

# Feeding preferences of estuarine mysids *Neomysis integer* and *Rhopalophthalmus tartessicus* in a temperate estuary (Guadalquivir Estuary, SW Spain)

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## Abstract

Mysid shrimps are an important component of estuarine food webs because they play a key role in energy transfer as intermediate prey. We investigated the seasonal, tidal and depth specific variation in the diet of the estuarine mysids *Neomysis integer* and *Rhopalophthalmus tartessicus* and explored its implications for the planktonic community structure of a temperate estuary (Guadalquivir Estuary, SW Spain). *Neomysis integer* is an opportunistic omnivore feeding mainly on mesozooplankton and on members of the detrital–microbial loop, shifting prey seasonally according to availability. In contrast, *R. tartessicus* showed a more carnivorous diet and shifted its target prey during seasons of low resource availability. Despite statistically significant differences in diet composition, both species shared prey of similar size, particularly juvenile *Mesopodopsis slabberi*, the most abundant mysid species in this estuary, and copepods. Although these similarities imply inter-specific resource competition, their co-existence is achieved by niche partitioning and spatial segregation: the higher osmoregulatory capacity and foraging plasticity of *N. integer* confers a broader niche breadth for this species allowing *N. integer* to inhabit the more stressful oligohaline region of the estuary where *R. tartessicus* cannot survive. We propose that this mechanism relaxes the potential for competition between *N. integer* and *R. tartessicus*.

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## 1. Introduction

Estuaries are highly productive ecosystems where high species diversity and abundance translate into complex food web dynamics (Day et al., 1989; Attrill and Rundle, 2002; Adams and Bortone, 2005; Alfaro et al., 2006). Mysids, a major component of estuarine and coastal zooplankton communities, play a key role in structuring estuarine communities (Mauchline, 1980; Mees and Jones, 1997). As intermediate-sized prey,

between mesozooplanktonic ( $\mu\text{m}$ ) and endo- or epibenthic (cm), mysids progressively replace copepods in the diet of small fishes and crustaceans as the latter grow (Baldó and Drake, 2002; González-Ortegón, unpublished data). Consequently, mysids can play an important role as a resource for many species that use estuaries as nurseries. As consumers, mysids are generally considered omnivorous, feeding on a wide range of items including detritus, phytoplankton and zooplankton. Mysids are also known to adjust their feeding behaviour to resources availability (Mauchline, 1980). They can feed selectively on species of different sizes and thus can modify the structure of the estuarine zooplankton (Fulton, 1982; Wooldridge and Webb, 1988; Rudstam et al., 1989; Kouassi et al., 2006) and phytoplankton (Kost and Knight, 1975; Siegfried and Kopache, 1980).

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*Neomysis integer* Leach 1814, *Mesopodopsis slabberi* van Beneden 1861 and *Rhopalophthalmus tartessicus* n.sp. (previously named “*Rhopalophthalmus mediterraneus*” (Vilas-Fernández et al., in press) dominate the hyperbenthos of Guadalquivir Estuary, where they show a clear spatial segregation along the estuarine salinity gradient: *N. integer* is abundant in the oligohaline zone (salinity <12), *R. tartessicus* is abundant in the more marine areas in the estuary (12–35), and *M. slabberi* inhabits the intermediate areas of mesohaline and polyhaline waters (6–29) (Vilas et al., 2006). The lower stretch of the Guadalquivir estuary (32 km) acts as a nursery area for many marine fish and crustacean decapods species. The analysis of the feeding habits of the most abundant fish species in that estuarine stretch revealed that the three mysid species all together represented about 60% of the mass in all stomachs analyzed (Baldó and Drake, 2002). Despite the relevance of mysids in the Guadalquivir estuary food web, information on their feeding habits in this area did not exist prior to this study.

*Neomysis integer* is a hyperbenthic and euryhaline mysid commonly found in Atlantic–European coasts and estuaries. Fockedey and Mees (1999) defined *N. integer* as an omnivore, which mainly utilizes mesozooplankton and detritus carbon pools. *Rhopalophthalmus tartessicus* is a hyperbenthic and euryhaline mysid, probably endemic to the Gulf of Cádiz and the unique species of its genus in Europe (Vilas-Fernández et al., in press). Although there is no information available about its diet, feeding studies on the congeneric African species *Rhopalophthalmus terranatalis* and *Rhopalophthalmus africana* suggest that *Rhopalophthalmus* species are also omnivorous with a clear tendency towards carnivory (Jerling and Wooldridge, 1995; Kouassi et al., 2006).

Through extensive stomach content analysis, we describe diet composition and major seasonal diet changes for *Neomysis integer* and *Rhopalophthalmus tartessicus*. We use multivariate analysis to study possible diet differences and their implication for the observed spatial segregation between these species, as well as their roles in the estuarine food web. This provides the first quantitative and qualitative analysis of trophic status for these two key mysid species in the Guadalquivir Estuary.

## 2. Materials and methods

### 2.1. Study area

The Guadalquivir river estuary (SW Spain: 37°15′–36°45′N, 6°00′–6°22′W) is vertically mixed and shows a longitudinal salinity gradient. As most temperate and well-mixed estuaries, its maximum turbidity zone is in the oligohaline region. The tidal influence reaches about 110 km up stream from the river mouth and the mean tidal range is 3.5 m in the outer estuary. We located our sampling sites at Tarfia (36°57′24″N–6°11′07″W), in the oligohaline estuarine region (mean ± SE salinity = 4.12 ± 0.4 and turbidity = 120.8 ± 16.4 NTU, nephelometer turbidity units), and Bonanza (36°52′25″N–6°20′46″W), in the polyhaline region (mean ± SE salinity = 19.7 ± 1.5 and turbidity = 76.8 ± 18.5

NTU), situated at 32 and 8 km respectively from the river’s mouth, both approximately being 3 m in depth at low tide. The temperature was spatially homogeneous throughout the estuary (mean ± SE temperature = 19.3 ± 0.4 °C), but showed a marked seasonal variation (Table 1).

### 2.2. Sampling protocols

During 2002, we collected seasonal samples (a single sampling date each season, see Table 1) at each site during new moon spring tide diurnal ebbs and floods. Two zooplankton nets (diameter 0.5 m; 250 µm mesh size) were submerged simultaneously for 10 min, one at the surface and the other just above the bottom. Collected material was immediately fixed in 10% formaldehyde solution. During each sampling event, temperature, turbidity and salinity (Refractometer ATAGO S/Mill) were measured.

Because of there were not enough specimens of both species collected from surface tows, we only used individuals collected from the bottom samples to eliminate depth variation. Since *Neomysis integer* and *Rhopalophthalmus tartessicus* populations are spatially segregated, we studied individuals from both Tarfia and Bonanza sampling sites. No *R. tartessicus* specimens were found in winter flood samples. Additionally, we studied samples in October 2002 (distinct that samples from autumn 2002), when both species coincided downstream at Bonanza. In this case, we selected specimens from surface and bottom samples, irrespective of tidal phases. These data allow us to compare feeding in an overlapping habitat use event and detect possible depth diet variation.

In the laboratory, adults were sexed according to Mauchline (1980) and the carapace length (CL) was measured with a calibrated binocular microscope as the distance from the tip of the rostrum to the posterior mediodorsal margin of the carapace. Following Fockedey and Mees (1999), we used two methods to characterize diet: counting the amount of different food items (diet composition) and weighing the bulk of the food material present in the stomach (fullness index).

