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EFFECTS OF TEMPERATURE ON SURVIVAL AND GROWTH OF *ARTEMIA* FROM TUZ LAKE, TURKEY

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Key words: *Artemia*, growth, survival, temperature effects, thermal constant

Abstract

The thermal constant, growth and survival of *Artemia* from Tuz Lake, Central Anatolia, were evaluated under laboratory conditions. *Artemia* nauplii (24-48 hours after hatching) were stocked into a 60 ppt salinity medium which contained *Dunaliella* and *Oocystis.* The *Artemia* were cultured at one of eight temperatures (2-degree intervals from 18° to 32°C) for 30 days, in two replicates. The animals grew faster but survival was lower as the temperature increased. Complete development from the nauplius stage to the adult stage required 30 days at 18°C, 20 days at 24°C and 15 days at 30°C. The thermal constant was 356±6 day-degrees and the developmental zero value was 6.3±1.9°C.

Introduction

Artemia cysts are commercially used in hatcheries as a live food for fish and crustaceans (Sorgeloos and Léger, 1992). *Artemia* is a non-selective phagotophic filter feeder that can be raised on various types of live and inert food (Provasoli and Shriashi, 1959; Barker-Jörgensen, 1966). As far as growth rate and production are concerned, the best results have been obtained with a live diet of *Dunaliella, Chlamydomonas* and *Chlorella* (Dobbeleir et al., 1980; Rosowski, 1989). The production of *Artemia* nauplii by incubation of cysts in sea water is a simple procedure and many studies document the best salinity, temperature and food conditions for its growth and survival. *Artemia* is distributed worldwide, inhabiting inland saltwater lakes, coastal lagoons and solar saltworks (Vanhaecke et al., 1987). Specific biotopes harbor populations with different tolerance ranges (Vanhaecke et al., 1984). Characteristics such as the cyst diameter, survival and growth of the nauplii are strain-dependent (Vanhaecke and Sorgeloos, 1980 a,b). The present study

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aims to determine the effect of temperature on survival and growth of a strain from Tuz Lake, Turkey.

Tuz Lake is located at 38°45' N, 33°22' E in Central Anatolia, Turkey. Its surface area is 1600 km^2 and maximum depth is 1.5 m (DPT, 1990). According to the terminology reported by Persoone and Sorgeloos (1980), *Artemia* biotopes have been characterized as thalassohaline and athalassohaline. Thalassohaline water is concentrated sea water (also called "chloride waters") with NaCl as the major salt. Most, if not all, of the coastal *Artemia* habitats (e.g., lagoons and bays) contain such water. There also are, however, *Artemia* habitats of the thalassohaline type which are located inland. One of them is Tuz Lake with a high concentration of sodium (100 g/l) and chloride (180 g/l; Uygun and Sen, 1978).

The mean depth of Tuz Lake is 30-40 cm, causing considerable seasonal salinity and temperature fluctuations. The lake level is highest in winter and early spring and lowest in July or August. The salinity of the lake ranges 35-395 ppt. In summer, the water temperature approaches 35°C and in autumn it drops to -3.8°C. Electrical conductivity and pH range 74-976 mS/cm and 5.8-9.5, respectively (Demirkalp and Basbug, 1995; Basbug and Demirkalp, 1997; Basbug, 1999).

Materials and Methods

A parthenogenetic *Artemia* population from Tuz Lake was tested at different temperatures to determine survival and growth performance. Non-processed cysts were stored in lake water in a refrigerator. To obtain nauplii, cysts were incubated in 1000 ml of a 30 ppt saline solution. Water temperature was kept at 30ºC during the hatching process, and strong aeration was provided by an air filter from the bottom.

Algae culture. For algae culture, 1000 ml glass separation funnels were disinfected with an HCl solution, rinsed in clean fresh water and filled with 60 ppt MGSL saline medium (Table 1). Phytoplankton samples, including *Dunaliella* and *Oocystis*, were collected from Tuz Lake with a plankton net of 55 µm mesh. STP+ES enrichment medium (Table 2) and

Table 1. Components of the MGSL medium*.

