Field distribution and osmoregulatory capacity of shrimps in a temperate European estuary (SW, Spain)

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Abstract

The spatial distribution of the six most common crustacean decapods in the benthos and hyperbenthos of the Guadalquivir estuary (SW, Spain) have been analysed in relation to their osmoregulatory capacities (at 20 °C). Field densities along the estuarine salinity gradient revealed that, although the species studied showed salinity tolerance ranges in the field that do overlap to some extent, there was a considerable spatial and/or salinity related segregation between several of them. Concerning their isosmotic points and their osmoregulatory salinity ranges, two main groups of species were distinguished: species with higher isosmotic points and tight ranges in osmoregulation, represented by marine species that entered the estuary from open sea and remained there for only part of the year (*Crangon crangon*, *Melicertus kerathurus* and *Palaemon serratus*); and those with slightly lower isosmotic points and wide ranges in osmoregulation, represented by estuarine species which completed their life cycle in brackish water (*Palaemon longirostris, Palaemon macrodactylus* and *Palaemonetes varians*). For all the species studied, their field distributions were clearly biased towards the lower end of the salinity ranges within which they osmoregulate. Nevertheless, individuals of the less euryhaline species (*Melicertus kerathurus* and *Palaemon serratus*) were mainly found in less saline water when the estuarine gradient was displaced downstream and low salinities occurred close to the river mouth.

Keywords: crustacean decapod; estuary; osmoregulation; isosmotic-point; field distribution; SW Spain, Guadalquivir Estuary.

1. Introduction

Estuarine environments are probably among the most exciting and stressful aquatic biotopes, where abiotic variables such as salinity can abruptly change across both spatial and temporal scales. Hence, since organisms need to maintain specific osmotic gradients between their body fluids and the ambient medium to remain in good physiological condition, their ability to tolerate osmotic stress can be considered as a mandatory requirement for their establishment and maintenance in estuaries (Péqueux, 1995). Euryhalinity is, consequently, a mandatory characteristic in estuarine inhabitants (Kinne, 1971). Nevertheless, under the extreme conditions existing in these ecosystems, slight differences in osmoregulatory capability among species may have large influence on the estuarine community structure. That is why salinity is often the main environmental factor governing to a large extent the horizontal distribution of marine, brackish and freshwater invertebrates living in estuaries (Mees and Hamerlinck, 1992; Cunha et al., 1999; Drake et al., 2002).

Previous studies of the osmoregulatory ability of crustaceans have revealed that species inhabiting estuaries display several patterns of osmoregulation (Péqueux, 1995). Such interspecific differences may occur even in closely related species of the same genus. Furthermore, intraspecific differences in osmoregulatory abilities of populations from different habitats have also been reported for several estuarine crustacean decapods (Kinne, 1971; McLusky et al., 1982). Hence, provided they persist enough, human alterations of the original salinity gradient of an estuary (because freshwater management from dams, for example) could lead to changes in the osmoregulatory abilities of their aquatic populations.

The Guadalquivir estuary (southern Europe) is a vertically well-mixed system, but with a gradual horizontal salinity gradient (Vannéy, 1970). Because the region is relatively dry, it experiences periods of drought, which may last several years. During these dry periods, the freshwater flow is completely controlled at the dam (110 km upstream) causing a greater saltwater intrusion. Furthermore, the freshwater input to the estuary is usually increased in late spring-early summer and early autumn to permit irrigation of cultivated land along the course of the river. This man-depending extra freshwater input during the summer causes an unnatural salinity reduction during the warm period. Among its macrofauna, there are twenty five decapod crustacean species but only a few of these are dominant in terms of density; the shrimps *Crangon crangon*, *Palaemon longirostris, Palaemon macrodactylus*, *Palaemon serratus* and *Palaemonetes varians*, and the prawn *Melicertus kerathurus* together comprise more than 99 % of total individuals (Cuesta et al., 2005). All dominant species are native to European waters, except *Palaemon macrodactylus*; this is an alien species, recently introduced in European estuaries (Ashelby et al., 2004; Cuesta et al., 2004; d´Udekem d´Acoz et al., 2005), whose original distribution was around Japan and along the northern coast of China and Korea (Newman, 1963).

