

Evaluation of the Copepod *Eurytemora affinis* Life History Response to Temperature and Salinity Increases

Anissa Souissi¹, Sami Souissi^{1,*}, and Jiang-Shiou Hwang^{2,3}

¹Univ. Lille, CNRS, Univ. Littoral Cote d'Opale, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, F 62930 Wimereux, France. E-mail: anissa.ben-radhia@univ-lille1.fr

²Institute of Marine Biology, National Taiwan Ocean University, 202 Keelung, Taiwan

³Department of Biomedical Science and Environmental Biology, Kaohsiung Medical University, Kaohsiung 80708, Taiwan. E-mail: jshwang@mail.ntou.edu.tw

(Received 11 April 2015; Accepted 29 September 2015; Published 20 January 2016)

Anissa Souissi, Sami Souissi, and Jiang-Shiou Hwang (2016) Zooplankton and particularly copepods have a key role in the functioning of aquatic ecosystems. However, the mechanisms involved in the physiological responses of copepods to temperature and salinity increases are little understood, and the role of plasticity involved in facing environmental changes has rarely been demonstrated experimentally. In this study, the copepod *Eurytemora affinis*, widely distributed in the northern hemisphere, was selected as a biological model to test the effect of a 4°C temperature increase at two salinities. In addition to the optimal salinity (15 psu), a stressful condition of salinity 25 psu was also verified. Copepods from the Seine estuary were acclimated in laboratory to their optimal temperature of 15°C at salinity 15 PSU and then they were acclimated during several generations to their upper thermal limit (20°C) at two salinities (15 and 25 PSU), after which the temperature was raised by 4°C. This experiment revealed that after long-term acclimation and under unlimited food conditions, *E. affinis* maintained good fitness at 20°C and at both optimal and stressful salinities. After temperature increase to 24°C, the population remained viable but copepod size was significantly decreased as well as female's fecundity. The decrease of fitness was accentuated under the additional stressful condition of salinity 25 psu. This study demonstrated that the mechanisms of response to temperature and salinity increases (i.e. global warming) are complex, and should be investigated through experimental studies that consider acclimation and multigenerational factors. Our results will enrich the development of Individual-Based Models (IBMs) capable to test the role of microevolution and plasticity of *E. affinis* in the framework of future climate scenarios.

Key words: *Eurytemora affinis*, Temperature, Salinity, Fitness, Multi-generation.

BACKGROUND

Temperature is a key external factor affecting biological processes at the individual, population, and community levels (Pörtner and Knust 2007). Although many studies have examined the effect of temperature on invertebrate ectotherms, the key evolutionary processes involved in acclimation and adaptation to global warming are not well documented. Only species with high plasticity may be able to adapt to the rapid temperature increases predicted with global warming (Berg et al. 2010).

At the upper thermal limit of organisms, most climate-change scenarios predict negative effects such as decreases in individual fitness and population size, in some cases leading to local extinctions (Pörtner and Farrell 2008). The positive effects of global warming noted in some studies were associated with improved population recruitment or a species' evolutionary response to climate change (Reed et al. 2011). However, current climate-change scenarios and their associated predictive models (that is, 'climate envelope' models) often neglect the role

*Correspondence: Tel: +33321992908. Fax: +33321992901. E-mail: sami.souissi@univ-lille1.fr

of plasticity and adaptation of living organisms to the rate of environmental change (Bradshaw and Holzapfel 2006; Hof et al. 2011). Organisms living in highly variable habitats, such as most aquatic ecosystems, offer a variety of biological models to study the possible pathways of adaptation to global warming.

Although many recent papers treat physiological responses to global warming (Pörtner and Farrell 2008; Hoffmann 2010; Somero 2010), a single standardized protocol that can generalize these emerging relationships is still lacking. The often-used individual approach is effective in assessing potential variability, but may induce artifacts in phenotype expression by removing the effect of interactions among individuals (Brown and Shine 2009). The sensitivity of many organisms to experimental manipulation can stress individuals and bias estimates of life-history traits. Thus, a compromise is necessary between individually oriented experimental observations (Devreker et al. 2007; Devreker et al. 2009) and large-scale experiments such as in a mesocosm (Heuschele et al. 2014) where information on the history of individuals and/or their parents is often ignored. Evaluation of potential maternal effects on the response of aquatic ectotherms such as copepods (Ianora et al. 2004) to external factors requires observations through several generations. While studies of terrestrial ectotherms have evaluated several generations (Beldade et al. 2002; Klok et al. 2009), only a few studies already observed copepods over several generations (Lee et al. 2007; Lee et al. 2012).

We used a multigenerational protocol (Souissi et al. 2015) to test the effects of temperature and salinity increases on an estuarine copepod, focusing on the importance of plasticity in its acclimation to environmental stress. In global warming, estuaries which are sensitive to hydroclimate regimes (Kimmel et al. 2006), could experience increased intrusion of more saline ocean waters due to sea level rise and this will induce significant changes in the food web structure and function.