Component	*Weight (g/l)*
NaCl	44.41
KCl	1.64
$MgSO_4 \cdot 7H_2O$	8.74
$MgCl_2 \cdot 2H_2O$	4.90
$Na_2CO_3 \cdot H_2O$	0.08
$CaCl_2 \cdot 2H_2O$	0.23

* Prepared according to D'Agostino and Provasoli, 1968.

Table 2. Components of the STP+ES enrichment medium* used for algae culture.

Component	*Weight (mg/l)*
$NaNO_3$	180
K_2HPO_4	10
Na_2 glicerophosphate	25
Fe (EDTA)	10
Thiamin	10
Biotin	5
Vitamin B12	20
Sucrose	100
Na-H glutamate	25
DL-alanine	20
Glycine	20
Tris buffer	250
Triptose	20
Metal U2	1 ml

* Prepared according to D'Agostino and Provasoli, 1968.

the phytoplankton samples were added simultaneously to the funnels. The culture was maintained at 20ºC, by cool white fluorescent bulbs for 24 h and aerated from the bottom. After preparation, the final solutions were counted under an inverted microscope and stored in the refrigerator until use.

Culture conditions. All tests were conducted at a salinity of 60 ppt and at one of eight temperatures (18º, 20º, 22º, 24º, 26º, 28º, 30º or 32±0.2ºC). Water temperature was regulated by a thermostat submerged in the water bath. After hatching, 300 nauplii were carefully transferred to the culture flask with 500 ml of the 60 ppt MGSL solution. The flasks were covered with Petri dishes, perforated to minimize evaporation, and mild aeration was applied from the bottom. Animals were fed a mixed diet of *Dunaliella* and *Oocystis* once a day or every other day *ad libitum* (initial density averaged 1.63 x 10^4/ml), depending on consumption. The culture medium was completely renewed every five days. Survival and growth were monitored when the medium was renewed. To determine survival, all live animals in the culture flask were counted. Eight randomly sampled animals from each temperature were anesthetized in chloroform-saturated sea water and measured under a microscope. All experiments were repeated twice.

The thermal constant and developmental zero were calculated as follows: K = Y(T-a), where K is the thermal constant, Y is the number of days required for development to the adult stage, T is the temperature and "a" is the developmental zero (Andrewartha and Birch, 1954). To calculate the thermal constant (K), the maturation time (time required to develop from the nauplius stage to the adult stage; $Y_1....Y_n$) was determined for each temperature (T). The developmental zero (a) was obtained when $K_1=K_2$. In the next step, the (a) value was fitted to the Andrewartha and Birch equation. The average was obtained after K was calculated for each temperature (K_1, $K_2...K_n$).

Statistical analysis. The survival data were normalized by arcsin transformation. For all treatments, temperature effects on survival and growth were analyzed statistically with one-way ANOVA. The Post Hoc LSD Comparison test was used to identify significant differences between sample means at a significance level of $p<0.05$ (Sokal and Rohlf, 1981). Standard deviations of growth values were calculated from eight samples from each treatment. The effect of temperature on development time was tested by the Kruskal-Wallis test.

Results

The time required for development from the nauplius stage to the adult stage (Fig. 1) was 30 days at 18°C, 23 days at 22°C, 18 days at 26°C and 15 days at 30°C. The effect of temperature was significant ($p=0.047$). The average thermal constant was 356±6 day-degrees (Table 3) and the developmental zero was 6.3±1.9°C.

Survival is presented in Fig. 2 and Table 4. Temperature significantly affected survival throughout the culture period ($p<0.05$). The temperature of 32ºC negatively affected the *Artemia*; survival was less than 12% on the tenth day of culture and no larvae survived at 15 days. At 30ºC, total mortality occurred by day 25 and at 26º and 28ºC no larvae survived at 30 days. Survival was higher at temperatures of 18-26ºC, but significantly differed among these treatments.

Growth is shown in Fig. 3 and Table 5. Temperature significantly ($p<0.05$) affected growth except on day 30. However, the Post Hoc test showed that growth at neighboring temperatures did not differ significantly. Generally, growth was very similar at 18-20-22° and at 24-26° and at 28-30-32°. Growth significantly differed between low and high temperatures, e.g., between 18° and 28°C or between 20° and 30°C ($p<0.05$). Low temperatures caused slower growth, especially in the early larval stage. For example, on day 5, the average total length was 0.82 mm at 18ºC, 1.32 mm at 24ºC and 2.30 mm at 32ºC, but there were no striking differences at 30 days ($p>0.05$).