### 2.3. Stomach analysis

Stomach contents were analysed with light microscopes (magnification ×250) and image-analysis techniques (Image Analyzer Leica Quantimet 500+ connected to a microscope). To obtain semi-permanent microscopic slides of stomach contents, each mysid was first dehydrated, the stomach removed and its content distributed on a microscopic slide in a drop of glycerine (Fockedey and Mees, 1999). For each tide and date, we processed 6–10 adult individuals. Total mean CL was 3.28 ± 0.06 mm for *Neomysis integer* and 3.42 ± 0.06 mm for *Rhopalophthalmus tartessicus* (Table 1).

We identified and counted different prey present in the stomachs according to Fockedey and Mees (1999). Items were gathered into main categories (between brackets single items): unidentifiable and macrophytal detritus, colonial and unicellular phytoplankton (*Pediastrum*, *Staurastrum*, and *Spirulina*), pollen (gymnosperm and angiosperm), pelagic

Table 1  
Number, sex ratio (F, females; M, males) and mean ( $\pm$ SE) carapace length (CL, in mm) of *Neomysis integer* and *Rhopalophthalmus tartessicus* specimens used for stomach contents analysis. Data are presented for different seasons, sites and tidal situations, including the overlapping event in October 2002. Sampling dates appear between brackets. Water temperature (T), salinity (S) and turbidity (Tb) at each sampling are also shown

|                                     | Tarifa              |                 |                    |                 |                        |                 | Bonanza               |                 |                    |                 |                 |                 |
|-------------------------------------|---------------------|-----------------|--------------------|-----------------|------------------------|-----------------|-----------------------|-----------------|--------------------|-----------------|-----------------|-----------------|
|                                     | Spring (April 2002) |                 | Summer (July 2002) |                 | Autumn (November 2002) |                 | Winter (January 2003) |                 | October 2002 flood |                 |                 |                 |
|                                     | Flood               | Ebb             | Flood              | Ebb             | Flood                  | Ebb             | Flood                 | Ebb             | Surface            | Bottom          | Surface         | Bottom          |
| <i>Neomysis integer</i>             |                     |                 |                    |                 |                        |                 |                       |                 |                    |                 |                 |                 |
| N                                   | 8                   | 10              | 10                 | 10              | 9                      | 9               | 6                     | 10              | 10                 | 10              | 10              | 10              |
| Sex ratio (F:M)                     | 6:2                 | 4:6             | 4:6                | 5:5             | 7:2                    | 3:6             | 5:1                   | 8:2             | 6:4                | 6:4             | 5:5             | 5:5             |
| T (°C)                              | 19.8                | 20.0            | 27.1               | 25.5            | 19.4                   | 19.3            | 14.4                  | 14.2            | 21.5               | 21.5            | 21.5            | 21.5            |
| Tb (NTU)                            | 78.5                | 30.9            | 0.0                | 32.4            | 299.9                  | 49.5            | 222.6                 | 138.2           | 60.2               | 60.2            | 60.2            | 60.2            |
| S                                   | 2                   | 3               | 2                  | 8               | 4                      | 9               | 1                     | 2               | 15                 | 15              | 15              | 15              |
| CL (mm)                             | 3.09 $\pm$ 0.11     | 3.54 $\pm$ 0.10 | 3.15 $\pm$ 0.05    | 3.27 $\pm$ 0.03 | 3.32 $\pm$ 0.04        | 3.44 $\pm$ 0.03 | 3.27 $\pm$ 0.09       | 3.33 $\pm$ 0.07 | 2.99 $\pm$ 0.04    | 2.99 $\pm$ 0.04 | 2.99 $\pm$ 0.04 | 2.88 $\pm$ 0.05 |
| <i>Rhopalophthalmus tartessicus</i> |                     |                 |                    |                 |                        |                 |                       |                 |                    |                 |                 |                 |
| N                                   | 10                  | 9               | 9                  | 10              | 10                     | 10              | —                     | 11              | 9                  | 9               | 10              | 10              |
| Sex ratio (F:M)                     | 10:0                | 6:3             | 5:4                | 6:4             | 6:4                    | 6:4             | —                     | 8:3             | 9:0                | 9:0             | 5:5             | 5:5             |
| T (°C)                              | 20.0                | 20.5            | 26.5               | 25.5            | 19.0                   | 18.5            | —                     | 14.2            | 21.5               | 21.5            | 21.5            | 21.5            |
| Tb (NTU)                            | 68.8                | 29.6            | 4.1                | 48.5            | 37.2                   | 5.9             | —                     | 14.5            | 60.2               | 60.2            | 60.2            | 60.2            |
| S                                   | 9                   | 25              | 19                 | 19              | 20                     | 35              | —                     | 14              | 15                 | 15              | 15              | 15              |
| CL (mm)                             | 4.10 $\pm$ 0.04     | 3.40 $\pm$ 0.06 | 3.83 $\pm$ 0.11    | 3.20 $\pm$ 0.07 | 3.45 $\pm$ 0.06        | 3.08 $\pm$ 0.04 | —                     | 3.55 $\pm$ 0.04 | 3.37 $\pm$ 0.04    | 3.37 $\pm$ 0.04 | 3.37 $\pm$ 0.04 | 2.91 $\pm$ 0.06 |

copepods (Cyclopoid: *Acanthocyclops robustus*; Calanoid: *Acartia clausi*, *Calanipeda aqua-dulcis*), copepodites, copepod nauplii, benthic copepods (Harpacticoid), cladocerans (*Daphnia galeata*), rotifers (*Keratella quadrata*, *Keratella cochlearis*, *Brachionus*), 'Others' (yeast, *Halacaridae*, insect fragments, nematods *Kinorhyncha* sp., larvae of gastropod, *Euglena* sp.) (see pictures in Supplementary material Appendix 1).

Numerical abundance of each dietary item was determined, but for detritus only its surface area (mm<sup>2</sup>) was determined. When necessary, the total detritus area found in 10 random observations per slide was measured and then referred to the total slide area. We calculated frequency of occurrence (FO) of each item as the proportion of analysed stomachs containing it. Numerical percentage of each item was also calculated as: number of the specific item/total number of items in the stomach  $\times$  100 (except for those items only quantified as surface area).

To estimate prey size, we related prey's body part (copepod mandible width and length, and mysid statocyst diameter and telson width) measures to original body size, using regression models calculated from laboratory measurements on individuals collected in the estuary and treated with bleach. For copepods: mandible length =  $2.022 \times$  mandible width + 34.461 ( $n = 44$ ,  $r = 0.87$ ,  $p < 0.01$ ); carapace length =  $13.858 \times$  mandible length<sup>0.856</sup> ( $n = 15$ ,  $r = 0.79$ ,  $p < 0.01$ ). To estimate biomass for copepods, we extrapolated data from the literature for *Acartia tonsa*: biomass ( $\mu$ g C) =  $0.15 \times e^{3.04 \times \text{cephalotorax length}}$  ( $R^2 = 0.62$ ), carbon weight = 45% dry weight (Pastorinho et al., 2003). For *Mesopodopsis slabberi*: telson width =  $1.415 \times$  statocyst diameter + 36.882 ( $n = 7$ ,  $r = 0.92$ ,  $p < 0.01$ ); carapace length =  $11.336 \times$  telson width<sup>0.921</sup> ( $n = 10$ ,  $r = 0.99$ ,  $p < 0.01$ ); dry weight =  $0.030 \times$  carapace length<sup>3.453</sup> ( $n = 285$ ,  $r = 0.99$ ,  $p < 0.01$ ).

