J. Agr. Sci. Tech. (2012) Vol. 14: 811-820

Hatching Requirements of *Daphnia magna* Straus, 1820, and *Daphnia pulex* Linnaeus, 1758, Diapausing Eggs from Iranian Populations *In vitro*

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ABSTRACT

Considering the crucial importance of *Daphnia* species in aquaculture, in particular for artificially- cultured sturgeon fry, a comprehensive study on the hatching requirements of their diapausing eggs seemed to be inevitable in order to obtain the ideal hatching technique. To do so, the ephippial eggs were collected from live food ponds in the cultivation and breeding centre of Gorgan and after isolation, were kept in dry and wet conditions at 4°C for 2 months in darkness. Following the pre-incubation period, the ephippia in each group were subdivided into two parts each treated with NaOCl 1% and distilled water. The effect of temperature levels (20, 25, and 30˚C) and photoperiod levels (12L: 12D, 24L: 0D) on hatching percent and the rate of egg hatching were investigated in artificial daphnia medium (AdaM) for 15 days. Results indicated that wet pre-treatment of *Daphnia magna* diapausing eggs with 1% NaOCl solution and subsequently exposure to continuous illumination at 20 and 25˚C was effective to reach the maximum number of hatchlings and the maximum egg hatching rate (P< 0.05). Exposure of the wet diapausing eggs of *Daphnia pulex* to 12 hours illumination and 25ºC without soaking in NaOCl 1% maximized the number of hatchlings and the rate of egg hatching (P< 0.05).

Keywords: Diapausing egg, *D. magna, D. pulex*, Hatching.

INTRODUCTION

Because the water flea Daphnia (Cladocera: Crustacea) has a crucial importance in fish feeding and development at early stages of life, it is widely used in aquaculture. The fat and protein contents of body weight in Daphnia are estimated to be 3.4-3.5 and 45-46% of dry weight, respectively (Zenkevich, 1962). In fish feeding, this miniature and nutritious live food organism is economically profitable because of its high availability and low expenditure in production. Acipenseridae represent a unique and relict lineage of fish, in danger of extinction in the Caspian Sea. To improve the stock recruitment of sturgeon, it is advisable to feed the manually propagated larvae well with adequate live food in order to increase their adaptive ability and survival in the natural environment. It has been shown that in sturgeon rearing ponds with Cladocera biomass of 10-20 g m^{-3}, the survivorship of fish larvae is enhanced to 60-80% (Aghaei Moghadam, 1999). There are several centers for breeding and cultivation of sturgeons in the North of Iran, established to propagate five commercially valuable species over spring season. Some species of sturgeon such as *Acipenser guldenstadti* and *Acipenser persicus,* are capable of late-autumn propagation when natural conditions tend to become unfavorable for cyclic

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[DOR: 20.1001.1.16807073.2012.14.4.17.3]

parthenogenetic reproduction of *Daphnia* (Niyani, 1998). Newly-developed fry of these two species need to feed on Daphnia one week after hatching because of the bigger size of their mouth, which enables them to feed on live food organisms larger than *Artemia* spp. Most species of *Daphnia* can undergo sexual reproduction resulting in the production of diapausing eggs which can survive harsh environmental conditions such as winter or drought periods that remove the adult ones (Gyllström and Hansson, 2004). In freshwater lakes or fish ponds, masses of diapausing eggs are abundantly found soaking on the water surface, deposited on the sediment of the bottom or being trapped by the vegetation in littoral zones. The application of this salient source in aquaculture has been neglected for years. It is possible either to keep the collected ephippial eggs dry or wet in a special synthetic culture medium for a period of time and subsequently to hatch them in an appropriate situation. Thus, it seems necessary to develop an ideal hatching technique to create artificially stock tanks rich in parthenogenetic adults even in winter to ensure adequate feeding of sturgeon fry propagated in autumn or winter seasons. Available literature has confirmed that the critical stimuli responsible for the hatching of diapausing eggs may differ between con-specifics for various geographical regions. In this study, we aimed to find the optimal hatching conditions of diapausing eggs of *D. magna and D. pulex* isolated from the live food ponds.