Discussion

Despite their wide geographical distribution, brine shrimp populations are isolated from one another. This isolation can result in marked differences between populations,

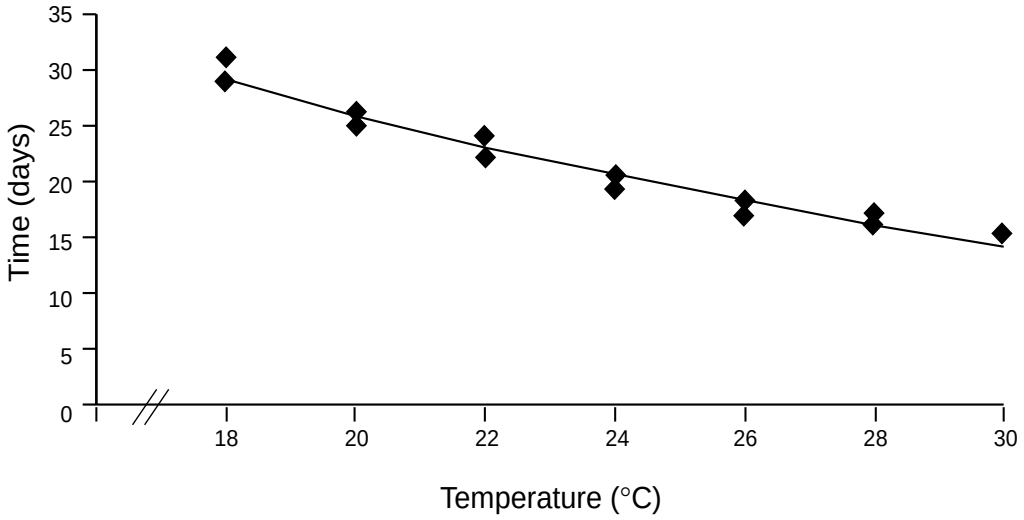

Fig.1. Time required for development of Tuz Lake *Artemia* from hatching to the adult stage at different temperatures.

Table 3. Average (± standard deviation, n = 7*) thermal constant values (day-degree) for Tuz Lake *Artemia* reared at different temperatures.

Temperature (°C)	*Thermal constant (K)*
18	349.86
20	355.21
22	360.23
24	353.24
26	353.92
28	368.24
30	354.93
Average	356.52±6.02

* No adults were obtained in the 32°C treatment.

even those separated by relatively short distances. Temperature and salinity significantly affect survival, with the effect of temperature more pronounced (Vanhaecke et al., 1984). Prior to carrying out our study, we tested various conditions for culturing algae, i.e., enrichment with STP, E1, E2, E3 and STP+ES at three salinities (5, 30 and 60 ppt). The algae did not develop well at 5 ppt and our nauplii were not resistant to low salinities. In contrast, D'Agostino and Provasoli (1968) found that *Artemia salina* from Commachio, Italy, tolerated 5 ppt salinity well. In their study, algae density was high using STP+ES enrichment at 5 and 60 ppt. We obtained similar results when using STP+ES enrichment and 60 ppt and therefore chose this medium for our study.

The temperature necessary to reach a certain point in development is called the "thermal constant" (Andrewartha and Birch, 1954). The developmental zero value in our laboratory experiments confirms the observation of Persoone and Sorgeloos (1980) that most *Artemia* strains do not survive below 6°C. Our calculations also agree with the

