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The Influence of Light (Intensity and Duration) on the Cysts Hatching Parameters and Nauplii Growth of *Artemia urmiana* (Günther 1890)

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Abstract: Light has important effects on egg hatching, nauplii growth and reproduction of Crustaceans. In this study, effects of four light intensities (00, 100, 2000 and 5000 Lux) and four photoperiods (24:00, 00:24, 12:12 and 2:22; Light: Dark) have separately conducted on cysts hatching parameters and nauplii growth of *Artemia urmiana*. Results showed that the light intensity and photoperiod have significant effects on hatching of cysts. The 2000 Lux illumination and photoperiods of 02L: 22D and 12L:12D had good results in hatching percentage and efficiency of cysts. The effect of photoperiod on *A. urmiana* growth was found to be stronger and more immediate than that of light intensity. Our results recommended that 02L: 22D photoperiod and 2000 Lux illumination for cysts hatching parameters and 12L:12D and 2000 Lux illumination for nauplii and juveniles growth in *A. urmiana* cultures.

Key words: *Artemia urmiana* · Light Intensity · Photoperiod · Cyst Hatching and Efficiency · Growth

INTRODUCTION

Artemia urmiana is one of the eight bisexual *Artemia* species known to date in the Lake Urmia that reproduces predominantly through oviparity. It is nothing that the *A. urmiana* population displayed high percentages of encysted offsprings, which makes it attractive for commercial exploitation [1]. Several parameters as hydration, cyst disinfection or decapsulation prior to incubation and hatching under optimal conditions (constant temperature of 25-28°C, 15-35 ‰ salinity, pH of 8-8.2, near saturated oxygen levels, maximum cyst densities of 2 g l^{-1} and strong illumination) are critical for hatching of *Artemia* cysts [2]. Hatching capability of *Artemia* cysts differ in function of the genotypic factors, culture conditions, food quantity available to the reproducing adults, exploitation, methods of harvest and post-harvest, hydration of cysts and environmental factors. Among these factors, light affects functions and structures of aquatic animals through one or more its modalities; intensity, spectral composition, angular distribution, polarization and duration of light or dark period [3]. Light also is one of the most important factors that in some way trigger the hatching mechanisms. A number of studies focused on the effects of light intensity, spectrum and photoperiod on hatching percentage and efficiency of cysts of *Artemia* [4-5] and growth of nauplii [6]. Although much research has been devoted to the *A. urmiana*, few attentions have been paid to the influence of light (intensity and photoperiod) on the hatching parameters and growth of nauplii prior to adulthood stages. The present study, therefore, deals with the effects of separately light intensities and photoperiods on ability of cyst hatching and nauplii growth to adulthood stages.

MATERIALS AND METHODS

The cysts of *A. urmiana* were supplied from Artemia and Aquatic Animals Research Institute, Urmia University (Iran) and incubated under standard hatching conditions [7]. The cysts (1.6 g) were hydrated and incubated in 800 ml hatching containers with a conical bottom to start the embryonic developments (3 replicates for each treatment). The incubation conditions were included at 28°C, 15 % and pH 8.2.

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In the experiment 1, white illuminations light of 00, 100, 2000 and 5000 Lux were applied at the water surface at 24 hours hatching period. The light intensity was altered by regulating the power supply of the lamp and also through changing the lamp position. The approximate light levels at the water surface were supplied by lamps (25 and 90 W) and measured by Luxmeter (Model LM-120). In the dark treatment, the whole place was covered by black partitions. In the experiment 2, photoperiods of 24:00, 00:24, 12:12 and 02:22 (Light: Dark) were applied at 2000 Lux constant illumination. The hatching percentage (HP) and efficiency (HE) were calculated with formulas of $HP\% = (N\times100) / (N+U+E)^{-1}$ and $HE = N \times 2000$ respectively; in which, 6 subsamples 250 µl were taken from each containers to determine numbers of nauplii (N), umbrella (U) and un-hatched embryos (E) [7]. Both experiments have been done in different times with sampling from 12 to 24 hours at 2 h intervals after initiation of hatching process.

After hatching, 500 instar-I nauplii were transferred directly to three cylindroconical flasks containing 1 and 2 L (for nauplius and metanauplius stages, respectively) diluted lake water with a salinity of 35 g L^{-1} and 24-25°C in treatments that above mentioned. The density of the animals at initial stage was 0.5 nauplii per mL which was reduced to one individual (metanauplius) per 4 ml after day 5 [7]. The animals were fed on unicellular halotolerant green algae *Dunaliella salina* according to feeding schedule proposed by [8-9] during the experiments. Mild aeration was applied from the bottom of the flasks. Total length of nauplii and juveniles were determined weekly using a stereomicroscope equipped with micrometer during three weeks in laboratory cultures. Statistical analysis was carried out using one-way analysis of variance (ANOVA) using SPSS (Version, 17). To determine the relationships among light intensities and photoperiod's treatments, the differences between means were determined and compared by the Duncan's test that used a significant level of $P \leq 0.05$ [10].

