Effect of salinity on the swimming behaviour of the estuarine calanoid copepod *Eurytemora affinis*

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**INTRODUCTION**

The calanoid copepod, *Eurytemora affinis* (Poppe, 1880) is the dominant zooplankton species in most of European and North American estuaries, and their population maintenance within an environment of net seaward flow has mainly been explained by endogenous rhythms of circatidal swimming activity. However, no attention has been paid to the potential link between the swimming behaviour of this species and salinity. The swimming behaviour of males, non-ovigerous females and ovigerous females from a continuous culture was investigated under different salinity conditions. Increase and decrease in salinity, respectively, increased and decreased the overall swimming activity of both males and non-ovigerous females. The complexity of the swimming paths of males and non-ovigerous females significantly increased with salinity. In contrast, ovigerous females were less motile and mainly sank. These observations suggest an endogenous behavioural adaptive strategy to salinity fluctuations and the intrinsic ability of *E. affinis* to undergo short-scale vertical migration triggered by changes in salinity. This supports field observations reporting increased abundance of *E. affinis* in the water column during flood tides and provides a behavioural basis for the maintenance of viable populations under net outflow conditions.
the link between the swimming behaviour of this species and salinity. In particular, it is still unclear whether the observed vertical migrations are triggered by endogenous rhythms of circatidal swimming activity (Hough and Naylor, 1992) or by tidally induced changes in salinity. This issue prompted the present investigation; it was designed to test for the existence and extent of the effect of salinity on E. affinis’ swimming behaviour.

METHODS

Eurytemora affinis were collected from the Seine estuary using a WP2 net (200-μm mesh size) at a temperature of 15.5°C in the low-salinity zone (S = 2.5–5) at low tide near the ‘Pont de Normandie’ (49°28’26” N, 0°27’47” W) where previous studies have shown a maximum abundance in this estuary (Mouny and Dauvin, 2002). A continuous culture was established under the optimum conditions of temperature (15°C) and salinity (15) for naupliar survival and development (Devreker et al., 2004) and reared on a mixed diet of the phytoplankton Isochrisis galbana and Nannochloropsis oculata in exponential growth phase with a 3/4:1/4 number ratio at a concentration of 10^7 cell L⁻¹.

The swimming properties of males, non-ovigerous females and ovigerous females were investigated for salinities ranging from 0 to 35. Increasing and decreasing salinities were used to mimic the effect of alternation between flood and ebb tides on E. affinis. To avoid the potential influence of mating interactions on swimming behaviour (Katona, 1973), observations were conducted on groups of males, non-ovigerous females and ovigerous females only. For each salinity treatment, 50 new individuals were moved from the continuous culture, brought together in a 2-L (i.e. 20 × 20 × 5 cm³) Plexiglas behavioural container and left in the experimental filming set-up to acclimatize for 10 min. The salinity treatments were randomized, and the resulting sequence of salinity treatments was 35, 2.5, 10, 5, 25, 0 and 15. Activity was then filmed during ~30 min. During the experiments, the animals were fed on the rearing phytoplankton mixture. All the experiments were conducted at 15°C in the dark and at night to avoid any potential behavioural artefact related to the diel light cycle of the reared organisms. Seven salinity treatments were conducted on consecutive nights, consistently between 10 p.m. and 1 a.m., and for each salinity treatment, sex group observations were arbitrarily ordered as ovigerous females, males and non-ovigerous females.

The two-dimensional (2D) trajectories of E. affinis were recorded at a rate of 25 frame s⁻¹ using one infrared digital camera (DV Sony DCR-PC120E) facing the experimental container (Fig. 1). Two arrays of 72 infrared light-emitting diodes (LEDs), each mounted on a printed circuit board about the size of a business card (i.e. 9.3-cm long and 4.9-cm wide) connected to a 12-V DC power supply, provided the only light source. Swimming paths were subsequently analysed using a home-made Java script. The program allows setting thresholds for contrast and background noise, ensuring that only the copepods are tracked. The x and y coordinates of the swim paths were automatically extracted and subsequently combined into a 2D picture. The time step was always 0.04 s (i.e. 1/25 s), and the output sequences of (x, y) coordinates were subsequently used to characterize the motility. Crossing paths and subsequent trajectory artefacts were identified and not included in further analysis. From the 21 films available (seven salinity treatments and three sex groups), we only considered the swimming paths in which the animals were at least two body lengths away from any of the walls of the experimental chamber or the surface. To work on statistically consistent swimming paths, paths of similar durations (i.e. 30–35 s) were selected according to the above-mentioned criterium, and the same number (N = 35) of swimming paths was considered for the behavioural analysis.