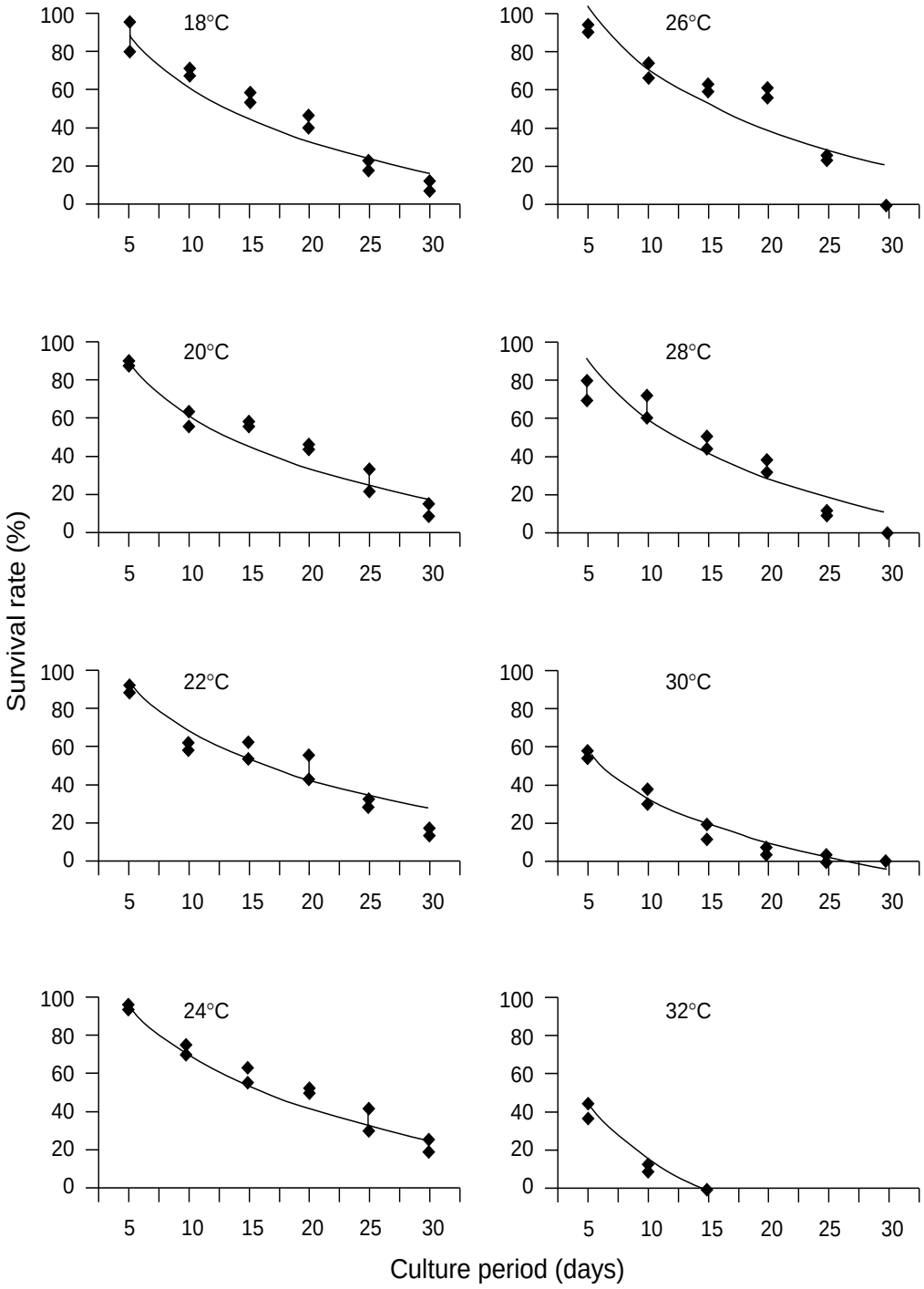

Fig. 2. Survival of *Artemia* from Tuz Lake reared at different temperatures.

Table 4. Average survival (%) of Tuz Lake *Artemia* reared at different temperatures for 30 days.

	Rearing temperature							
Day	*18°C*	*20°C*	*22°C*	*24°C*	*26°C*	*28°C*	*30°C*	*32°C*
5	88.35^{ab}	89.83^{ab}	90.00^{ab}	95.33^{ab}	91.66^{ab}	75.00^{c}	55.83^{d}	42.00^{d}
10	69.30^{de}	60.16^{cde}	60.02^{cde}	72.33^{def}	70.00^{de}	66.50^{de}	34.83^{a}	11.66^{b}
15	56.17^{ad}	57.20^{ad}	58.16^{a}	59.33^{a}	61.17^{a}	47.66^{ac}	16.00^{b}	-
20	43.00^{abd}	46.33^{abc}	49.33^{abc}	51.50^{bce}	57.50^{ce}	35.83^{a}	5.50^{f}	-
25	20.33^{bc}	28.49^{cd}	29.80^{cd}	35.50^{d}	25.33^{ce}	11.00^{ab}	-	-
30	9.16^{abc}	12.49^{ab}	15.33^{b}	22.00^{d}	*	-	-	-

Values in a row sharing the same letter are not significantly different (Post Hoc LSD, $p<0.05$).
* Only seven of the originally stocked 300 animals survived to 30 days.

observations of Demirkalp and Basbug (1995) that nauplii, metanauplii and adults do not survive in Tuz Lake below 6°C.

