Inter- and intra-specific differences in euryhalinity determine the spatial distribution of mysids in a temperate European estuary

Cesar Vilas *, Pilar Drake, Emilio Pascual

Instituto de Ciencias Marinas de Andalucía (CSIC). Polígono Río San Pedro s/n, Apartado Oficial, 11510 – Puerto Real, Cádiz, Spain

A R T I C L E   I N F O

Article history:
Received 25 August 2008
Received in revised form 17 November 2008
Accepted 18 November 2008

Keywords:
Estuary
Mysids
Mesopodopsis slabberi
Neomysis integer
Rhopalophthalmus tartessicus
Salinity
Spatial distribution
Tolerance

A B S T R A C T

Mysids are an important component of estuarine hyperbenthos and a major prey item in the food web of many estuaries. Understanding the abiotic and biotic mechanisms determining mysid distributions is therefore important to comprehend the general processes structuring estuarine communities. We carried out field surveys and exposure-survival experiments for three species of mysids, Neomysis integer, Leach Mesopodopsis slabberi van Beneden and Rhopalophthalmus tartessicus Vilas-Fernandez, Drake and Sorbe, to link salinity tolerances of different sex and life stages (adults and juveniles) to their spatial distributions within the Guadalquivir estuary, SW Spain. Despite being euryhaline, the three species of mysids were unevenly distributed along the saline gradient, with salinity being the environmental variable which best explained structure changes in the estuarine mysid assemblage. R. tartessicus remained confined to the outer and more marine part of the estuary and showed a higher temporal variation in its salinity-related distribution (position within the salinity gradient). M. slabberi and N. integer displayed wider estuarine distributions but remained associated with intermediate and low salinities, respectively. We found considerable inter- and intra-specific differences in tolerance to sudden salinity changes: N. integer, and juveniles of M. slabberi and R. tartessicus, showed a high tolerance to sudden salinity changes, whereas adults of M. slabberi and R. tartessicus were only tolerant to salinities close to their isosmotic points. For the less euryhaline species M. slabberi and R. tartessicus acclimation to unfavourable salinities decreased survival after exposure to sudden salinity changes. Both location and strength of the salinity gradient were important factors in determining spatial distribution, either directly to avoid osmotic stress and mortality risk (R. tartessicus and M. slabberi) or indirectly to reduce inter-specific mysid competition (N. integer). We suggest inter- and intra-specific euryhalinity differences determine the spatial distribution of mysids and the specific strategies they use to maintain this spatial structure in a highly variable environment.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Mysids (Crustacea: Mysidae) are a typical and often dominant component of estuarine hyperbenthos and play a key role both as consumers and prey in the food web of many estuaries (Elliott et al., 2002; Mauchline, 1980; Mees and Jones, 1997). Therefore, mysid communities are fundamental to the nursery function that estuaries provide for many crustacean and fish species (Beck et al., 2001; Fernandez-Deldago et al., 2007). In the Guadalquivir Estuary, a tidal estuary with regulated freshwater inflow on the southern Atlantic coast of Spain, three mysid species (Neomysis integer, Mesopodopsis slabberi and Rhopalophthalmus tartessicus) make up 59% of the macroinvertebrate biomass living in the water column (Drake et al., 2002), 67% of prey biomass found in small fish guts (Baldó and Drake, 2002) and 84% of the diet of decapod crustaceans (Gonzalez-Ortegón, personal communication). N. integer and M. slabberi are hyperbenthic, euryhaline and very abundant mysids commonly found in Atlantic-European coasts and estuaries (Fockedey et al., 2006; San Vicente, 1996), the latter also being found throughout the Mediterranean, Marmara, Black and Azov Sea’s (Wittmann, 1992). Contrary to these two widespread species, R. tartessicus is a hyperbenthic and euryhaline mysid, probably endemic to the Gulf of Cádiz, and the only representative of this genus in European waters (Vilas-Fernandez et al., 2006).

The importance of physicochemical conditions to the distribution of estuarine inhabitants is well documented. Among the many factors, such as temperature, that act as metabolic determinants in marine invertebrates (Vernberg, 1983), salinity plays a relevant role and has long been recognised as significant in the distribution of crustacean macrofauna (Kinne, 1964, 1971). As temperate estuarine environments are exposed to large tidal, seasonal and yearly fluctuations in salinity, species distributions within these estuaries are frequently limited by their salinity tolerances (McKenney, 1994b; Vlasblom and Elgershuizen, 1977; Whitfield et al., 2006).

