive and life span characteristics for *P. spinosa* at different temperatures (±SD).[a]

	Temperatures (°C)			
Reproductive Characteristics	15	20	25	30
Life span	52.6 ± 3.4 [c]	48.6 ± 7.8 [c]	38.0 ± 8.1 [b]	8.2 ± 3.0 [a]
Reproductive period (Day)	-	12.5 ± 9.9 [a]	10.6 ± 1.6 [a]	-
Pre reproductive (Day)	-	13.6 ± 6.8 [b]	8.0 ± 6.5 [a]	-
Post reproductive (Day)	-	3.5 ± 3.1 [a]	4.5 ± 4.7 [a]	-
Number of clutch	-	2.8 ± 1.9 [a]	2.5 ± 1.5 [a]	-
Number of offspring per3 day	-	57.9 ± 15.3 [b]	37.9 ± 17.4 [a]	-
Offspring per clutch	-	47.6 ± 21.0 [a]	57.5 ± 20.2 [a]	-
Cyst production	-	173.8± 20.1 [b]	113.7 ± 26.3 [a]	-

[a] Values in each row that share the same superscript are not significantly different (P< 0.05).

cysts). The higher values for total number of cyst produced and number of offspring were observed at 20˚C.

DISCUSSION

In standardized laboratory conditions, we observed in *P. spinosa* from Iran significant difference in growth rate, longevity and reproductive capacity at different temperatures. Life history studies on other fairy shrimps have shown that temperature plays an important role on growth (Atashbar *et al*., 2012). Growth rates increased with increasing temperature in lab experiments for *Chirocephalus diaphanus* Prevost, 1803 (Lake 1969), *Branchinecta gigas* Lynch, 1937 (Daborn 1975), *Branchinecta mackini* Dexter, 1956 (Daborn 1977) and *Branchinecta orientalis* Sars, 1901 (Atashbar *et al*., 2012). Since anostracans are poikilotherms, higher temperatures can accelerate their metabolic rate, reflected in their faster growth under warmer conditions (Atashbar *et al*., 2012). Our results prove the life strategy of *P. spinosa* is adapted to warm water, ranging between 20 and 25°C, of vernal pools that stay inundated for 10 to 20 days at least.

Based on the results of this study, *P. spinosa* increased in length rapidly in the first 9 days after hatching at 25 and 30°C, while little growth was observed at 15°C. However, growth continued for a much longer period at 15°C compared to 25 and 30°C. The same observations have been made by Atashbar *et al.* (2012) in *B. orientalis*. We observe a positive correlation between temperature and body length in *P. spinosa*. Total length was significantly longer at high temperature (13.2±2.0 mm at 30°C compared to 5.3±1.3 mm at 15°C) on day 12. The results are in agreement with

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finding of other research, for example, Lake (1969) in *C. diaphanus* (14.99 mm at 10°C and 21.20 mm at 25°C), Anderson and Hsu (1990) in *Streptocephalus seali* Ryder, 1879, Beladjal *et al*. (2003b) in *Tanymastigites perrieri* Daday, 1910 and Atashbar *et al*. 2012, in *B. orientalis* Sars, 1901.

Survival and life span were significantly different at various experimental temperatures. We observed a reverse correlation between temperature and survival. In *P. spinosa*, all specimens at 30°C died within 10-12 days after birth. Survival was significantly highest at low temperature (86% at 15°C) and lowest at high temperature (0% at 30°C) on day 12 of culture period. Also longer life span was detected at 15°C (52 days) and shortest life span observed at 30°C (12 days). These results are in agreement with findings of other research, for example animals at the coldest temperature had a longer life span (63.00±5.29 days at 12°C) (Atashbar *et al*., 2012). Also a similar pattern of life span was observed by Beladjal *et al.* (2003b) who reported the longest life expectancy (100 days) for *T. perrieri* cultured at 10°C, while the minimum life span (20 and 18 days) was observed at 30 and 35°C. These findings reveal that these species are generally sensitive to high temperatures and perform better at low temperatures.

In our study, fairy shrimps reached sexual maturity and produced cysts at 20 and 25°C, but the animals never reached sexual maturity at 15 and 30°C. *P. spinosa* produced a significantly ($P< 0.05$) higher number of cysts at 20°C (173.8 cysts) compared with those at 25°C (113.7 cysts). These results are in agreement with findings of other research, for example maximum reproductive output of *Branchinecta orientalis* observed at 18°C was significantly higher than that produced at higher and lower temperatures (Atashbar *et al*., 2012). *T. perrieri* produced a significantly higher number of cysts at 20°C compared to those at 10 and 30-40°C (Beladjal *et al*., 2003b) quite similar to the findings of the current study. However we were unable to find a significant relationship between reproduction and temperature. Therefore we need to study more about temperature and reproductive characteristics.

In addition, this study provides updated life history data with important implications for natural populations of *P. spinosa*. Our data revealed that the temperature range of 20-25°C provides the best conditions for growth, life span and cyst production of the species.

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چرخه زندگی پریان میگوی *Phallocryptus spinosa* در شرایط دمایی مختلف

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چکیده

این تحقیق درصد ماندگاری، نرخ رشد، طول عمر و ویژگی‌های تولیدمثلی گونه (دوره تولید مثل، تعداد تخم، تعداد نوزادان *Phallocryptus spinosa* را در تیمارهای دمایی مابین 15-30°C از ایران را نشان می دهد. ناپلی های تازه تفریخ شده(با تعداد ۲۰۰ عدد در سه تکرار) در محیط کشت با تیمارهای دمایی (۱۵، ۲۰، ۲۵ و ۳۰ درجه سانتیگراد) تقسیم گردیدند. در طول آزمایش، پریان میگو با جلبک تک سلولی دونالیلا ترتیولکتا (*Dunaliella tertiolecta*) و مخمر فرموله شده لنسی پی زد (Lansy PZ) غذادهی شد. درصد ماندگاری و نرخ رشد لاروها هر سه روز یکبار در روزهای پرورشی ۱۸-۳ اندازه گیری گردید. بعد از بالغ شدن پریان میگوها، ۱۶ جفت پریان میگو از هر تیمار به ظرف‌های جداگانه حاوی ۳۰۰ میلی لیتر آب لب شور (۵ گرم در لیتر) با دمای مشابه منتقل گردید تا ویژگی‌های تولید مثل و طول عمر آنها در تیمارهای دمایی مورد مقایسه قرار گیرد. بر مبنای نتایج، حداکثر رشد در کوتاه ترین زمان در دمای 30 °C (2/13 ± 0/2 mm) در روز ۱۲) دیده شد ولی تا قبل از روز ۱۵ همگی تلف شدند. بیشترین درصد بقا (۸۶ درصد) در دمای 15 °C دیده شد ولی حداقل رشد نیز در این تیمار دیده شد و هیچ یک از پریان میگوها در این دما به بلوغ جنسی نرسیدند. پریان میگوها در دو دمای 20 °C و ۲۵ به بلوغ جنسی رسیدند و در اکثر پارامترهای تولید مثلی خصوصا از نظر تولید سیست توسط هر ماده (۱۷۴ سیست)، نتایج بهتری در 20 °C به دست آمد. لذا دمای 20 °C برای پرورش این گونه به عنوان دمای بهینه معرفی می شود. مطالعات آینده باید بر روی امکان تولید انبوه این گونه به عنوان یک غذای زنده با ارزش در صنعت آبزی پروری تمرکز کند.

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