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Effects of salinity on survival, and embryonic and postembryonic development of *Eurytemora affinis* from a freshwater lake

AKIRA ISHIKAWA[1], SYUHEI BAN & NAONOBU SHIGA

Faculty of Fisheries, Hokkaido University, 3–1–1 Minato-machi, Hakodate, Hokkaido 041–0821, Japan
[1]Present address: Faculty of Bioresources, Mie University, 1515 Kamihama-cho, Tsu, Mie 514–8507, Japan

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Abstract: The dominant copepod, *Eurytemora affinis*, in a freshwater lake, Lake Ohnuma, Hokkaido, Japan is supposed to have invaded the lake almost 20 years ago. Effects of salinity on survival, and embryonic and postembryonic development of this freshwater-acclimated copepod that primarily colonizes the upper reaches of estuaries, were examined in the laboratory to evaluate its salinity tolerances. Seven different salinities (0, 5, 10, 15, 20, 25, and 30‰) were tested in this study and all experiments were carried out under the conditions of 15°C and 12L : 12D light cycle. A short-term test for salinity tolerance of adult females revealed that the optimum salinity for the *E. affinis* specimens tested was in the range from 5 to 15‰, even though they inhabited a freshwater lake. While this copepod died immediately at 25 and 30‰, they were able to survive at these salinities after they were osmotically acclimated. The eggs hatched at salinities from 0 to 20‰ but not above. However, osmotically acclimated eggs could hatch even at 30‰. The embryonic development time was estimated at nearly 2 d at salinities of 0 and 5‰ but increased with increasing salinity above 10‰. The development time of the eggs spawned by osmotically acclimated females at salinities of 20 and 25‰ also approached 2 d. Survival and the development index (%) of the juveniles decreased remarkably at higher salinities, especially above 25‰. These results suggest that the physiological characteristics of the *E. affinis* in Lake Ohnuma are not substantially different from those of specimens inhabiting brackish waters, and therefore support the hypothesis that the animals invaded the lake approximately 20 years ago from some distant brackish water.

Key words: salinity tolerance, survival, postembryonic development, *Eurytemora affinis*, invasion of freshwater lake

Introduction

Eurytemora affinis (Poppe, 1880) is a widely distributed calanoid copepod occurring in pure sea water through freshwater areas in most parts of the northern hemisphere (Gurney 1931; Katona 1970; Vaupel-Klein & Weber 1975). In Europe and North America, this species often dominates at the upper reaches of estuaries (0–18‰ salinity) where environmental variables vary significantly over short-term intervals (Cronin et al. 1962; Jeffries 1962; Bradley 1975; 1978; Vaupel-Klein & Weber 1975; Pauw 1973; Collins & Williams 1982) and the population occasionally exceeds 3 $\times 10^6$ indiv. m^{-3} (Heinle & Flemer 1975). According to Jeffries (1967), the plankton community in estuaries is regarded as 'true-estuarine', 'estuarine and marine', 'euryhaline marine' and 'stenohaline marine' over a gradient from low to high salinities. For example, in the Bristol Channel and Severn Estuary, England, these subcommunities are characterized by four copepods, i.e., *E. affinis*, *Acartia bifilosa* var. *inermis* (Rose, 1929), *Centropages hamatus* (Lilljeborg, 1853) and *Calanus helgolandicus* (Claus, 1863), respectively (Collins & Williams 1981). There are many studies on salinity and temperature tolerances for the *E. affinis* populations inhabiting estuaries (Bradley 1975, 1978; Vaupel-Klein & Weber 1975; Roddie et al. 1984). Roddie et al. (1984) reported that *E. affinis* has higher salinity-temperature tolerance, and is a hyper/hypo-osmoregulator. They suggested that this physiological ability enables the species to inhabit the upper part of the estuary. Apart from estuaries, there are also many reports on the introduc-

Corresponding author: Akira Ishikawa; e-mail, ishikawa@bio.mie-u.ac.jp

tion of this copepod into rivers and inland lakes from brackish waters, possibly due to human activities in North America, e.g., in the Great Lakes (Engel 1962; Faber & Jermolajev 1966; Robertson & Gannon 1981) and in the Ohio River (Bowman & Lewis 1989).