This paper analyses the field salinity-related distribution of the six most abundant prawns and shrimps of the Guadalquivir estuary in relation to their osmoregulatory capacities.

2. Materials and methods

2.1 *Studied populations*

The studied populations of *Crangon crangon*, *Palaemon longirostris, Palaemon macrodactylus*, *Palaemon serratus, Palaemonetes varians* and *Melicertus kerathurus* inhabit the Guadalquivir Estuary (SW Spain: 36° 45'- 37° 15' N, 6° 00'- 6° 22' W). This is a vertically mixed estuary but with a gradual horizontal change in salinity, except when there is a considerable increase in river flow from heavy rainfall, and freshwater almost reaches the mouth of the estuary (Drake et al., 2002). In addition, to permit the irrigation of cultivated fields along the river, during the warm season, there are sudden increases of the freshwater inflow (from a dam 110 km upstream) that cause a considerable decline in salinity (Baldó et al., 2005). Under such environmental conditions, *P. serratus* and C*. crangon* are marine species that use the estuary both as nursery and adult-feeding grounds, while *M. kerathurus* only uses it as nursery ground; In contrast, *P. longirostris, P. macrodactylus* and *P. varians* are permanent residents of the Guadalquivir Estuary, where they complete their life cycles (Cuesta et al., 2005).

2.2 Shrimp collection

Field salinity-related distribution of studied species were estimated by monthly sampling (at each new moon), between May 1997 and January 2005, at three sampling stations. The stations, locally known as Tarfía, La Esparraguera and Bonanza, are situated at 32, 20, and 8 km up-stream from the river mouth, respectively (Figure 1). Samplings were conducting from a boat anchored on the East bank of the Guadalquivir Estuary and equipped with a local fishing gear: length 10 m, opening area 7 x 3 m, and mesh size 1 mm. At each sampling date, four samples were taken during 24 h at each site. Each

sample consisted of a passive haul made during the first 2 hours of diurnal/nocturnal flood and ebb tides. During sampling, the current speed was measured (digital flow meter HYDRO-BIOS, 438 110) and later used to calculate sampled water volumes. Water temperature and salinity (Refractometer ATAGO S/ Mill) were also measured. Samples were fixed in 5 % formalin immediately after collection; in the laboratory, individuals were sorted into species and counted. Since only adults were used in osmoregulation experiments (see next section), juveniles were discarded by counting only individuals retained in a 5 mm light mesh sieve. In the case of *M. kerathurus*, due to the larger size of this species, individuals retained by the sieve were young stages.

(←Fig. 1)

2.3 Osmolality experiments

To estimate the osmoregulatory capacities of the species studied, specimens collected in the estuary were carried alive to the laboratory in the water in which they had been collected. Specimens of *M. kerathurus*, *C. crangon* and *P. serratus* were collected from the lower reaches at salinities between 20 and 30; while *P. longirostris*, *P. macrodactylus* and *P. varians* were collected from the upper reaches at salinities between 6 and 15. For these experiments, individuals were collected between May and September 2004 (the water temperature: 20-24 °C). In the laboratory, animals were kept in aerated aquaria at the salinity of the water in which they had been collected and at 20 (±1) ºC (annual average temperature at the estuary), under natural light and dark conditions, and daily supplied with commercial dry pellets (Trow España).

The day before hemolymph extraction, specimens were not fed. Only adult males and females in the intermoult condition were used in osmoregulation experiments, except for the prawn *M. kerathurus*. This species only uses the estuary as nursery area of its young stages and, consequently, its osmoregulatory capability was estimated in juveniles.

