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Journal of Experimental Marine Biology and Ecology
303 (2004) 31–46

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

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Development and mortality of the first naupliar stages of *Eurytemora affinis* (Copepoda, Calanoida) under different conditions of salinity and temperature

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Received 1 May 2003; received in revised form 20 August 2003; accepted 1 November 2003

Abstract

As a prevalent species complex in temperate estuaries and salt marshes of the Northern Hemisphere, populations of *Eurytemora affinis* that inhabit these environments must be adapted to salinity fluctuations. Some populations have invaded freshwater environments. In this work, we focus on the combined effects of temperature and salinity fluctuations on mortality rates and development time of the first naupliar stages under starvation. Two temperatures (10 and 15 °C) and eight salinities, ranging from 0 to 35 psu are investigated. We show (i) that among all experimental conditions the optimal temperature and salinity for naupliar survival and development are 15 psu and 15 °C, and (ii) that only the most extreme salinities (i.e. 0 and 35 psu) have a negative effect on naupliar survival. Nauplii develop faster and reach a higher developmental stage at 15 than at 10 °C, independent of salinity. The relevance of this metabolic adaptive pattern is discussed in the general framework of in situ behavior, tidal forcing and biogeographic variability, as well as the potential sources of the observed individual variability.

Keywords: *Eurytemora affinis*; Individual variability; Mortality; Naupliar development; Salinity; Temperature

1. Introduction

Estuarine copepods are often exposed to fluctuating environmental factors such as temperature, salinity and quality and/or quantity of food. All of these factors may affect

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doi:10.1016/j.jembe.2003.11.002

copepod life history. Copepod viability in such fluctuating environments thus requires both physiological and behavioral adaptations (Fiksen and Giske, 1995). In order to understand the adaptive strategies, several experimental studies have been conducted on both the development and reproduction of copepods (Ban, 1994; Caramujo and Boavida, 1999; Cervetto et al., 1999; Lopez, 1996). However, some critical processes, such as mortality, still remain poorly studied, leaving a gap in contemporary literature. Even though some aspects of mortality, such as predation, on several developmental stages are difficult to identify in the laboratory (Bernard and Souissi, 1998), mortality rates due to environmental stress can still be examined under controlled conditions. Such experimental work would allow the study of early naupliar stages, neglected in previous studies mainly devoted to late developmental stages (Kiørboe and Sabatini, 1995; Peterson and Kimmerer, 1994; Vidal, 1980). Even though early developmental stages are difficult to sample and identify, they play a fundamental role in the pelagic food web (Green et al., 1992; Merrell and Stoecker, 1998; Ohman and Hirche, 2001). Moreover, because of the extreme sensitivity of copepod population models to the parameterization of mortality (Souissi and Ban, 2001), a more complete understanding of the mortality of early developmental stages is important.

Among estuarine copepod species, *Eurytemora affinis* is dominant in the temperate estuaries of the Northern Hemisphere (Andersen and Nielsen, 1997; Gasparini et al., 1999; Hough and Naylor, 1992), is now widely acknowledged as having high capacity of osmoregulation in response to fluctuating environmental conditions (Kimmel and Bradley, 2001; Roddie et al., 1984), and is a good biological example for mortality studies under different experimental conditions. Its life cycle and the effect of temperature, salinity and food quality and quantity on its post-embryonic development and reproduction have indeed been the subject of many studies (Andersen and Nielsen, 1997; Ban, 1994; Koski et al., 1999; Vijverberg, 1980). Experimental studies conducted on late copepodite stages have shown that population of *E. affinis* from Forth estuary (Scotland) has a greater affinity for low salinity waters, as suggested by its in situ distribution (Roddie et al., 1984). This species can nevertheless be found under higher salinity conditions (20–25 ‰) because of their osmoregulation capacities (Kimmel and Bradley, 2001; Roddie et al., 1984).

In the Seine estuary, the distribution of *E. affinis* is mainly influenced by salinity (Mouny, 1998), and restricted to the oligo- and mesohaline waters (0–20 ‰) despite the megatidal regime (Mouny and Dauvin, 2002). Because salinity stress is often a major driving process in the distribution of many estuarine species, the knowledge of the behavioral response of *E. affinis* populations and individuals to different salinity regimes could be useful in explaining the distribution of the species in estuaries. However, most studies explaining the role of behaviorally induced movements in the population-retention mechanisms of *E. affinis* are based on late developmental stages (Kimmerer et al., 1998; Morgan et al., 1997). We nevertheless stress that the early developmental stages have very limited swimming capacities and thus could be mainly passively transported to higher salinity zones (Le Hir et al., 2001). In addition, while the maintenance cost of individual survival can be affected by temperature and salinity fluctuations (Roddie et al., 1984), their effects on the early developmental stages remain unknown.

The objective of this study is to understand the combined effects of temperature and salinity on the development and mortality of the early naupliar stages of *E. affinis*. The experimental protocol used is based on individual observation, allowing for the quanti-

fication of individual variability (Ban, 1994; Souissi and Ban, 2001). In addition, compared to the above-mentioned studies, the frequency of observations was increased in order to obtain finer measurements within shorter naupliar stage durations. In order to investigate specifically the effects of salinity and temperature on metabolism, no food supply was provided aside from some control experiments.