* Corresponding author. Present address: Marine Science Institute, University of California Santa Barbara, CA 93106-6150. Tel.: +11 805 893 7397; fax: +1 805 893 8062.
E-mail addresses: vilas@msi.ucsb.edu (C. Vilas), pilar.drake@icman.csic.es (P. Drake), emilio.pascual@icman.csic.es (E. Pascual).
Despite the fact that most species living in estuaries are euryhaline, they can exhibit variable osmoregulatory capacities (Gonzalez-Ortegon et al., 2006), as well as ontogenic changes in their salinity tolerances (Anger et al., 2008; Guerin and Stickle, 1997; McKenney, 1994b; Rupp and Parsons, 2004; Torres et al., 2007; Wada et al., 2000). Vilas et al. (2006) determined oxygen consumption and body fluid osmolality (only for adults) at different salinities of present mysid species, suggesting an extraordinary osmoregulatory capacity of *N. integer* (extremely efficient hyperregulator at salinities from 3 to 32) and *M. slabberi* (strong hyper- and hyporegulator between 7 and 29) compared with *R. tartessicus* (hyperregulated only at salinities from 19 up to 36). Irrespective of species, oxygen consumptions of mysid juveniles at different salinities suggested that they are more euryhaline than adults (Vilas et al., 2006). Observed salinity-related mysid distributions could be caused by active or passive transport towards their preferred salinity or by differences in their survivorship at different salinities. From a population dynamics perspective it is important to understand how salinity-related mortalities determine the observed distribution of mysid species. This is particularly relevant in an estuary with regulated freshwater inflow where salinity can decrease abruptly in the warm seasons (because of dam release for agriculture irrigation) when high mysid production occurs (Baldó et al., 2005; Drake et al., 2002; Fernandez-Delgado et al., 2007). However, the relationship between the physiological capacities of each species and the spatial distribution of mysids in the estuary is still unknown. Field distributions should be restricted within the salinity ranges tolerated by species, but distributions can vary within the salinity range tolerated due to interactions between salinity and other factors (Gilles and Pêqueux, 1983), as shown for mysids *Mysidopsis bahia* (De Lisle and Roberts, 1986) and *Paramyxis lacustris* and *Limnomyysis benedeni* (Ovcarenko et al., 2006). Furthermore, acclimation is a relevant factor in determining salinity tolerance of organisms (Bhattacharya, 1982; De Lisle and Roberts, 1987); although acclimation to a given environmental condition is generally assumed to enhance the physiological performance of individuals in this environment, sometimes it may cause a decreased fitness of acclimated organisms (Leroi et al., 1994; Wilson and Franklin, 2002).

In the present study we investigate how salinity tolerance may determine field distributions of mysid species in a temperate estuary with regulated freshwater inflow. Specifically, we (1) compare lethal effects of salinity changes in the laboratory for each life stage-species combination of mysids *N. integer, M. slabberi* and *R. tartessicus* through exposure to sudden changes of salinity and survival measurements at different times; (2) for the less tolerant stage-species combinations, we also exposed individuals acclimated to different salinities to sudden changes of salinity; and (3) we compare their range of salinity tolerance with their observed field distribution and explore spatial and salinity-related changes in mysid distributions in relation to salinity changes within the estuary.

2. Material and methods

2.1. Area description

The Guadalquivir Estuary (SW Spain: 37° 25′-36° 47′ N, 4° 58′-7° 00′ W) is a non-stratified estuary with a gradual horizontal change in salinity, except when increased river flow causes freshwater to reach the mouth of the estuary (Drake et al., 2002). During the warm season sudden high freshwater inflows are induced from a dam 110 km upstream to irrigate cultivated fields along the river, which has the effect of reducing both salinity and mysid abundances (Baldó et al., 2005). Hence, the salinity gradient shows both long-term (seasonal) and short-term (tidal and dam management-related) displacements along the river course (Drake et al., 2002). On average, the five isoaline, boundary between oligohaline (salinity between 0.5-5) and
mesohaline (5-18) zones (McLusky, 1999), is situated at 25 km upstream from the river mouth at low tide (35 km at high tide); the 18 isohaline, boundary between the mesohaline and polyhaline (18-30) zones, is situated at 5 km at low tide (15 km at high tide). Temperature was spatially homogeneous throughout the estuary during the current study period (annual mean±SE temperature=19.3±0.4 °C), although there was marked seasonal variation (11.3 °C to 27.6 °C, Fig. 1A).

2.2. Field mysid collection

Mysids were sampled monthly between April 2001 and June 2003. Sampling was carried out on the spring tide during diurnal ebb and flood tide at three stations situated at 8, 20 and 32 km upstream from the mouth of the estuary. Two zooplankton nets (diameter 0.5 m; 250 µm mesh size), one positioned at the surface and the other on the bottom, passively sampled simultaneously for 10 minutes. Collected material was immediately fixed in 10% formaldehyde solution. Temperature, turbidity, salinity (Refractometer ATAGO S/Mill) and current speed (Digital flow meter HYDRO-BIOS 438 110, to calculate sampled water volumes and then densities as individuals/m³) were also measured. In the laboratory mysids were sorted into species, sex and life stages (adult male and female and juvenile), and then measured and counted.

2.3. Salinity tolerance experiments

In order to collect enough individuals to carry out experiments, adults used to estimate salinity tolerances were collected April through June 2003 (field temperature: 17 to 23 °C), from the 8 (for M. slabberi and R. tartessicus) and 32 km (for N. integer) sites and transferred to the laboratory in ambient water (salinity ranged 5-10 for N. integer and 18-25 for M. slabberi and R. tartessicus). Conversely, juveniles were obtained in the laboratory from gravid females collected in the field. Specimens were kept at 20 °C (near the annual mean estuarine temperature), under natural light and dark conditions and supplied with Artemia salina nauplii and micro-algae enriched rotifers ad libitum. When original (field) and target acclimation salinity (initial salinity in salinity tolerance experiments) differed by more than 5, individuals were gradually transferred (in 5 units salinity steps) from the original to the target acclimation salinity. Individuals were kept in target acclimation-salinity water for at least one week prior to experiments (De Lisle and Roberts, 1986; Simmons and Knight, 1975). Acclimation salinities were chosen according to the field distribution and osmoregulatory capacity of each species: 6 for M. slabberi and 24 °C for each sampling date and summarized in a summary of salinity tolerance data. We used a one-way ANOVA analysis of mysid samples was carried out (based on Bray-Curtis similarity matrix); first axis coordinates of samples were displayed versus salinities at which samples have been collected and Spearman correlation coefficient (r) between both variables was estimated. BEST and MDS analyses, as well as permutation tests, were carried out using the computer software PRIMER v6 package (Clarke and Gorley, 2006).