#### 2.4. Fullness index

To quantify the fullness indices (FI) we processed 17 individuals per sample. For each freshly fixed mysid, the stomach was carefully dissected after removing the carapace. Stomach (and its content) and the rest of mysid body were dried separately in small aluminium pans for 4 days at 60 °C, after which they were weighed with a microbalance METTLER U3 ( $\pm 1 \mu$ g). Dry weight of stomach content itself ( $DW_{\text{content}}$ ) was calculated as follows:  $DW_{\text{content}} = DW_{\text{stomach}} - DW_{\text{empty}}$ , where  $DW_{\text{stomach}}$ , dry weight of the stomach with its content;  $DW_{\text{empty}}$ , dry weight of the empty stomach derived from  $DW_{\text{mysid}}$  (dry weight of the mysid without its stomach) according to regressions:  $\ln(DW_{\text{empty}}) = 5.047 + 0.513 \times \ln(DW_{\text{mysid}})$  ( $n = 89$ ,  $r = 0.67$ ,  $p < 0.01$ ) for *Neomysis integer* (Fockede and Mees, 1999); and  $\ln(DW_{\text{empty}}) = -5.399 + 0.932 \times \ln(DW_{\text{mysid}})$  ( $n = 67$ ,  $r = 0.71$ ,  $p < 0.01$ ) for *Rhopalophthalmus tartessicus*. To get the latter linear regression, the empty stomach (after carefully emptied) and mysid body of 67 adults of *R. tartessicus* were weighed separately. The amount of food present in the stomachs at a given time ( $t$ ) was expressed as:  $FI_t = (DW_{\text{content}}/DW_{\text{mysid}}) \times 100$ .

## 2.5. Data analysis

We used least-squares regressions and ANOVA to assess possible inter-specific, tidal, depth and seasonal differences in FI. Prior to analyses, data were arcsine  $\sqrt{x}$ -transformed to reduce heteroscedasticity of variance (Zar, 1984).

To study inter- and intra-specific diet composition differences, we performed multivariate analyses using PRIMER (Plymouth Routines In Multivariate Ecological Research) (Clarke and Warwick, 1994). Prior to analyses, the prey were grouped into major categories and data were root–root transformed to down-weight the importance of the most dominant prey items. Pair-wise Bray–Curtis similarity coefficients were calculated, which provide a rough measure of dietary breadth of each species and of inter-specific differences. Main prey categories responsible for similarity and dissimilarity in each considered group were identified using SIMPER (Clarke and Warwick, 1994). In addition, ANOSIM tests (Clarke and Green, 1988) were carried out to determine significant differences between species, tidal situations and seasons. Differences in diet composition were analyzed by Cluster analysis and graphically represented by means of non-metric multidimensional scaling (MDS) using group-average linking based on Bray–Curtis similarities. Stress value ( $S$ ) was included as a measure of the ordination fitness ( $S < 0.05$  means an excellent representations;  $S < 0.10$  corresponds to a good ordination;  $S < 0.20$  gives a useful picture).

## 3. Results

### 3.1. Stomach fullness index

Mean FI values of *Neomysis integer* ranged between 0.8 and 9.4, showing a marked seasonal variation with higher FI during the winter-spring (Fig. 1A). FI of *Rhopalophthalmus tartessicus* oscillated between 0.2 and 0.4, except in winter (3.1), and were always lower than those of *N. integer* (Fig. 1). ANOVA analysis showed that such seasonal and intra-specific differences were statistically significant (Table 2). Conversely, we found no significant differences between flood and ebb samples for *R. tartessicus*; while for *N. integer*, they were

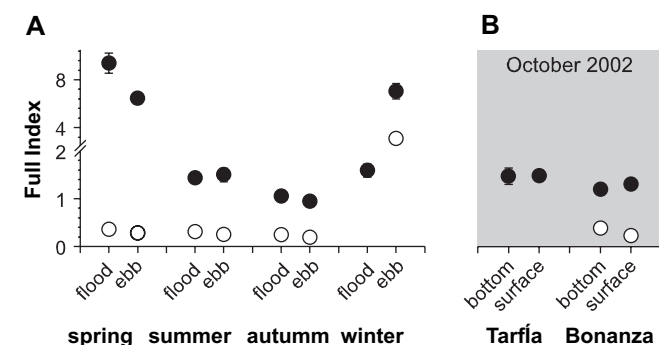


Fig. 1. Stomach fullness indices (mean  $\pm$  SE) of *Neomysis integer* (●) and *Rhopalophthalmus tartessicus* (○); by season and tide (A); and by sampling site and depth in both populations spatial overlapping at October 2002 (B).

significant only in spring and winter, showing dissimilar patterns (Table 2). When both species occurred simultaneously at Bonanza (October 2002), FI of *N. integer* individuals were again significantly higher than those of *R. tartessicus*, both at the surface and at the bottom (Table 2; Figure 1B). For *N. integer*, no significant differences in stomach fullness were found for individuals collected in October 2002 at different sampling sites (Tarfia and Bonanza). No significant differences in FI were observed between individuals collected at surface and bottom for both species.

### 3.2. Diet composition

*Neomysis integer*'s most frequent prey were: unidentified and macrophytal detritus, colonial phytoplankton, pollen, pelagic copepods and rotifers (FO > 60%, except for rotifers in autumn). *Neomysis integer* consumed juveniles of *Mesopodopsis slabberi* in summer, autumn and winter, although FO were usually below 45%. Other items less frequent were unicellular phytoplankton (FO > 20%) and, except in spring, some additional prey (FO 10–30%) classified as "Others" (alien organisms from the subtidal estuary, such as insects, and/or meiobenthos, and members of microbial-loop) (Supplementary material Appendix 2). *Rhopalophthalmus tartessicus* consumed frequently (FO > 60%) unidentified detritus, juveniles of *Mesopodopsis slabberi* and copepods (especially *Acartia clausi*); and with FO of 10–60%, pollen grains, colonial phytoplankton and macrophytal detritus; other items, such as the rotifers, cladocerans, phytoplankton and halacarids, were occasionally encountered, especially in winter and spring (Supplementary material Appendix 2). A certain temporal pattern for some prey items were observed in both species, mainly related to winter and spring, as Cladocera *Daphnia galeata*, Cyclopoid *Acanthocyclops robustus* and Calanoid *Calanipeda aqua-dulcis* (Supplementary material Appendix 2).

*Neomysis integer* consumed a high abundance of unidentified and macrophytal detritus. Colonial phytoplankton was the main item among primary producers, especially in winter, although pollen grains and unicellular phytoplankton were also consumed throughout the year. Conversely, *Rhopalophthalmus tartessicus* only consumed significant amounts of detritus and primary producers in winter (Fig. 2). Although both species showed maximum animal prey consumption in spring, for *N. integer* this was mainly due to the ingestion of rotifers and for *R. tartessicus* of pelagic copepods. *N. integer* showed a more varied diet than *R. tartessicus*, with 3–6 different prey items per stomach vs. 1–3, respectively (Fig. 2). While pelagic copepods and rotifers were the main animal prey for *N. integer*, for *R. tartessicus* important prey were pelagic copepods and juveniles of *Mesopodopsis slabberi* (Fig. 2), this item being less frequently consumed by *N. integer*.