MATERIALS AND METHODS

Sampling Procedure of the Diapausing Eggs

Diapausing eggs were collected from 5 live food ponds during 5 months from late winter to early summer in Sturgeon Breeding and Cultivation Center of Gorgan, near Voshmgir Dam, Golestan, north of Iran (54°, 44´ E; 37°, 13´ N). The ponds, which were dried out for over 3 months immediately before sampling, were not significantly different in size, nutrient load and macrophyte cover. Depth and area of live food ponds averaged around 1.5 m and 0.5 ha, respectively. Macrophyte cover in live food ponds was dominated by lake marginal plants of typha, submerged plants of *Lemna minor* (Duckweed), and leaf-floating species of azolla. Syanophyta algae (blue-green bacteria) was also found to feed Daphnia and other zooplankton species inhabiting in the ponds including Ostracoda, Conchostraca and Cyclops.

Isolation of diapausing eggs from the sediment was conducted by means of the sugar floatation method (Onbé, 1978; Vandekerkhove *et al*., 2004C). Diapausing eggs in five randomly chosen pelagic zones were obtained by plankton sampling nets with different mesh sizes (20 and 50 µm) in each pond. In addition, five locations were randomly chosen to collect the ephippia trapped by the vegetation in the littoral zone. The vegetation debris from littoral zone was dried under sunlight and then crushed into small parts by physical pressure to give particles with an average size of 10mm. The resulting mixture was strained with small mesh-sized strains (50, 63 and 120 µm) to separate diapausing eggs of *Daphnia*. Ephippia samples isolated from pelagic and littoral zones of the five ponds were mixed thoroughly into a total sample to be used in the trial.

Experimental Procedure

Diapausing eggs of Daphnia investigated in this study, consisted of two embryos. Those ephippia identified with the occurrence of bacterial, fungal or physical damage under laboratory sterio microscope were refused to be used in the experiment.

A proportion of the total sample was kept dry during a period of dark incubation at 4ºC for 2 months while the remainder was transferred to screw-capped vials to be stored wet in a few ml of distilled water.

[DOR: 20.1001.1.16807073.2012.14.4.17.3]

Following pre-incubation in darkness, the treatment of the ephippia consisted of incubating under a continuous or 12 hours light day^{-1} at 3 temperature levels (20, 25, 30ºC) with or without preliminary soaking in a 20 percent solution of commercial Clorox (i.e. approximately 1.0 percent sodium hypochlorite) as described by Pancella and Stross (1963). For this, the ephippia were shipped into a stoppered test tube with 20 ml of the soaking solution and agitated gently. After soaking for 5 minutes, the contents of the tube were poured onto a silk screen and washed with distilled water to remove the hypochlorite. In case of sets without sodium hypochlorite pre-treatment, they were handled in the same manner at which distilled water was used as a substitute for the hypochlorite solution. Illumination was provided by using cool white fluorescent lamps above the ephippia in laboratory incubator. Light intensity was determined by using a lux meter (Lutron LX-101, Taiwan) and fixed at 1,700 lux at the level of each beaker. Treatments were provided by using sets of ephippia (the number of ephippia varied among sets) in oval beakers containing 250 ml of previously aerated AdaM medium (200 µS cm^{-1}, Kluttgen *et al*., 1994) for 12 hours, as shown in Table (1). The incubation medium was renewed every 5 days. The test beakers were examined once each day up to 15 days and the newly-hatched individuals were counted and then removed. This was carried out by piping the hatched individuals with a glass pipette and then transferring them to the cultivation tanks.

Table 1. AdaM[a] used for incubating diapausing eggs (Kluttgen *et al*., 1994).

$CaCl_2\,2H_2O$ (117.6 g l^{-1})	23 ml
SeO_2 (0.0035 g l^{-1})	1 ml
$NaHCO_3$ (25.2 g l^{-1})	22 ml
Fresh distilled water (20°C)	10 l
Sea salt	3.33 g

[a] Artificial daphnia Medium.

Hatching Indices

The difference in the number of diapausing eggs in each treatment was taken into account when computing the hatching indices.

Hatching percentage

Hatching percentage was measured according to N_H/N_e, where N_H is the total number of hatchlings and N_e is the multiplied number of ephippia by two (Schwartz and Hebert, 1987).

Hatching rate

The hatching index of each control day was determined with regard to the first hatch on day 3, as shown in Table 2. Hatching rate was calculated with the following equation, as proposed by Pahlevani (1999), where I_i is the hatching index and N_i is the number of hatchlings on a specific control day.

$$\textit{Hatching rate}=\sum_{i=3}^{15}\frac{N_i}{N_e}\times I_i$$

Statistical Analysis

The experimental design was a factorial 2×2×3×2×3 (2 pre-incubation conditions, 2 preliminary treatments by NaOCl, 3 levels

Table 2. Hatching index of each control day.