Spatial and salinity-related variation in the field distribution of each life stage-species combination were expressed as the distance (D) from the river mouth (upstream) and salinity value (S), respectively, at which the population center of mass (CM) was located: DCM=Σ i pi Di ; SCM=Σ i pi Si , where pi is the proportion of individuals collected at the sampling i, and D and S are the distance and salinity, respectively, at which each sample was taken (Gonzalez-Ortegon et al., 2006). DCM and SCM were estimated during high mysid density period (T>15 °C) for each sampling date and summarized in box plot graphs of DCM and SCM values. Additionally, to analyse changes in mysid distribution in relation to changes in estuarine salinity, we plotted DCM and SCM values versus mean estuarine salinity (0-36) zones, is situated at 25 km at low tide (15 km at high tide). Temperature was spatially homogeneous throughout the estuary during the current study period (annual mean±SE temperature=19.3±0.4 °C), although there was marked seasonal variation (11.3 °C to 27.6 °C, Fig. 1A).

2.2. Field mysid collection

Mysids were sampled monthly between April 2001 and June 2003. Sampling was carried out on the spring tide during diurnal ebb and flood tide at three stations situated at 8, 20 and 32 km upstream from the mouth of the estuary. Two zooplankton nets (diameter 0.5 m; 250 µm mesh size), one positioned at the surface and the other on the bottom, passively sampled simultaneously for 10 minutes. Collected material was immediately fixed in 10% formaldehyde solution. Temperature, turbidity, salinity (Refractometer ATAGO S/Mill) and current speed (Digital flow meter HYDRO-BIOS 438 110, to calculate sampled water volumes and then densities as individuals/m³) were also measured. In the laboratory mysids were sorted into species, sex and life stages (adult male and female and juvenile), and then measured and counted.

2.3. Salinity tolerance experiments

In order to collect enough individuals to carry out experiments, adults used to estimate salinity tolerances were collected April through June 2003 (field temperature: 17 to 23 °C), from the 8 (for M. slabberi and R. tartessicus) and 32 km (for N. integer) sites and transferred to the laboratory in ambient water (salinity ranged 5-10 for N. integer and 18-25 for M. slabberi and R. tartessicus). Conversely, juveniles were obtained in the laboratory from gravid females collected in the field. Specimens were kept at 20 °C (near the annual mean estuarine temperature), under natural light and dark conditions and supplied with Artemia salina nauplii and micro-algae enriched rotifers ad libitum. When original (field) and target acclimation salinity (initial salinity in salinity tolerance experiments) differed by more than 5, individuals were gradually transferred (in 5 units salinity steps) from the original to the target acclimation salinity. Individuals were kept in target acclimation-salinity water for at least one week prior to experiments (De Lisle and Roberts, 1986; Simmons and Knight, 1975). Acclimation salinities were chosen according to the field distribution and osmoregulatory capacity of each species: 6 for M. slabberi and 24 °C for each sampling date and summarized in a summary of salinity tolerance data. We used a one-way ANOVA analysis of mysid samples was carried out (based on Bray-Curtis similarity matrix); first axis coordinates of samples were displayed versus salinities at which samples have been collected and Spearman correlation coefficient (r) between both variables was estimated. BEST and MDS analyses, as well as permutation tests, were carried out using the computer software PRIMER v6 package (Clarke and Gorley, 2006).

Spatial and salinity-related variation in the field distribution of each life stage-species combination were expressed as the distance (D) from the river mouth (upstream) and salinity value (S), respectively, at which the population center of mass (CM) was located: DCM=Σ i pi Di ; SCM=Σ i pi Si , where pi is the proportion of individuals collected at the sampling i, and D and S are the distance and salinity, respectively, at which each sample was taken (Gonzalez-Ortegon et al., 2006). DCM and SCM were estimated during high mysid density period (T>15 °C) for each sampling date and summarized in box plot graphs of DCM and SCM values. Additionally, to analyse changes in mysid distribution in relation to changes in estuarine salinity, we plotted DCM and SCM values versus mean estuarine salinities (poled across sampling sites) and estimated Spearman correlation coefficients. Since SCM and DCM data did not meet the assumptions of ANOVA, statistical differences in spatial and salinity-related field distributions of different mysid categories were asserted by using the Kruskall-Wallis one way analysis and the Notched Box- and-Whisker plot for further pair-wise comparisons.