The estuarine copepod *Eurytemora affinis* was selected as a model to test effects of global warming on body size, reproduction, and survival. *E. affinis* is a widely distributed species in most temperate estuaries in the northern hemisphere, and has invaded freshwater ecosystems (Lee 1999; Winkler et al. 2008). *E. affinis* is eurythermal and could potentially show high plasticity in the face of future environmental changes, but its

sensitivity to temperature increase has not been studied.

We selected an already well-studied population of *E. affinis* from the Seine estuary, where it is present year-round in the salinity gradient zone (middle estuary), reaching maximum densities and dominating the zooplankton community in late spring and early summer (Mouny and Dauvin 2002; Devreker et al. 2010). Consequently this species can be considered as an appropriate ecological model to extrapolate experimental results to a real field situation. As in most northern-hemisphere estuaries, the *E. affinis* population in the Seine decreases during summer when temperature approaches 20°C (Mouny and Dauvin 2002; Devreker et al. 2010), suggesting that 20°C is its upper thermal limit. The previous studies on *E. affinis* from the Seine estuary used individual-based protocols and focused on the effects of salinity variation at spring like temperatures (10-15°C) on several life-history traits (Devreker et al. 2007; Devreker et al. 2009). But nevertheless these individual-observations contributed to the calibration of an individual-based model that confirmed the high sensitivity of *E. affinis* population to temperature increase in the Seine estuary (Dur et al. 2013). In addition to temperature increases, *E. affinis* may potentially be exposed to extreme fluctuations of salinity (Kimmel et al. 2006) through variable river flow discharge and increased sea level, as projected in all global-warming scenarios (IPCC 2007). We examined the simultaneous effects of temperature and salinity on the life-history traits of *E. affinis*. Copepods previously acclimated to the experimental conditions of summer temperature (20°C) were used to test the effect of a temperature increase (+4°C) at two salinities. This represents a realistic upper thermal limit shift of +4°C, as projected by IPCC climate models (IPCC 2007), and has been used to test genetic adaptation to temperature in *Daphnia* (Van Doorslaer et al. 2009).

MATERIALS AND METHODS

Copepod sampling

Eurytemora affinis was collected in late November 2006 in the oligohaline part of the Seine estuary (English Channel, France). Zooplankton samples were collected with standard WP2 nets of 200 µm mesh size, under the Tancarville bridge (49°26'N-00°16'W). Water temperature and salinity

were 12°C and 5 PSU, respectively. The copepods were pre-sorted and transported to the laboratory within a few hours. Then, large numbers of late developmental stages of *E. affinis* were carefully isolated for subsequent steps of the experiments.

Initiation of copepod cultures

A common copepod batch culture in a 25 L aquarium was started with large numbers of late developmental stages of *E. affinis* collected in the Seine estuary. The copepod culture was acclimated to laboratory conditions (12 h light / 12 h dark, salinity 15 PSU, and 15°C) during two generations, to remove the historical imprint of maternal and grand maternal effects. Then, the batch culture was transferred to 20°C and salinity 15 PSU and maintained during two generations. Copepods were fed every two days with an excess of the alga *Rhodomonas marina*.

Procedure and acclimation for the multigenerational experiment

In order to initiate the multigenerational protocol 2 L beakers were filled with autoclaved filtered seawater that was adjusted to the experimental salinity with deionized water. To start the first generation (F0) at 20°C, 40 ovigerous females were randomly sorted from the batch culture and immediately transferred to 2L beaker. Then, the females were incubated for a sufficient period to allow the nauplii to hatch, since embryonic development is temperature-controlled (Andersen and Nielsen 1997). Adult females were removed before they formed the second egg sac. The nauplii produced were followed in the beaker until adulthood. The cohort was fed daily with excess *R. marina*, in its exponential growth phase, at a mean concentration in copepod culture of ~5000 cells ml⁻¹, and the water was changed once the copepodite stages developed. During the experimental periods, copepod cultures were continuously fed with algae in the same physiological status (exponential growth phase). After ovigerous females appeared, all individuals produced were fixed in buffered formalin, except for 40 live ovigerous females which were used to start the next generation in a new beaker. The same experimental steps were repeated for each subsequent generation. The methods used to maintain the algal culture were described by Souissi et al. (2010).

After one generation at 20°C and salinity

15 PSU, 40 additional ovigerous females were used to start a new culture at the higher salinity of 25 PSU (Fig. 1). At this stage, two experiments were run for the acclimation phase, to test the multigenerational effects of the different conditions. One experiment at 20°C and 15 PSU (T20S15), and the second, started one generation later, was run at 20°C and 25 PSU (T20S25). These experiments were followed for an acclimation period of ten generations for T20S15 (Fig. 1) and nine generations for T20S25 (Fig. 1). We reared a large number of generations to better acclimate the population to its upper thermal limit conditions. To remove any potential effect of genetic heterogeneity in the starting batch, the high-salinity treatment at T20S25 was initiated from the first generation at T20S15 (this explains the lag in the number of generations between salinity treatments).

Temperature increase experiment

After the pre-experimental acclimation period, eight successive generations were reared under T20S15 and T20S25 (Fig. 1). Then, all cultures were simultaneously transferred to 24°C and followed during five more generations (Fig. 1). The feeding of copepods was the same as described above in the acclimation phase.