After a 24 hour acclimation period, animals were transferred from the original salinity to salinities of 2, 5, 15, 25 and 35. When original and target salinities differed in more tan 5 units, individuals were gradually (5 units per 12 hr) transferred until the target salinity was reached. Sea water, diluted with distilled water, was used in experiments at different salinities. The osmotic concentration of the hemolymph was determined after 24 hours of acclimation to the experimental salinity. From each shrimp, a single hemolymph sample was extracted from the pericardial cavity by puncturing the pericardium with a 50 µl syringe. Hemolymph and water osmolalities were measured using a Wescor 5520 vapour pressure osmometer. The carapace length (CL) of animals, measured from postorbital to posterior carapace edge, was estimated after extracting hemolymph.

2.4 Data Analysis

Constancy of each species is estimated as the percentage of months in which a species was collected at each sampling site, and its field density is expressed as the number of individuals per 100 m^3 of water filtered. For each sampling date, spatial and salinity-related distributions of each species are expressed as both the distance (D) from the river mouth (upstream) and salinity

value (S) at which the centre of mass ($_{cm}$) of its estuarine distribution was located:

$D_{cm} = \Sigma\ p_i\ D_i$ and $S_{cm} = \Sigma\ p_i\ S_i$

where p_i is the proportion of individuals collected at the sampling i, and D_i and S_i the distance and salinity respectively at which the sample was taken (expressions derived from Fortier and Leggett, 1982).

Since water temperature showed considerable seasonal changes, monthly estimates of D_{cm} and S_{cm} were grouped in three different sets according to water temperature (T): $T < 18$ °C, $18 \leq T \leq 22$ °C and $T > 22$ °C.

For the range of salinity tested in osmoregulation experiments (2-35), the relationship between hemolymph and water osmolalities was examined by least-squares linear regression analysis. Depending on the osmoregulatory pattern shown by each species, one, two or three regression equations were separately calculated for each of the different portions observed in the hemolymph osmolality curve. Then, isosmotic points were obtained directly from the regression line intersections with the isosmotic lines.

In this study, shrimp and prawn field distributions were analysed in relation to the estuarine water salinity, while hemolymph osmolalities were referred to the medium osmolality. For convenience, all through the text water osmolality (mmol Kg^{-1}.) was converted to salinity (S) according to the equation:

Osmolality = -23.22 + 27.95 x Salinity (R= 0.999; N=30).

3. Results

3.1 Environmental conditions

Water temperature showed a consistent seasonal pattern all through the studied period, while salinity only did so in rainy years (Figure 2). Spatially, at each sampling date, temperature was quite homogenous throughout the estuary, while salinity displayed a clear horizontal gradient between sampling sites (Figure 3). According to average salinities, the two outer sampling stations were located in the polyhaline and mesohaline zones, respectively; while the inner sampling station was between the mesohaline and oligohaline stretches. In addition, at each sampling date, tidal salinity changes (measured as increase or decrease of salinity per hour) were about four times greater at the outer station (8 km from river mouth) than at the inner one (32 km), on average. This inter-station pattern was consistently observed despite that some intra-station differences existed among the cold (T < 18°C), intermediate (18 °C < T < 22°C) and warm (T < 22 °C) periods (Figure 3). Namely, maximal tidal salinity changes tended to occur in spring and autumn at the outer sampling station and minimal in the warm period at the inner sampling station.

(←Fig. 2)

(←Fig. 3)

3.2 Spatial and salinity-related distribution

From May 1997 to January 2005, the constancy of the species studied along the estuary displayed three different spatial patterns (Table 1): a) increasing values from the inner sampling sites to the outer estuarine zone (*M. kerathurus, P. serratus* and *C. crangon*); b) decreasing values from the inner sampling sites to the outer estuarine zone (*P. macrodactylus* and *P. varians*);

and c) values that remained more or less constant throughout the study zone (*P. longirostris*). For the two first groups of species, the constancy changed evenly along the estuary, except for *P. varians* whose constancy dropped abruptly at the outer sampling site.