Acute lethal effects of sudden salinity changes on mysids were analysed using SIGMAPLOT software package. For each mysid life stage-species combination, we estimated the two extreme salinities causing a 50% mortality of individuals after a specific exposure time as lethal concentration (lower and upper LC50). That is, curves of mortality caused by a single exposure time were fitted by automatic iterations to the equation y=50+{100/[1+/exp(−x/b)]} (Arntz, 1986). When operating in radians, the parameter b is the LC50, while a is a parameter related to the shape of the curve (the higher value of a, the lower the curve slope). For each life stage-species combination, LC50 values versus exposure time were graphically represented as a summary of salinity tolerance data. We used a one-way ANOVA (factor=life stage-species combination) to test statistical differences in calculated LC50 values of different life stage-species combinations,
spring to autumn, with a reduced gradient in winter when large freshwater inflows from the dam reduce salinities throughout the estuary (Drake et al., 2007) (Fig. 1B). Mean estuarine salinities (pooled across all sampling sites at each date) displayed considerable variation over time fluctuating between 3.5 in April 2002 and 18.7 in July 2001 (Fig. 1B). The overall mean estuarine salinity was also strongly positively related to the range or gradient in salinity throughout the estuary (shaded area). During periods of strong freshwater inflow, when the mean estuarine salinity is ≤5, salinities at each sampling site indicate that the two outer sampling stations, which are usually located in the polyhaline (30 to 18) and mesohaline (<18 to 5) zones, were in the oligohaline (≤5) zone (Fig. 1C). The inner sampling station (32 km) was consistently oligohaline or in the lower range of the mesohaline zone.

During the peak mysid density period (~March-October, temperature >15 °C), tidal salinity changes (measured as increase or decrease of salinity per hour at each sampling date) were considerably different along the salinity gradient, with the rate of change about four times greater at the outer station (8 km from river mouth) than at the innermost site (32 km). Moreover, tidal salinity changes were greatest at times when mean estuarine salinity and the overall salinity gradient were high (Fig. 1D).

### 3. Results

#### 3.1. Estuarine salinity gradient

Salinities recorded within the estuary from April 2001 to June 2003 oscillated between 0 and 36, showing the largest gradient from late and Student-Newman-Keuls post-hoc tests to carry out further pairwise comparisons. Additionally, we used a paired two sample signed rank test to assess the null hypothesis that, in fitted equations, parameter values were consistently higher at higher salinities.

#### 3.2. Spatial and salinity-related distribution

According to BEST analysis results, salinity was the single environmental variable which best explained changes observed in the field mysid assemblage structure ($r=0.49; p<0.01$). Although the best environmental variable combination also involved temperature, the correlation coefficient between mysid assemblage and salinity-temperature environmental patterns hardly increased ($r=0.50; p=0.01$). This dominant role of salinity in determining mysid assemblage structure was confirmed by the high significant correlation ($r=0.81; p<0.01$) found between salinity and the first axis coordinates of each sample in the MDS ordination analysis (Fig. 2A). Even if mysid samples from the outer sampling sites tended to be located in the left-upper part of the ordination plot and those from the inner estuarine area in the right-lower extreme, changes in estuarine salinity caused a displacement of sample positions along this diagonal irrespectively of sampling sites; nevertheless, the smaller displacements observed at higher salinities suggest a weaker effect of salinity changes on mysid assemblage at such salinities.

In general, the three mysid species displayed a considerable spatial and salinity-related segregation: *N. integer* occurred mainly in the inner part of the estuary (≥20 km from mouth) where salinities are below 10; *R. tartessicus* inhabited the outer part of the estuary (<15 km from mouth) at salinities above 10; while *M. slabberi* population was generally intermediate (Fig. 2B, C). The temporal variation in salinity-related distribution (measured as $S_{CM}$) was highest for *R. tartessicus*. However, when the spatial distribution was taken into account ($D_{CM}$), *R. tartessicus* was confined to the outer estuary throughout the year, whereas the other two species showed wider estuarine distributions (Fig. 2B). Within each species, males tended to be located slightly closer to the mouth of the estuary than females, although intra-specific differences concerning sex were not statistically significant (Fig. 2B). Juveniles of *M. slabberi* and *N. integer* had similar distributions to that of their females, while juvenile *R. tartessicus* were found exclusively near the mouth of the estuary (Fig. 2B).

When spatial and salinity-related distributions were analysed with respect to levels of estuarine salinity (Fig. 3) we distinguished two broad patterns. Pattern 1, individuals moved upstream as salinity increased (significant correlation between mean salinity and $D_{CM}$) and, consequently, their positions along the salinity gradient did not change significantly (no significant correlation between mean salinity and $S_{CM}$). This occurs at all life stages of *M. slabberi* and adults of *N. integer*.
Regressions and respective Spearman correlation coefficients between distance ($D_{CM}$) and salinity ($S_{CM}$) at which centre of mass of different life stages, adult males, adult females and juveniles of *Rhopalophthalmus tartessicus*, *Neomysis integer* and *Mesopodopsis slabberi* populations were found at each sample time, and mean salinity in the estuary. *, $p<0.05$; **, $p<0.01$. 

Fig. 3.
Fig. 4. Survival (%) response curves for Rhopalophthalmus tartessicus, Neomysis integer and Mesopodopsis slabberi adult females, adult males and juveniles, exposed to sudden salinity changes after different acclimation salinities and measured at different times (hours) after exposure.
(although N. integer females were significantly correlated they were restricted to a narrow 0–10 range of salinity). Pattern b, individuals that did not moved upstream as salinity increased (no significant correlation between mean salinity and D_CM) and, consequently, their positions along the salinity gradient changed significantly (significant correlation between mean salinity and S_CM). This was seen for all life stages of R. tartessicus and juveniles of N. integer.