Measurements of morphological and life-history traits

From each generation in each set of conditions, 20 to 40 adult ovigerous females and

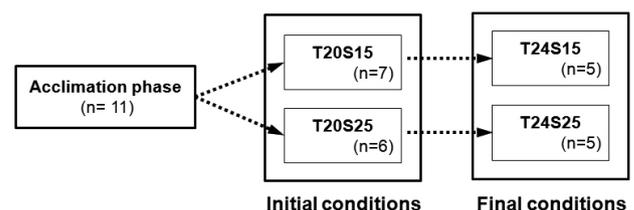


Fig. 1. Schematic illustration of the different steps of the experimental culture conditions. All experiments were initiated with the same culture initiated at 20°C and two salinities 15 and 25 PSU and acclimated during several generations. Then these copepod cultures were followed in two initial conditions (2 salinities) at 20°C and simultaneously transferred to the final conditions at 24°C. TXY: corresponds to the experimental temperature condition at X°C and salinity condition Y PSU. n: indicates the number of generations followed in each experimental treatment. The dashed arrows indicate the temporal progress of the experiment through the three phases mentioned by rectangles with heavy lines.

males were sorted from the fixed sample. The prosome length and width were measured under an inverted microscope (OLYMPUS IX71), using the image analysis software package Image J 1.41 (Rasband 1997-2008) (Souissi et al. 2010; 2015). Because of the high correlation observed between prosome length and width in all experiments, only prosome length data will be presented in the results section.

Fecundity was estimated by counting the eggs in each female's ovisac (clutch size). The protocol used the first clutch from the female copepod for all generations, to avoid any age effect, as may occur in the reproduction of this species (Devreker et al. 2009).

We calculated the survival of individuals

during each generation i (S_i) by the equation:

$$S_i = 100 \times \frac{Ntot_i}{\overline{CS}_{i-1} \times NFemOV_{i-1}}$$

Where $Ntot_i$ is the total number of individuals produced by generation i ; \overline{CS}_{i-1} is the mean clutch size during generation $i-1$, and $NFemOV_{i-1}$ is the number of ovigerous females from generation $i-1$ incubated to start generation i (fixed at 40 ovigerous females).

Statistical analyses

As prosome length of both females and males were distributed normally (see Table 1 and Fig. 2), a two-sample T-test was used to evaluate the

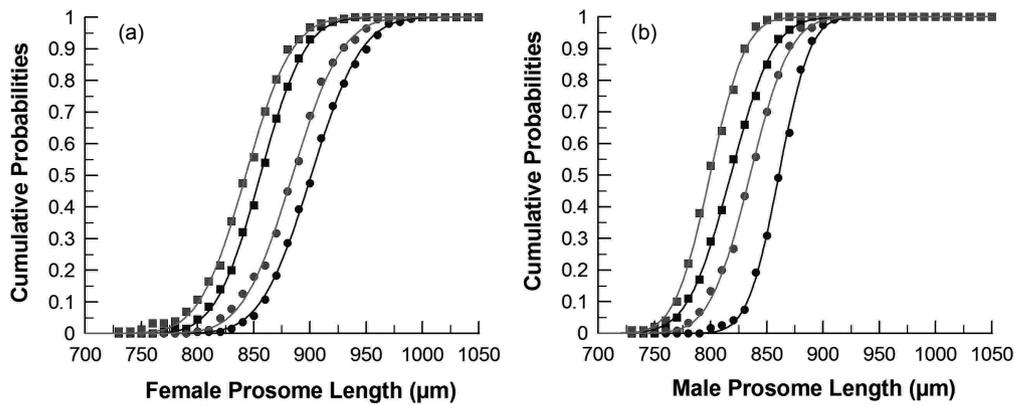


Fig. 2. Distribution of the cumulative probabilities of the female (a) and male (b) prosome lengths of *Eurytemora affinis* over the different experimental conditions. Circles indicate data from the initial experimental conditions at 20°C and squares indicate data after the transfer to 24°C in each experimental condition. Black lines and symbols designate data from the experiments carried out at the optimal salinity of 15 PSU. Gray lines and symbols designate data from the experiments carried out at the stressful salinity of 25 PSU. The observed data (symbols) were fitted by a normal cumulative probability density function (solid lines). The fitted parameters and their associated statistics are shown in Table 1.