### 3.3. Dietary breadth and inter-specific differences

Although *Neomysis integer* diet was temporally more homogeneous than that of *Rhopalophthalmus tartessicus* (average similarity 83.8% vs. 73.7%, respectively), the former

Table 2

ANOVA results comparing fullness indices (values per 1 and arcsin $\sqrt{x}$  transformed) for *Neomysis integer* and *Rhopalophthalmus tartessicus*. Values over the same line were not significantly different ( $p > 0.05$ ; test Student–Newman–Keuls). df, degrees of freedom; MS, sum of squares;  $p$ , significance level; R, *R. tartessicus*; N, *N. integer*; sp, spring; s, summer; a, autumn; w, winter; E, ebb; F, flood

| Variable = FI                       | df  | MS     | $p$   | Student–Newman–Keuls                    |
|-------------------------------------|-----|--------|-------|---|
| Seasonal samples                    |     |        |       |   |
| Species                             | 1   | 2351.8 | <0.01 | R < N                                   |
| Residual                            | 237 | 15.3   |       |   |
| <i>Neomysis integer</i>             |     |        |       |   |
| Season                              | 3   | 685.9  | <0.01 |   |
| Tide                                | 1   | 45.0   | <0.01 |   |
| Interactions                        |     |        |       |   |
| Season $\times$ tide                | 3   | 125.4  | <0.01 | aE < aF < sF < sE < wF < spE < wE < spF |
| Residual                            | 123 | 3.2    |       |   |
| <i>Rhopalophthalmus tartessicus</i> |     |        |       |   |
| Season                              | 3   | 143.3  | <0.01 | a < s < sp < w                          |
| Tide                                | 1   | 1.6    | >0.05 |   |
| Residual                            | 103 | 1.5    |       |   |
| October 2002 sample                 |     |        |       |   |
| Species                             | 1   | 286.8  | <0.01 | R < N                                   |
| Residual                            | 98  | 1.4    |       |   |
| <i>Neomysis integer</i>             |     |        |       |   |
| Sampling sites                      | 1   | 1.0    | >0.05 |   |
| Depth                               | 1   | 3.0    | >0.05 |   |
| Residual                            | 64  | 1.5    |       |   |
| <i>Rhopalophthalmus tartessicus</i> |     |        |       |   |
| Depth                               | 1   | 4.2    | >0.05 |   |
| Residual                            | 31  | 1.1    |       |   |

showed higher prey diversity than *R. tartessicus*, with 7 vs. 4 different prey categories contributing to the average diet similarity in more than 5% (Table 3). With regards to diet dissimilarity between both species (average dissimilarity 41.5%), there were 7 different prey that contributed to it in more than 5%; all these items were much more frequent in the diet of *N. integer* than in that of *R. tartessicus*, except *Mesopodopsis slabberi* juveniles, which were more frequent in the latter (Table 3).

MDS ordination showed two major groups: samples of *Neomysis integer* (black) and *Rhopalophthalmus tartessicus* (white) (Fig. 3). Similarly, samples corresponding to each species appeared separated into two distinct groups in the CLUSTER analysis at 80% similarity level, corresponding with observed groups at MDS. Multivariate ANOSIM analysis showed that inter-specific differences in the diet composition of both species were statistically significant ( $R = 0.72$ ,  $p = 0.01$ ).

ANOSIM analyses also revealed no statistically significant differences in diet composition between tides ( $R = -0.15$ ,  $p > 0.05$ , for *Neomysis integer*;  $R = -0.30$ ,  $p > 0.05$ , for *Rhopalophthalmus tartessicus*) nor between seasons for *R. tartessicus* ( $R = 0.37$ ,  $p > 0.05$ ). Conversely, for *N. integer*, seasonal differences in diet were statistically significant ( $R = 0.85$ ,  $p < 0.05$ ). In MDS ordination plot, *R. tartessicus* samples appeared distributed irrespective of tidal situation and season, while samples of *N. integer* from a same season were closer irrespective of the tidal situation (Fig. 3).

### 3.4. Diet patterns of spatially overlapping populations

In the overlapping situation (October 2002), only two prey types (juvenile *Mesopodopsis slabberi* and copepod *Acartia clausi*) were ingested with similar FO by both species (Table 4). *Neomysis integer* also consumed prey which were not or rarely found in the *Rhopalophthalmus tartessicus* stomachs: mainly benthic copepods, phytoplankton, pollen, rotifers and nematods (Table 4 and Fig. 4). Similarly, *N. integer* ingested a higher quantity of detritus more frequently than *R. tartessicus* (Fig. 4). Average dissimilarity in diet between both species in October 2002 (41.5%) was similar to that obtained when individuals of each species were collected at different sampling sites (40.5%), suggesting that inter-specific differences in diet remained even in spatially overlapping situation.

Regarding depth variation, no evident differences were observed for *Neomysis integer* (Table 4; Fig. 4). Conversely, *Rhopalophthalmus tartessicus* collected at bottom consumed more colonial phytoplankton than those collected at the surface, as well as they ingested detritus more frequently (bottom vs. surface: 77.8% vs. 20% and 44.4% vs. 10% for unidentified and macrophytal detritus, respectively) (Table 4). However, Calanoid copepods were relatively more abundant in stomachs of *R. tartessicus* individuals collected at the surface than at the bottom (Fig. 4). Such inter-specific differences were also evident in the MDS ordination plot, where the samples of *R. tartessicus* corresponding to surface and bottom in October 2002 appeared more separated from each other (similarity of 68.6%) than those of *N. integer* (similarity of 91.6%) (Fig. 3).