3.3. Lethal effects of sudden salinity changes

Survival experiments with individuals of R. tartessicus acclimated to 24 of salinity indicated that all its life stages were highly susceptible to low salinities exposure: about 50% of juveniles and most adult specimens died within 24 h below 10 (Fig. 4). Males and females of R. tartessicus acclimated to salinity 24 showed an abrupt increase of mortality at high salinities (≥30) after 24 h of exposure; after 48 and 72 h of exposure, survivals of males and females were very low at all salinities tested, while survivals of juveniles were still close to 80% at salinities between 12 and 36. Nevertheless, experiments with adults of R. tartessicus acclimated to 30 of salinity indicated that, after 72 h, survivals of males were above 80% at salinities between 12 and 42, and survivals of females above 60% at salinities between 12 and 36. Conversely, all life stages of N. integer were highly tolerant to sudden salinity changes within the entire range of salinity found in the estuary (1 to 36). In fact, juveniles, males and females of N. integer acclimated to 6 salinity showed survivals above 80% at salinities between 1 and 36 after 72 h of exposure (Fig. 4).

Irrespective of the acclimation salinity, juveniles of M. slabberi showed a relatively high tolerance to salinity changes, with survival above 80% at salinities between 6 and 42 after 24 h of exposure (Fig. 4). For adults of M. slabberi acclimated to 6 salinity, with the exception of a 100% of mortality observed in less than 4 h for individuals exposed to freshwater, mortality occurred more rapidly at high salinities (≥30); moreover, both males and females showed very high mortality at all salinities tested after 72 h of exposure (except for females at 6 and 12 of salinity). In contrast, when adults of M. slabberi were acclimated to 24 salinity, survival was higher than those of individuals acclimated to 6 at both high and low salinities; furthermore, after 72 h of exposure, males and females showed survival above 50% for a wide salinity range (6 to 36).
Survival and salinities in which 50% of the individuals died (LC_{50} values) (Tables 1 and 2; Fig. 5) suggest considerable intra- and interspecific differences in mysid tolerance to sudden salinity changes. In particular, all life stages of N. integer, juveniles of M. slabberi and, to a lesser extent, juveniles of R. tartessicus showed a high tolerance to sudden salinity changes, while adults (males and females) of M. slabberi and R. tartessicus only did when they were acclimated to relatively high salinities (to 24 and 30, respectively). Parameter w denotes the shape of the survival logistic curve: low values indicate a steep transition from low to high survivals; high values indicate a steady pattern of survival. This consistently showed lower values (signed rank test statistic=2.31; p<0.05) in curves fitted to low salinity transitions (0.0-1.5) than in those fitted to high salinity transitions (1.3-15.1). We also found an additional effect of acclimation for the less euryhaline species, R. tartessicus and M. slabberi (Figs. 4, 5). Males and females of R. tartessicus acclimated to 30 salinity showed significantly higher values of upper LC_{50} than individuals acclimated to 24; similarly, upper LC_{50} values were significantly higher for adults of M. slabberi acclimated to 24 than for those acclimated to 6 (Table 2). Conversely, a significantly higher tolerance to low salinities was not observed for individuals of R. tartessicus and M. slabberi which were acclimated to lower salinities (Table 2).

### 4. Discussion

#### 4.1. Inter- and intra-specific euryhalinity differences

Since temperate estuaries are exposed to large tidal and seasonal fluctuations in salinity, euryhalinity is an almost universal rule in mysids and many other estuarine taxa (De Lisle and Roberts, 1986; Gonzalez-Ortegon et al., 2006; Guerin and Stickle, 1997; McKenney, 1994a). Spatial and salinity-related distributions found for Rhopalophthalmus tartessicus, Neomysis integer and Mesopodopsis slabberi in the field confirm their euryhalinity. However, R. tartessicus and M. slabberi are marine species which enter the estuary and remain confined to its outer zone or associated with intermediate salinities, respectively; while N. integer is a genuine estuarine species mainly associated with the oligohaline zone of the estuary (Fig. 2).

Despite general euryhalinity, we found significant intra (ontogenic) and/or inter-specific (marine vs. estuarine species) differences in mysids tolerance to sudden salinity changes. In particular juveniles of marine species (R. tartessicus and M. slabberi) were more tolerant than their respective adults, and all N. integer stages were clearly more euryhaline than R. tartessicus and, to a lesser extent, M. slabberi. These differences mainly occurred at low salinities, as clearly displayed by parameter w (related to the shape of the curve). Within the natural range of salinity (0 to 36) (Tables 1 and 2) while survival increased sharply at low salinities, suggesting strong physiological constraints, at upper salinities we found gradual or no decrease in survival. Similarly, results of the MDS ordination analysis suggested that field salinity changes also caused stronger effects on the estuarine mysid assemblage at low as opposed to high salinities (Fig. 2A).

