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Temperature Tolerance of Loliginid Squid (*Loligo vulgaris* Lamarck, 1798) Eggs in Controlled Conditions

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Abstract

Temperature tolerance of common squid (*Loligo vulgaris* Lamarck, 1798) eggs in controlled conditions was investigated between May 21, 2002 and March 04, 2003. The treatments were performed as 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, and 28°C at 37 ppt. Illumination was kept at dim light for 13-49 days before hatching. A mean dorsal mantle length of hatchlings was measured as 2.74 mm ± 0.1 SD. In the trials, the eggs developed and hatched normally after 44-49 d at 12°C, 32-39 d at 14°C, 23-30 d at 16°C, 23-29 d at 18°C, 18-25 d at 20°C, 14-20 d at 22°C, and 13-19 d at 24°C, except at 6, 8, 10, 26, and 28°C. In the experiments, hatching rates and hatching success of the eggs ranged from 15.4 to 99.6% and from 9.3 to 98.5%, respectively, and significant differences were found between the treatments (χ^2, $p<0.05$).

Key Words: Squid, *Loligo vulgaris*, egg, hatching, temperature.

Introduction

Loligo vulgaris Lamarck, 1798 spawns monocyclic and egg-laying occurs in separate batches during the spawning period (Rocha *et al*., 2001). Egg capsules of *L. vulgaris* are laid on the underside of rocky overhangs, on branched sessile organisms (Boletzky, 1998) or on fishing lines (Villenueva, 2000; Villanueva *et al*., 2003; see also "Materials and methods" of present study) and hang down in the water. The egg masses comprise dozens to hundreds of finger-like egg capsules, 60-160 mm in length, and each capsule containing 50-130 eggs of 1.92-2.88 mm in diameter (Mangold-Wirz, 1963; Worms, 1983; Marthy and Aroles, 1987; Martins, 2001; Şen, 2003, 2004).

Temperature is the main factor that regulates the length of embryonic development in cephalopods (Boletzky, 1987). Embryogenesis of *L. vulgaris* depends on the experimental water temperature, ranging from a few weeks to a few months (Naef, 1928; Jecklin, 1934; Mangold-Wirz, 1963; Boletzky, 1974; 1987; Şen, 2003; 2004). Villanueva *et al*. (2003) reported that 12 and 24.7°C are unusual for *L. vulgaris* eggs because of producing irregular statolith growth and low embryo survival in the laboratory.

The objective of the present study was to determine the optimum level of temperature for the incubation of *L. vulgaris* eggs by comparing the embryogenesis, hatching rate and hatching successes of the eggs at different incubation temperatures.

Material and Methods

Egg masses of *Loligo vulgaris* were collected from fishing lines deployed 24 h before egg collection at depths of 25 to 48 m from Izmir Bay (38°41'10"N, 26°75'35"E) between May 21, 2002 and March 04, 2003. Egg strings were transported to the indoor facilities of the Fisheries Faculty of Ege University on the same day. After obtaining the egg masses, they were sorted according to embryonic development stages based on Naef's (1928; Roman numerals) and Arnold's (1965; Arabic numerals) criteria, and then their morphometric characteristics were measured. During the experimental period, embryonic development stages of the eggs were observed under a stereo microscope (20-40X magnification) at regular intervals. The embryonic development stage was 13 (IV) at the initial of the treatments, which is the gastrula stage.

All trials were performed with acclimation process at ±1°C. A total of 60 egg capsules and 3758 eggs of which 124 eggs were used for embryonic observations were used, and separated into clusters of five capsules. They were divided into 12 groups based on experimental temperature levels: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, and 28°C. The egg capsules suspended in the water column were introduced into 4.2 l transparency plastic beakers containing 4 l of filtered seawater, and placed in a 10 l water bath. Temperature was adjusted by using ice or thermostatic aquarium heater. For maintenance of the eggs, seawater was changed every two days by replacing 100% of the beaker volume with fresh seawater that was regulated to experimental salinity (37 ppt) and temperature, and supplied with continuous aeration at 35 ml/min, directly to the egg capsules. Illumination of experimental designs was kept at dim light (<2 lux).

The length of the incubation period, hatching rates (no. of hatching eggs x 100/ no. of incubated

eggs), and hatching success (no. of viable paralarvae x 100/ no. of incubated eggs) of the eggs were estimated for each experimental group.