2. Materials and methods

2.1. Field collection and animal acclimatization

Zooplankton was collected in the Seine estuary near the 'Pont de Normandie' (49° 26′065N, 00°16′920W) where previous studies have shown a maximum abundance of *E. affinis* at low salinities (e.g. Mouny and Dauvin, 2002). At each sampling date (25/03/2002, 23/04/2002, 18/05/2002 and 04/10/2002), where water temperature was 10.5, 13, 15.5 and 15.5 °C, respectively, zooplankton was collected with a WP2 net (200-μm mesh size) from the NO 'Côte d'Aquitaine'. A Solomat™ probe was used in order to identify the low salinity zone (2.5–5 psu) where zooplankton samples were taken. Specimens were stored in 30-l isotherm tanks and transported to the laboratory, where adults (mostly ovigerous females) were sorted out by pipette and acclimated in 2-l beakers containing freshly prepared treatment water at the experimental conditions of temperature and salinity (50 individuals/beaker). The individuals were kept in two temperature-controlled rooms at 10 and 15 °C, respectively. Treatment water was made from filtered seawater (GF/C Whatman filter; 0.45 μm) from the English Channel and deionised water. Preliminary tests using filtered water from the Seine estuary showed no significant differences on naupliar development and mortality. A large range of salinities (0, 2.5, 5, 10, 15, 20, 25, 30 and 35 psu) was used at 15 °C, the supposed optimal temperature for *E. affinis* reproduction and development (Gasparini et al., 1999; Mouny, 1998). For the 10 °C experiments, the copepods were collected on the 25/03/2002 and only the first five previous salinities were considered. Copepods collected at the other dates were used in the experiments at 15 °C. The first five salinities were tested using the samples of 23/04/2002 and the 18/05/2002. Copepods collected on 04/10/2002 were used for the later higher salinities, 25, 30 and 35 psu only.

2.2. Female selection

After 24 h of acclimatization, 5–10 ovigerous females were individually sorted and placed in a 30-ml beaker containing the same treatment water (Fig. 1). All experiments were performed under a light cycle representative of each sampling day. Females were observed five to six times per day in order to assess the hatching of nauplii.

2.3. Nauplii selection

After hatching, nauplii were sorted and placed individually in 15-ml beakers. One to three females, giving 12–55 nauplii, were used under all experimental conditions (Table 1).

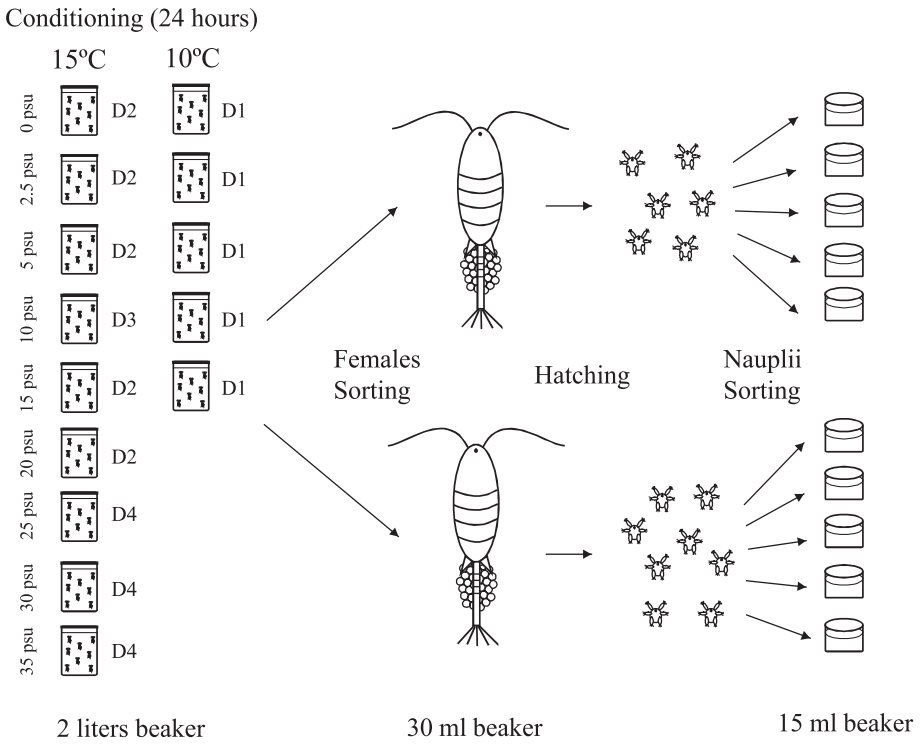


Fig. 1. Schematic representation of the experimental protocol used for conditioning *E. affinis* adults, sorting gravid females in 30-ml beakers for the egg hatching survey. The nauplii are isolated in smaller beakers (15 ml) as soon as they appeared. This protocol was used for each experimental combination of salinity and temperature. Sampling dates are indicated for each experiment D1 (25/03/2002), D2 (23/04/2002), D3 (18/05/2002) and D4 (04/10/2002).

The nauplii were transferred from one beaker to another with freshly prepared water only twice a week to minimize manipulation stress. Each nauplius was observed three to six times per day under a binocular microscope until its death. At each observation, the time and the naupliar condition (developmental stage or dead) was recorded. The different naupliar stages were identified using Katona's (1970) description.