(←Table 1)

To illustrate the spatial and salinity-related distributions of studied species within the estuary, the average abundance of each species at each sampling site has been represented by salinity intervals (Figure 4). It is important to keep in mind that, at the inner sampling site (Tarfía), water salinity never reached values above 17. Although there were some additional interspecific differences, two broad categories of species can be distinguished with respect to their spatial and salinity-related distributions: species that were more abundant in the outer part of the estuary and showed maximal densities at intermediate values of salinity, such as *M. kerathurus, P. serratus, C. crangon* and *P. longirostris*; and species whose density slightly increased up-stream and showed peak values at the lowest salinities, such as *P. macrodactylus* and *P. varians*.

(←Fig. 4)

Irrespective of water temperature, the temporal variation in salinity-related distribution (measured as S_{cm}) was considerable for all studied species (Figure 5). Conversely, when the spatial distribution was taken into account (measured as D_{cm}), two different patterns again arose: *M. kerathurus, P. serratus* and, to a less extent, *C. crangon* were confined to the outer estuary throughout the year, while the other three species showed a wider estuarine

distribution (Figure 5). In addition, although with some intra-specific differences depending on water temperature, there seems to be a general pattern of partial segregation of the different species along the estuary (inter-specific shift in D_{cm}) and/or salinity gradient (inter-specific shift in S_{cm}).

(←Fig. 5)

In summary, salinity-related distributions found in the field confirmed that the six species studied are euryhaline. Nevertheless, while *M. kerathurus*, *P. serratus* and *C. crangon* correspond to marine species which enter the estuary and remain more or less associated with salty water and/or confined to its outer zone. *P. longirostris*, *P. macrodactylus* and *P. varians* are genuine estuarine species largely associated with brackish waters and with a wider spatial distribution within the estuary.

3.3 Osmotic regulation

Concerning the curve which describes the relationship between hemolymph and water osmolalities of each species, three distinct patterns of hypo/hyperosmoregulation were observed (Figure 6): species whose curve can be described by a single regression equation over all the tested salinity range (*P. longirostris, P. macrodactylus* and *P. varians*); species whose curve can be divided into two portions and two regression equations were necessary to describe their osmoregulation pattern (*M. kerathurus* and *P. serratus*); and species whose curve can be divided in three portions (*C. crangon*) and three regression equations were required to describe their osmoregulation pattern (Table 2 and Figure 6).

(←Fig. 6)

(←Table 2)

The shrimps *P. longirostris, P. macrodactylus* and *P. varians* were extremely efficient hyper-hypo-osmoregulators over all the experimental salinity range (Figure 6). Thus, their hemolymph was maintained hyperosmotic to the medium in salinities below 21 and hypo-osmotic at external salinities higher than 23. To be more exact, their isosmotic points were 21.5, 21.7 and 22.5, respectively.

The hemolymph concentration of *P. serratus* was practically independent of changes in the medium, at salinities between 16 and 35, remaining constant at about 25 (Figure 6). Similarly, the hemolymph osmolality of *M. kerathurus* was maintained at about 28 in medium salinities between 19 and 35. In contrast, the hemolymph of both species was hyperosmotic and roughly paralleling the iso-osmotic line at salinities between 3 and 16, for *P. serratus*, and between 4 and 19, for *M. kerathurus*. It should also be noted that *P. serratus* and *M. kerathurus* individuals showed a significantly reduced vitality at 3 and 10 of salinity, respectively.

In salinities between 29 and 35, the hemolymph of *C. crangon* was maintained hypo-osmotic to the medium and paralleling the iso-osmotic line (Figure 6). In contrast, the hemolymph concentration was practically independent of changes in the medium in salinities between 13 and 29, remaining almost constant at about 26. Finally, in salinities between 3 and 13, the hemolymph was also maintained hyperosmotic, but its osmolality showed a significant decrease as the medium salinity went down.