During this study, salinity in the Guadalquivir Estuary showed a marked horizontal gradient, except during high rainy periods and/or high dam freshwater inflow events (estuarine mean salinity ~5) when the sea water effect was restricted to the outer estuary. In addition, the stronger the salinity gradient the greater the short-term tidal variation in salinity (Fig. 1C, D). Though specimens inhabiting the outer estuary (mainly marine species) were exposed to larger and more sudden tidal salinity changes than those inhabiting the inner estuarine stretch, these changes occurred within a less acute salinity range and with higher rates of tidal salinity change (Fig. 1D). This may produce a

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Lower</th>
<th>Upper</th>
<th>Lower</th>
<th>Upper</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>Females</td>
<td>Males</td>
<td>Juveniles</td>
<td>Females</td>
<td>Males</td>
<td>Juveniles</td>
</tr>
<tr>
<td>R. tartessicus</td>
<td>24</td>
<td>9.1±3.9</td>
<td>28.6±2.0</td>
<td>11.6±0.4</td>
<td>37.2±0.7</td>
<td>6.0±0.1</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>11.5±0.4</td>
<td>37.8±1.1</td>
<td>12.0±0.0</td>
<td>43.0±1.2</td>
<td>1.0±0.0</td>
</tr>
<tr>
<td>M. slabberi</td>
<td>6</td>
<td>2.0±0.0</td>
<td>25.5±1.3</td>
<td>4.8±0.8</td>
<td>28.9±1.4</td>
<td>2.0±0.1</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>4.0±0.2</td>
<td>39.5±1.2</td>
<td>1.7±0.2</td>
<td>36.9±0.5</td>
<td>3.6±0.4</td>
</tr>
<tr>
<td>N. integer</td>
<td>6</td>
<td>1.1±0.4</td>
<td>42.6±1.1</td>
<td>0.6±0.0</td>
<td>41.3±1.2</td>
<td>0.2±0.0</td>
</tr>
</tbody>
</table>

S0: acclimation salinity.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>R. tartessicus</th>
<th>M. slabberi</th>
<th>N. integer</th>
</tr>
</thead>
<tbody>
<tr>
<td>F24</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>F30</td>
<td>**</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>M24</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>M30</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>J24</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>J24</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>F6</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>F30</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>M24</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>M30</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>J24</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>J6</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

Lower and upper significance of LC_{50} at low and high salinities respectively. 6, 24 and 30, acclimation salinities of individuals. n.s, no significant; *, p<0.05; **, p<0.01.
shorter residence time of mysids in physiologically unfavourable low salinities. Both facts could partially explain the apparent paradox that, despite their relatively restricted spatial field distributions and their minor tolerance to salinity changes, *R. tartessicus* adult individuals showed wider salinity-related field distributions than individuals of the other two mysid species (Figs. 2, 5).

Due to short-term tidal salinity variations in estuaries, osmotic equilibrium between organism and environment may never be attained, and consequently estuarine inhabitants may be exposed to almost continuous osmotic stress (Kinne, 1971). While macrozooplankton (as adult mysids and amphipods) may control their estuarine position by vertical tide-related migrations, the movement of smaller zooplankton (as copepods) seems to be insufficient to totally control their estuarine distribution (Kimmerer et al., 1998). Thus, observed ontogenetic changes for the less euryhaline species could indicate alternative size-dependent mechanisms of overcoming unfavourable conditions within estuaries. While juvenile mysids with lower ability to escape from unfavourable low salinities present high tolerance to sudden salinity changes without additional energetic cost (Vilas et al., 2006), adult mysids with higher mobility reduce osmotic stress by reducing their residence time in physiologically unfavourable salinities, as previously described. Therefore, distance phenomena (distance from individuals to their more favourable water mass) interact with salinity tolerances to determine the spatial distribution of highly mobile specimens within estuaries (Wagner and Austin, 1999).

Broad tolerance to salinity in young developmental stages has been observed in many crustaceans (Charmantier and Charmantier-Daures, 2001) and other marine invertebrates, such as the invasive mussel *Mytilopsis leucophaeata*, whose larval stages are highly resistant to variable environmental salinity and temperature conditions (Verween et al., 2007). High osmoregulation abilities of earlier developmental stages let them survive when hatching occurs in highly variable systems, like salt marshes and estuaries, and, consequently, such ability may have an important adaptive value to inhabit in these systems (Charmantier and Anger, 1999; Charmantier et al., 2002). Recent work on ontogenetic patterns of osmoregulation in the crab *Arama* spp. shows how differences in the ontogeny of salinity tolerance matched with life-history strategies, highlighting the crucial role of early juvenile hyperosmoregulation in dilute media (Anger et al., 2008). Although osmoregulation ontogeny was not studied in the present work, high euryhalinity observed for mysid juveniles could be related with high hyper and/or hypo-osmoregulation abilities, and interspecific differences on that trait explain differences in life-history strategies. Further studies of this trait in estuarine mysids will enhance our understanding of their population dynamic and community structure.