Table 1. Values of the fitted parameters of the Normal cumulative density function of prosome lengths of *Eurytemora affinis* females and males and their associated statistics (see also Figure 2)

Condition	μ	σ	n	R^2	SSE	RMSE
Females						
T20S15	896.3 ± 0.95	35.43 ± 1.35	196	0.9996	0.0017	0.0091
T24S15	850.8 ± 0.40	31.55 ± 0.54	200	0.9999	0.00029	0.0038
T20S25	879.7 ± 1.60	34.25 ± 2.23	167	0.9989	0.0047	0.0153
T24S25	838.6 ± 1.00	32.01 ± 1.38	158	0.9995	0.0019	0.0098
Males						
T20S15	854.7 ± 0.45	21.52 ± 0.62	120	0.9999	0.00067	0.0054
T24S15	811.7 ± 0.45	28.21 ± 0.69	100	0.9999	0.00063	0.0052
T20S25	828 ± 0.75	29.27 ± 1.06	120	0.9997	0.0015	0.0079
T24S25	793.7 ± 0.80	24.89 ± 1.08	100	0.9996	0.0018	0.0088

μ : the mean of the Normal distribution (values are given with 95% of confidence intervals), σ : the standard deviation of the Normal distribution (values are given with 95% of confidence intervals), n: the number of individuals, R^2 : coefficient of determination, SSE = the sum of squares due to errors, RMSE = the root mean squared errors. A value closer to 0 indicates a better fit.

statistical significance of the differences between all experimental treatments.

To study the effects of experimental conditions on the measured life-history traits (clutch size and survival), we performed non-parametric statistical analyses. Because the main objective of the study was to investigate the effects of a global-warming scenario, the initial conditions (T20S15 and T20S25) and final conditions (T24S15 and T24S25) were first analyzed separately. The differences within each set of conditions (initial or final) were tested using the non-parametric test Kruskal-Wallis. Finally, two-sample comparisons between initial and final conditions were performed using the Wilcoxon rank sum test for equal medians. All statistical analyses considered generations as replicates for the same experimental conditions and were performed with Matlab Software (Mathworks Inc., Version, 7.5).

RESULTS

Size responses to experimental treatments

The cumulative probability distributions of prosome length (Fig. 2) in all experimental treatments were computed and fitted for females (Fig. 2a) and males (Fig. 2b). Distributions were symmetrical, with all values of R^2 for Gaussian distributions exceeding 0.99 (Table 1). For prosome length, the values of μ were similar to the observed means for each experiment (data not shown here), and showed a negative effect of temperature and/or salinity increase on prosome length (Table 1). The use of an unpaired two-sample T-test to compare all combinations of two treatments confirmed that the means of prosome lengths in all experimental conditions were significantly different in both males and females ($p < 0.001$).

Figure 3a shows the distribution of the mean prosome length of females obtained in each generation during all experimental treatments. The mean size of females decreased significantly when temperature was increased to 24°C (Wilcoxon rank sum test, $p < 0.001$). On the contrary no significant difference between salinities was observed.

Fecundity and survival responses to experimental treatments

After the transfer to 24°C, clutch size varied least in T24S15, and most in T24S25, which also showed the smallest median clutch size (Fig.

3b). The mean clutch sizes of the generations obtained during the initial and final conditions were all significantly different (Wilcoxon rank sum test, $p < 0.05$). These observations indicate that the transfer of copepods from all the initial conditions to 24°C significantly decreased the fecundity of *Eurytemora affinis*. The lowest value of mean clutch size was observed in the population at T24S25, i.e., under conditions of double stress (high salinity and high temperature).

Figure 3c shows the percentage of survival per generation during all experimental conditions. The narrowest range of variation in survival was

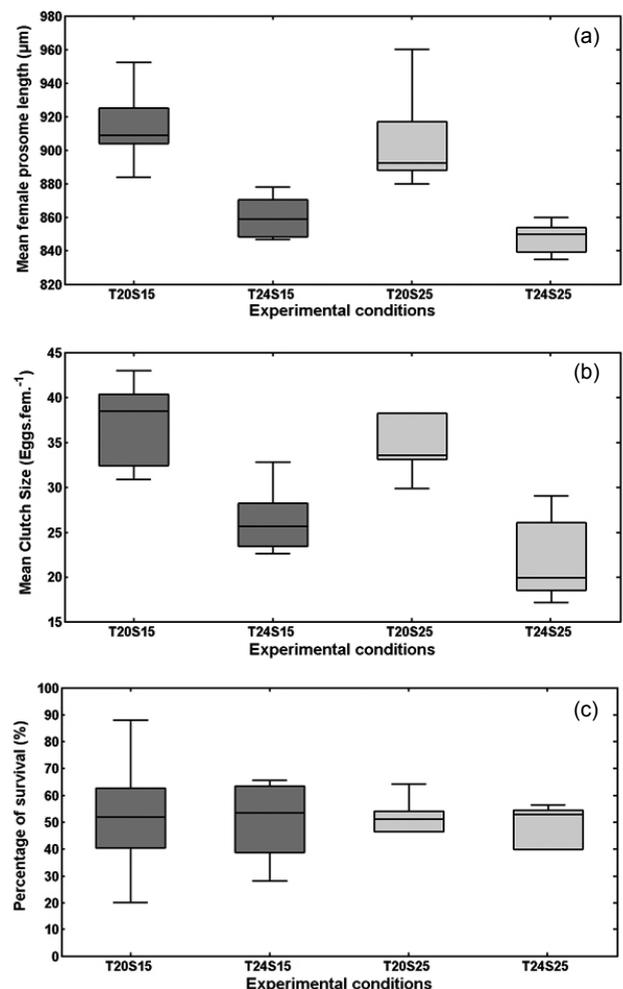


Fig. 3. Female prosome length, clutch size and survival of *E. affinis* under the different experimental conditions. Box-and-whiskers plots describing the distributions of the mean prosome length of copepod females (a), clutch size per generation of *E. affinis* (a) and the percentage of survival during one generation (c) in all experimental conditions (T20S15, T24S15, T20S25 and T24S25). Boxes extend from the lower to the upper quartile with an internal segment for the median; the whiskers extend to the most extreme data points.

obtained at T20S25. However, no significant differences between treatments were obtained.