When the osmoregulatory capability was expressed as change in hemolymph osmolality as a function of water osmolality (☜hemolymph

osmolality/water osmolality), among the estuarine species, *P. longirostris* was the strongest regulator (0.10), followed by *P. varians* (0.13) and *P. macrodactylus* (0.17). Similarly, the marine species were also strong regulators (0.09 for *P. serratus* and *M. kerathurus*; 0.14 for *C. crangon*), but only in a narrower range of external salinity; the extent of the salinity ranges at which *P. serratus*, *M. kerathurus* and *C. crangon* were efficient osmoregulators were 19, 16 and 16 salinity units, respectively. In addition, *C. crangon* seems to go on osmoregulating at salinity values between 3 and 13, although it presents a less efficient osmoregulatory capability (0.46).

3.4 Field distribution and osmoregulatory capacity

Since a synergistic relationship between temperature tolerance and salinity tolerance of estuarine species could exist, and the osmoregulatory capabilities of prawns and shrimps were tested at 20 °C, only field distributions at intermediate temperatures (18 to 22 °C; mean: 19.9 °C) are considered in this section (Figure 7).

(←Fig. 7)

All through the tested salinity range (3 to 35), the studied populations of *P. longirostris, P. macrodactylus* and *P. varians* displayed an equally strong osmoregulatory capability. However, the three species showed field distributions clearly biased to the less saline extreme of the salinity range within which they osmoregulate. Similarly, *C. crangon*, *P. serratus* and *M. kerathurus* also showed a field distribution clearly biased to lower salinities, even further than expected according to their osmoregulatory capabilities. Nevertheless, they were mainly found in less saline water when the estuarine gradient was

displaced downstream and low salinities occurred close to the river mouth (Figure 5).

Thus, although all studied species tended to be distributed at the less saline extreme of their salinity range of tolerance, a considerable salinity-related and/or spatial segregation was observed between the genuine estuarine species (*P. longirostris, P. macrodactylus* and *P. varians*) and two of the marine species: *P. serratus* and *M. kerathurus*. However, due to its peculiar osmoregulatory pattern (Figure 6), *C. crangon* displayed an intermediate field distribution between the two groups of species.

4. Discussion

As expected, the salinity-related field distribution of the six species studied indicated that all of them were euryhaline species. Nevertheless, as inhabitants of estuaries need to maintain a more or less pronounced hyperosmotic state in the usually dilute estuarine media, a more limited capability in hyperregulation may considerably influence their natural distribution. According to Péqueux (1995), there are two broad categories of euryhaline invertebrates in respect of hyperosmotic regulation: the “strong regulators” which are capable of maintaining their hemolymph osmolality almost constant over a wide range of external salinity values; and the “weak regulators” that only osmoregulate within a narrow range of external salinity values and whose hemolymph osmolality drops, more or less in parallel with the isosmotic line, when the medium salinity falls below a certain limit. Indeed, osmoregulatory patterns exhibited by the studied populations of *P. longirostris, P. macrodactylus* and *P. varians* clearly conformed to the first category, while

those exhibited by *M. kerathurus, P. serratus* and *C. crangon* fitted the second category better. Nevertheless, for salinities below 13, the osmoregulatory capability of *C. crangon* does not decrease sharply (Figure 6), but displays an intermediate osmoregulatory behaviour (line slope: 0.46) between that of estuarine (0.1 – 0.17) and marine species (0.76 – 0.83).

It is worth noting that all species included within each osmoregulatory category exhibit a similar cycle of life in the study area. That is, the first group corresponds to marine species that use the estuary as nursery (*M.* kerathurus, *P. serratus* and *C. crangon*) and adult-feeding grounds (*P. serratus* and C*. crangon*) and, hence, their larval stages were not collected within the estuary; in contrast, *P. longirostris, P. macrodactylus* and *P. varians* are permanent residents of the Guadalquivir Estuary, being their larva present in the estuarine plankton during most the year (Cuesta et al., 2005).