The length of egg capsules was measured with measurement board to the nearest mm, egg dimensions (length and width) were measured by millimetric ocular to the nearest 0.01 mm, and number of eggs per capsule was counted by macrometric observations. During hatching, newly hatchlings (paralarvae) were harvested from the beakers and counted daily. Dorsal mantle lengths (DML) of the viable paralarvae were also measured by millimetric ocular to the nearest 0.01 mm.

The obtained data were given as mean ±SD values. Differences between grouped data were analyzed by χ^2 test.

Results

The egg capsules were transparent, gelatinous, finger-like in shape, whitish in colour with the egg clearly visible, and with negative buoyancy. Eggs were wound in a spiral within the egg capsule and were ovoid and yolky in appearance. About one-third of the egg was covered by the growth of the blastoderm over the embryo's surface.

Descriptive analysis of morphometric characters of egg capsules, eggs and DML of paralarvae are shown in Table 1.

The eggs in the treatments achieved normal embryogenesis and hatching, except at 6, 8, 10, 26, and 28°C. At the 6°C treatment, the eggs died in the initial stage on day 30. At 8°C treatment, embryos in the eggs developed abnormally from stage 17 (VII-VIII) throughout to stage 21 (X-XI), and died on day 49. At 10°C treatment, the embryos developed throughout at stage 25 (XIII), but fully deformed from stage 24 (XII), and all of them died on day 41. At 26°C treatment, although the embryos developed very fast reaching stage 28 (XVIII) by 10 days, all individuals deformed from this stage and died on day 19. At 28°C treatment, the eggs reached stage 16 (VI-VII), but all died in the third day of the experiment. The embryonic development of eggs, according to the criteria of Arnold (1965), is summarized in Figure 1.

Length of the embryonic development, total hatching rate, and hatching successes for the trials are given in Table 2. There were significant differences in total hatching rate, hatching success between the treatments (χ^2, $p<0.05$). The hatching periods lasted for 6-8 days, and the percentages of hatchlings are shown in Figure 2 by treatments.

Table 1. Morphometric characteristics of egg capsules, eggs and newly hatchlings

Values	Capsule length (mm)	Eggs per capsule (no.)	Egg length (mm)	Egg Width (mm)	Dorsal mantle length (mm)
N	60	3758	100	100	100
Min	75	35	2.2	1.56	2.48
Max	165	105	2.88	2.12	2.92
Mean	117	62.6	2.49	1.83	2.74
SD	27.8	18.4	0.16	0.10	0.10

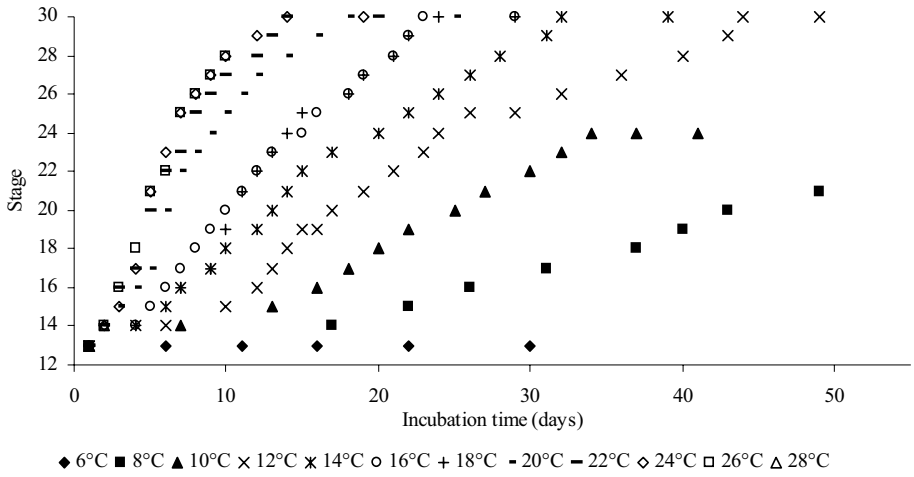

Figure1. Embryonic development of *L. vulgaris* eggs based on the criteria of Arnold (1965).