Control day	3	4	5	6	7	8	9	10	11	12	13	14	15
Hatching index (I_i)	1	0.092	0.85	0.77	0.7	0.61	0.54	0.47	0.4	0.31	0.23	0.15	0.1

[DOR: 20.1001.1.16807073.2012.14.4.17.3]

of temperature, 2 levels of light exposure, 3 replicates). Data of hatching indices were subjected to one way ANOVA. Comparisons of mean within each significant factor and interaction were performed by least significant difference (LSD) test with statistical analysis system (SAS) program when p was < 0.05 (Zar, 1996).

RESULTS AND DISCUSSION

A number of factors altering the hatching success at a given day length, such as temperature (Vandekerkhove *et al*., 2004), light intensity (Vanhaecke *et al*., 1981), oxygen concentration, salinity level and food quality (Irigion *et al*., 2002) have been investigated by several researchers. In the present trial, the effects of soaking in NaOCl and different levels of temperature and photoperiod on the hatching response of *D. magna* and *D. pulex* diapausing eggs after 2 months pre-incubation period while stored wet and/or dry in darkness were simultaneously studied.

Table 3 shows the analysis of variance of factors affecting the hatching indices of *D. magna* and *D. pulex* diapausing eggs. As indicated in Table 3, interactions between more than 2 variables were not significant for both hatching indices whereas the interaction between the pre-incubation condition and hatching temperature were statistically significant in determining the rate of egg hatching and hatching percentage ($P< 0.05$).

Means of hatching percentage and the rate of egg hatching in daphina diapausing eggs are depicted in Table 4. As deduced from Table 4, variations in conditions required inducing egg development in *D. pulex* and *D. magna* were observed. The highest amount of hatching percentage in *D. magna* and *D. pulex* ephippial eggs were 53.05 and

Table 3. ANOVA of factors affecting the percentage of hatching and hatching rate for *D. magan* and *D. pulex* diapausing eggs.

		Sum of Squares				F value			
	Degrees of freedom	Hatching (%)		Hatching rate		Hatching (%)		Hatching rate	
Source		*D. magna*	*D. pulex*	*D. magna*	*D. pulex*	*D. magna*	*D. pulex*	*D. magna*	*D. pulex*
PIP[a]	1	1390.2	695.3	0.1	0.1	35.43 **	21.42**	86.5 **	161.5 **
F[b]	1	179.3	19.1	0.0	0.0	4.57 *	0.59 [n.s]	8.47 **	0.40 [n.s]
LL[c]	1	80.49151	64.1	0.0	0.0	2.05 [n.s]	1.97 [n.s]	11.77 **	4.06 *
T[d]	2	22633.1	14242	0.5	0.4	288.4**	219.3**	186.1 **	277.4 **
PIP×F	1	3.7	40.1	0.0	0.0	0.09 [n.s]	1.24 [n.s]	0.80 [n.s]	0.76 [n.s]
PIP×LL	1	1.1	21.8	0.0	0.0	0.03[n.s]	0.67 [n.s]	2.85 [n.s]	2.53 [n.s]
PIP×T	2	417	188.5	0.0	0.0	5.31 **	2.90 **	13.07**	26.85 **
F×LL	1	25.4	41.4	0.0	0.0	0.65 [n.s]	1.27 [n.s]	0.03 [n.s]	3.10 [n.s]
F×T	2	64.7	48.2	0.0	0.0	0.83 [n.s]	0.74 [n.s]	1.41 [n.s]	4.03 *
LL×T	2	33	78.2	0.0	0.0	0.42 [n.s]	1.20 [n.s]	2.52 [n.s]	2.54 [n.s]
PIP×F×LL	1	5	25.6	0.0	0.0	0.13 [n.s]	0.79 [n.s]	0.85 [n.s]	0.01 [n.s]
PIP×F×T	2	13.2	105.6	0.0	0.0	0.17 [n.s]	1.63 [n.s]	0.19 [n.s]	0.84 [n.s]
PIP×LL×T	2	12.7	124.8	0.0	0.0	0.16 [n.s]	1.92 [n.s]	0.32 [n.s]	0.31 [n.s]
F×LL×T	2	4.7	28.8	0.0	0.0	0.06 [n.s]	0.44 [n.s]	0.91 [n.s]	0.59 [n.s]
PIP×F×LL×T	2	2	37.4	0.0	0.0	0.02 [n.s]	0.58 [n.s]	0.19 [n.s]	1.10 [n.s]
Error	48	1883.1	1558.4	0.0	0.0	35.43 **	0.79 [n.s]	86.58 **	
Total	71	26748.7	17319	0.7	0.6	4.57 *	1.63 [n.s]	8.47 **	