In our study, the best growth and survival were obtained at 22-26°C. Tuz Lake *Artemia* tolerated low temperatures very well. However, low temperatures caused a delay in development (e.g., *Artemia* reached 8.3 mm in 20 days at 30°C but required 30 days to reach the same length at 18°C). On the other hand, a rapid increase in mortality occurred at 30-32°C and no larvae in the 30°C treatment survived until day 30.

Temperature resistance correlates with genetic differences between and within sibling species; parthenogenetic strains are more tolerant to high temperatures (Vanhaecke et al., 1984). But variation in temperature resistance between parthenogenetic strains may be attributed to different degrees of ploidy. Diploid strains such as Salins de Giraud and Shark Bay are clearly less resistant than parthenogenetic strains which are mixed polyploid populations (Vanhaecke and Sorgeloos, 1989). Barata et al. (1996) studied a bisexual diploid strain, *Artemia salina* (Bonmati Saltwork, Spain), a parthenogenetic diploid population (La Mata Lagoon, Spain) and a parthenogenetic tetraploid population (Trinidad Saltworks, Spain). Survival of the three strains markedly dropped as the temperature increased from 15° to 24° to 30°C. The bisexual strain was least tolerant to high temperatures, with high mortality at 30°C. The two parthenogenetic strains had a similar survival pattern. However, the tetraploid strain had a higher survival at 15°C (Barata et al., 1996).

Consequently, as the Tuz Lake *Artemia* population is parthenogenetic (Basbug and Demirkalp, 1997), higher resistance to temperature increases was expected. Nevertheless survival of this strain decreased at 30° and 32°C. This confirms field observations of Basbug and Demirkalp (1997) and Basbug (1999). The reproduction season of *Artemia* in Tuz Lake is approximately three months, shorter than for many other *Artemia* strains. Cysts begin to hatch in early May. Biomass is most abundant in late May/early June, and