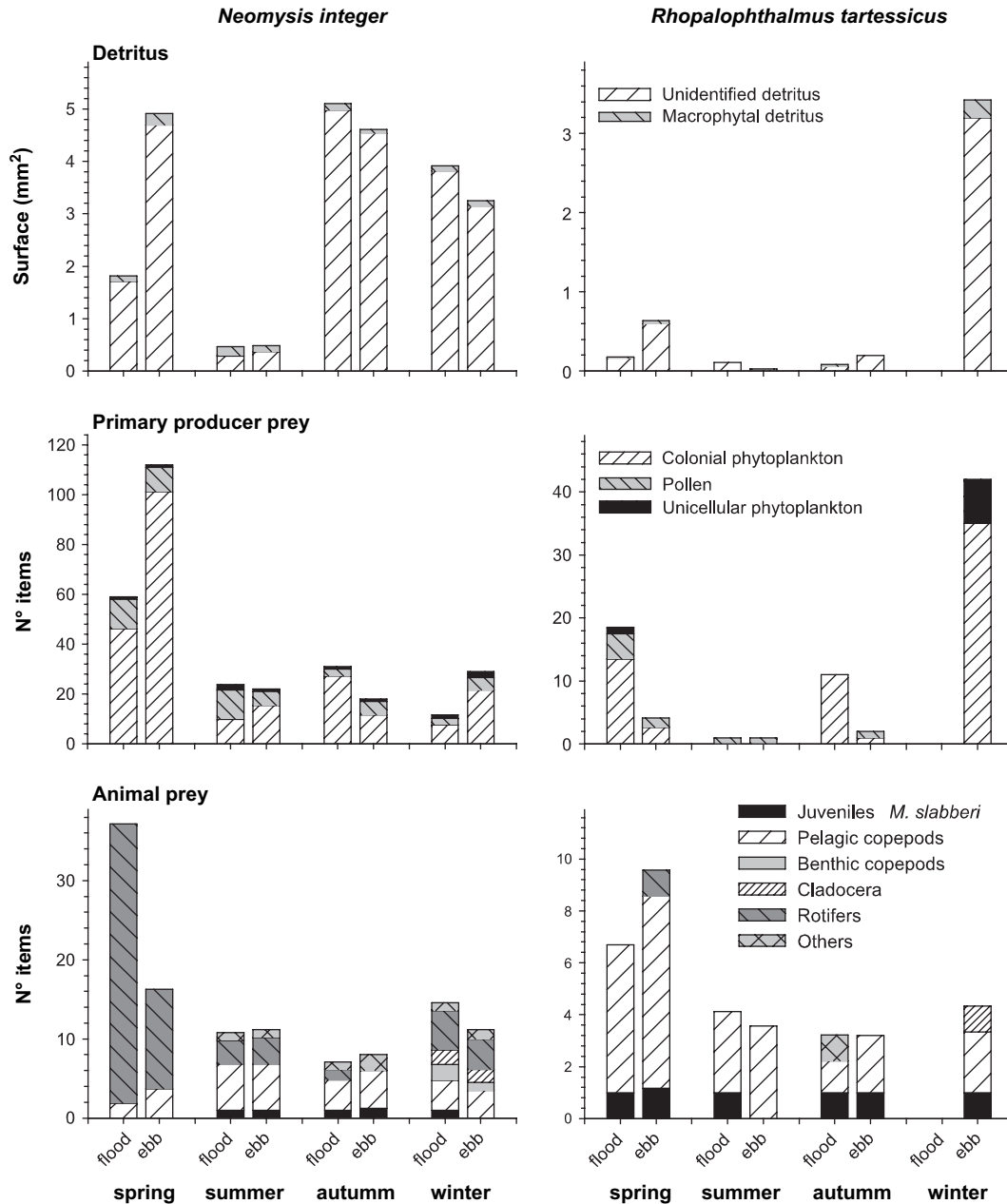


Fig. 2. Quantitative diet composition of *Neomysis integer* and *Rhopalophthalmus tartessicus* by season and tidal situation. Detritus was estimated as the mean surface area ( $\text{mm}^2$ ); primary producers and animal prey were estimated as mean number per stomach.

### 3.5. Size of ingested copepods and *Mesopodopsis slabberi* juveniles

For each season, copepods and mysids ingested by both species were within the same size ranges (Fig. 5). Cephalothorax length (CL) of consumed copepods ranged from 0.39 to 1.57 mm (mean  $0.74 \pm 0.01$  mm) for *Neomysis integer* and from 0.45 to 1.15 mm (mean  $0.80 \pm 0.01$  mm) for *Rhopalophthalmus tartessicus*; carapace length (CL) of *Mesopodopsis slabberi* ranged from 0.60 to 1.51 mm (mean  $1.14 \pm 0.10$  mm) for *N. integer* and from 0.52 to 1.95 mm (mean  $1.17 \pm 0.06$  mm) for *R. tartessicus*. This body sizes coincide with *M. slabberi* juveniles CL lengths (0.47–2.06 mm) (C. Vilas, unpublished data).

Mean individual dry weight biomass of copepods ingested by *N. integer* and *R. tartessicus* ranged between 2.24 and 5.64  $\mu\text{g}$  (mean  $3.72 \pm 0.33$   $\mu\text{g}$ ) and between 2.81 and 5.90  $\mu\text{g}$  (mean,  $4.16 \pm 0.28$   $\mu\text{g}$ ), respectively. Similarly, individual mean dry weight of *M. slabberi* juveniles ingested by *N. integer* and *R. tartessicus* oscillated between 4.4 and 102.8  $\mu\text{g}$  (mean  $50.8 \pm 13.6$   $\mu\text{g}$ ) and between 5.0 and 111.4  $\mu\text{g}$  (mean  $61.8 \pm 14.3$   $\mu\text{g}$ ), respectively.

Total mean individual dry weight of juvenile mysids and copepods ingested by both species (pooled stomach contents of *Neomysis integer* and *Rhopalophthalmus tartessicus*) were estimated as 56.3  $\mu\text{g}$  and 4.0  $\mu\text{g}$ , respectively. Thus, although juveniles of *Mesopodopsis slabberi* were numerically less

Table 3

Prey categories contributing (up to 90%) to intra-specific similarity and to inter-specific dissimilarity in the diet composition of *Neomysis integer* and *Rhopalophthalmus tartessicus*. Frequency of occurrence (%) data (square-root transformed). Av. O%, average frequency of occurrence; Av. Sim, average similarity; Sim/SD, similarity/standard deviation; Av. Diss, average dissimilarity; Diss/SD, dissimilarity/standard deviation; Contrib., contribution

| Species   | Prey categories                        | Similarity                                 |           |         |              |
|---|--|--|-----------|---------|--------------|
|   |  | Av. O%                                     | Av. Sim   | Sim/SD  | Contrib. (%) |
| <i>Neomysis integer</i>   | Unidentified detritus                  | 100.0                                      | 13.5      | 12.6    | 16.3         |
|   | Macrophytal detritus                   | 96.3                                       | 13.0      | 9.6     | 15.6         |
|   | Pelagic copepods                       | 92.7                                       | 12.6      | 7.4     | 15.0         |
|   | Total pollen                           | 88.2                                       | 12.0      | 6.4     | 14.3         |
|   | Colonial phytoplankton                 | 86.3                                       | 11.7      | 6.2     | 14.0         |
|   | Total rotifers                         | 58.7                                       | 6.6       | 1.5     | 7.9          |
|   | Unicellular phytoplankton              | 34.7                                       | 6.2       | 4.6     | 7.4          |
| Average percentage similarity                                   |  | 83.82                                      |           |         |              |
| <i>Rhopalophthalmus tartessicus</i>                             | Unidentified detritus                  | 88.6                                       | 22.9      | 3.9     | 31.1         |
|   | Pelagic copepods                       | 71.9                                       | 21.3      | 5.6     | 28.9         |
|   | <i>Mesopodopsis slabberi</i> juveniles | 59.4                                       | 15.6      | 1.8     | 21.2         |
|   | Macrophytal detritus                   | 21.7                                       | 6.5       | 1.0     | 8.8          |
| Average percentage similarity                                   |  | 73.66                                      |           |         |              |
|   | Dissimilarity                          |  |           |         |              |
|   | Av. O% <i>Neomysis integer</i>         | Av. O% <i>Rhopalophthalmus tartessicus</i> | Av. Diss. | Diss/SD | Contrib. (%) |
| <i>Neomysis integer</i> and <i>Rhopalophthalmus tartessicus</i> |  |  |           |         |              |
| Total pollen  | 88.2                                   | 13.8                                       | 6.0       | 2.3     | 14.5         |
| Total rotifers  | 58.7                                   | 1.2  | 6.0       | 1.9     | 14.4         |
| Colonial phytoplankton  | 86.3                                   | 14.8                                       | 5.9       | 2.0     | 14.2         |
| Macrophytal detritus  | 96.3                                   | 21.7                                       | 5.3       | 2.1     | 12.9         |
| Unicellular phytoplankton                                       | 34.7                                   | 2.1  | 4.4       | 2.4     | 10.7         |
| Others  | 29.8                                   | 2.2  | 3.7       | 1.5     | 8.9          |
| <i>Mesopodopsis slabberi</i> juveniles                          | 32.0                                   | 59.4                                       | 3.6       | 1.2     | 8.6          |
| Harpacticoids   | 14.7                                   | 0.0  | 2.0       | 0.8     | 4.8          |
| Cladoceran <i>Daphnia galeata</i>                               | 19.0                                   | 1.0  | 1.7       | 0.6     | 4.2          |
| Average percentage dissimilarity                                | 41.5                                   |  |           |         |              |

abundant than copepods, their gravimetric contribution to their diet was probably the most important among all animal prey.