[a] Pre incubation period; [b] Soaking in NaOCl; [c] Length of light, [d] Temperature.

[DOR: 20.1001.1.16807073.2012.14.4.17.3]

Table 4. Means of hatching percent (a) and rate of hatching (b) in *D. magana* and *D. pulex* diapausing eggs under different levels of studied factors.

			Wet pre-incubation period				Dry pre-incubation period			
			Soaked in NaOCl 1%		Non-soaked in NaOCl 1%		Soaked in NaOCl 1%		Non-soaked in NaOCl 1%	
			12 hr Light/Day	24 hr Light/Day	12 hr Light/Day	24 hr Light/Day	12 hr Light/Day	24 hr Light/Day	12 hr Light/Day	24 hr Light/Day
D. magna	(a)	20°C	33.33±8.78	36.78±2.81	30.83±8.78	32.5±6.614	45.83±10.1	53.05±6.36	41.67±9.46	43.89±2.55
		25°C	33.33±8.03	37.92±3.15	30.83±10.1	32.78±4.34	45.83±14.2	48.75±7.5	43.33±5.2	43.33±2.6
		30°C	1.67±1.443	1.25±1.25	0.55±0.962	0.55±0.00	2.08±0.722	4.17±0.722	3.05±1.735	2.78±0.962
	(b)	20°C	0.13±0.034	0.13±0.01	0.08±0.031	0.14±0.023	0.22±0.049	0.28±0.066	0.18±0.032	0.24±0.022
		25°C	0.14±0.056	0.16±0.016	0.12±0.075	0.12±0.014	0.23±0.083	0.3±0.046	0.2±0.016	0.24±0.018
		30°C	0.01±0.008	0.00±0.006	0.00±0.004	0.00±0.000	0.01±0.004	0.03±0.007	0.02±0.013	0.02±0.007
D. pulex	(a)	20°C	25.0±9.014	27.5±7.5	25.83±10.1	25.55±7.51	30.83±3.82	36.67±3.82	30.83±7.64	35.0±4.409
		25°C	17.08±1.91	31.67±6.29	31.67±7.64	33.33±3.33	39.17±10.1	38.33±5.2	37.5±9.014	36.67±3.82
		30°C	0.83±1.443	0.83±0.721	1.11±1.924	0.55±0.962	2.92±3.146	1.25±1.25	4.16±4.018	2.22±1.924
	(b)	20°C	0.08±0.021	0.12±0.032	0.07±0.03	0.09±0.024	0.19±0.025	0.23±0.036	0.17±0.027	0.16±0.008
		25°C	0.08±0.007	0.15±0.024	0.13±0.025	0.15±0.041	0.25±0.04	0.26±0.044	0.26±0.048	0.27±0.043
		30°C	0.00±0.007	0.00±0.003	0.00±0.007	0.00±0.003	0.02±0.023	0.01±0.01	0.03±0.033	0.02±0.016

39.17%, respectively (Table 4). Davinson (1969) obtained the maximum rate of hatching (100%) in decapsulated diapausing

eggs of *D. pulex* during exposure to a wide range of photoperiods. De Meester and De Jager (1993) determined the hatching rate of 81.9% in *D. magna* ephippial eggs after pre-incubation in darkness for 2 to 5 months at 4ºC and subsequent exposure of decapsulated eggs to continuous illumination with an intensity of 103×10^3 w m^{-2} at 21ºC. In contrast to previous studies, the reduction detected in the present experiment may be due to the application of non-decapsulated eggs and not-checking the presence of activated diapausing embryos covered in ephippium. In addition, this might be attributed to the possibly long term interactive diapausing phase during which the embryos stay inactive in spite of providing a desirable condition for hatching; the stimuli could not be efficient to terminate this phase. The threshold of hatching reaction for the possible affecting factors varied in diapausing eggs of species settling at various habitats and the importance of these stimuli to the reaction may be varied according to the type of species and geographical situation of its endemic habitat (Carvalho and Wolf, 1989).