Three different kinds of behaviours were considered for males, non-ovigerous females and ovigerous females: active swimming, passive sinking (downward vertical motion, with tail down) and hovering (i.e. no motion). Each behaviour...
was expressed in terms of time allocation percentage for each category of organisms. The swimming and sinking speeds were estimated as the distance \( d_t \) between two successive copepod positions divided by the time interval between those positions (i.e. 40 ms). The distance \( d_t \) (mm) was computed as:

\[
\text{distance } d_t = \left[ (x_{t} - x_{t+1})^2 + (y_{t} - y_{t+1})^2 \right]^{1/2},
\]

where \((x_{t}, y_{t})\) and \((x_{t+1}, y_{t+1})\) are the positions of a copepod at time \( t \) and \( t + 1 \), respectively. Average swimming and sinking speeds and their SDs were measured over the duration of individual tracks \( N = 33 \) for males, non-ovigerous females and ovigerous females.

Swimming tracks were further quantified by their fractal properties (Seuront et al., 2004a, 2004b, 2004c; Uttieri et al., 2005). As extensively discussed by Seuront et al. (Seuront et al., 2004c), fractals provide a reliable quantification of the degree of complexity of a swimming track and overcome the scale-dependent issues related to more traditional metrics. The fractal dimension \( D \) of a swimming path is bounded between \( D = 1 \) and \( D = 2 \). When a copepod is moving along a completely linear path, \( D = 1 \). In the opposite extreme instance of curviness, when the motions are so complex that the path fills the whole available space (i.e. for the case of Brownian motion), \( D = 2 \). \( D \) thus provides a measure of path complexity bounded between the linear and Brownian movements. The fractal dimensions of \( E. \) affinis swimming paths were estimated by superimposing a regular grid of boxes of length \( l \) on the paths and counting the number of ‘occupied’ boxes (Seuront et al., 2004b, 2004c). The surface occupied by a path is then estimated with a series of counting boxes spanning a range of subsurfaces down to some small fraction of the entire surface. The number of occupied boxes increases with decreasing box size, leading to the following power-law relationship \( N(l) \propto l^{-D} \), where \( l \) is the length of the box and \( N(l) \) is the number of boxes occupied by the path. \( D \) is estimated from the slope of the linear regression of the log–log plot of \( N(l) \) versus \( l \). Because slight reorientation of the overlying grid can produce different values of \( N(l) \), the fractal dimension \( D \) was estimated for the rotation of the initial 2D grid of 5° increments from 0 to 45° (Seuront et al., 2004c).

Preliminary video recordings of the swimming behaviour of \( E. \) affinis were conducted in the experimental container under the conditions of temperature and salinity of the continuous culture to infer the presence of any endogenous rhythms of circatidal activity previously observed in this species (Hough and Naylor, 1992). Every hour during a period of 24 h, 50 individuals of each sex group were recorded swimming freely in the experimental container during 15 min. The number of individuals located in the upper and bottom layers of the container was averaged for every 1-min segments and the swimming behaviour quantified as described earlier.

This experiment was repeated three times over a period of 1 month.