Table 1
Total number of females (*f*) and nauplii (*n*) used for each combination of salinity and temperature

Salinity (psu)									
Temperature (°C)	0	2.5	5	10	15	20	25	30	35
10	3*f* 55*n*	2*f* 36*n*	3*f* 52*n*	2*f* 32*n*	2*f* 31*n*				
15	1*f* 16*n*	2*f* 51*n*	3*f* 55*n*	2*f* 32*n*	2*f* 37*n*	2*f* 39*n*	2*f* 37*n*	2*f* 24*n*	1*f* 12*n*

A mixed culture of *Isochrysis galbana* and *Nannochloropsis oculata* was used (final concentration 10,000 cells ml^{-1}) to conduct control experiments to study the development of first naupliar stages at 15 °C and 15 psu.

2.4. Data standardization and statistical analysis

The times of molting and death were normalized and standardized to obtain the absolute age of the nauplii and to allow comparisons between datasets irrespective of the starting time of the experiment. Development time in a stage n was calculated only when the nauplius had molted to the stage $n+1$.

When the number of females used for the same experimental condition was greater or equal to two, an inter-female comparison was carried out using a non-parametric Wilcoxon–Mann–Whitney (WMW) test because of the low number of nauplii per female. Only few combinations of temperature and salinity showed a significant difference between females, so lifespan of nauplii were pooled for each experimental condition. Afterwards, the null hypothesis that the medians of all treatments were equal was tested using a Kruskal–Wallis test. Then box-and-whisker plots were used to compare the distribution of lifespan lengths under all of the experimental conditions. Moreover, differences between lifespan lengths in all experimental combinations were quantified using a Wilcoxon–Mann–Whitney test with a significance level set at $P<0.05$. All statistical analyses were performed with the MATLAB Software.

3. Results

3.1. Effects of salinity and temperature on survival of nauplii, and influence on individual variability

Fig. 2 shows the patterns of mortality of nauplii for different combinations of temperature and salinity. The variability observed in age at death, for all experimental conditions, can be attributed to individual variability. Except for the lowest salinity (0 psu) where individual nauplii died rapidly (Fig. 2), the general pattern shows a phase of low mortality corresponding to the use of internal reserves for metabolism. This phase of resistance increases with salinity until 15 psu at 10 and 15 °C, but is longer at 10 °C. Above 15 psu at 15 °C, the mortality due to starvation occurs earlier (Fig. 2). These results suggest that nauplii of *E. affinis* from this population are not capable of osmoregulatory activity at 0 psu, as more than 70% of individuals died in the first day at this salinity. The decreasing phase of the survival curve (Fig. 2) can be explained by inter-individual variability of mortality due to starvation. The presence of some resistant individuals was responsible for the sigmoid shape of the curve at the end. Median lifespan ranged from 0.28 to 8.08 days and from 0.25 to 6.12 days at 10 and 15 °C, respectively.

Fig. 3 shows the patterns of lifetimes of different individuals within the different experiments. This allowed for the representation of the dispersion of data as a result of inter-individual variability, as well as the shape of the distribution of lifespan data (e.g. a non-centered median is indicative of skewness). The medians in all trials were signifi-

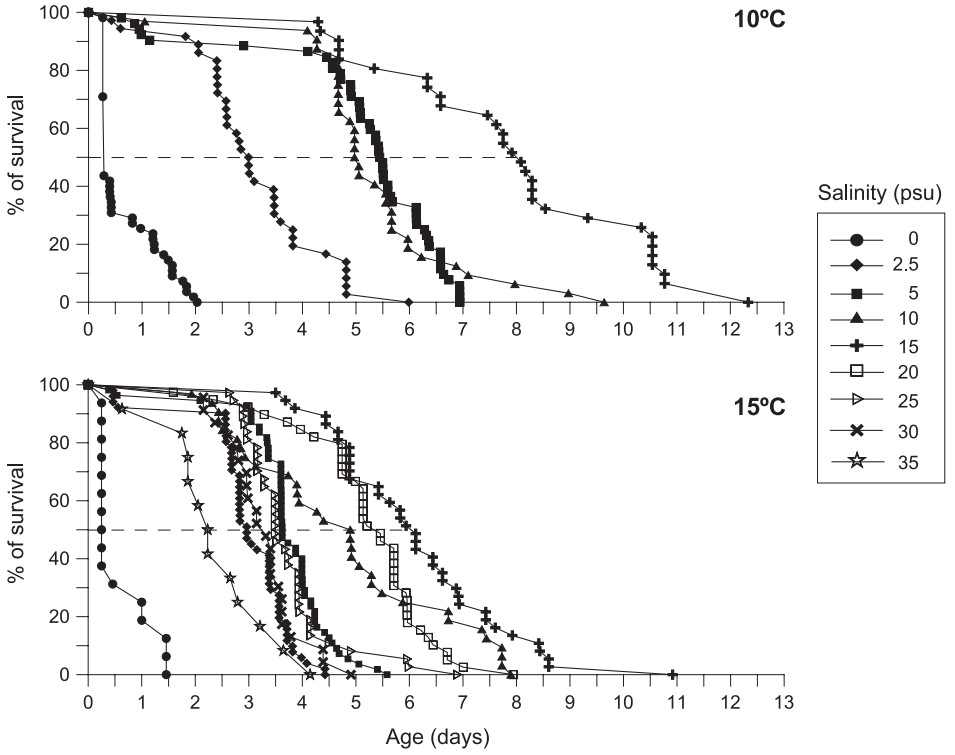


Fig. 2. Survival percentage of *E. affinis* first naupliar stages as a function of age for the different salinities at 10 and 15 °C. The survival medians shown by discontinuous lines correspond to ages of 50% dead nauplii. Each salinity was represented by a different symbol according to the indicated scale. Each point represents one individual.