In Japan, *E. affinis* is distributed in both fresh- and brackish waters in Hokkaido and a few brackish waters in the northern part of Honshu (Mizuno 1984). In the freshwater inland lake, Lake Ohnuma in Hokkaido, *E. affinis* was first observed in 1981 (Hokkaido Fish Hatchery 1982). This copepod is now one of the dominant species among the crustacean zooplankters in the lake (cf. Ban & Minoda 1989). Lake Ohnuma is a volcanic dammed lake, completely isolated from the sea, so that the intrusion of *E. affinis* into the lake is caused by neither marine traffic nor penetration of brackish waters. Ban & Minoda (1989) suggested that this copepod was introduced accidentally, with the transportation of the eggs of pond smelt, *Hypomesus transpacificus nipponensis* McAllister, 1963, from some distant brackish water, but evidence is lacking. While the transplantation of pond smelt eggs has been carried out every year since 1927 from several brackish or freshwater lakes in Hokkaido, invasion of the copepod into the lake would not have occurred for the last 15 years, because the pond smelt eggs were collected from lakes where *E. affinis* was not present during that period (Ban, personal communication). Therefore, the *E. affinis* population that inhabits this lake may have been isolated from saline waters for around two decades. In order to clarify the actual level of salinity tolerance in this population of the copepod, we have examined in this study the effects of salinity on its survival, embryonic and postembryonic development.

Materials and Methods

Short-term test for salinity tolerance

Salinity tolerance in adult females of *Eurytemora affinis* was examined by a short-term test in which survival was observed under starvation conditions. The experimental copepods were obtained from a stock culture, continuous for over three generations, that originated from Lake Ohnuma, and were kept in the laboratory at 15°C and on a 12L : 12D cycle (cf. Ban 1994). The temperature of 15°C is within the optimal range of 5 to 19°C for this copepod (Roddie et al. 1984). Ten adult females were placed in a number of 2-ml vials containing saline water which was obtained by diluting filtered sea water taken from off the coast of Hakodate, Hokkaido, with filtered lake water from Lake Ohnuma, and held at 15°C, 12L : 12D cycle. Water of six different salinities (5, 10, 15, 20, 25 and 30‰) was prepared for the experiments. In addition, filtered lake water, i.e., a salinity of 0‰, was used as a control. Survival of the animals was checked every day at 24 h intervals until no survivors remained. Within the first 24 h, an additional check was made at 6 h after the start of the experiment. All treatments were carried out in duplicate. The effect of osmotic acclimation to the higher salinities 25 and 30‰ was also tested. Prior to transferring the 10 adult females into 2-ml vials each containing saline water of 25 and 30‰, the animals for the former vials were acclimated at 15 and 20‰, and 15, 20 and 25‰ for the latter vials, at 12-h intervals, respectively. Then survival was observed as described above.

Effects of salinity on hatching success and embryonic development time

In this experiment, egg sacs detached from adult females which were collected from the lake on 20 September 1989, were used. Twenty-seven to 30 egg sacs were placed individually in wells of a 96-well tissue culture plate (Corning Co. Ltd.) filled with diluted sea water under conditions of 15°C and 12L : 12D light cycle. Seven different salinities were prepared as described above. Hatching was then checked under a stereo-microscope at 3- to 45-h intervals until 93 h from the start of the experiments. All treatments were carried out in duplicate. The embryonic development times at the various salinities were estimated from a regression analysis of incubation time on percentages of unhatched egg sacs (number of unhatched egg sacs/total number of hatched egg sacs) according to Edmondson (1965). Data obtained from the duplicate experiment for each salinity were pooled prior to analysis. Osmotic acclimation experiments at 20, 25, and 30‰ were also conducted. Ten pairs of males and egg carrying females to be used for the experiment were first transferred from lake water (0‰) to diluted filtered sea water (15‰), and then to 20, 25, and 30‰ in that order, allowing 12-h intervals for each acclimation. The acclimated pairs of males and females were each placed in experimental bottles filled with 50 ml of water at the salinity to be tested, i.e. 20, 25 and 30‰. They were fed a sufficient amount of euryhaline algae, *Pavlova* sp. and *Tetraselmis tetrathele* (G. S. West) Butcher, 1959 at a density of $>10^4$ cells ml^{-1}. The females were allowed to develop and spawn eggs in the bottles twice. The eggs the female originally carried with her were named the 1st clutch and the newly spawned eggs were labeled the 2nd and 3rd clutches, in chronological order. Hatching of the eggs was checked every 12 h. In this experiment, the embryonic development times were calculated by subtracting the time of spawning from the time that the eggs hatched and these values were averaged for 9–10 egg sacs during each treatment. The times were obtained only for the 2nd and the 3rd clutches, because we could not determine the accurate embryonic development time of the 1st clutch. In all these experiments, we defined hatching time as the time at which the first egg hatched within an egg sac.