#### 4. Discussion

*Neomysis integer* and *Rhopalophthalmus tartessicus* differ widely in their foraging preferences in the Guadalquivir estuary. This dietary segregation appears to be driven largely by feeding choices as even during occasions when the populations were spatially overlapping, consumed prey differences remained. Both mysid species shared the juvenile of another mysid species (*Mesopodopsis slabberi*) as a prey item suggesting strong interactions within the guild. The diet composition of *N. integer* and *R. tartessicus* in the Guadalquivir Estuary was analysed only for adult individuals of similar size irrespective of sex, so that neither possible ontogenic shifts in diet, reported for other mysid species (Siegfried and Kopache, 1980; Viherluoto et al., 2000; Froneman, 2001), nor sexual differences (although not observed in a previous study by Focke and Mees, 1999) could be ruled out. Generally, since gut passage time reported for mysids is on the order of 30–90 min, the particles present in the stomach provide a good measure of recently ingested food (Zagursky and Feller, 1985). However, rigid zooplankton parts (e.g., mandibles) can stay in the stomachs for more than 12 h (Rudstam et al.,

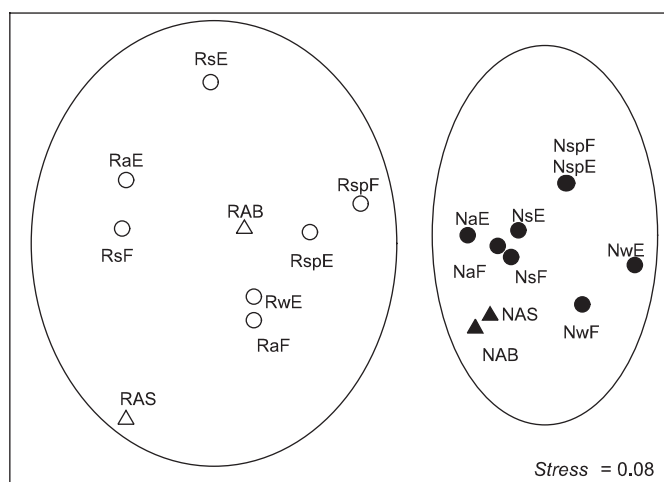


Fig. 3. Multidimensional scaling (MDS) ordination of Bray–Curtis similarity matrix based on prey frequency of occurrence (%) data (square root transformed) for *Neomysis integer* (black) and *Rhopalophthalmus tartessicus* (white). Cluster groupings at the 80% similarity level are superimposed. N, *N. integer*; R, *R. tartessicus*; sp, spring; s, summer; a, autumn; w, winter; F, flood; E, ebb; S, surface; B, bottom; A, autumn 2002. Samples during the overlapping situation of October 2002 shown as triangles.

Table 4  
For each depth, mean frequency of occurrence (%) of each item found in *Neomysis integer* and *Rhopalophthalmus tartessicus* stomachs at sampling site Bonanza in October 2002. The mean numerical proportion per stomach (%) (excluding unidentified detritus and macrophytal) is also given in brackets. Major prey categories in bold gathering items above

| Prey items                             | <i>Neomysis integer</i> |                  | <i>Rhopalophthalmus tartessicus</i> |                    |
|--|-------------------------|------------------|-------------------------------------|--------------------|
|  | Bonanza                 |                  | Bonanza                             |                    |
|  | Surface                 | Bottom           | Surface                             | Bottom             |
| <b>Unidentified detritus</b>           | <b>100</b>              | <b>100</b>       | <b>20</b>                           | <b>77.8</b>        |
| <b>Macrophytal detritus</b>            | <b>100</b>              | <b>80</b>        | <b>10</b>                           | <b>44.4</b>        |
| <b>Colonial phytoplankton</b>          | <b>80 (35.9)</b>        | <b>50 (33.6)</b> | <b>0 (0.0)</b>                      | <b>11.1 (90.9)</b> |
| <i>Mesopodopsis slabberi</i> juveniles | <b>60 (3.6)</b>         | <b>50 (4.0)</b>  | <b>50 (16.2)</b>                    | <b>77.8 (2.2)</b>  |
| <i>A. clausi</i>                       | 50 (7.9)                | 60 (7.3)         | 60 (67.6)                           | 77.8 (5.0)         |
| <i>A. robustus</i>                     | 40 (3.1)                | 20 (4.0)         | 0 (0.0)                             | 0 (0.0)            |
| <b>Pelagic copepod</b>                 | <b>80 (11.0)</b>        | <b>80 (11.3)</b> | <b>60 (67.6)</b>                    | <b>77.8 (5.0)</b>  |
| <b>Benthic copepod</b>                 | <b>40 (4.6)</b>         | <b>60 (7.3)</b>  | <b>0 (0.0)</b>                      | <b>0 (0.0)</b>     |
| <b>Nauplii</b>                         | <b>10 (6.1)</b>         | <b>0 (0.0)</b>   | <b>0 (0.0)</b>                      | <b>0 (0.0)</b>     |
| <i>K. quadrata</i>                     | 10 (6.1)                | 0 (0.0)          | 0 (0.0)                             | 0 (0.0)            |
| <i>Brachionus</i>                      | 20 (6.1)                | 10 (4.0)         | 0 (0.0)                             | 0 (0.0)            |
| <b>Rotifers</b>                        | <b>20 (12.2)</b>        | <b>10 (4.0)</b>  | <b>0 (0.0)</b>                      | <b>0 (0.0)</b>     |
| Angiosperm pollen                      | 70 (6.5)                | 50 (9.6)         | 0 (0.0)                             | 11.1 (1.9)         |
| Gymnosperm pollen                      | 40 (5.3)                | 30 (8.0)         | 0 (0.0)                             | 0 (0.0)            |
| <b>Pollen</b>                          | <b>70 (11.9)</b>        | <b>50 (17.6)</b> | <b>0 (0.0)</b>                      | <b>11.1 (1.9)</b>  |
| <i>Pediastrum</i>                      | 0 (0.0)                 | 10 (4.0)         | 0 (0.0)                             | 0 (0.0)            |
| <i>Staurastrum</i>                     | 0 (0.0)                 | 10 (4.0)         | 0 (0.0)                             | 0 (0.0)            |
| <i>Spirulina</i>                       | 10 (3.1)                | 0 (0.0)          | 0 (0.0)                             | 0 (0.0)            |
| <b>Unicellular phytoplankton</b>       | <b>10 (3.1)</b>         | <b>20 (8.0)</b>  | <b>0 (0.0)</b>                      | <b>0 (0.0)</b>     |
| <i>Halacaridae</i>                     | 10 (3.1)                | 10 (4.0)         | 0 (0.0)                             | 0 (0.0)            |
| Nematod                                | 60 (8.6)                | 60 (10.0)        | 10 (16.2)                           | 0 (0.0)            |
| <b>Others</b>                          | <b>60 (11.7)</b>        | <b>70 (14.0)</b> | <b>10 (16.2)</b>                    | <b>0 (0.0)</b>     |

1989). Hence, counting the number of rigid prey parts present in the stomach could result in an overestimation of the actual number of copepods and mysids consumed.