### RESULTS

Preliminary observations did not show any alternation between aggregation of individuals near the bottom and even distribution throughout the whole water column. Instead, the percentage of individuals located in the upper and bottom layers of the container was stable for each sex group over the duration of the experiment (Kendall’s \( \tau \)-test, \( P > 0.05 \)), not significantly different between the three sex groups and the three sets of experiments (\( \chi^2 \)-test, \( P > 0.05 \)). Similarly, no significant differences were identified in the time allocated to swimming, hovering and sinking behaviours (\( \chi^2 \)-test, \( P > 0.05 \)) nor in the swimming and sinking speeds (Kruskal–Wallis H-test, \( P > 0.05 \)) of each sex group. No significant differences were identified in the fractal dimensions estimated for each sex group (\( P > 0.05 \)). As a consequence, the results cannot be biased by the endogeneous rhythms of circatidal activity previously observed in \( E. \) affinis (Hough and Naylor, 1992).

The swimming activity of \( E. \) affinis males, non-ovigerous females and ovigerous females is shown in Fig. 2 as a function of salinity. Male swimming and sinking activities, respectively, significantly increased (Kendall’s \( \tau \)-test, \( P < 0.01 \)) from 3.2 to 86.7% and decreased (\( P < 0.01 \)) from 88.5 to 5.1% when salinity increased from 0 to 35 (Fig. 2A). In contrast, no significant differences were detected between the hovering behaviour that remained bounded between 7.9 and 9.2% (8.3 ± 0.4, \( \bar{x} \pm \text{SD} \)). Non-ovigerous female swimming and sinking activities were similar to those observed for the males (Fig. 2B) and respectively significantly increased from 5.3 to 70.5% (\( P < 0.01 \)) and decreased from 88.5 to 2.2% (\( P < 0.01 \)) when salinity increased from 0 to 35. However, their hovering activity significantly increased from 6.2 to 27.3% for salinity increasing from 0 to 35. Ovigerous female swimming and sinking activity significantly decreased from 92.0% at \( S = 0 \) to 40.7% at \( S = 35 \) (Fig. 2C) but at a significantly lower rate than that for males and non-ovigerous females (\( F \)-test, \( P < 0.01 \); Zar, 1996). Their swimming activity significantly increased with salinity (\( P < 0.01 \)) but at a much lower rate than that for males and non-ovigerous females (\( F \)-test, \( P < 0.01 \)). Their hovering activity significantly increased (\( P < 0.01 \)), with salinity from 3.4 to 34%, at a significantly higher rate than that for non-ovigerous females (\( t \)-test, \( P < 0.01 \), Zar, 1996).
The swimming speed of *E. affinis* was also influenced by changes in salinity (Fig. 3, Table I). *Eurytemora affinis* male and non-ovigerous female swimming speeds exponentially increased from 1.2 mm s\(^{-1}\) at \(S = 0\) to 3.3 mm s\(^{-1}\) at \(S = 35\) (\(\nu_{\text{swim}} = 1.04e^{0.03S}, r^2 = 0.99, P < 0.01\)) and were not significantly different over the range of salinities considered (Wilcoxon–Mann–Whitney U-test, \(P > 0.05\); Fig. 3A). Male and non-ovigerous sinking speeds were significantly lower than their swimming speeds (Wilcoxon–Mann–Whitney U-test, \(P < 0.01\); Fig. 3B) and did not exhibit any significant difference between salinity treatments (Kruskal–Wallis H-test, \(P > 0.05\); Fig. 3B). Ovigerous female swimming speed increased linearly with increase in salinity from 0.31 to 0.76 mm s\(^{-1}\) (\(\nu_{\text{swim}} = 0.33 + 0.01S, r^2 = 0.99, P < 0.01\); Fig. 3A), whereas their sinking velocity (\(\nu_{\text{swim}} = 0.79 \pm 0.01 \text{ mm s}^{-1}\)) did not exhibit any significant difference between salinity treatments (H-test, \(P > 0.05\); Fig. 3B).
Males and non-ovigerous females exhibited very comparable curvilinear swimming paths developed similarly in the vertical and horizontal planes (Fig. 4A–D). In high-salinity treatments, *E. affinis* swimming paths were characterized by highly convoluted paths mostly (e.g. 60% and 74% at \(S = 25\) and \(S = 35\); \(\chi^2\) test, \(P < 0.05\)) directed upwards (Fig. 4B and D), whereas in low-salinity treatments, swimming paths were less complex and mainly (65, 68, 71, 83 and 86% at \(S = 15, 10, 5, 2.5\) and 0; \(\chi^2\) test, \(P < 0.05\)) oriented downwards (Fig. 4A and C). In contrast, ovigerous females always exhibited rectilinear trajectories mainly restricted to the vertical plane and characterized by the alternation of sinking and swimming bouts.