Hatching Response to Variations in Pre-incubation Condition

The pre-incubation period in darkness and the period of light exposure (hatching stage) are two major stages in hatching of Daphnia diapausing eggs. The rate of egg initiating development and final hatching was found to be dependent on both the temperature and duration of pre-incubation period (Schwartz and Hebert, 1987). Table 5 indicates the combined perception of pre-incubation condition and temperature for hatching percentage and the rate of egg hatching in *D. magna* and *D. pulex*. The diapausing eggs of these species were reluctant to hatch at 30°C under any of the conditions but a remarkable

Table 5. Comparison of the hatching success % (a) and the rate of hatching (b) of *D. magna* and *D. pulex* diapausing eggs in different pre-incubation condition and hatching temperatures.

			Hatching temperature (ºC)		
			20	25	30
D. magna	(a)	Wet	$46.11\pm2.297^{a}_{A}$	$45.31\pm2.209^{a}_{A}$	$3.02\pm0.356^{a}_{B}$
		Dry	$33.36\pm1.882^{b}_{A}$	$33.71\pm1.89^{b}_{A}$	$1.01\pm0.3^{a}_{B}$
	(b)	Wet	$0.23\pm0.016^{a}_{A}$	$0.24\pm0.017^{a}_{A}$	$0.02\pm0.003^{a}_{B}$
		Dry	$0.12\pm0.009^{b}_{A}$	$0.14\pm0.013^{b}_{A}$	$0.0\pm0.001^{a}_{B}$
D. pulex	(a)	Wet	$33.33\pm1.49^{a}_{A}$	$37.92\pm1.868^{a}_{A}$	$2.64\pm0.76^{a}_{B}$
		Dry	$25.97\pm2.136^{b}_{A}$	$28.44\pm2.378^{b}_{A}$	$0.83\pm0.336^{a}_{B}$
	(b)	Wet	$0.19\pm0.019^{a}_{A}$	$0.26\pm0.011^{a}_{B}$	$0.02\pm0.006^{a}_{C}$
		dry	$0.09\pm0.008^{b}_{A}$	$0.13\pm0.011^{b}_{B}$	$0.0\pm0.001^{a}_{C}$

Different superscripts (a-b) in each column indicate significant difference (P< 0.05). Different subscripts (A-B) in each raw indicate significant difference (P< 0.05).

proportion of ephippial eggs completed the development rather easily at 20 or 25°C under different conditions (Tables 5). In the present experiment, the significant interaction of these two factors implies a positive and synergic effect between wet pre-incubation of diapausing eggs and an increase in temperature to 25ºC. As indicated in Table 5, wet pre-incubation period followed by treating at 30ºC had an undesirable effect, demonstrating the dominance of higher temperature (30ºC) over wet storage of diapausing eggs on hatching response. The combined effect of wet pre-incubation or dry at low temperature (< 5ºC) on delaying or anticipating the reaction has been investigated as a primary research in this trial. Based on the obtained results, it can be stated that wet pre-incubation of diapausing eggs in darkness and lower temperature (4ºC) can precipitate their hatching response. In this sense, Schwartz and Hebert (1987) reported the shifted color and decayed texture of *D. pulex* sexual eggs when incubated wet at 35°C as compared to the normal appearance of the majority of ephippial eggs after dry pre-incubation at the same temperature. The results presented here, are in accordance with those reported on the precipitation of seed germination in plants immediately after a wet pre–incubation period in darkness and at lower temperature namely as stratification process (Frankland and Wareing, 1966; Pinfield, 1968; Zarska-Maciejewska and Lewak, 1976). They proved that physiological changes such as extending oxygen absorbance area and energy, increasing enzymatic activities, changes in hormone inhibitors and stimulators release in stratified seeds are responsible for the termination of dormancy.