Relationship between clutch size and body length

A linear relationship between clutch size and female prosome length was obtained ($y = 0.196x - 142.11$, $R^2 = 0.986$, $p < 0.02$) (Fig. 4). The clutch size varied more widely than the prosome length. In contrast, no significant relationship was found between the survival rate and the size of *E. affinis*.

DISCUSSION

Role of acclimation and effect of temperature increase

The application of temperature increase by 4°C to the population of *E. affinis* acclimated to its upper thermal limit revealed that individuals of this species remain viable at an elevated temperature of 24°C. In the current climate conditions, *E. affinis* may not encounter such warm temperatures in the Seine estuary (Devreker et al. 2010). However, in the laboratory, this summer temperature, which was predicted to occur by the end of the 21st

century (IPCC 2007), and may be earlier, was not lethal to *E. affinis* populations. Although 20°C seems to be its upper thermal limit in the Seine estuary, *E. affinis* populations were observed in higher summer temperatures ($\geq 24^\circ\text{C}$) in other habitats, such as the Chesapeake Bay in USA (Kimmel et al. 2006) and Lake Onhuma in Japan (Ban and Minoda 1989). Caution must be taken in making such comparisons, because *E. affinis* is a species complex composed of genetically divergent populations (Lee 1999; Winkler et al. 2011). While high genetic diversity exists among *E. affinis* populations, the species is fundamentally cold-temperate and shows a significant decrease in fitness at elevated temperatures (Devreker et al. 2010). The fitness and plasticity of this species have been studied only with regard to the effects of salinity variation (Lee et al. 2007). Thus, its response to temperature variation alone remains unclear. It is possible that populations situated in the southernmost parts of its range (i.e., Chesapeake Bay) may be better adapted to higher temperatures, but experimental data that support such a hypothesis have not been reported. In our study, the period of acclimation to the upper thermal limit was considered a crucial step in simulating a global-warming scenario, because temperature increase will occur progressively. Therefore, we maintained several generations in the laboratory before an experimental increase of temperature. Data gathered over 16 generations revealed that at 20°C, the upper thermal limit of *E. affinis* was a physiologically adequate temperature for its development (this study). These observations indicate that the decrease in *E. affinis* density observed in the Seine estuary could be caused by other factors, such as the presence of predators during summer (Winkler and Greve 2004), the production of diapausing eggs (Ban and Minoda 1994), or other external factors (pollution, competition, etc.).

Effect of salinity increase

The population of *E. affinis* from the Seine estuary shows a preference for low salinity (Devreker et al. 2010), which is most likely associated with its adaptation to local hydrodynamics. However, our test of a high-salinity treatment revealed that *E. affinis* was not strongly affected by this stress. The high capacity for osmoregulation in this species (Roddie et al. 1984) allowed it to maintain fitness at a salinity of 25 PSU at two relatively high temperatures (20°C

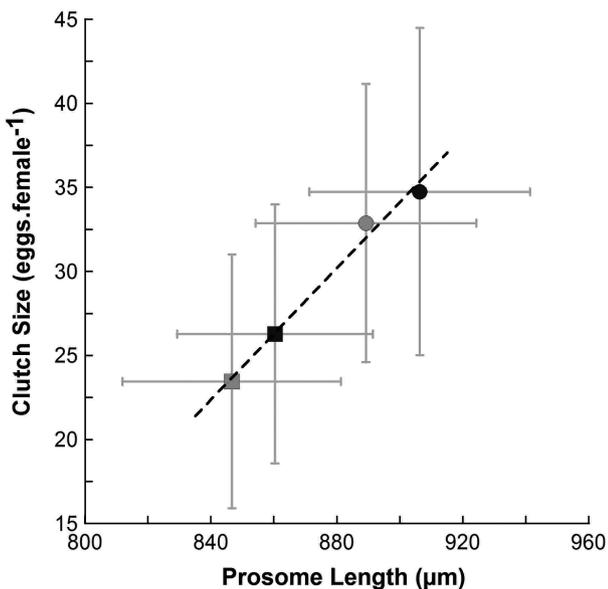


Fig. 4. Relationship between the mean female prosome length and clutch size of *Eurytemora affinis* under the different experimental conditions. The labels used to designate the data from the different conditions are the same as in Figure 2. Error bars show the standard deviation. The dashed line corresponds to the linear regression ($CS = 0.196PL - 142.11$, $R^2 = 0.986$).