Effects of salinity on survival of juveniles and postembryonic development

Effects of salinity on survival and postembryonic devel-

opment were examined at all salinities described above, except for at 0‰ (filtered lake water) because no growth of the food algae, *Pavlova* sp. and *T. tetrathele*, occurred at zero salinity. Prior to obtaining nauplii used in the experiment, several pairs of males and females were reared at the six different salinity conditions and fed sufficient amounts of both algal species ($>10^4$ cells ml^{-1}). Thirty-six to 40 nauplii, hatched within 24 h in each culture, were placed into new 500-ml jars filled with corresponding water of the salinity and algae, at 15°C and on a 12L : 12D light cycle. The water in the jars was changed once a week. During the experiment, algal density was maintained at 5×10^4 cells ml^{-1} and dead bodies and fecal pellets were removed with a pipette. Survival number of the animals and their developmental stage was determined under a stereo-microscope and recorded 10 and 17 d from the start of the experiments. All experiments were carried out in triplicate.

Statistical analysis

To test for differences under each salinity regime, data obtained were analyzed statistically with the log-rank test of Peto & Peto (cf. Tango 1993) for salinity tolerance of the adult females, with analysis of covariance (ANCOVA) for embryonic development time, and with one-way analysis of variance (ANOVA) for juvenile survival and postembryonic development. Multiple comparison was then conducted with Fisher's PLSD method when ANCOVA and ANOVA indicated significant differences among treatments. The differences in the percentages of juvenile survival between 10 and 17 d from the start of the experiments were tested by the Student's *t*-test.

Results

Short-term test for salinity tolerance

The survival curves for adult females varied for the seven different salinities (Fig. 1A). There was no significant difference between the survival curves for the three salinities from 5 to 15‰ (log-rank test, $X^2<3.20$, $p>0.05$) and the percentages of survivors at these salinities were relatively high throughout each experiment, compared to the other salinities. All females at 25 and 30‰ died immediately at the start of the experiment (<6 h). Percentages of survivors at 20‰ declined drastically to 40% within 2 d and thereafter the percentage gradually decreased, reaching 0% at 12 d from the start of the experiments. The survival curve at 0‰ was intermediate to those at 5, 10 and 15‰, and 20‰. Survival rates at 0 and 20‰ were significantly lower than those at 5, 10 and 15‰ (log-rank test, $X^2>6.39$, $p<0.05$), although there was no significant difference in survivorship between 0 and 20‰ (log-rank test, $X^2=0.34$, $p>0.1$). The day when the percentage of survivors reached 50%, i.e. LT_{50}, at the salinities from 0 to 20‰, was estimated graphically from the results obtained in this experiment (Table 1). LT_{50} was longer at 5 to 15‰ (4.53 to 5.63 d) than at 0‰