### Table I: *Eurytemora affinis*

<table>
<thead>
<tr>
<th>Salinity</th>
<th>Males</th>
<th></th>
<th>Non-ovigerous females</th>
<th></th>
<th>Ovigerous females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\nu) (mm s(^{-1}))</td>
<td>(L) (mm)</td>
<td>(Re)</td>
<td>(\nu) (mm s(^{-1}))</td>
<td>(L) (mm)</td>
</tr>
<tr>
<td>0</td>
<td>1.10 (0.08)</td>
<td>0.82 (0.01)</td>
<td>0.90</td>
<td>1.11 (0.10)</td>
<td>0.86 (0.02)</td>
</tr>
<tr>
<td>2.5</td>
<td>1.12 (0.09)</td>
<td>0.82 (0.01)</td>
<td>0.92</td>
<td>1.15 (0.07)</td>
<td>0.85 (0.01)</td>
</tr>
<tr>
<td>5</td>
<td>1.21 (0.08)</td>
<td>0.83 (0.02)</td>
<td>1.01</td>
<td>1.18 (0.07)</td>
<td>0.85 (0.01)</td>
</tr>
<tr>
<td>10</td>
<td>1.38 (0.10)</td>
<td>0.81 (0.02)</td>
<td>1.12</td>
<td>1.40 (0.10)</td>
<td>0.84 (0.02)</td>
</tr>
<tr>
<td>15</td>
<td>1.69 (0.11)</td>
<td>0.83 (0.01)</td>
<td>1.40</td>
<td>1.63 (0.12)</td>
<td>0.86 (0.02)</td>
</tr>
<tr>
<td>25</td>
<td>2.46 (0.18)</td>
<td>0.83 (0.02)</td>
<td>2.04</td>
<td>2.37 (0.16)</td>
<td>0.87 (0.01)</td>
</tr>
<tr>
<td>35</td>
<td>3.30 (0.15)</td>
<td>0.82 (0.02)</td>
<td>2.71</td>
<td>3.27 (0.17)</td>
<td>0.86 (0.01)</td>
</tr>
</tbody>
</table>

\(\nu\), mean swimming speed; \(L\), prosome length; \(Re\), related Reynolds number. The Reynolds number is defined as \(Re = \nu L/\nu\), where \(\nu\) is the kinematic viscosity (\(\nu = 10^{-5}\) m\(^2\) s\(^{-1}\)). SDs are given in parenthesis.

\[ \begin{align*}
\chi^2 \text{ test, } P < 0.05
\end{align*} \]

\[ \begin{align*}
L. SEURONT & \text{ SALINITY AND EURLTEMORA AFFINIS BEHAVIOUR}
\end{align*} \]

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Fig. 4. Illustrations of trajectories recorded for *Eurytemora affinis* males (A and B), non-ovigerous females (C and D) and ovigerous females (E and F) under conditions of low (A, C and E; \(S = 5\)) and high (B, D and F; \(S = 35\)) salinities. The grey and black arrows indicate the beginning and the end of the swimming paths, respectively.
observed percentages of trajectories directed upwards and downwards are consistent with the significant ($P < 0.01$) negative correlation found between salinity and the number of individuals observed in the bottom half of the experimental chamber. In addition, once males and non-ovigerous females got close to the bottom or the surface of the experimental chamber, they consistently decreased their motility and remained in proximity with the interface.