cantly different (WMW test, $P>0.001$). At 10 and 15 °C, the maximum lifespan was observed at 15 psu salinity (Fig. 3). The highest dispersions around the median lifetime were observed for three experiments: $S_{10}T_{15}$, $S_{15}T_{10}$ and $S_{15}T_{15}$ (Fig. 3). In each of these experiments, the inter-female differences were not significant, which highlighted a high individual variability at these optimal conditions. The highest median of survival was obtained at $S_{15}T_{10}$, which is significantly different from all other experiments. On the other hand, the following three sets of experiments ($S_{2.5}T_{10}$, $S_{2.5}T_{15}$ and $S_{30}T_{15}$), (S_5T_{10}, $S_{10}T_{10}$, $S_{10}T_{15}$ and $S_{20}T_{15}$) and (S_5T_{15} and $S_{25}T_{15}$) were not significantly different and, consequently, had similar effects on naupliar survival.

3.2. Stage-specific mortality and mortality schedule

The percentages of stage-specific mortality as a function of salinity and temperature are shown in Table 2. Except in the case of the lowest salinity treatments (0 psu), where the salinity stress stopped the naupliar development at stage N1, most individuals molted into N2 and sometimes N3 before death. At 15 °C, the majority of individuals reached stage

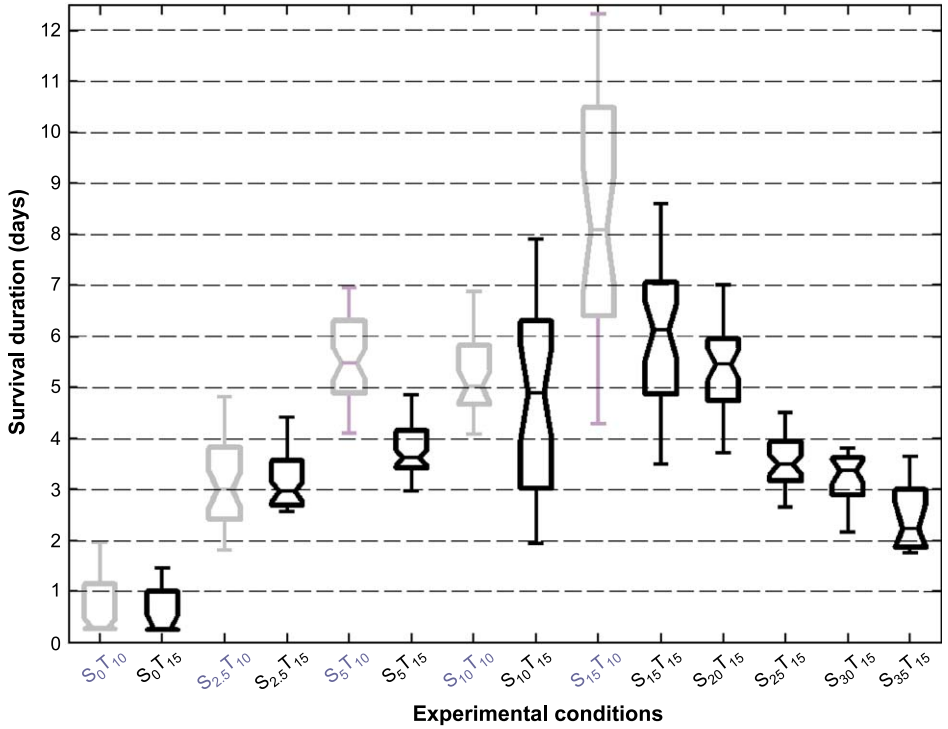
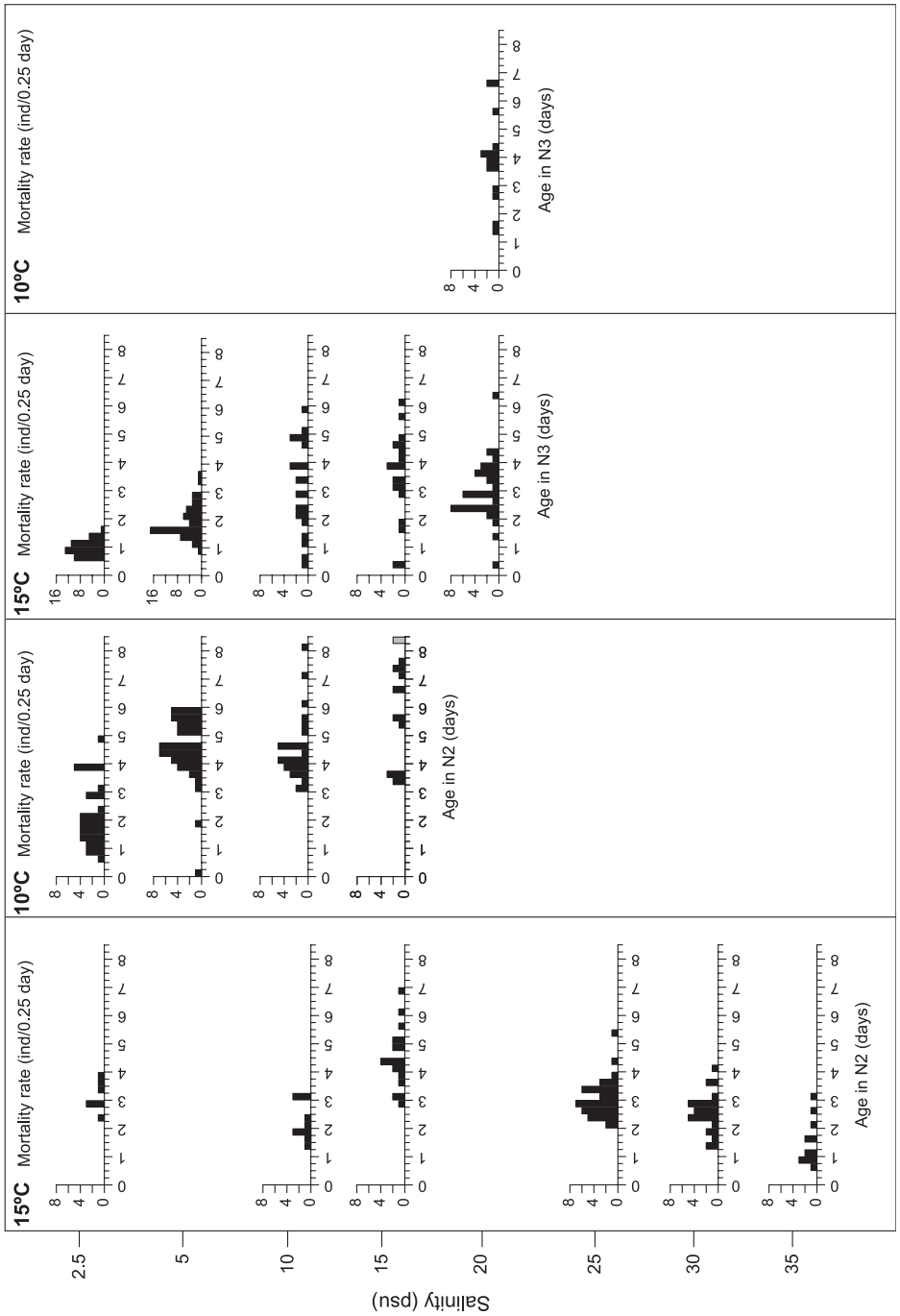