RESULTS

Effect of Illumination Levels on HP, HE of Cysts and Total Body Length of *A. Urmiana*: Hatching percentage in 2000 Lux was significantly higher compared to other treatments after 24 h ($P\leq0.05$). At complete darkness and 100 Lux, hatching percent was not significantly different ($P\geq0.05$). Hatching percentage increased by increasing light intensity from total darkness to 2000 Lux. In higher intensities (5000 Lux), hatching percent dropped. Hatching efficiencies were obtained between from 58463 (5000 Lux) to 118853 (2000 Lux) with significant differences between treatments after 24 h ($P\leq0.05$). There were no significant differences between treatments in total body length after three weeks ($P\geq0.05$).

Table 1: Hatching percentage (HP) and efficiency (HE) (mean ± SE) of *A. urmiana* cysts at different illuminations

Treatments	HP (%) at 14 hours	HP (%) at 24 hours	HE at 14 hours	HE at 24 hours
00	3.36±0.25^{bc}	39.38±6.11^{b}	6554.1±931.9^{c}	82900±14158.1^{b}
100	6.30±1.35^{ab}	35.24±9.47^{b}	10750±1507.4^{b}	76216.7±18485.3^{b}
2000	8.73±1.97^{a}	58.67±7.1^{a}	15976.7±2471.7^{a}	118853.3±22855.5^{a}
5000	4.09±0.5^{c}	36.01±0.87^{b}	7403.3±659.6^{c}	58463.3±7157.7^{c}

Significant differences were determined using ANOVA test ($P \leq 0.05$). Values in each line that share the same superscript letters are not significantly different.

Table 2: Total body length (µm) (mean ± SE) of *A. urmiana* at different light intensities after three weeks of experiment

Day Treatments	Day 7	Day 14	Day 21
00 Lux	5.60±0.28 ^{a}	9.60±0.50^{a}	10.82±0.68^{a}
100 Lux	5.44±0.35^{a}	9.40±0.42^{a}	10.60±0.73^{a}
2000 Lux	5.10±0.22^{a}	9.00±0.61^{a}	11.84±0.55^{a}
5000 Lux	5.13±0.15^{a}	8.80±0.4^{a}	12.00±0.43^{a}

Significant differences were determined using ANOVA test ($P \leq 0.05$). Values in each line that share the same superscript letters are not significantly different

Table 3: Hatching percentage (HP %) and efficiency (HE) (mean ± SE) of *A. urmiana* cysts at different photoperiod levels

Treatments	HP (%) at 14 hours	HP (%) at 24 hours	HE at 14 hours	HE at 24 hours
24L:00D	18.52±3.21^{a}	63.1±11.56^{a}	22350±2301.6^{a}	121833.4±14937^{a}
00L:24D	8.70±5.78^{b}	32.7±15.82^{c}	8263.3±1151.5^{b}	58183.3±9164.5^{b}
12L:12D	12.2±2.15^{b}	47.34±12.44^{b}	16883.3±2358.9^{a}	92063±15030.8^{a}
02L:22D	11.33±4.44^{b}	46.42±18.24^{b}	15646.7±3362.2^{a}	100586.7±11310^{a}

Significant differences were determined using ANOVA test ($P \leq 0.05$). Values in each line that share the same superscript letters are not significantly different

Table 4: Total body length (μm) (mean ± SE) of *A. urmiana* at different photoperiod levels after three weeks of experiment

Day Treatments	Day 7	Day 14	Day 21
24:00	4.60±0.14[a]	8.92±0.38[a]	9.20±0.61[b]
00:24	5.45±0.18[a]	9.28±0.45[a]	11.42±0.68[ab]
12:12	5.82±0.26[a]	9.92±0.46[a]	13.21±0.80[a]
02:22	4.84±0.10[a]	9.81±0.52[a]	10.53±0.54[b]

Significant differences were determined using ANOVA test (P ≤ 0.05). Values in each line that share the same superscript letters are not significantly different