These observations are specified by the significant positive correlation observed between salinity and the fractal dimensions estimated from the swimming paths of males ($D_{\text{male}} = 1.12 + 0.04S$, $r^2 = 0.98$, $P < 0.01$) and non-ovigerous females ($D_{\text{female-no}} = 1.11 + 0.03S$, $r^2 = 0.98$, $P < 0.01$; Fig. 5). This quantitatively shows that the complexity of the swimming paths significantly increased with salinity. In contrast, no significant correlation was found between salinity and the fractal dimensions of the ovigerous female swimming paths (Fig. 5). The fractal dimensions of ovigerous females were always significantly smaller than those of the males and non-ovigerous females ($P < 0.01$). Except for the lowest two salinity levels ($S = 0$ and $S = 2.5$), male fractal dimensions were significantly higher than those of non-ovigerous females ($P < 0.01$, Fig. 5).

**DISCUSSION**

Vertical migration behaviour of zooplankton in estuarine environments and the role of behaviour in maintaining populations within the MTZ are still poorly understood. Zooplankton may use active and passive mechanisms to enhance retention in particular estuarine regions. For instance, the shift of the *E. affinis* population from brackish water towards lower salinities in the Schelde estuary during the 1990s has been associated with an increase in the oxygen concentration in the freshwater zone (Appeltans et al., 2003). In the estuaries of Gironde (Castel and Veiga, 1990), Columbia (Haertel and Osterberg, 1967) and Chesapeake Bay (Roman et al., 2001), the abundance of *E. affinis* as well as other copepod species (Morgan et al., 1997; Kimmerer et al., 1998) is highly correlated with temporal and spatial patterns of turbidity, suggesting that the same physical processes resuspend, advect, trap and concentrate sediments and zooplankton. In the Conway estuary (Hough and Naylor, 1991) and Columbia estuary (Morgan et al., 1997), *E. affinis* is also thought to migrate vertically in response to the tidal cycle to decrease advection loss from the MTZ region. The present observations are congruent with the hypothesized role of behavioural strategies in the maintenance of viable populations of *E. affinis* under net outflow conditions (Morgan et al., 1997).

Under conditions of increasing salinity, males, non-ovigerous females and ovigerous females increased their overall swimming activity (see Figs 2 and 3). Males and non-ovigerous females were mainly observed swimming upwards (Fig. 4B and D). This is consistent with the observed increase in the dispersal of *E. affinis* into the upper water column during flood tides (Morgan et al., 1997; Roman et al., 2001; Fig. 6) and suggests an endogenous behavioural adaptive strategy independent of the previously described circatidal activity (Hough and Naylor, 1992). Males and non-ovigerous females increased their swimming path complexity under high-salinity conditions (Figs 4A–D and 5), and although ovigerous females were mainly sinking (Fig. 2C), they increased the extent of their horizontal movements when salinity increased (Fig. 4E and F). *Eurytemora affinis* graze selectively on living and non-living particles (Chervin et al., 1981; Sellner and Bundy, 1987, Irigoien et al., 1996; Gasparini and Castel, 1997) and increase their feeding activity in the water column because their feeding rates are much lower in the MTZ (Tackx et al., 1995, 2003). As an increase in swimming path complexity has previously been related to an increase in foraging activity related to feeding (Tiselius, 1992), it is tempting, although highly speculative, to relate the observed increase in swimming path complexity (Fig. 5) to another potential endogenous behavioural adaptive strategy, namely one related to the increase in feeding activity in the water column.