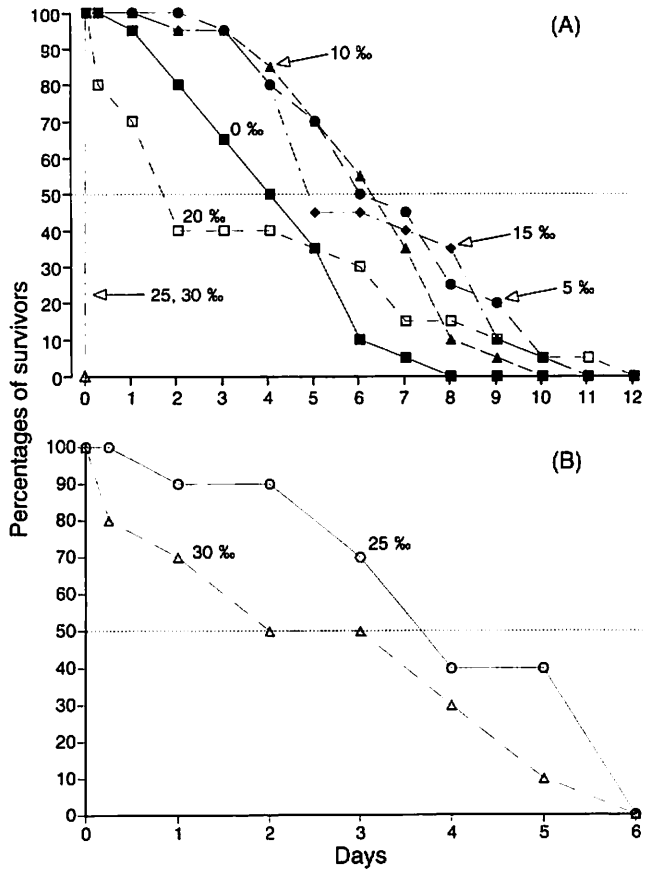

Fig. 1. A. Survival of adult females of *Eurytemora affinis* from Lake Ohnuma at seven different salinities from 0 to 30‰. Values are given as averages of duplicate treatments. **B.** Survival of adult females of this copepod at two different salinities (25 and 30‰) after acclimation at 15 and 20‰, and at 15, 20, and 25‰, in this order, at 12-h intervals. Dotted horizontal lines represent 50% survival.

Table 1. LT_{50} in adult females of *Eurytemora affinis* from Lake Ohnuma at seven different salinities from 0 to 30‰ without acclimation and at 25 and 30‰ with acclimation. Data are given as mean and range for the treatment without acclimation. Acclimation was carried out at the salinityies as indicated (arrow) at 12-h intervals.

Salinity (‰)	No. of replicates	LT_{50} (days)
0	2	3.35 (2.7–4.0)
5	2	5.5 (5.0–6.0)
10	2	5.63 (5.0–6.25)
15	2	4.53 (4.2–4.86)
20	2	1.74 (1.67–1.8)
25	2	—*
30	2	—*
15→20→25	1	3.67
15→20→25→30	1	2

* not calculated.

(3.35 d). LT_{50} at 20‰ (1.74 d) was the shortest of the salinities from 0 to 20‰. Once a female was acclimated from 15‰ to a higher salinity, the animal survived for up to 5 d even at 25 and 30‰ (Fig. 1B). In this case, the LT_{50} at 25 and 30‰ (3.67 and 2.00 d, respectively) was longer than that estimated at 20‰ without acclimation (Table 1).

Effects of salinity on hatching success and embryonic development time

The percentages of hatched egg sacs were high, above 73.1%, at salinities below 15‰, but very low (28.1%) at 20‰ (Table 2). At 25 and 30‰, the eggs were unable to hatch and collapsed within 24 h. The embryonic development times were calculated as the y-intercept of the estimated regression lines, i.e. the time when 0% of egg sacs remain unhatched (Fig. 2, Table 2). They were significantly different for the five salinities from 0 to 20‰ (ANCOVA, DF=4, 77, F=24.18, p<0.001). The embryonic development times were not significantly different between 0 and 5‰ (Table 2), being less than 50 h, but tended to increase with increasing salinity at >10‰.

The osmotically acclimated eggs, i.e. 1st clutch, hatched at all salinities from 20 to 30‰. The hatching success rate for 1st clutch eggs was highest at 20‰ with a value of 80% (Table 3). The percentage decreased with increasing salinity, but it was relatively high (60%) even at 30‰. Although an increase in hatching success from 1st to 3rd clutches was not observed at all of the three salinities, the embryonic development time for the 3rd clutch, which was produced by females acclimated for a longer time, tended to be shorter than that of the 2nd clutch at all salinities. The embryonic development times of the eggs in the 3rd clutch at both 20 and 25‰ (Table 3) were similar to those estimated at 0 and