Specific differences in euryhalinity and tolerance at low salinities could lead to the two distinct strategies adopted by the three species to cope with estuarine salinity gradient variations. Adults of most euryhaline species *N. integer* and all life stages of *M. slabberi* move their position together with the water mass (described as pattern a) trying to remain within their more favourable salinity zone (mesohaline and mesohaline) in agreement with their tolerance limits and/or less interspecific competition and predation, respectively (Vilas et al., 2008: see ahead Section 4.3). In contrast, the least euryhaline species *R. tartessicus* maintains its position in the outer part of the estuary in polyhaline waters, where less acute salinity changes occur and it avoids low salinity water masses (described as pattern b). When low salinity (0-10) water reaches the outer estuary we observe a decrease of *R. tartessicus* densities, due either to mortality or seaward movement out of the sampled water mass. Both juveniles of *N. integer* and *R. tartessicus* also maintain their position at the most inner and outer parts of the estuary respectively (pattern b). Their extreme euryhalinity allows them to maintain their position regardless of strong salinity changes in the low and high salinity zones respectively, although showing a highly skewed salinity-related field distribution compared with their wide salinity tolerance (Fig. 5). This segregation is most evident when the salinity gradient is strongest and population densities are maximal. Because adults of *N. integer* and *R. tartessicus* prey on juveniles of *M. slabberi* (Vilas et al., 2008) and they are morphologically similar to juveniles of *N. integer* and *R. tartessicus*, avoidance of cannibalism by adults (mainly males) (Johnston and Ritz, 2001; Mauchline, 1980; Richoux et al., 2004) could explain this intra-specific segregation. Similarly, the frequent occurrence of *SCM* values for *M. slabberi* juveniles at water masses where *N. integer* and *R. tartessicus* adult densities decreased suggests an attempt to avoid predation or just the result of a lower mortality by predation at such water masses (Fig. 2).

### 4.2. Acclimation

Acclimation is an important factor in determining salinity tolerance of organisms (Bhattacharya, 1982; De Lisle and Roberts, 1986; Kefferd et al., 2007), so stepwise (vs. direct) transfer of individuals to different salinities may increase their tolerance (Baylon and Suzuki, 2007; Bhattacharya, 1982). But stepwise acclimation can not occur in the field because of large short-term tidal salinity variations in estuaries. Furthermore, repeated salinity changes may have a cumulative effect and increase mortality at salinities that are otherwise tolerated. Thus, for the less euryhaline organisms, the additional energetic cost (osmoregulation energy expenditure) of being exposed to less favourable conditions within their tolerance range may cause the observed poor physiological conditions of less euryhaline mysids when acclimated to unfavourable salinities. When the less euryhaline adults of *R. tartessicus* and *M. slabberi* were acclimated to more favourable salinity conditions (closer to their isosmotic points) (Vilas et al., 2006) they showed broad tolerance ranges, while these were shortened by acclimation to less saline conditions. Even if mysids acclimated to less saline waters displayed an initial shift of their low tolerance limit (low LC50) towards lower salinities, any advantage in tolerating less saline conditions disappeared progressively (Table 2; Fig. 5).

A considerable saving of the energy required for osmoregulation may be obtained by reducing the differences in osmolality between body fluids and environment (Born, 1968; Hagerman, 1970). While adults of *R. tartessicus* hyperegulated at salinities between 19 and 36, and *M. slabberi* were strong hyper and hyperegulators at salinities between 7 and 29, both showed minimal oxygen consumption at salinities around their isosmotic point (35 and 25, respectively) (Vilas et al., 2006). This indicates an optimal performance at these water masses with lower energetic costs, and contrary higher consumptions at low salinities (Vilas et al., 2006). High euryhalinity of all life stages of *N. integer* and juveniles of *R. tartessicus* and *M. slabberi*, with constant oxygen consumption regardless of salinity (Vilas et al., 2006), could explain their better physiological conditions when acclimated to low salinities and their observed wider salinity tolerance ranges. For the mysids *Myisidopsis bahia* and *Neomyisys integer*, acclimation to lower salinities also shortened salinity tolerance ranges (De Lisle and Roberts, 1986; Vl Aslom and Elgerus Unden, 1977), and salinity conditions close to isosmotic point accounting for optimal growth in *M. bahia*, suggesting a worse physiological conditions concurrent with osmotic stress (McKenney and Celestial, 1995). A detrimental effect of acclimation to nonoptimal environmental conditions on the physiological performance of individuals has been also reported for other relevant environmental factors, such as temperature (Leroy et al., 1994; Wilson and Franklin, 2002; Woods and Harrison, 2002).

### 4.3. Biotic interactions

According with our survival (this study) and physiological (Vilas et al., 2006) results, the observed mysid field distributions can not be explained as a result of a salinity-dependent mortality along the
estuarine salinity gradient. Under such assumption, extreme values of S_{CM} should have been associated to lower mysid densities and that relationship was not observed. The association between S_{CM} values and salinity ranges at which M. slabberi and R. tartessicus adults osmoregulate reinforces the general idea that field distributions are centered on the region, in this case salinity range, where species perform optimally (McGill et al., 2006). However, the highly euryhaline adults of N. integer do not follow this pattern and are confined to the oligohaline zone of the estuary despite its isosmotic point at 30 (Vilas et al., 2006). Therefore, other than just physiological reasons, biotic interactions may be leading species to actively choose a particular salinity range and/or stretch of the estuary to live in.

Regarding biotic interactions, several features suggest inter-specific resource-competition between adults of N. integer and R. tartessicus in the Guadalquivir Estuary: although N. integer showed a more omnivore diet than the more carnivorous R. tartessicus, both species shared prey of similar size, particularly copepods and juveniles of M. slabberi (Vilas et al., 2008). Thus, their estuarine coexistence may be achieved by spatial segregation such that the higher euryhalinity and foraging plasticity of N. integer allows it to inhabit the more osmotic-stressful oligohaline region where R. tartessicus cannot survive.