Effect of Hatching Temperature

Many investigators have confirmed the need of changing temperature in order to induce hatching reaction in diapausing eggs of different zooplankton species (Mayer, 1990; Yurista, 1997; Tsitsials and Barry, 2002). The presence of interaction effects of temperature with NaOCl 1% pre-treatment on the rate of hatching in *D. pulex* ephippial egg may reflect that dipping in this oxidizing solution as an un-natural stimulus could interfere with the effect of the major proximate cues such as temperature for the termination of dormancy (Table 6). Not only do these results indicate the temperature tolerance of the diapausing eggs to hatch, but also it could be concluded that the influence of temperature on hatching outweighs other examined stimuli (Tables 5 and 6).

[DOR: 20.1001.1.16807073.2012.14.4.17.3]

Table 6. Comparison of hatching rate in *D. pulex* diapausing eggs under different levels of temperature and pre-treatment with NaOCl 1%.

Hatching temperature (ºC)			
30	25	20	
$0.01\pm0.006^{a}{}_{C}$	$0.2\pm0.021^{a}{}_{A}$	$0.12\pm0.014^{a}{}_{B}$	Non-soaked in NaOCl 1%
$0.01\pm0.004^{a}{}_{C}$	$0.19\pm0.024^{a}{}_{A}$	$0.15\pm0.02^{b}{}_{B}$	Soaked in NaOCl 1%

Different superscripts (a-b) in each column indicate significant difference (P< 0.05). Different subscripts (A-C) in each raw indicate significant difference (P< 0.05).

Effect of Pre-treatment by NaOCl 1%

A more readily observed effect of hypochlorite is its ability to permit an immediate response to light (Pancella and Stross, 1963). In the present trial, soaking of *D. magna* ephippial eggs in NaOCl 1% solution increased the rate of egg hatching and hatching percentage, significantly (P< 0.05) (Table 7) while the reaction of *D. pulex* ephippial eggs to this factor was negative (Table 3). Brendonk *et al* (1996) demonstrated that pre-treatment with NaOCl 7.5% for 5 to 10 minutes had a significant effect (P< 0.05) on hatching percentage of *Streptocephalus proboscideus* ephippial eggs in comparison with other decapsulating methods. As reported by Pancella and Stross (1963), pre-treatment of *D. pulex* ephippial eggs from wild and artificially-cultured clones prior to light exposure significantly shortened the latent period.

Effect of the Length of Light Exposure

Based on field studies on masssive hatching of zooplankton resting eggs during spring, irrespective of the local prevailing biotic and abiotic conditions, light is one of the most important proximate stimuli for the activation of resting eggs (Wolf and Carvalho, 1989) and photoperiod is likely to be a key factor explaining the seasonal pattern of emergence observed for many temperate zooplankton populations (Herzig, 1974; Hairston *et al*., 2000). Different photoperiod levels, their intensity and wavelength have shown various effects on hatching reaction of the diapausing eggs (Pancella and Stross, 1963; Davinson, 1969).

As indicated in Table 8, different photoperiod levels had a similar effect on the hatching percentage of both *Daphnia magna* and *Daphnia pulex* ephippial eggs, while the rate of egg hatching differentiated

Table 8. Comparison of hatching rates in *D. magna* and *D. pulex* ephippial eggs under different photoperiods.

	Length of light exposure	
	24 hr Light/Day	12 hr Light/Day
D. magna	$0.14\pm0.018_{A}$	$0.11\pm0.015_{B}$
D. pulex	$0.123\pm0.017_{A}$	$0.11\pm0.015_{B}$

Values are expressed as Mean±Standard error. Different subscripts in each raw indicate significant difference (P< 0.05).

Table 7. Comparison of the effect of NaOCl 1% pre-treatment on hatching % and hatching rate of *D. magna* diapausing eggs.

	Hatching (%)	Rate of hatching
Soaked in NaOCl 1%	$28.66\pm3.431_{A}$	$0.14\pm0.015_{A}$
Non-soaked in NaOCl 1%	$25.51\pm3.051_{B}$	$0.11\pm0.018_{B}$

Values are expressed as Mean±Standard error. Different subscripts in each column indicate significant difference (P< 0.05).