and 24°C). Field populations of *E. affinis* show a preference for low-salinity zones, and some are capable of invading freshwater habitats (Lee 1999). In the Scheldt estuary, for example, the bulk of the *E. affinis* population shifted upstream to the freshwater zone in recent years (Mialet et al. 2010). We suggest that this pattern may also have been mediated by long-term hydroclimatic variability, particularly the decrease of river discharge observed in the Scheldt during the last decade (Souissi S, unpub. data). Previous studies in the Gironde and Elbe estuaries showed that the upstream-downstream position of *E. affinis* populations is directly related to river discharge levels (Castel 1995, Peitsch et al. 2000). These studies found that during summer months, *E. affinis* moves to the freshwater areas of the estuary, which allow the species to avoid marine water intrusion, and also to avoid competitors (for example, *Acartia* sp.) and predators that prefer high salinities. Hence, *E. affinis* seems to alter its upstream-downstream position in the estuary to improve its fitness. Since 2001, an observed trend of river discharge in the Seine estuary (Massei et al. 2010) may favor the upstream migration of *E. affinis*. According to (IPCC 2007) and the regional climate, river discharge may decrease 10-30 % during summer (Ducharne et al. 2007). Consequently, a reorganization of the freshwater planktonic ecosystem in the Seine estuary is to be expected, and *E. affinis* could come to dominate the mesozooplankton community, as observed recently in the Scheldt (Mialet et al. 2011).

Ecological implications

Temperature ranges in the Seine estuary are favorable for the growth and development of *E. affinis*. Ovigerous females are observed year-round (Ben Radhia-Souissi 2010). However, when temperature approaches 20°C during the summer months the density of *E. affinis* declines contrary to that of the broadcast-spawning members of the genus *Acartia* (David et al. 2007). Our field observations (Souissi, unpublished data) did not confirm an overlap between the habitats of *E. affinis* (preferring low salinities) and *Acartia* sp. (preferring higher salinities), which suggests that the seasonality of each species is related to differences in life-history strategies and in thermal optima. *Neomysis integer*, the main predator of *E. affinis* in the oligohaline zone, is highly abundant during the summer months (Mouny and Dauvin 2002; David et al. 2006). The risk of high

predation at this season could explain the temporal distribution of *E. affinis* in the field, with maximum abundance observed at temperatures below 20°C. Other factors, such as food availability and quality during summer, may negatively affect *E. affinis* (Ask et al. 2006). Generally, European populations of *E. affinis* flourish as far south as the Gironde estuary, with low abundances recorded in other Spanish estuaries (Albaina et al. 2009). This may indicate that *E. affinis* has thermal limitations that reduce its competitiveness compared to other copepod species, such as *Acartia tonsa* (David et al. 2007).

CONCLUSIONS

Our study confirmed that the upper thermal tolerance limit (see Somero 2010) of *Eurytemora affinis* in the Seine estuary is above its actual maximal field temperature (see Fig. 9A in Devreker et al. 2010). Despite the high capacity of *E. affinis* to survive conditions that mimic global warming, the populations of this species may be challenged to expand their geographical position upstream, as it was observed in the Scheldt estuary. In spite of the relatively high resilience of *E. affinis* at high temperatures, it is a weak competitor for species of the genus *Acartia*, which prefer warmer temperatures and may thus enlarge their distributional range as a result of global warming. Field studies of populations of these two copepod species (and genera) could provide important information regarding the present-day impact of climate change on synecological and competitive patterns of these two species. It is expected that abundance and distribution of *Eurytemora* and *Acartia*, and their relative dominance in northern-hemisphere estuaries, will be mediated by future climate change (see also Kimmel et al. 2006).

Our study confirmed that a long term acclimation of copepods in the laboratory allowed obtaining copepod line well adapted to their upper thermal limit at two different salinities. Then this copepod line was used to test a scenario of temperature increase of 4°C. In real conditions temperature will fluctuate at different scales and this will help the population to maintain its genetic heterogeneity (Bradley and Ketzner 1982). This means that the plasticity of copepods is a key issue and should take all attention in future studies dealing with climate change.

Modeling studies of potential evolutionary responses (such as improvement of physiological performance or fitness during global warming) of

aquatic organisms to climate changes should be emphasized (Reed et al. 2011). Individual-based models (e.g., Dur et al. 2013) offer appropriate tools for the integration of these experimental results, to test the role of microevolution and plasticity of *E. affinis* in the framework of future climate scenarios.

Acknowledgments: This paper is part of the doctoral thesis of A. Souissi. It is a contribution to the Seine-Aval ZOOSEINE and ZOOGLOBAL projects and to the bilateral project between the National Science Council (NSC) of Taiwan and the National Centre for Scientific Research (CNRS) of France. We are grateful to J. Dodson for his comments on an early version of the manuscript. We thank D. Ballenghien and H. Derdar for their assistance during the experiments and D. Devreker for his contribution to the study. We thank J. Reid for the English editing. We thank Chih-Ming Lin for his help in formatting the manuscript. Conceived and designed the experiments: SS AS JSH. Performed the experiments: AS. Analyzed the data: AS SS. Wrote the paper: AS SS JSH.