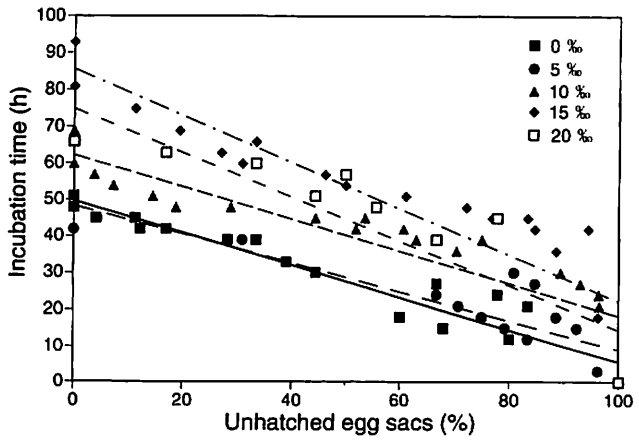

Fig. 2. The embryonic development time of *Eurytemora affinis* from Lake Ohnuma at different salinities from 0 to 20‰, estimated by Edmondson's (1965) methods. Percentages of unhatched egg sacs (number of unhatched egg sacs/total number of hatched egg sacs) are plotted against the time axis. The intercept of the regression line on the time axis gives the embryonic development time. Values obtained from duplicate experiments are pooled for each salinity. No eggs hatched at 25 and 30‰.

Table 2. Percentages of hatched egg sacs and embryonic development time in *Eurytemora affinis* from Lake Ohnuma at seven different salinites from 0 to 30‰, and results of ANCOVA and multiple comparison by Fisher's PLSD method. All treatmens were carried out in duplicate and the data were pooled for the following analysis. The embryonic development time was estimated from a regression analysis between both the parameters of incubation time and percentages of unhatched egg sacs. r^2 represents the coefficient of determination. Dashes denote no data.

Salinity (‰)	No. egg sacs tested	% egg sacs hatched	Embryonic development time (h)	r^2
0	59	73.1	49.7	0.92
5	57	87.8	48.5	0.84
10	57	96.7	62.3	0.81
15	57	78.2	85.6	0.82
20	53	28.1	75	0.8
25	57	0	—	—
30	57	0	—	—
	ANCOVA between the salinities from 0 to 20‰			
		DF	4, 77	
		F	24.18**	
	Multiple comparison* by Fisher's PLSD		15 20 10 0 5 (‰)	

* Treatments not underscored by the same line are significantly different at p<0.05 level.
** Significant difference, p<0.001.

Table 3. Percentages of hatched egg sacs and embryonic development time in *Eurytemora affins* from Lake Ohnuma at high salinities from 20 to 30‰. Female carrying egg sacs, i.e. 1st clutch, were transferred into 20, 25 and 30‰ after acclimation in 15‰, in 15 and 20‰, and in 15, 20 and 25‰ in this order, respectively, at 12-h intervals. Second and 3rd clutches were produced by females exposed at each salinity. Dashes denote no date, because the embryonic development time of the 1st clutch could not be estimated.

Salinity (‰)	Clutch	No. egg sac tested	% hatched egg sac	Embryonic development time (h)
20	1st	10	80	—
	2nd	10	100	52.8
	3rd	10	90	50.4
25	1st	10	70	—
	2nd	9	66.7	55.2
	3rd	9	77.8	48
30	1st	10	60	—
	2nd	9	77.8	81.6
	3rd	9	66.7	60

5‰ without acclimation (see Table 2).

Effects of salinity on survival of juveniles and their postembryonic development

The survival percentages at 10 d from the start of the experiments (Day 10) at salinities from 5 to 20‰ were in the range of 41.7 to 51.7% (Table 4). A remarkable decrease in survival was recorded above 25‰, and the percentage of survivors after 10 d was lowest (6.7%) at 30‰. However, the differences in percentages of survival of the copepod at 10 d were not statistically significant between the treatments (Table 4). The percentages of survivors at 17 d from the start of the experiments (Day 17) at salinities from 10 to 30‰ were not significantly different from those at Day 10 (t-test, $t<1.51$, $p>0.1$) (Table 4), but were significantly different at 5‰ (t-test, $t=7.51$, $p<0.05$). At a salinity of 5‰, the algae that the animals were fed on did not grow actively and high mortality of the algae was observed. The low percentage of survivors at Day 17 at a salinity of 5‰ may have been caused by a decrease in food quality due to the low salinity. Although no significant differences in the percentages of survivors at Day 17 were found between the five salinities from 5 to 25‰, survival rates decreased above 25‰ as was also observed on Day 10 (Table 4).