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by the application of continuous illumination or half day photoperiod, such that the hatching rate of both daphnia ephippial eggs increased significantly ($P< 0.05$) in continuous illumination. As evidenced by Pancella and Stross (1963), pre-treatment of *D. pulex* ephippial eggs from cultured and wild clones shortened the latent period prior to their exposure to the continuous illumination. In this research, maximum requirement of light (photoperiod) to induce and initiate the development of daphnia ephippial eggs might be attributed to the effect of pre-incubation in darkness at 4ºC. This finding is consistent with the result of Davinson (1963), who reported that ephippial eggs of *D. pulex* pre-incubated in cold and darkness required two folds of light energy to reach a maximum rate of 100% activation as compared to those stored at 24ºC. Moreover, this might be due to the use of ephippia with upper average age (the interval between egg releases from the female until their application in the experiments). It has been confirmed that photosensitive compounds in ephippia could be degraded or decomposed with time, making ephippia less responsive to light stimuli that induce hatching (De Mester *et al* 1998). No significant interaction ($P> 0.05$) was found between photoperiod and temperature in the present trial. This result is in agreement with the findings of Vandekerkhove *et al*. (2005) demonstrating the direct effect of decreasing within-year variation in temperature along north-to-south gradient on decreasing a photoperiod by temperature interaction effects.

CONCLUSIONS

Our results suggest the occurrence of pre-incubation condition and hatching temperature interaction effect on hatching percentage and hatching rate of both species. In this study, successful hatching of the ephippial eggs occurred at continuous illumination with hatching temperature of 20 or 25°C and wet pre-incubation period. The technique described in this paper, indicates that with a few simple experiments, it is generally possible to identify conditions under which the development of a considerable proportion of ephippial eggs, if not all, can be elicited. With this technique, we could solve the difficulty of hatching diapausing eggs that has undoubtedly been the greatest impediment to feed artificially propagated larvae of autumn sturgeon broodstocks in breeding and cultivation centers of the country.

ACKNOWLEDGEMENTS

Thanks are due to Prof. Joschen Vandekerkhove and Dr. Tome Debie researchers of the laboratory of Aquatic Ecology and Biology of Evolution for critically going through the manuscript and suggesting improvements. The authors are grateful to all staff working in the Fishery Faculty of Agricultural Sciences and Natural Resources University of Gorgan.

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بررسی شرایط لازم جهت تخم‌گشایی در تخم‌های خفته دافنی ماگنا *Daphnia magna* Straus, 1820 و دافنی پولکس *Daphnia pulex* Linnaeus, 1758 در شرایط آزمایشگاهی

س. حق‌پرست، ع. شعبانی، ب. شعبانپور و س. ع. حسینی

چکیده

با توجه به اهمیت حیاتی گونه‌های دافنی در آبزی پروری، به_ویژه در تغذیه بچه‌ماهیان نورس تاس-ماهی که در شرایط مصنوعی پرورش یافته‌اند، انجام مطالعه‌ای جامع در زمینه شرایط لازم جهت تخم-گشایی تخم‌های خفته به منظور دستیابی به تکنیکی بهینه امری اجتناب‌ناپذیر می‌نمود. بدین‌منظور، تخم-های خفته از استخرهای پرورش غذای زنده واقع در مرکز تکثیر و پرورش گرگان جمع‌آوری شده و پس از جداسازی، به صورت خشک و مرطوب به مدت ۲ ماه در محیط تاریک با دمای ۴°C نگهداری شدند. پس از طی دوره انکوباسیون اولیه، تخم‌های هر گروه به ۲ زیر گروه تقسیم و هر یک به‌طور مجزا تحت تیمار غوطه‌وری در محلول NaOCL 1 % و آب مقطر قرار گرفتند. اثر سطوح متفاوت دما (۲۰°C، ۲۵°C و ۳۰°C) و دوره روشنایی (۲۴L:۰ D، ۱۲L :۱۲D) بر درصد و سرعت تخم‌گشایی تخم‌های خفته در محیط کشت AdaM طی ۱۵ روز بررسی گردید. نتایج نشان دادند که تیمار اولیه تخم‌های مرطوب دافنی ماگنا با محلول 1%NaOCL و قرار دادن متوالی آن‌ها در معرض روشنایی مداوم و دمای ۲۰° C و ۲۵° C در رسیدن به حداکثر میزان هچلینگ‌ها و سرعت تخم‌گشایی موثر بود ($P<0.05$). قرار دادن تخم‌های مرطوب دافنی پولکس در معرض ۱۲ ساعت روشنایی و دمای ۲۵°C بدون نیاز به غوطه‌وری در NaOCL 1 % تعداد هچلینگ‌ها و سرعت واکنش تخم‌گشایی را به حداکثر رساند($P<0.05$).

[DOR: 20.1001.1.16807073.2012.14.4.17.3]