REFERENCES

- Albaina A, Villate F, Uriarte I. 2009. Zooplankton communities in two contrasting Basque estuaries (1999-2001): reporting changes associated with ecosystem health. *J Plankton Res* **31**:739-752.
- Ask J, Reinikainen M, Båmsted U. 2006. Variation in hatching success and egg production of *Eurytemora affinis* (Calanoida, Copepoda) from the Gulf of Bothnia, Baltic Sea, in relation to abundance and clonal differences of diatoms. *J Plankton Res* **28**:683-694.
- Andersen CM, Nielsen TG. 1997. Hatching rate of the egg-carrying estuarine copepod *Eurytemora affinis*. *Mar Ecol Prog Ser* **160**:283-289.
- Ban S, Minoda T. 1989. Seasonal distribution of *Eurytemora affinis* (Poppe, 1880) (Copepoda; Calanoida) in freshwater lake Ohnuma, Hokkaido. *BFFHU* **40**:147-153.
- Ban S, Minoda T. 1994. Induction of diapause egg production in *Eurytemora affinis* by their own metabolites. *Hydrobiologia* **292/293**:185-189.
- Beldade P, Koops K, Brakefield PM. 2002. Developmental constraints versus flexibility in morphological evolution. *Nature* **416**:844-847.
- Ben Radhia-Souissi A. 2010. Study of the reproductive plasticity and morphology of an estuarine copepod: intercontinental comparison. PhD dissertation. France: University Lille 1 Sciences and Technologies.
- Berg MP, Kiers ET, Driessen G, Van Der Heijden M, Kooi BW, Kuenen F, Liefing M, Verhoef H, Ellers J. 2010. Adapt or disperse: understanding species persistence in a changing world. *Glob Chang Biol* **16**:587-598.
- Bradley BP, Ketzner PA. 1982. Genetic and nongenetic variability in temperature tolerance of the copepod *Eurytemora affinis* in five temperature regimes. *Biol Bull* **162**:233-245.
- Bradshaw WE, Holzapfel CM. 2006. Evolutionary responses to rapid climate change. *Science* **312**:1477-1478.
- Brown GP, Shine R. 2009. Beyond size-number trade-offs: clutch size as a maternal effect. *Philos Trans R Soc Lond B Biol Sci* **364**:1097-1106.
- Castel J. 1995. Long-term changes in the population of *Eurytemora affinis* (Copepoda, Calanoida) in the Gironde estuary (1978-1992). *Hydrobiologia* **311**:85-101.
- David V, Chardy P, Sautour B. 2006. Fitting a predator-prey model to zooplankton time-series data in the Gironde estuary (France): Ecological significance of the parameters. *Estuar Coast Shelf Sci* **67**:605-617.
- David V, Sautour B, Chardy P. 2007. Successful colonization of the calanoid copepod *Acartia tonsa* in the oligomesohaline area of the Gironde estuary (SW France) - Natural or anthropogenic forcing? *Estuar Coast Shelf Sci* **71**:429-442.
- Devreker D, Souissi S, Forget-Leray J, Leboulenger F. 2007. Effects of salinity and temperature on the post embryonic development of *Eurytemora affinis* (Copepoda; Calanoida) of the Seine estuary: a laboratory study. *J Plankton Res* **29**:i117-i133.
- Devreker D, Souissi S, Molinero JC, Beyrend-Dur D, Gomez F, Forget-Leray J. 2010. Tidal and annual variability of the population structure of *Eurytemora affinis* in the middle part of the Seine Estuary during 2005. *Estuar Coast Shelf Sci* **89**:245-255.
- Devreker D, Souissi S, Winkler G, Forget-Leray J, Leboulenger F. 2009. Effects of salinity and temperature on the reproduction of *Eurytemora affinis* (Copepoda; Calanoida) from the Seine estuary: a laboratory study. *J Exp Mar Biol Ecol* **368**:113-123.
- Ducharne A, Baubion C, Beaudoin N, Benoit M, Billen G, Brisson N, Garnier J, Kieken H, Lebonvallet S, Ledoux E, Mary B, Mignolet C, Poux X, Sauboua E, Schott C, Théry S, Viennot P. 2007. Long term prospective of the Seine river system: Confronting climatic and direct anthropogenic changes. *Sci Total Environ* **375**:292-311.
- Dur G, Jiménez-Melero R, Beyrend-Dur D, Hwang JS, Souissi S. 2013. Individual-based model of the phenology of egg-bearing copepods: Application to *Eurytemora affinis* from the Seine estuary, France. *Ecol Model* **269**:21-36.
- Heuschele J, Ceballos S, Andersen-Borg, CM, Bjærke O, Isari S, Lasley-Rasher R, Lindehoff E, Souissi A, Souissi S, Titelman J. 2014. Non-consumptive effects of predator presence on copepod reproduction: insights from a mesocosm experiment. *Mar Biol* **161**:1653-1666.
- Hoffmann AA. 2010. Physiological climatic limits in *Drosophila*: patterns and implications. *J Exp Biol* **213**:870-880.
- Hof C, Levinsky I, Araújo MB, Rahbek C. 2011. Rethinking species' ability to cope with rapid climate change. *Glob Chang Biol* **17**:2987-2990.
- IPCC. 2007. Climate change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge UK: Cambridge University Press.
- Ianora A, Miralto A, Poulet SA, Carotenuto Y, Buttino I, Romano G, Casotti R, Pohnert G, Wichard T, Colucci-D'Amato Terrazzano G, Smetacek V. 2004. Aldehyde suppression of copepod recruitment in blooms of a ubiquitous planktonic diatom. *Nature* **429**:403-407.
- Kimmel DG, Miller WD, Roman MR. 2006. Regional scale