Furthermore, predation of both species on juveniles of M. slabberi is a clear example of intraguild predation. This interaction has relevant consequences in ecosystem structure (Holt and Polis, 1997; Polis et al., 1989) and, as explained before, could lead to the observed mysid juveniles segregation (from adult mysids) despite their high euryhalinity. In the case of M. slabberi individuals, they feed selectively on phytoplanckton and detritus (David et al., 2006; Tackx et al., 2004) and present ontogenic-diet shift towards microzooplankton and copepods (Fromeman, 2001). Moreover, this species feeds on smaller size prey than similar co-occurring mysids (Jerling and Wooldridge, 1994), suggesting a lower key trophic level within the guild than co-occurring mysids species and a reduction of adult inter-specific competition with N. integer and R. tartessicus. It would allow adults of M. slabberi to spread their distribution close to their physiological limits (Fig. 5). All these features suggest that potential (limited by salinity tolerance) field distributions of mysids could have been reduced to their actual field distributions by interaction of biotic factors (De Lisle and Roberts, 1986; Gilles and Péqueux, 1983; Ovcarenko et al., 2006).

Similar field distribution is observed in other estuarine mysids communities: Neomysis americana and Mysidopsis tortonsei in Rio de la Plata (Calliari et al., 2007), N. americana and Mysis stenolepis in St. Lawrence Estuary (Winkler et al., 2007). In Lough Furnace N. integer chooses high turbidity water masses for feeding to avoid resource competition with other mysid species by spatial segregation (Köpcke and Kausch, 1996; Parker and West, 1979). This diet-related spatial segregation is also seen in the Baltic Sea, where Mysis mixta eats more zooplankton and pelagic material than Mysis relictica (Vihervuoto et al., 2000). A parallel situation is found for amphipod sandhoppers Talitridae ugalini and Talitrus saltator; the former is absent close to low salinity water bodies compared to T. saltator which shows greater capacity to respond to external osmotic changes (Calosi et al., 2007).

5. Conclusion

Salinity is the main environmental variable sorting the mysid community in the Guadalquivir Estuary. In summary, R. tartessicus inhabits the less osmotic-stressing outer estuary, M. slabberi lives within the salinity range at which their adults are strong osmoregulators (Vilas et al., 2006), and the more euryhaline N. integer occupies the inner estuary where adults of R. tartessicus (potential competitors) are excluded because of their intolerance to low salinities. Two main strategies are adopted to maintain this spatial segregation: pattern a) species or life-stages that remain within a constant salinity zone and move together with the water mass along the estuary (M. slabberi and adults of N. integer), and pattern b) those that maintain their position in the same part of the estuary (R. tartessicus and juveniles of N. integer). When salinity-related field distributions and salinity-related physiological features are compared, salinity determines the estuarine distribution of males and females of R. tartessicus and, to a lesser extent, those of M. slabberi. In contrast, the field distribution of the most euryhaline N. integer was clearly biased toward lower estuarine salinities and is not directly determined by salinity. As hypothesized in a previous publication (Vilas et al., 2006), the estuarine distribution of the less euryhaline species (marine species) tends to occur at physiologically more favourable salinities (lower osmotic stress and mortality risk); whereas that of the most euryhaline species (estuarine species) occurred at ecologically more favourable salinities (lower interspecific mysid competition and predation). In conclusion, either directly (R. tartessicus and M. slabberi) or indirectly (N. integer), the existence of an estuarine gradient of salinity and its strength represent relevant features in determining the spatial distribution of mysid species within the Guadalquivir Estuary.

Considering the essential role that estuaries play for human and marine life (Harley et al., 2006; Lotze et al., 2006), results presented in this work will be valuable to increase our ability to predict how mysid communities could be affected by future environmental changes promoted by anthropogenic global climate change (IPCC, 2001). Especially, those promoted by the alteration of freshwater and tidal influence through the construction of water control structures (Ritter et al., 2008) and seasonal changes in river discharges (Milliman et al., 2008). Mysids serve as a link between detritus (terrestrial and estuarine carbon), phyto- and microzooplankton, and larger predators such as fishes and crustaceans (Baldó et al., 2005; Vilas et al., 2008). Because of their importance in the estuarine trophic web and their role in the nursery function of estuaries, mysids are likely to transmit anthropogenic effects from land, rivers and marshes to estuarine and marine systems. The understanding of their community ecology is essential to develop efficient ecosystem based management plans in estuaries.

Acknowledgements

We express our thanks to Manuel Ruiz for sampling assistance and Mariana Espigares for her assistance in processing samples. We thank Nick Shears, Kate Shears, Carlos J. Melián, Julio Lorda and anonymous reviewers for their suggestions and corrections that clearly improved this manuscript. The study was supported by the Spanish MCYT project REN2000-0822 MAR, the Environmental and Fishery Agency of Autonomous Andalucía Government (“Consejería de Medio Ambiente” and “Consejería de Agricultura y Pesca: Junta de Andalucía”) and grants to C.V. from Spanish MCYT Pre-doctoral Fellow Program (FPI 2001–2005) and Post-doctoral Fellow Program (2006–2008) from the Spanish foundation Fundación Alfonso Martin Escudero. [SS]

References