Fig. 3. Box-and-whiskers plot of the 14 experiments of mortality realized in different salinity conditions at 10 °C (grey boxes) and 15 °C (black boxes). S_xT_y in the abscissa axis corresponds to the experiment realized at a salinity x psu and at a temperature y °C. The centre horizontal line in the box is the median, the top and the bottom of the box are the 25th and 75th percentiles (quartiles), and the ends of the whiskers are the 5th and 95th percentiles. The notch in the box represents the 95% confidence interval of the median.

N3 for salinities ranging from 2.5 to 20 psu (Table 2). However, the majority of individuals died in stage N2 at 10 °C. No individual reached stage N3 under the highest salinity conditions (i.e. S>30 psu; Table 2). Even if the maximal lifespan was generally longer at 10 °C than at 15 °C (Fig. 3), the proportion of individuals reaching N3 was higher at 15 °C than at 10 °C (Table 2).

Table 2
Percentage of mortality in the first naupliar stages (N1, N2 and N3) for each experimental salinity at 15 and 10 °C

T (°C)	Stages	Salinity								
		0 psu	2.5 psu	5 psu	10 psu	15 psu	20 psu	25 psu	30 psu	35 psu
10	N1	100	5.60	7.69	3.13	0				
	N2	0	94.40	92.31	84.38	51.61				
	N3	0	0	0	12.50	48.39				
15	N1	100	7.84	3.64	0	0	0	0	0	8
	N2	0	13.73	3.64	31.25	48.65	12.82	95	100	92
	N3	0	78.43	92.73	68.75	51.35	87.18	5	0	0


15°C
10°C
15°C
10°C
Mortality rate (ind/0.25 day)
Age in N2 (days)
Age in N3 (days)
Salinity (psu)
2.5
5
10
15
20
25
30
35

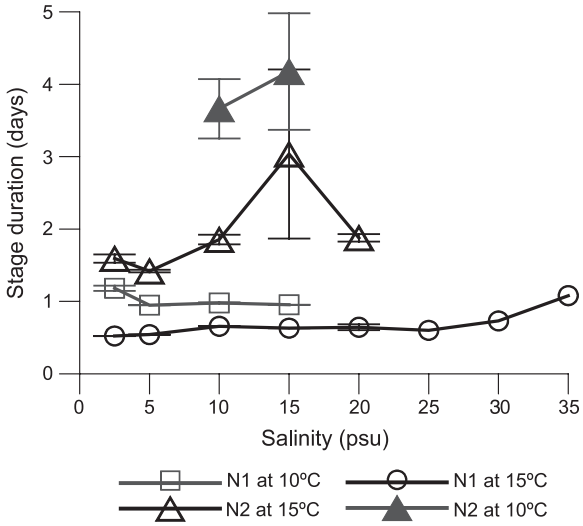


Fig. 5. Mean development time in stage N1 and N2 as a function of different temperature and salinity combinations. The stage N3 is not represented because no individual reached N4. Error bars represent the variability (standard deviation) centres on the medium. Error bars of N1 development are not visible because the variability is very low.