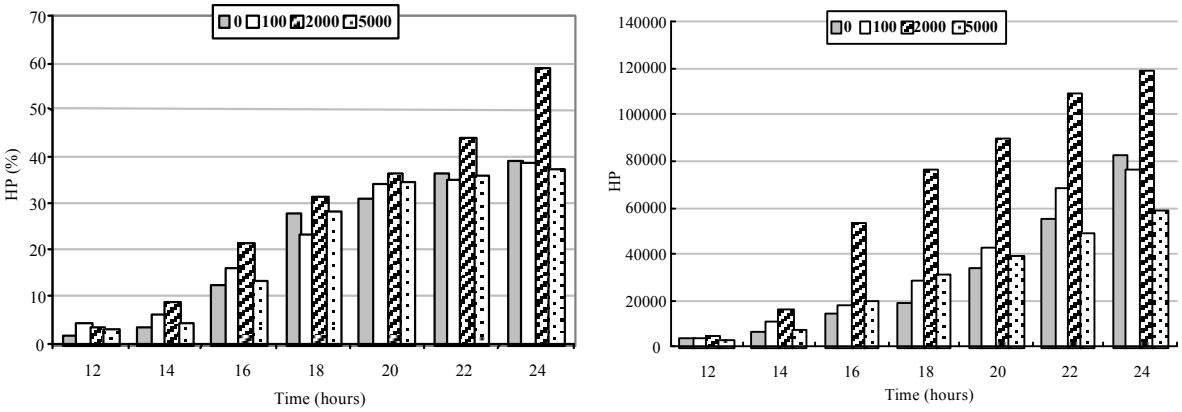

Fig. 1: Hatching percentage (HP) and efficiency (HE) of *A. urmiana* cysts in 2 hours intervals at different illuminations

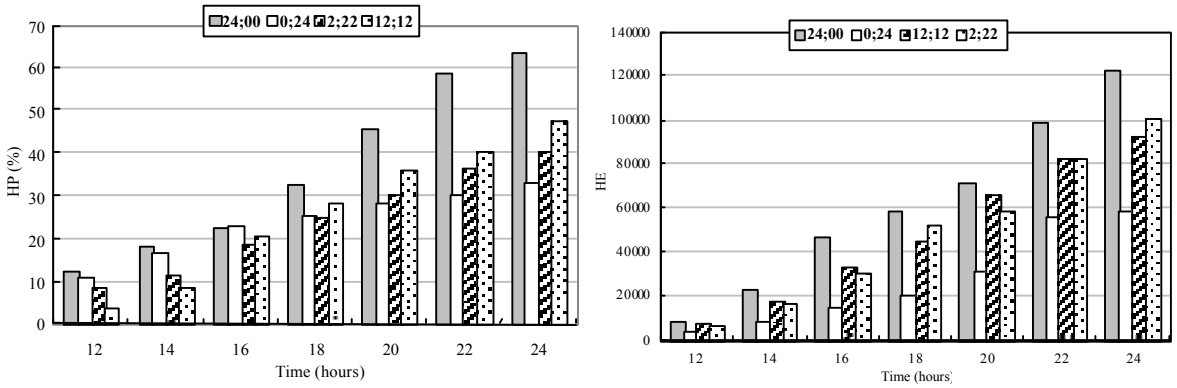

Fig. 2: Hatching percentage (HP) and efficiency (HE) of *A. urmiana* cysts in 2 hours intervals at different photoperiods

Effect of Photoperiod Levels on HP, HE of Cysts and Total Body Length of *A. Urmiana*: There were significant differences between 24L: 00D with other treatments on hatching percentages of cysts after 14 h (P≤0.05); however, values were in the range from 8.7% (00L: 24D) to 18.52 % (24L: 00D). Maximum and minimum hatching percentage obtained in 24L: 00D (63.1%) and 00L: 24D (32.7%) after 24 h, respectively. The relatively lower hatching percentage was obtained in cysts that incubated at 00L:24D (32.7%) after 24 h. Hatching efficiencies of cysts were different between 00L:24D with other treatment (P≤0.05) after 24 h,; however, differences were not significant between two treatments of 24L:00D and 02L:22D (P≥0.05). The results of total body length showed significant differences between photoperiods of 12L: 12D and other treatments (P≤0.05). Maximum and minimum of total body length after three weeks of experimental period were observed at photoperiods of 12L: 12D and 24L: 00D, respectively.

DISCUSSIONS AND CONCLUSION

As for other crustaceans, light directly or indirectly modify locomotors patterns, feeding, reproduction, molt cycle, egg hatching and development. Diapausing stages of many arthropods are known to resume active development when exposed to one or more exogenous stimuli. The effect of light on the hatching process was first described in [11]; revealed that apart from light exposure time, hatching increases up to 50% were demonstrated for San Francisco Bay and Bulgarian *Artemia* cysts hatched in light compared to complete

darkness. It is well known that the hatching percent, efficiency and rates of *Artemia* cysts vary from one strain to another and even variations may be expected among batches from the same strain. As a consequence, a high degree of interference exists between the different sets of parameters which are involved in cyst hatching [11].