- climate forcing on zooplankton dynamics in Chesapeake Bay. *Estuar Coast* **29**:375-387.
- Klok CJ, Hubb AJ, Harrison JF. 2009. Single and multigenerational responses of body mass to atmospheric oxygen concentrations in *Drosophila melanogaster*: evidence for roles of plasticity and evolution. *J Evol Biol* **22**:2496-2504.
- Lee CE. 1999. Rapid and repeated invasions of fresh water by the copepod *Eurytemora affinis*. *Evolution* **53**:1423-1434.
- Lee CE, Remfert JL, Chang YM. 2007. Response to selection and evolvability of invasive populations. *Genetica* **129**:179-192.
- Lee KW, Dahms HW, Lee JS. 2012. Multigenerational life history traits of the copepod *Tigriopus* from the Northwest Pacific rim in crossbreeding experiments. *J Exp Mar Biol Ecol*. pp. 436-437, 56-62.
- Massei N, Laignel B, Deloffre J, Mesquita J, Motelay A, Lafite R, Durand A. 2010. Long-term hydrological changes of the Seine River flow (France) and their relation to the North Atlantic Oscillation over the period 1950-2008. *Int J Climatol* **30**:2146-2154.
- Mialet B, Azémar F, Maris T, Sossou C, Ruiz P, Lionard M, Van Damme S, Lecerf A, Muylaert K, Toumi N, Meire P, Tackx M. 2010. Spatial spring distribution of the copepod *Eurytemora affinis* (Copepoda, Calanoida) in a restoring estuary, the Scheldt (Belgium). *Estuar Coast Shelf Sci* **88**:116-124.
- Mialet B, Gouzou J, Azémar F, Maris T, Sossou C, Toumi N, Van Damme S, Meire P, Tackx M. 2011. Response of zooplankton to improving water quality in the Scheldt estuary (Belgium). *Estuar Coast Shelf Sci* **93**:47-57.
- Mouny P, Dauvin JC. 2002. Environmental control of mesozooplankton community in the Seine estuary (English Channel). *Oceanol Acta* **25**:13-22.
- Peitsch A, Kopcke B, Bernat N. 2000. Long-term Investigation of the Distribution of *Eurytemora affinis* (Calanoida; Copepoda) in the Elbe Estuary. *Limnologica* **30**:175-182.
- Pörtner HO, Knust R. 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* **315**:95-97.
- Pörtner HO, Farrell AP. 2008. Ecology, Physiology and Climate Change. *Science* **322**:690-692.
- Reed TE, Schindler DE, Hague MJ, Patterson DA, Meir E, Waples RS, Hinch SG. 2011. Time to Evolve? Potential Evolutionary Responses of Fraser River Sockeye Salmon to Climate Change and Effects on Persistence. *PLoS One* **6(6)** e20380. doi:10.1371/journal.pone.0020380.
- Roddie BD, Leakey RJG, Berry AJ. 1984. Salinity-temperature tolerance and osmoregulation in *Eurytemora affinis* (Poppe) (Copepoda: Calanoida) in relation to its distribution in the zooplankton of the upper reaches of the forth estuary. *J Exp Mar Biol Ecol* **79**:191-211.
- Somero GN. 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *J Exp Biol* **213**:912-920.
- Souissi A, Souissi S, Devreker D, Hwang JS. 2010. Occurrence of intersexuality in a laboratory culture of the copepod *Eurytemora affinis* from the Seine estuary (France). *Mar Biol* **157**:851-861
- Souissi A, Souissi S, Hansen BW. 2015. Physiological improvement in the copepod *Eurytemora affinis* through thermal and multigenerational selection. *Aquaculture Research*, in press, doi:10.1111/are.12675.
- Van Doorslaer W, Stoks R, Duvivier C, Bednarska A, De Meester L. 2009. Population dynamics determine genetic adaptation to temperature in *Daphnia*. *Evolution* **63**:1867-1878.
- Winkler G, Greve W. 2004. Trophodynamics of two interacting species of estuarine mysids, *Praunus flexuosus* and *Neomysis integer*, and their predation on the calanoid copepod *Eurytemora affinis*. *J Exp Mar Biol Ecol* **308**:127-146.
- Winkler G, Dodson JJ, Lee CE. 2008. Heterogeneity within the native range: population genetic analyses of sympatric invasive and noninvasive clades of the freshwater invading copepod *Eurytemora affinis*. *Mol Ecol* **17**:415-430.
- Winkler G, Souissi S, Poux C, Castric V. 2011. Genetic heterogeneity among *Eurytemora affinis* populations in Western Europe. *Mar Biol* **158**:1841-1856.