Fig. 4 shows the distribution of the mortality rates related to age within stages N2 and N3 when computation was possible (i.e. for individuals that had molted from stage *n* to *n* + 1). The shapes of the distributions shown in Fig. 4 are not regular, some distributions are uniform and others showed a clear mode (e.g. Fig. 4; N3 at 15 °C). For the same experimental conditions, individuals reaching stage N3 died earlier in this stage than those who died in stage N2 (see 2.5, 10, 15 and 20 psu in Fig. 4). This longer survival in stage N2 was influenced by salinity, it was maximal at 15 psu (Fig. 4). However, the temperature effect on the distribution of mortality rates was less obvious since there was no particular pattern of variation between temperatures.

3.3. Effects of temperature, salinity and starvation on stage duration

Stage durations were never computed in N1 at 0 psu and in N3 as all individuals died in these stages. Fig. 5 shows the mean development time in stages N1 and N2 computed for the other experimental conditions. The stage N1 was always significantly shorter than the stage N2, and the duration in N1 was only affected by temperature and highest salinities. The mean stage duration of N1 doubled when temperature decreased from 15 to 10 °C and

Fig. 4. Mortality rate distribution as a function of age within stage N2 and N3 for each tested salinity at 10 and 15 °C. The mortality rate is expressed in individuals per 0.25 day. In order to make all histograms legible, the *y*-scale in the third column (2.5 and 5 psu) was modified and the two individuals survived more than 8.5 days in one experiment (second column, 15 psu) are represented by a grey bar.

Table 3
Development time (days) of the first naupliar stages (N1, N2 and N3) of the copepod *E. affinis* as a function of temperature and food conditions

Study	Control experiments	Present		Ban (1994)	
Geographical zone	Seine estuary	Seine estuary		Lake Ohnuma (Japan)	
Food	*Isochrysis galbana*, *Nannochloropsis oculata*	Without		*Chlamydomonas reinhardii*, *Cryptomonas tetrapyrenoidosa*	
Salinity	15 psu	15 psu	15 psu	near 0 psu	near 0 psu
Temperature	15 °C	15 °C	10 °C	15 °C	10 °C
Food condition	10,000 cells ml^{-1}	without food	without food	50,000 cells ml^{-1}	50,000 cells ml^{-1}
Stages					
N1	0.53	0.6	0.95	0.5	1.5
N2	1.06	3.0	4.2	1.5	2.4
N3	1.77			1.1	1.9

Comparison between this study and data of previously realized experiments on the same species. These development times are for optimal salinity for *E. affinis* living in brackish water and for *E. affinis* living in freshwater (Lake Ohnuma). Development times in N3 are not given because no nauplii completed this stage.

is the highest at 10 °C and 2.5 psu, and at 15 °C and 35 psu (Fig. 5). The salinity had no effect on stage N1 duration around the optimal salinity between 10 and 25 psu. Individuals were synchronous in the stage N1 as the individual variability was very low (Fig. 5). Alternatively, the mean development duration in stage N2 varied with both temperature and salinity (Fig. 5). The mean development time was maximal at the optimal salinity of 15 psu at both temperatures. Individual variability of the development time in N2 depended on salinity as it was maximal at 15 psu at both temperatures (Fig. 5), so the temperature effect on individual variability was more ambiguous since variability was higher at 10 °C than at 15 °C for 10 psu but was inverted for 15 psu. The control

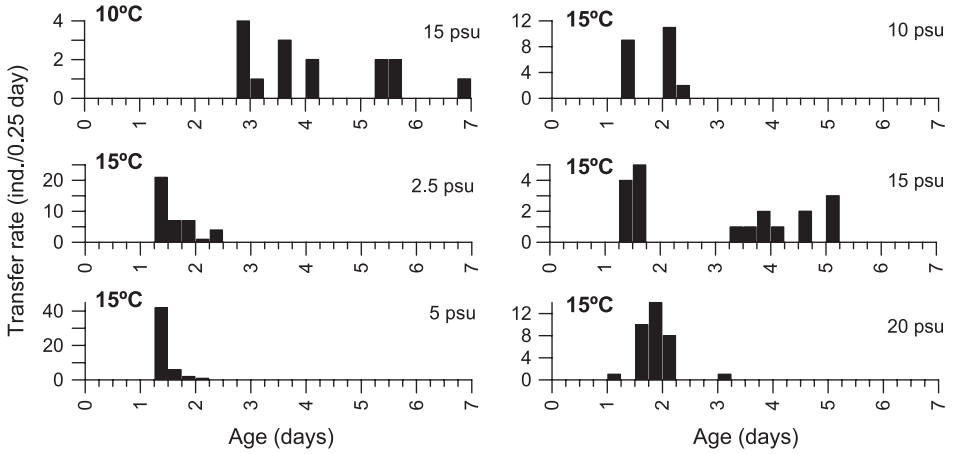


Fig. 6. Transfer rate distribution of naupliar moulting from stage N2 to stage N3 as a function of age within the stage N2 at 10 and at 15 °C. Transfer rate is expressed as individuals per 0.25 day.

experiment in the presence of food showed that starvation affects the development of the stage N2, which is three times shorter when food is present (Table 3).