When osmoregulatory patterns of the populations studied were compared to those previously found for other populations of the same species (Panikkar, 1941, 1968; Born, 1968; Spaargaren, 1972; McLusky et al., 1982; Hagerman and Uglow, 1983; Mantel and Farmer, 1983; Campbell and Jones, 1989), it is concluded that all of them conform to the overall osmoregulatory patterns previously described for each species. Even so, there are some intra-specific differences suggesting that the studied populations of *P. serratus* and *C. crangon* could hyper-osmoregulate better within more dilute saline regions than populations from more saline environments. As previously suggested for several crustacean species (McLusky et al., 1982), such differences in osmorregualtion could be phenotypic acclimation responses to different environmental conditions. Nevertheless, they could just be spurious differences

due to variations in experiment protocol and/or biological material between the present and previous studies.

A considerable saving of the energy required for osmoregulation may be obtained by reducing the differences in osmolality between body fluids and medium (Born, 1968). For example, in *C. crangon*, the oxygen consumption is increased in salinities below its isosmotic point irrespective of water temperature, and at low temperature (6 to 13 °C) it also increased at higher salinities (Hagerman, 1970). Despite that, osmoregulatory responses of estuarine species to salinity changes have revealed that almost all species inhabiting estuaries show isosmotic points at salinities considerably higher than those in which they usually live (Guilles and Pequeux, 1983; Mantel and Farmer, 1983). In the Guadalquivir estuary, both estuarine and marine species conform to this general pattern: on most sampling occasions, the centre of mass of their field distributions was situated in more dilute water than their isosmotic points (Figure 6). However, marine species only showed field distributions with their centre of mass in more dilute water when the latter was close to the river mouth (Figure 5). Mobile organisms may penetrate temporally in physiologically unfavourable environments in order to access an additional resource. Marine species make such attempts from their favourable habitat preferably if they are not excessively demanding (Wagner, 1999; Wagner and Austin, 1999). In estuaries, due to the short-term tidal salinity variations, osmotic equilibrium between organism and environment may never be attained (Kinne, 1971). Hence, marine species may reduce osmotic stress by reducing their residence time in physiologically unfavourable salinities. The higher tidal salinity changes per hour in the outer estuary may also produce a shorter

residence time in lower salinities; thus, this could explain why, in the field, the spatial distribution of marine species were more restricted than their salinity-related distribution.

Despite the studied populations of *P. longirostris, P. macrodactylus* and *P. varians* displaying a similarly strong osmoregulatory capability at salinities between 3 and 35, there was a partial segregation by species along the estuary (Figure 3 and 4). In particular, *P. longirostris* was more abundant at the outer estuarine zone and in intermediate salinities, while most of the population of *P. macrodactylus* and *P. varians* were clearly displaced to the inner estuary and were more abundant in lower salinities. When the salinity-related distributions of these species are analysed with regard to their isosmotic points (Figure 6), intuitively, the distribution of *P. longirostris* seems to be slightly more suitable from an energetic point of view (lower osmoregulatory cost). It is important to keep in mind that, of these three strong osmoregulators, *P. longirostris* was the only one living in the main course of the Guadalquivir Estuary until recently. In fact, the typical habitats of *P. varians* are salt marshes, ponds and channels connected to the estuary, where this species is the dominant shrimp; it explains its low density in the studied estuarine habitat, the main course of the river, where higher densities were usually related with rainfall and/or freshwater inflow to the estuary. On the other hand, *P. macrodactylus* is a non-native species which has been introduced recently in the estuary (Cuesta et al., 2004). At present, its population is still increasing and the observed distribution should be considered provisional. Hence, it is still premature to ascertain if this foreign species will or will not be displaced to the oligohaline zone because of competition with its ecologically closer native species (*P. longirostris*).