Under low-salinity conditions, *E. affinis* spent more time sinking (Fig. 1), decreased their swimming speed (Fig. 3A) and, when active, males and non-ovigerous females were mainly swimming downwards (Fig. 4A).
and C) in convoluted ways (Fig. 5). In the field, these behavioural traits would allow *E. affinis* to avoid massive losses related to seaward advection of individuals in the water column during the ebb tide (i.e. under conditions of decreasing and/or low salinity; Fig. 6). In tidally forced estuaries, the major advective losses occur during the ebb tide. This implies that the greater the proportion of the population in the water column, the greater the advective losses (Fig. 6). The present observations are then congruent with the hypothesis that settling and/or swimming to a position lower in the water column or into the bottom detritus and sediments would minimize losses during ebb tides (Fig. 6). Males and non-ovigerous females mainly swimming downwards at 1.1–1.6 mm s\(^{-1}\) under low-salinity conditions (Fig. 3, Table I) may then relocate themselves 2–5.8 m deeper in the water column in <0.5–1 h and therefore limit seaward losses. The behavioural traits of *E. affinis* ovigerous females should also favour the retention of the population in the MTZ. The net movements of ovigerous females were always directed downwards, and they were mainly observed sinking (Fig. 3C). Even if the observed sinking velocities (\(v_{\text{sink}} = 0.79 \pm 0.01 \text{ mm s}^{-1}\)) are much lower than the previous estimates (i.e. 0.340 ± 0.008 cm s\(^{-1}\); Castel and Veiga, 1990), an individual ovigerous female may sink from 1.5 to 3 m during a slack tide period (ca. 0.5–1 h) to avoid peak ebb velocities on the surface. This is also consistent with previous studies, showing that peak abundances of *E. affinis* ovigerous females and nauplii are usually associated with the bottom waters of the MTZ (Herman et al., 1968; Heinle and Flemer, 1975; Morgan et al., 1997). However, this may be the combination of the previous behavioural traits and carrying eggs in a sac where they develop until hatching that maintain *E. affinis* population in the MTZ. In contrast, *Acartia tonsa*, another calanoid copepod found in estuarine waters (Tester and Turner, 1991; Cervetto et al., 1999), release eggs in the water column which predisposes them to be advected out of the MTZ region. Finally, the retention of the population in the MTZ can also potentially reduce predation by visual predators.

The changes in salinity that *E. affinis* experience in the present laboratory experiments would be associated with changes in turbulence and flow velocity in the field. Other calanoid copepods have been shown to modify their swimming behaviour in response to hydrodynamic disturbances and velocity gradients (Buskey et al., 2002; Woodson et al., 2005). Changes in flow velocity and turbulence intensity might therefore as well trigger swimming activity in *E. affinis* and help to maintain populations in place. This is a critical issue that should be considered in further studies to complement the present work because previous laboratory experiments demonstrate that *E. affinis* could not resist a current speed >2 cm s\(^{-1}\) (Castel and Veiga, 1990).

The relevance of behavioural investigations based on 2D projections of 3D swimming paths as implicitly done here has widely been discussed elsewhere (Seuront et al., 2004c). It has been concluded that 2D records are not sufficient to characterize 3D swimming behaviour if the swimming paths are not isotropic. It is acknowledged that the swimming behaviour of *E. affinis* is very unlikely to be three-dimensionally (3D) isotropic, especially considering the observed variability in time allocation to
swimming, hovering and sinking behaviours. A 3D approach might then lead to slightly different values of swimming speed and fractal dimension (Seuront et al., 2004c). However, the conclusions drawn here from 2D behavioural experiments are based on the relative differences in the vertical movements of *E. affinis* triggered by changes in salinity, which do not hinder the generality and the ecological relevance of our results.

The observed behavioural response to decreasing salinities suggests that in the Seine estuary, the populations of *E. affinis* have the intrinsic potential to undergo short-scale (i.e. 2–6 m) vertical migration triggered by changes in salinity. In particular, the specific behaviour of ovigerous females is suggested as an adaptive strategy to maintain population in the MTZ. *Eurytemora affinis* is a sibling species complex characterized by morphologically and genetically different populations around the world (Lee, 2000; Lee and Frost, 2002), which occurs in saline and hypersaline salt marshes (S: 25–40), brackish estuaries and lakes (S: 0.5–25) and has invaded freshwater lakes and reservoirs (S ≤ 0.5; Lee, 1999). Considering the genotypic control of *E. affinis* temperature and salinity tolerance (Bradley, 1986) and the presence of populations of each genetically divergent sibling species in diverse habitats that vary in salinity (Lee, 2000), future investigations are needed to understand whether the observed behavioural adaptivity to salinity changes is a general feature or instead the result of phenotypic plasticity (i.e. a change in the average phenotype expressed by a genotype in different environment) or natural selection.

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