In *A. urmiana*, a relative hatching percentages and efficiencies were obtained in the cysts in complete darkness in our experiments (Tables 1, 3). This phenomenon has not been observed in other *Artemia* strains cysts; that a very small percentage of the cysts hatched in complete darkness [2, 5, 6, 11]. The embryological development in the cysts, when hydrated, is related to some triggering light stimulus [12]. Other findings detected no light sensitivity in cysts from San Francisco Bay, that is; a part of the encysted embryos do hatch in darkness [12]. Although the physiological role of light during the hatching process is poorly understood, *Artemia* cysts, when hydrated and in aerobic conditions, need a minimal light triggering for the onset of the hatching process, related to light intensity and/or exposure time. There are several evidences that a minimal dose of light energy is needed to trigger the onset of embryonic metabolism that vary from strain to strain. Our results revealed that an initial exposure 2 hours (at a relatively shorter photoperiod in this experiment) could be an adequate time for hatching of *A. urmiana* cysts after complete hydration. These variations may be attributed to differences in shell characteristics. Chorion thickness of cysts in *A. urmiana* ranged from 1.2 to 9.3 um showing great variability [3]. Therefore, this method (02L:22D photoperiod) could be an adequate method for the saving of costs of power for hatching of *A. urmiana* cysts in the large hatcheries of shrimps and marine fish larvae [11], during the hatching experiments with dry cysts of *A. salina* (Utah, USA) observed that light influences the hatching process, in which hatching efficiency is considerably higher under conditions of continuous light than in darkness.

Thickness of cyst and light intensity threshold ranges are important parameters for maximal hatching rate. Differences of chorion pigment haematin are known to be responsible for the light absorption. According to our study, hatching rate increases with increasing light intensity until from certain intensity [5]. The present study showed an increase in hatching rate from darkness to 2000 Lux, however more light intensities (5000 Lux) leads to decrease cysts hatchability. Therefore, for the optimal hatching rate of *A. urmiana* cysts using an illumination of 2000 Lux is recommended.

The ephippial eggs of *Daphnia pulex* are stimulated to hatch when exposed to fluorescent light [13]. The hatching mechanism evidently differs widely among the Cladocera. The majority of ephippia maintained in the dark failed to hatch, demonstrating the importance of light in development. Sensitivity to hatching cues may also vary with genotype as noted for the resting eggs of some monogonont rotifers and copepods. Genotypic variation may account for some of the differences observed in hatching times in the present and previous studies.

Light effects on the crustacean molt cycle have been known for many years and there has been ample demonstration that the molt cycle and ecdysis can be inhibited or accelerated by choice of the appropriate light regime. The effect of photoperiod on *A. urmiana* growth was found to be stronger and more immediate than that of light intensity in the current study. Although, the effect of light intensity on the growth of *A. urmiana* revealed that the animals in the dark were smaller in length at the end of three weeks than those raised at the three light intensities with no significant differences between the treatments. However, the larvae grown in complete darkness and low light intensity (100 Lux) showed a higher growth than those cultured under higher illuminations between days 6-17. This phenomenon is probably due to increments of growth between molts during days 6-17 under darkness and low illumination. Results of [6] indicated that the larvae in the dark surroundings swam less than illuminated larvae in days 7-10 of culturing conditions and had higher growth rates. More active nauplii in higher light intensity can not probably convert a higher amount of food energy into growth in two initial weeks. In some another crustaceans such as prawn and shrimp, [14] reported that photoperiods (24L/00D, 12L/12D and 00L/24D) did not significantly influenced molt interval and growth of *Penaeus indicus*; however, [15] revealed that the light intensity had stronger effects on prawn growth than that of photoperiod in *P. merguensis*. The period of preadult development of *Daphnia pulex* in darkness compared to that of animals in LD 14:10 photocycles detected no significant differences between treatments [16]. Results of [17] indicated that lengthening of instars of the prawn *Leaner serrutus* in both continuous illumination and continuous darkness compared to photocycle controls.

On the basis of our results, 2000 Lux illumination and 02L:22D photoperiod for better results in hatching percentages and efficiencies in *A. urmiana* cysts and 2000 Lux illumination with 12L:12D for growth of nauplii and juveniles of this species is recommended in the laboratory culture conditions.

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