The proportion of copepodites to total survivors, which can be used as an index of development (%), was very high at Day 10 at both 5 and 10‰ (88.2 and 97.0%, respectively) (Table 4). Although there were no significant differences in this proportion between 5, 15 and 20‰, it declined at 15‰ (55.1%). The proportion decreased to 12.1% at 25‰, and no copepods developed into the copepodite stage at 30‰. At Day 17, although no significant difference in the proportion of adults to total survivors was found between 5 and 10‰, the proportion at 5‰ was highest (75%) and there was a trend of decreasing proportion with increasing salinities (Table 4). The absence of a negative food effect on the development of the animals at 5‰, was probably due to a decrease in food competition at the low algal density, because of the low number of survivors at Day 17.

Discussion

Eurytemora affinis living in estuaries possesses the ability to hyper- or hypo-osmoregulate, enabling it to tolerate a wider range of salinities and, furthermore, to survive at even higher salinities, above 30‰, after being osmotically acclimated (Roddie et al. 1984; Vaupel-Klein & Weber 1975). It has been reported from field observations that the optimum salinity range of this copepod is from 5 to 15‰ (e.g. Jeffries 1962). Roddie et al. (1984) have confirmed experimentally that the animals prefer almost the same range of salinities as observed in the field. In this study, the optimum salinity for *E. affinis* collected from a freshwater lake, Lake Ohnuma, was in the range from 5 to 15‰ rather than the ambient salinity of 0‰. Furthermore, after they were osmotically acclimated, they were able to survive at both 25 and 30‰. This indicates that the *E. affinis* population that inhabits Lake Ohnuma possesses the same salinity tolerances as populations of *E. affinis* inhabiting brackish waters.

Hatching success of the eggs in this study drastically declined at 20‰ and no hatching occurred above 25‰. However, osmotically acclimated eggs (1st clutch, carried by the female during acclimation) hatched even at 30‰ with a hatching rate of 60%, indicating that the eggs can also be

Table 4. Percentages of survival for *Eurytemora affinis* from Lake Ohnuma at 10 and 17 d from the start of the experiments, and the proportion of copepodites or adults to total survivors at 10 and 17 d, as an index of development, at six different salinities from 5 to 30‰. The results of a one-way analysis of variance for survivorship and the development index are also shown. Data are shown as mean±standard deviation.

Salinity (‰)	No. of replicates	Day 10		Day 17	
		Survival (%)	Copepodite (%)	Survival (%)	Adult (%)
5	3	45.8±13.8	88.2±4.3	13.3±7.6	75.0±25
10	3	41.7±27.5	97.0±5.2	36.7±23.2	56.9±10
15	3	45.0±15.6	55.1±36.9	41.7±18.1	35.0±17.3
20	3	51.7±27.7	60.2±26.6	51.7±27.7	26.9±18.1
25	3	18.0±8.3	12.1±21	16.3±5.4	0
30	3	6.7±11.5	0	5.0±8.7	0
	ANOVA between the salinities from 0 to 30‰				
	DF	5, 12	5, 12	5, 12	5, 12
	F	2.72	10.84***	3.41**	12.12***
	Multiple comparison* by Fisher's PLSD		10 5 20 15 25 30 (‰)	20 15 10 25 5 30 (‰)	5 10 15 20 25 30 (‰)

* Treatments not underscored by the same line are significantly different at $p<0.05$ level.

** Significant difference, $p<0.05$.

*** Significant difference, $p<0.01$.

acclimatized to a broad range of salinities. According to Ban & Minoda (1991), the embryonic development time of this copepod from Lake Ohnuma was about 48 h at 15°C. In this study, the times estimated at salinities of 0 and 5‰ were very close to 48 h (49.7 and 48.5 h, respectively). Although the embryonic development time was prolonged above 10‰, the development time of the eggs spawned by osmotically acclimated females at 20 and 25‰ surprisingly approached 48 h (50.4 h and 48.0 h, respectively). The embryonic development times of *E. affinis* from Patuxent River Estuary, Maryland, U.S.A. (Heinle & Flemer 1975), from Halifax, Nova Scotia, Canada (McLaren et al. 1969) and of *E. hirundoides* (=*E. affinis*) from the Gironde estuary, France (Poli & Castel 1983) were 1.9, 2.2, and 2.7 d, respectively, at 15°C. These results indicate that the eggs produced by this population from Lake Ohnuma are not embryologically different from those from several estuaries.