Discussion

The present results demonstrated that temperature directly affected incubation time, embryonic development, hatching rate and hatching success of *L. vulgaris*. *L. vulgaris* eggs were incubated normally at 12 and 24°C. However, eggs developed unsuitably at 6, 8, 10, 26 and 28°C, because the eggs deformed and died. The embryonic development time is inversely related to the incubation temperature (Naef, 1928; Jecklin, 1934; Mangold-Wirz, 1963; Mangold and Boletzky, 1973; Boletzky, 1974; 1979; 1987; Worms, 1983; Segawa, 1987; Blackburn *et al*., 1998; Martins, 2001; Villanueva *et al*., 2003; Şen, 2003; 2004).

In this study, maximum length of egg capsule and egg were found bigger than those of Mangold-Wirz (1963), Naef (1928), Jecklin (1934), Martins (2001), and Şen (2004). Minimum number of eggs per capsule in this research was lower than that of Mangold-Wirz (1963), Boletzky, (1974; 1979; 1987), Marthy and Aroles (1987), Martins (2001), and Şen (2004). In the present investigation, mean DML of paralarvae was bigger than that of Şen (2004), but smaller than that of Turk *et al*. (1986), Sweeny *et al*. (1992), and Villanueva (1994). The size of hatchlings depends largely on the size of the spawned egg (Mangold *et al*., 1971; Segawa *et al*., 1988) and region (Villanueva *et al*., 2003).

The present findings on incubation periods in the trials were similar to those of Mangold-Wirz (1963) at 14°C, and of Boletzky (1974) at 20°C, but shorter than those of Naef (1928) and Mangold-Wirz (1963) at 22°C, and completely different from those of Boletzky (1974) at 10°C. Because extreme temperature levels (6, 8, 10, 26, 28°C, and even 12 and 24°C) cause a damage to gelatinous layer of egg capsule and then to water uptake function, the embryos at various egg stages deformed and died. The present results corresponded to Villanueva *et al*. (2003), in which unusual temperature levels for incubation and embryonic development of *L. vulgaris* eggs were shown as below 14°C and above 22°C.

Hatching period varies among the loliginids and apparently does not show any obvious patterns (Arkhipkin and Middleton, 2003). Baeg *et al*. (1992) pointed out that *L. bleekeri* hatchling hatched during one night; however, in *L. opelescens L. forbesi*, *L. vulgaris reynaudii* and *L. vulgaris*, the duration from the first paralarvae hatching to emergence of the last being 4-6 days, 7 days, 3 days, and 9-12 days, respectively (Yang *et al*., 1986; Segawa *et al*., 1988; Blackburn *et al*., 1998; Şen, 2004). This period was found as 6-8 days in the present experiment.

Table 2. The length of incubation time, hatching rate and hatching success for trials

Treatments (°C)	Incubation time (days)	Hatching rate (%)	Hatching success (%)
12	44-49	95	9.3
14	32-39	98	81.3
16	23-30	91.2	98.5
18	23-29	98.9	86.9
20	18-25	99.6	95.9
22	14-20	99.6	88.5
24	13-19	15.4	12.3

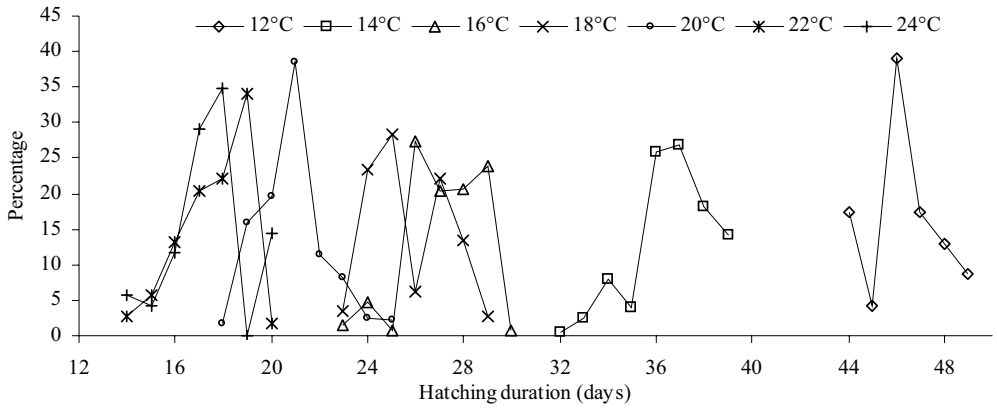

Figure 2. Hatching percentages in the treatments.

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