A synergistic relationship between temperature and osmoregulation ability has previously been reported for several crustacean decapod species, but no general trend of salinity-temperature interaction can be found in osmoregulation studies (Gilles and Péqueux, 1983). While some species show a higher osmoregulatory capability at high temperatures (Weber and Spaargaren, 1970), others can resist salinity changes better at low temperatures (Hagerman and Uglow, 1983; Rupp and Parsons, 2004). Furthermore, among different populations of the same species, one may not show salinity-temperature interaction in its osmoregulation ability (Hagerman and Uglow, 1983), while another may do so (Pannikkar, 1941), suggesting that such effects are phenotypic acclimation responses (Weber and Spaargaren, 1970). Nevertheless, large and quick fluctuations of both factors are generally worse tolerated than such changes of just one factor (Gilles and Péqueux, 1983). In the Guadalquivir estuary, water temperature was always fairly homogenous throughout the estuary and only short-term (tidal) salinity changes occurred. Conversely, water temperature showed a remarkable and consistent seasonal pattern (11 °C in late winter to 28 °C in late summer), while the seasonal salinity trend may change from one year to another. Furthermore, because of freshwater input for agriculture field irrigation, a sudden and "unexpected" decline in salinity took place during the warm period (Figure 2). In this study, osmoregulation experiments were carried out at a constant temperature (20 °C); thus the existence, or non-existence, of synergic temperature-salinity effects in the osmoregulatory capabilities of the populations studied cannot be assessed. However, under such an inter-yearly variable seasonal trend in water temperature vs. salinity, it is reasonable to expect that

permanent residents (estuarine species) would not have developed synergic temperature-salinity responses in osmoregulation. In contrast, for marine species, which mainly enter the estuary in the warm period, a more effective hyperregulation at high rather than at low temperatures would be expected.

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References

Ashelby, C.W., Worsfold, T.M., Fransen, C., 2004. First records of the oriental prawn *Palaemon macrodactylus* (Decapoda : Caridea), an alien species in European waters, with a revised key to British Palaemonidae. Journal of the Marine Biological Association of the United Kingdom 84, 1041-1050.

Baldó, F., Cuesta, J.A., Fernández-Delgado, C., Drake, P., 2005. Efecto de la regulación del caudal del río Guadalquivir sobre las características fisicoquímicas del agua y la macrofauna acuática de su estuario. Ciencias Marinas, 31, 467-476.

Born, J.W., 1968. Osmoregulatory Capacities of 2 Caridean Shrimps *Syncaris Pacifica* (Atyidae) and *Palaemon macrodactylus* (Palaemonidae). Biological Bulletin 134, 235-244.

Campbell, P.J., Jones, M.B., 1989. Osmoregulation of the Estuarine Prawn *Palaemon longirostris* (Caridea, Palaemonidae). Journal of the Marine Biological Association of the United Kingdom 69, 261-272.

Cuesta, J.A., González-Ortegón, E., Rodríguez, A., Baldo, F., Vilas, C., Drake, P., 2005. The Decapod Crustacean community of the Guadalquivir Estuary (SW Spain): seasonal and inter-year changes in community structure. Hydrobiologia, in press.

Cuesta, J.A., González-Ortegón, E., Drake, P., Rodriguez, A., 2004. First record of *Palaemon macrodactylus* Rathbun, 1902 (decapoda, caridea, palaemonidae) from European waters. Crustaceana 77, 377-380.

Cunha, M.R., Sorbe, J.C., Moreira, M.H., 1999. Spatial and seasonal changes

of brackish peracaridan assemblages and their relation to some enviromental variables in two tidal channels of the Ria de Aveiro (NW Portugal). Marine Ecology Progress Series. 190, 69-87.

Drake, P., Arias, A.M., Baldo, F., Cuesta, J.A., Rodríguez, A., Silva-García, A., Sobrino, I., García-González, D., Fernández-Delgado, C., 2002. Spatial and temporal variation of the nekton and hyperbenthos from a temperate European estuary with regulated freshwater inflow. Estuaries 25, 451-468.

Fortier, L., Leggett, W.C., 1982. Fickian Transport and the Dispersal of Fish Larvae in Estuaries. Canadian Journal of Fisheries and Aquatic Sciences 39, 1150-1163.

Freire, C. A., Cavassin, F., Rodrigues, E. N., Torres, A. H., McNamara J. C., 2003. Adaptative patterns of osmotic and ionic regulation and the invasión of freshwater by the palaemonids shrimps. Comparative Biochemistry and Physiology 136 A: 771-778.