Another way to express individual variability in development from one stage to another is to study the distributions of molting rates as a function of age within a given stage. Indeed, despite the high frequency of observations, the variance of development time in N1 was very low in all experiments (Fig. 5); the molting from N1 to N2 was quasi synchronous, all individuals had molted into N2 after 0.5–0.6 day at 15 °C and 1 day at 10 °C. The distribution of transfer rate was calculated only for the nauplii that had molted from N2 to N3. These distributions shown on Fig. 6 are not uniform. An amount of time spent in stage N2 is necessary before molting to the stage N3.

4. Discussion

4.1. Effects of temperature and salinity on naupliar metabolism

Our study showed that naupliar survival is maximal at 15 psu, indicating that this is the optimal salinity for *E. affinis* in the Seine estuary. Individuals survive longer at 10 °C but their development stopped between N2 and N3. On the contrary at 15 °C, several individuals reached the stage N3 at optimal and near optimal salinity. As it is known that the first naupliar stages (at least N1) of copepods do not feed (e.g. Ban, 1994; Peterson and Painting, 1990), the only source of energy is represented by their yolk reserves. The maintenance costs can be separated into three different sources of energy use: (i) routine metabolism (principally respiration), (ii) molting and (iii) osmoregulation. Roddie et al. (1984) showed that oxygen consumption of *E. affinis* increased considerably from 5 to 15 °C. In our starvation experiments, initial reserves were limited to the maternal yolk sources. Thus, the temperature increase from 10 to 15 °C accelerated the development (see Fig. 5), as well as the consumption of reserves, and consequently shortened lifetimes (see Fig. 3). On the other hand, salinity acts as a stress factor that increases mortality rates when it is not optimal (Roddie et al., 1984). Kimmel and Bradley (2001) have demonstrated that salinity variations induce synthesis or degradation of amino acids during osmoregulation. This generates an increase in the consumption of protein reserves as well as in energy requirements for enzymatic activity. Without energy renewal, this stress decreases nauplii survivorship and causes death of the nauplius in early stages. Our results suggest that there is no salinity stress at 15 psu and nauplii develop normally to N3. The stage N1 seems to be more sensitive to temperature than salinity. We can thus postulate that the metabolic activity of this developmental stage is principally internally controlled. One may also note here that only the lowest salinity (0 psu) was lethal for all nauplii, as the osmotic stress was strong enough to stop the development of individuals early in the stage N1.

We showed that salinity and temperature have combined effects on the physiology of *E. affinis* since the nauplii molted into N3 for a wider range of salinities at 15 °C than at 10 °C. The few previous experimental results on the effects of salinity and temperature on the survival of *E. affinis* were obtained from small numbers of individuals (i.e. 10–20 individuals in Roddie et al., 1984) belonging to late copepodite stages and adults.

Moreover, the observed optimal salinity for survival varied between 5 and 20 psu, but contrary to our results, adults survived better at 0 psu than at 30 and 35 psu where survival was very low. This discrepancy could be attributed to the physiological difference existing between first naupliar and late copepodite stages. On the other hand, Roddie et al. (1984) found higher survival rates at lower temperatures (8 °C), and the range of salinity tolerance was wider at 8 °C than at 18 °C. According to Kimmel and Bradley (2001), high salinities are most stressful for *E. affinis* at high temperatures. Also, median survival of *E. affinis* strongly decreased when high salinities are combined with high temperatures (Gonzalez and Bradley, 1994). Other studies have shown that the optimal temperature for egg production and survival of *E. affinis* is around 15 °C (Ban, 1994; Gasparini et al., 1999; Vijverberg, 1980). In a congeneric species, *Eurytemora velox*, Nagaraj (1988) found the same combined effects of salinity and temperature with higher survival rates in a higher range of salinities (up to 30–35 psu), when temperature decreased.

4.2. Physiological state and inter-molt development

In our study, individuals stopped their development in the stage N2 at 10 °C (except at 15 psu), but survived longer than at 15 °C. Salinity stress combined with low temperature, which slowed down metabolism, together with food starvation prevented individuals from molting into later stages. The same phenomenon is observed at 15 °C for salinities greater than 20 psu. Longer survival of individuals at 10 °C might be due to their low metabolism and because they did not use energy to molt. This energy cost is also shown by the rapid mortality of individuals that molted into stage N3 (Fig. 4), when compared to those remaining at the stage N2. Moreover, even for individuals that molted into N2, this stage is often shorter in the presence of food, as was shown by the control experiments and other studies (Ban, 1994) using *E. affinis* under the same salinities and temperatures (Table 3). This suggests that the energy lost into routine metabolism (i.e. respiration) delays the molting into N2. This crucial delay before molting, shown in Fig. 6, was not independent of temperature, but was independent of salinity. As shown for fish larvae, this energy loss can be related to weight losses (Letcher et al., 1996). Starvation studies using copepods have focused on the effects of food limitation on inter-molt development and survival of feeding stages (Crain and Miller, 2001; Lopez, 1996) and also on reproduction (Huggett, 2001).