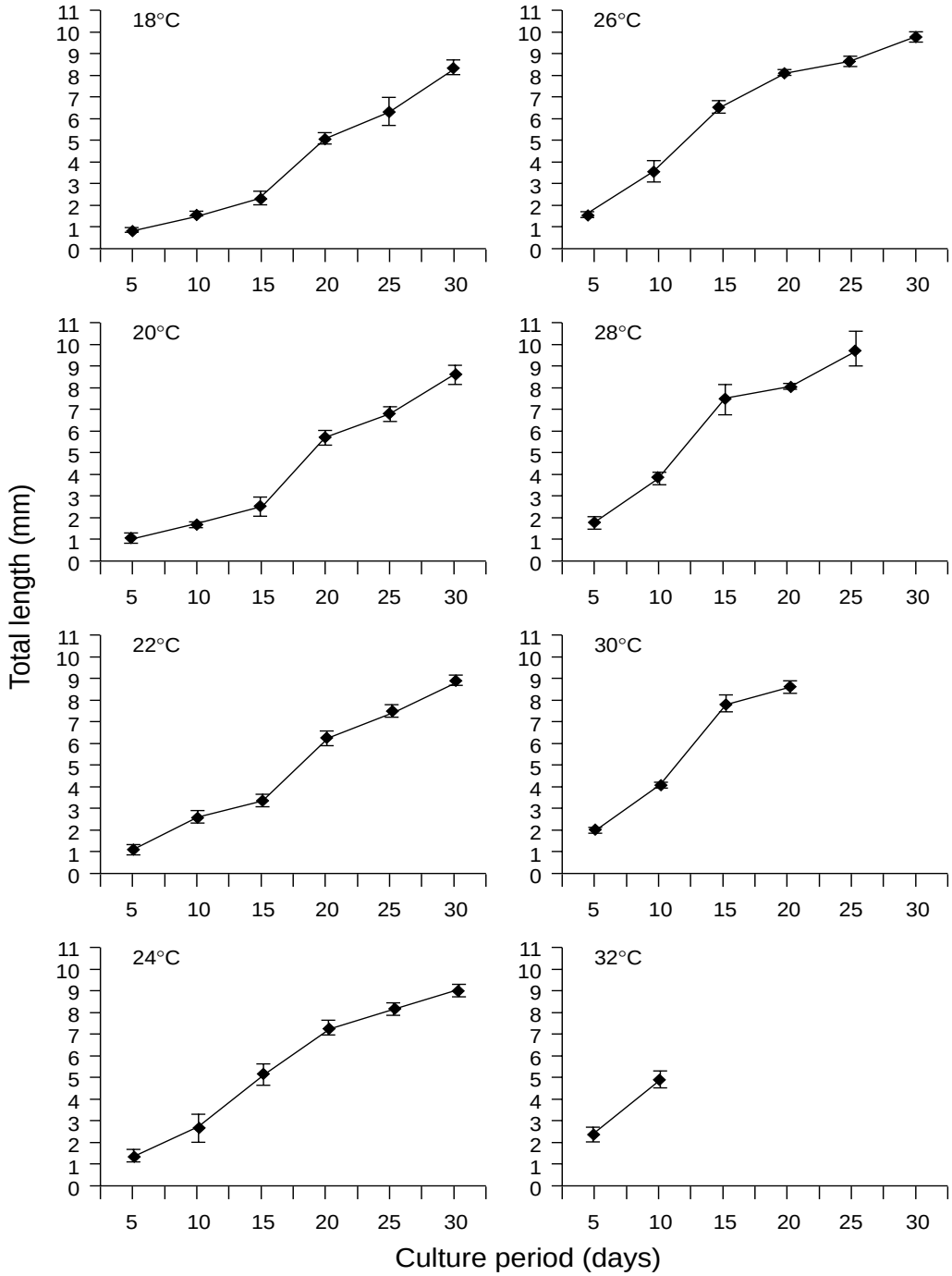

Fig. 3. Growth of *Artemia* from Tuz Lake reared at different temperatures. For each temperature and each sampling day, eight animals were measured.

Table 5. Average (± standard deviation, N = 8) length (mm) of Tuz Lake *Artemia* reared at different temperatures for 30 days.

	Temperature							
Day	*18°C*	*20°C*	*22°C*	*24°C*	*26°C*	*28°C*	*30°C*	*32°C*
5	0.82±0.04^{bcd}	0.98±0.33^{bcd}	1.15±0.28^{bcde}	1.32±0.45^{bcde}	1.50±0.14^{bcde}	1.75±0.36^{acde}	1.94±0.20^{ade}	2.30±0.43^{ae}
10	1.57±0.06^{ac}	1.65±0.14^{ac}	2.15±0.35^{ac}	2.60±0.85^{acd}	3.50±0.71^{bcd}	3.80±0.43^{bd}	3.98±0.12^{bd}	4.80±0.57^{b}
15	2.30±0.42^{a}	2.45±0.64^{a}	3.40±0.42^{a}	5.10±0.28^{b}	6.30±0.32^{c}	7.50±0.99^{cd}	7.70±0.57^{d}	-
20	5.03±0.31^{ab}	5.70±0.45^{abc}	6.25±0.49^{bc}	7.3±0.43^{de}	7.90±0.15^{def}	8.10±0.19^{def}	8.50±0.28^{ef}	-
25	6.30±0.85^{abc}	6.80±0.56^{abcd}	7.50±0.65^{abcde}	8.10±0.30^{bcde}	8.40±0.29^{cde}	9.80±1.14^{ef}	-	-
30	8.30±0.50^{bc}	8.60±0.60^{b}	8.90±0.28^{b}	9.20±0.35^{b}	9.50±0.43^{ab*}	-	-	-

Values in a row sharing the same letter are not significantly different (Post Hoc LSD, $p<0.05$).
* Only seven of the originally stocked 300 animals survived to 30 days.

sharply decreases in mid-June when the temperature approaches 30°C. The poor tolerance of this strain to high temperatures may be related to local adaptations. Indeed, the studies mentioned above confirm that lifespan characteristics of *Artemia* may vary, due to local adaptation.

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