The survival and postembryonic development level during naupliar and copepodite stages of *E. affinis* decreased with increasing salinity, especially above 25‰, indicating that juvenile stages of this copepod are vulnerable to high salinity environments. There are few reports of reproduction in *E. affinis* at higher salinities. Vaupel-Klein & Weber (1975) reported that reproductive populations of *E. affinis* did not occur in water at salinities above 22.5‰ in the western Dutch Wadden Sea, and suggested a greater susceptibility of juveniles to environmental variables, such as salinity. Soltanpour-Gargari & Wellershaus (1985) also suggested that salinity is probably a factor responsible for mortality in *E. affinis* collected from the Weser estuary, Germany, mainly in the more sensitive stages, such as the nauplius.

As a whole, the physiological characteristics of *E. affinis* from Lake Ohnuma, observed in this study, were similar to those of this copepod in several estuaries. This is indirect evidence that the population of *E. affinis* inhabiting the lake was introduced from brackish waters (cf. Ban & Minoda 1989). Furthermore, this indicates that the copepods did not lose the ability of hyper/hypo-osmotic regulation, even though they colonized this freshwater environment approximately two decades ago.

This copepod commonly becomes abundant in the upper reaches of estuaries at a salinity range from 0 to 18‰ (Cronin et al. 1962; Jeffries 1962; Pauw 1973). It has been proposed that the factors limiting distribution of *E. affinis* to upper estuarine areas are predominantly biotic factors such as food competition, predation, and parasitism, rather than abiotic factors, such as salinity and temperature (Vaupel-Klein & Weber 1975; Bradley 1975). On the other hand, Roddie et al. (1984) pointed out that temperature and salinity are important factors controlling its distribution in the estuaries. Likewise, decreases in survival rates and the development index (%) during the juvenile stages at higher salinities, especially above 25‰, such as those observed in this study, may directly explain why this copepod's distribution is restricted to upper estuarine areas. In other words, a high survival rate and rapid development at lower salinities could be advantageous in enabling the copepod to build up a large population in upper estuarine areas, allowing it to dominate over other populations of copepods. The ability to hyper-osmoregulate, coupled with a wider range of temperature tolerances enables this copepod to maintain its population in the upper estuary. This may be the most likely reason as to why *E. affinis* is regarded as a true estuarine copepod (Jeffries 1967), why it dominates in the freshwater lake, Lake Ohnuma (Ban & Minoda 1989), and also why it occurs from brackish to fresh waters in North America (Engel 1962; Faber & Jermolajev 1966; Robertson & Gannon 1981; Bowman & Lewis 1989). In addition, the production of diapause eggs in this copepod (Ban 1992) plays an important role in maintaining the population in these freshwaters, where the environmental conditions are very severe in winter.

It is generally accepted that the origin of crustaceans inhabiting freshwaters is marine. According to Brand & Bayly (1971), with respect to habitat and osmotic characteristics, the evolutionary progress of Australasian centropagids from marine to freshwater is as follows; (1) oceanic and inshore marine euryhaline conformers, (2) truly estuarine or brackish water hyper/hypo-osmotic regulators, (3) freshwater hyperosmotic regulators (some of which developed enough cellular tolerance or regulatory systems to enable existence in some athalassic saline waters), (4) athalassic saline water conformers with either cellular tolerance or cellular osmoregulation or both. In this scheme, *E. affinis* can be considered a 'truly estuarine or brackish water hyper/hypo-osmotic regulator' (cf. Roddie et al. 1984). Evolutionarily, *E. affinis* may be a hyper/hypo-osmotic regulator that is approaching a freshwater hyperosmotic regulator, since this copepod has lost its ability to reproduce in higher salinity waters or pure sea water (cf. Vaupel-Klein & Weber 1975).

Among prawns of the family Palaemonidae, the freshwater species *Palaemonetes antennarius* (H. Milne-Edwards, 1837) has often been described as a variety of a brackish species, i.e., *P. varians* (Leach, 1814), because of the relatively few differences in the morphological and embryological features between the two species (cf. Parry 1957). However, Parry (1957) revealed that salt concentrations in the blood and urine of *P. antennarius* are distinctly different from those of *P. varians*, while there are relatively few differences in physiological features between the two species. Finally, he mentioned that, if differences in morphology and physiology appear to be due to geographical isolation, it would be an interesting evolutionary study to await the development of further distinctions. This would also be the case for *E. affinis* populations inhabiting freshwater lakes, since *E. affinis* is considered to occupy an important position in an evolutionary sequence that follows habitat changes from brackish to fresh water.

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