In the Seine estuary, the growth of the population of *E. affinis* abundance starts to increase around 10 °C and reaches a maximum around 15 °C (Mouny, 1998). We specifically used these temperatures in our experiments. According to other studies, the development time of *E. affinis* nauplii increases significantly below 10 °C (Escaravage and Soetaert, 1993; Vijverberg, 1980). At very low temperatures, Escaravage and Soetaert (1993) have shown that *E. affinis* cannot achieve complete development when temperature decreases below 5 °C, as development is halted in naupliar stages at 2 °C. At high temperatures, stage duration decreased but mortality increased (Ban, 1994; Vijverberg, 1980). Development time can be less affected at high temperatures (20–25 °C) than at low temperatures, since reserve depletion related to oxygen consumption increases less between 15 and 25 °C than between 5 and 15 °C (Roddie et al., 1984). The asymmetric effect of temperature is a general rule for ectotherms (Atkinson, 1994) that has been shown

on another copepod species from the same family (*Temora longicornis*) for egg-production rates (Halsband-Lenk et al., 2002).

4.3. Sources of individual variability

The protocol used in this work has allowed for the observation of each nauplius during its development. Individual variability has been shown in each experimental condition and even within the nauplii of a single female. For *E. affinis*, Bradley (1986) suggested that tolerance to temperature and salinity stress is controlled by a group of regulatory genes. As the genotype controls the synthesis of proteins necessary for metabolic activity, a difference in genotype can modify the metabolic performance as a function of environmental conditions. Since this genotype is heritable (Bradley, 1986), salinity and temperature impose selection on this trait. This selection tends to reach the optimal tolerance for a given environment. Thus, optimal salinity varies among populations of *E. affinis* or seasonal cohorts (Halsband-Lenk et al., 2002; Lee, 1999). In our study, the significant differences found between the development of nauplii of two females in few combinations of temperature and salinity suggest that there is variation among clutches in optimal salinity (Lee and Petersen, 2002). This variability at the clutch level could be thought of as a 'reservoir of genetic diversity' to respond either to short- or long-term changes in salinity (i.e. in case of fresh- or seawater inflows, Lee, 1999). However, this adaptation is limited, as it needs more than one generation to be achieved (Lee and Petersen, 2002). There is a demonstrated temperature-salinity interaction effect on salinity and temperature tolerance, so that temperature could greatly affect optimal salinity (Lee and Petersen, 2002).

4.4. General metabolism and ecology

Our study has shown that nauplii of *E. affinis* of the Seine estuary (France) show a more efficient development at 15 °C than at 10 °C since development times are shorter and reach a higher developmental stage. In the Western Schelde estuary (Netherlands), development time of *E. affinis* nauplii with food is very low below 10 °C and is incomplete below 5 °C (Escaravage and Soetaert, 1993). The development seems to be stabilized between 15 and 20 °C (Escaravage and Soetaert, 1993), whereas Vijverberg (1980) showed that in the same estuary the naupliar development duration was two times faster at 20 °C than at 15 °C. In Lake Ohnuma (Japan), Ban (1994) observed higher mortality rates of *E. affinis* reared in the laboratory at 20 °C than at 15 °C. However, a general pattern based on a synthesis of all available results in the literature cannot be achieved, because some differences on phenotypic and genetic characters exist between the different populations of *E. affinis* found around the world (Lee, 1999; Lee and Frost, 2002). For European populations of *E. affinis*, 15 °C seems to be the optimal temperature for development and reproduction (Gasparini et al., 1999; Mouny, 1998).

The possibility for the nauplii to resist to high salinity (i.e. 30 psu and in a weaker extent 35 psu) could be related to a potential passive transport of first naupliar stages as estuarine passive particles (Le Hir et al., 2001). Adults of *E. affinis* are believed to alternate from swimming to sinking behavior in relation to flood or ebb currents to remain in a specific area of an estuary (Morgan et al., 1997). However, as nauplii have extremely

limited swimming capacities, they could be flushed into open seawater for a longer period of time. Their resistance to high salinities shows that nauplii could be adapted to this form of behavior in contrast to adults, which are more sensitive to high salinities (Roddie et al., 1984).

The patterns of metabolism reactions observed in the present work are fully congruent with in situ studies. Maximum abundances of *E. affinis* in the Seine and Wester Schelde estuaries occur respectively in April (Mouny and Dauvin, 2002) and May (Escaravage and Soetaert, 1995), when the temperature is optimal for development and reproduction (i.e. 15 °C). Salinity affects the abundance of *E. affinis* as a function of the tidal cycle, abundance maxima were observed in the 5–15 psu zone in the Seine estuary (Mouny, 1998). However, as tolerance to salinity is greater when temperature decreases, we emphasize that during the cold season copepods could be distributed more towards the oligohaline part of the estuary than during the warm season. Their abundance should nevertheless be lower, as temperature is not optimal for development and reproduction.

Finally, we emphasize that our experimental results based on the mortality of first naupliar stages without food could have resulted from external effects (i.e. salinity, temperature and pollution) on copepod metabolism and viability. These experiments allowed us to determine the optimal conditions for development and thus the potential effects of environmental variations on organism metabolism. The protocol introduced here might provide a valuable and promising method for studying the biological quality of aquatic environments and identifying sources of ecological stress on organisms.

Acknowledgements

Financial and infrastructure support was provided by the ‘Région Haute Normandie’ within the framework of the Seine-Aval II Programme, the Université des Sciences et Technologies de Lille and the Centre National de la Recherche Scientifique. We are grateful to Dr. C.E. Lee for her comments and improvements of the English. We also thank Dr. K. Ghertsos and J.R. Seymour who kindly improved the language. [**SS**]

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