*Neomysis integer* always showed higher FI than *Rhopalophthalmus tartessicus*, especially when detritus contributed to a high extend to the diet (spring and winter). Furthermore, the unique high FI for *R. tartessicus* occurred in winter, when both unidentified and macrophytal detritus were

consumed in a larger proportion. Such higher stomach fullness could be due to the combined effect of higher detritus content (winter and spring are rainy season in the region) and lower availability of other food resources in winter. Therefore, at least in this study and especially for *R. tartessicus*, FI are more related to the detritus content in the stomachs than with a real estimation of feeding patterns concerning other prey. For *N. integer* in the Guadalquivir estuary, we found

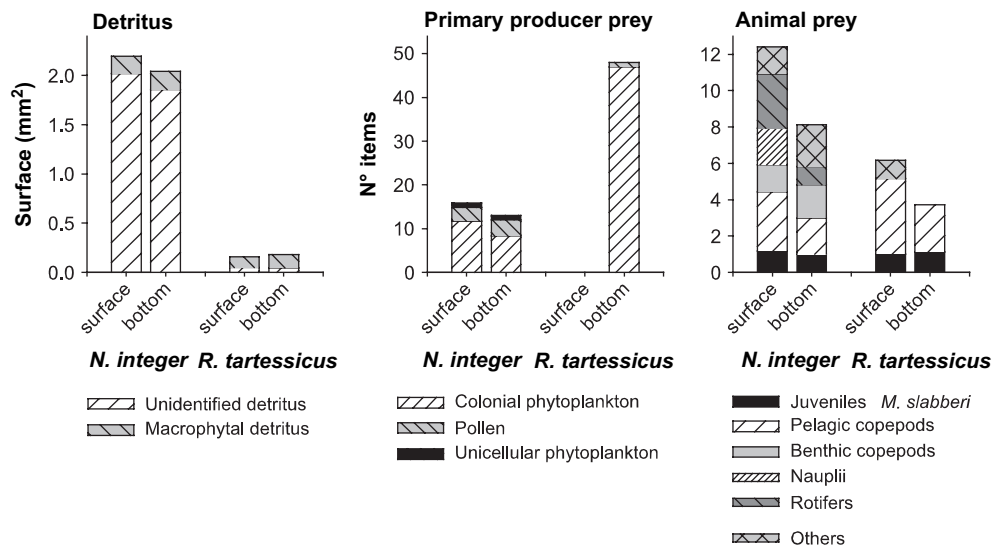


Fig. 4. Quantitative diet composition of *Neomysis integer* and *Rhopalophthalmus tartessicus* by depth at Bonanza in October 2002. Detritus was estimated as the mean surface area (mm<sup>2</sup>); primary producers and animal prey were estimated as mean number per stomach.



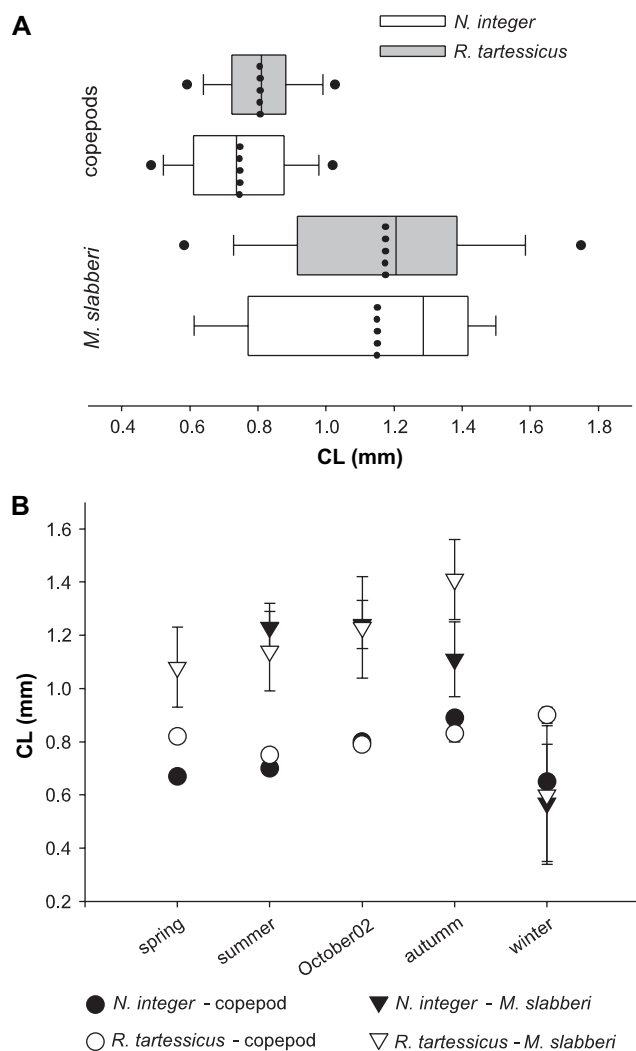


Fig. 5. Box-plot graph (A) and seasonal trends (B) in size of copepods and juvenile *Mesopodopsis slabberi* (cephalotorax and carapace length, CL, in mm, respectively) ingested by *Neomysis integer* (white) and *Rhopalophthalmus tartessicus* (grey). Box boundaries, 25th and 75th percentiles; black line, median; dotted line, mean; whiskers, 90th and 10th percentiles.

higher FI and higher detritus presence than in individuals from other European populations (Fockedey and Mees, 1999).

Numerous examples show a significant contribution of detritus in the diet of mysids (Lasenby and Langford, 1973; Foulds and Mann, 1978; Mauchline, 1980; Johnston and Lasenby, 1982; Webb and Wooldridge, 1989; Mees et al., 1995; Kibirige and Perissinotto, 2003), suggesting that mysids can be responsible for the remineralisation of an important proportion of the non-refractory detritus (Kost and Knight, 1975; Jansen, 1985). The great quantity and regularity of detrital items in *Neomysis integer* stomachs could show a selective ingestion of detritus, contrasting with the accidental ingestion suggested in other estuarine populations (Fockedey and Mees, 1999). Using stable isotopes, Winkler et al. (2007) found suspended organic matter was unlikely to have an important role in the nutrition of mysids. But the presence of organisms such as yeast cells, nematods and *Euglena* in some stomachs of *N. integer* indicate the use of the microbial

loop, which is typical of net heterotrophic systems (high dependence on allochthonous input of organic matter) such as estuaries (Sintes et al., 2004) and was also recently observed for fish larvae (Pepin and Dower, 2007). Conversely to *N. integer*, presence of detritus in *Rhopalophthalmus tartessicus* could be an accidental uptake, except in winter when, due to the scarcity of resources, they could consume detritus selectively. In that sense, consumption of macrophytal detritus, particularly by *N. integer*, suggests that it can serve as a nutritionally significant food item, especially in periods of low availability of other nutritionally more valuable food (Foulds and Mann, 1978; Wainwright and Mann, 1982; Zagursky and Feller, 1985). A similar diet shift in mysids according to food availability was previously reported (Viherluoto et al., 2000; Winkler et al., 2007). That is, in order to complete their diet, mysids show a more opportunistic behaviour when resources are more limited.

Mysids are able to digest pollen grains (Rudstam et al., 1989), a food item also ingested by mysids from other populations (Fockedey and Mees, 1999; Viherluoto et al., 2000). According to David et al. (2006), terrestrial plant and bacteria may contribute substantially to estuarine particulate organic matter composition (<200  $\mu\text{m}$ ), with phytoplankton being the smallest component. Furthermore, *Neomysis integer* and *Mesopodopsis slabberi* are able to assimilate these types of food items, although they preferred phytoplankton vs. vegetal detritus and bacteria in their diet (David et al., 2006). Phytoplankton contribution to diet was not gravimetrically evaluated, but it was found in the stomachs of both species, especially in those of *N. integer*. Even though it would have a low quantitative importance, phytoplankton could be relevant qualitatively because its oligo-elemental contribution (Fockedey and Mees, 1999). Although unicellular algae are generally more abundant in the water column than colonial algae, both *N. integer* and *Rhopalophthalmus tartessicus* consumed colonial algae more frequently, representing size selection towards larger particles.

Temporal replacement of species in the Guadalquivir estuary zooplankton (Guisande et al., 1986) was apparent in the consumption patterns of studied mysid species, suggesting selection depending on availability. Generally, most important animal prey items for each species were those which were abundant in their respective estuarine zones: for *Rhopalophthalmus tartessicus*, *Mesopodopsis slabberi* juveniles and copepods (*Acartia clausi*); for *Neomysis integer*, zooplanktonic species typical of the oligohaline area, such as rotifers, cladocerans and the copepod *Calanipeda aqua-dulcis*. Furthermore, *N. integer* consumed *M. slabberi* juveniles mainly when both prey and predator showed overlapping distributions (at oligomesohaline zones during summer and autumn). Similar positive correlations between prey densities and predation rates by mysids have been reported in other estuaries (Jerling and Wooldridge, 1995; Winkler et al., 2007).

Some abundant potential prey species were never or rarely ingested. As examples, the harpacticoid copepod *Euterpina* sp., abundant in the more marine zone of the estuary (Guisande et al., 1986) or *Acanthocyclops robustus*, an abundant

freshwater cyclopoid copepod, only consumed by both species in winter, when freshwater input likely swept it away to the outer estuary. Low predation on these species could be due to their higher escape response, as suggested by Fockedey and Mees (1999). Copepod nauplii are also scarce in mysid stomachs (Siegfried and Kopache, 1980; Bowers and Vanderploeg, 1982; Fockedey and Mees, 1999). Several hypotheses could explain such scarcity: (1) positive size selection by adult mysids towards bigger size stages (copepodites and adults) which are energetically more valuable; (2) more successful avoidance of the mysid feeding current by nauplii than by later copepod life stages (Fockedey and Mees, 1999); or (3) quick digestion of ingested nauplii, because of their small size, leading to underestimated ingestion rates by stomach content analyses (Rudstam et al., 1989).

In general, *Neomysis integer* presented a more omnivore and opportunistic diet than *Rhopalophthalmus tartessicus*, the latter with more tendencies to carnivory and specialization. Both species often consume the same type and size of prey, especially copepods and mysids, probably far more gravimetrically important in their diet. *A priori*, this feature suggests a high inter-specific competition for resources. However, the more flexible and opportunistic diet of *N. integer* combined with a higher osmoregulatory capacity at low salinities allows this mysid species to successfully colonize the oligohaline region of the estuary, where *R. tartessicus* can not survive (Vilas et al., 2006). In this way, *N. integer* avoids both direct resource competition and predation of their juveniles (similar size and look as *Mesopodopsis slabberi* juveniles, C. Vilas unpublished data) by adults of *R. tartessicus*. Moreover, the oligohaline region usually coincides with the maximum turbidity zone, where accumulation of detritus particles and other fluvial resources occurs, which means an increase in prey diversity for *N. integer*. In other estuaries, *N. integer* also chose high turbidity water masses for feeding, avoiding resource competition with other mysid species by spatial segregation (Parker and West, 1979; Köpcke and Kausch, 1996). A similar food niche-partitioning to reduce competition was found for *Neomysis americana* and *Mysis stenolepsis* (Winkler et al., 2007) in the St. Lawrence Estuary, and in the Baltic Sea, with *Mysis mixta* eating more zooplankton and pelagic material than *Mysis relicta*, influencing their spatial distribution (Viherluoto et al., 2000).

Results obtained in the Guadalquivir Estuary are in agreement with the general description of mysids as omnivorous organisms. They are micro and mesozooplankton consumers (Mauchline, 1980; Siegfried and Kopache, 1980), capable of feeding on a high variety of heterotrophic food items and adapting their diet according to prey availability (Wooldridge and Bailey, 1982; Wooldridge and Webb, 1988; Jerling and Wooldridge, 1995; Winkler et al., 2007). Consequently, they could have important implications for the trophic structure of estuarine ecosystems (Kibirige et al., 2003). Diet composition of *Neomysis integer* from the Guadalquivir was similar to those reported for other populations of this species (Vorstman, 1951; Kinne, 1955; Mauchline, 1971; Parker and West, 1979; Aaser et al., 1995; Fockedey and Mees, 1999). Also

*Rhopalophthalmus tartessicus* diet is comparable to that described for *Rhopalophthalmus terranatalis* and *Rhopalophthalmus africana*, both African species with similar habitats and life histories (Wooldridge and Bailey, 1982; Kouassi et al., 2006). These three *Rhopalophthalmus* species have a largely carnivorous diet and exhibit a similar unusual swimming behaviour, with the ventral side facing upwards. Since such swimming behaviour could allow mysids to see the prey against the light, it could represent an evolutionary advantage in the attack–capture process (see video in Appendix 3 of Supplementary material).

Moreover, both *Neomysis integer* and *Rhopalophthalmus tartessicus* prey on *Mesopodopsis slabberi* juveniles, suggesting age-structured intraguild predation. A pattern of intraguild predation (IGP) among mysids had only been reported before for *Rhopalophthalmus terranatalis* on *Mesopodopsis wooldridge* (Wooldridge and Bailey, 1982; Wooldridge and Webb, 1988) and for *Praunus flexuosus* on *N. integer* (Winkler and Greve, 2004). IGP is a widespread trophic interaction with a potential impact on community structure and stability (Arim and Marquet, 2004; Bascompte and Melian, 2005). In this case, it could be a determinant factor of its intermediate spatial position in the estuary, just between *N. integer* and *R. tartessicus* populations (Vilas et al., 2006). Since *M. slabberi* and, to a lower extent, *N. integer* and *R. tartessicus* are main prey for the estuary nursery function (Baldó and Drake, 2002; Drake et al., 2002, 2007; Fernandez-Delgado et al., 2007), this interaction in the Guadalquivir food web might be relevant to community stability and presents an interesting future line of research.

The ecological relevance of mysids has been reported in many other estuaries, where they are key prey for many young stages of fishes and crustaceans (Sorbe, 1981; Elliott et al., 2002). Due to the regular availability of detritus in estuaries, fish production may be periodically subsidized by the detritus food chain, in which mysids are important links (Elliott et al., 2002). In Guadalquivir estuary, *Neomysis integer* may play an important role in linking macrophyte and detrital production (due to its ability to feeding benthically and to feed on detritus) to higher trophic levels. *N. integer* and *Rhopalophthalmus tartessicus* (largest and most carnivorous mysid species in the estuary) serve as an important link between their small zooplankton prey (e.g. rotifers, copepods and *Mesopodopsis slabberi* juveniles) and its predators (e.g. fishes and crustaceans), by converting micro- and mesozooplankton to big zooplankton prey. Consequently, different stages of mysids species in this estuary, considered as a whole, provide a relatively wide spectrum of prey size (40 µg for *M. slabberi* juveniles to 4316 µg for adults *R. tartessicus*), which might be an essential part of the estuarine nursery function. In fact, the ontogenic shift in the diet of the most abundant fishes in the Guadalquivir estuary suggests a change from *M. slabberi* ingestion to *R. tartessicus* ingestion as they grow (Baldó and Drake, 2002).

Feeding behaviour and consequent intra-guild interactions, in combination with physiological capabilities, determine the spatial distribution and structure of the mysid community in the estuary. Their abundance, relevance as a key link in the food web and their potential power to stabilise zooplankton

communities make mysids a key guild for the stability and maintenance of estuarine ecosystems and the marine communities which depend on its nursery function.

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## Appendix. Supplementary information

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ecss.2007.09.025](https://doi.org/10.1016/j.ecss.2007.09.025).

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