Gilles, R., Péquex, A., 1983. Interactions of Chemical and Osmotic Regulation with the Environmental. *in* F. J. Vernberg and W. B. Vernberg, editors. Environmental Adaptations. Academic Press, New York, 8, pp. 109-177.

Hagerman, L., 1970. The oxygen consumption of *Crangon vulgaris* (Fabricius) (Crustacea, Natantia), in relation to salinity. Ophelia 7, 283-292.

Hagerman, L., Uglow, R.F., 1983. The influence of temperature on the osmoregulation of the brackish-water shrimp *Palaemonetes varians* Leach. Ophelia 22, 229-236.

Kinne, O., 1971. Salinity - Invertebrate. *in* Kinne, O., (Ed.), Environmental factors. Wiley-Interscience, London, 1 (2), pp. 821-995.

Mantel, L.H., Farmer, L.L., 1983. Osmotic and Ionic Regulation. *in* Mantel, L. H., (Ed.), Internal anatomy and physiological regulation. Academic Press, New York, 5, pp. 53-161.

McLusky, D.S., Hagerman, L., Mitchell, P., 1982. Effect of salinity acclimation on osmoregulation in *Crangon crangon* and *Praunus flexuosus*. Ophelia 21, 89-100.

Mees, J., Hamerlynck, O., 1992. Spatial community structure of the winter hiperbenthos of the Schelde estuary, The Netherlands, and the adjacent coastal waters. Netherlands Journal of Sea Research 29, 357-370.

Newman, W.A., 1963. On the introduction of an edible Oriental shrimp (Caridea, Palaemonidae) to San Francisco Bay. Crustaceana, 5, 119-132.

Panikkar, N. K., 1941. Osmoregulation in some palaemonid prawns. Journal of the Marine Biological Association of the United Kingdom 25, 317-359.

Pannikar, N. K., 1968. Osmotic behaviour of shrimps and prawns in relation to their biology and culture. Fisheries Reports F.A.O. 57, 527-538.

Pequeux, A., 1995. Osmotic Regulation in Crustaceans. Journal of Crustacean Biology 15, 1-60.

Rupp, G. S., Parsons, G. J., 2004. Effects of salinity and temperature on the survival and byssal attachment of the lion's paw scallop *Nodipecten nodosus* at its southern distribution limit. Journal of Experimental Marine Biology and Ecology 309, 173-198.

Spaargaren, D. H., 1972. Osmoregulation in the prawns *Palaemon serratus* and *Lysmata seticaudata* from the Bay of Naples. Netherlands Journal of Sea Research 5, 416-436.

Udekem d'Acoz C. d', Faasse M., Dumoulin E., De Blauwe H., 2005. Occurrence of the Asian shrimp, *Palaemon macrodactylus* Rathbun, 1902, in the Southern Bight of the North Sea, with a key to the Palaemonidae of North-West Europe (Crustacea, Decapoda, Caridea). Nederlandse Faunistische Mededelingen, 22: 95-111

Wagner, C. M., 1999. Expression of the estuarine species minimum in littoral fish assemblages of the lower Chesapeake Bay tributaries. Estuaries 22, 304-312.

Wagner, C. M., Austin, H. M., 1999. Correspondence between environmental gradients and summer littoral fish assemblages in low salinity reaches of the Chesapeake Bay, USA. Marine Ecology-Progress Series 177, 197-212.

Weber, R. E., Spaargaren, D. H., 1970. On the influence of temperature on the osmoregulation of *Crangon crangon* and its significance under estuarine conditions. Netherlands Journal of Sea Research 5, 108-120.

Vannéy, J. R., 1970. L´hydrologie du bas Guadalquivir. Instituto de Geografía aplicada del Patronato "Alonso de Herrera", Madrid, Spain, 176 pp.

FIGURE CAPTIONS

Figure 1. Map of The Guadalquivir Estuary (Southwest Spain) showing the location of the three sampling sites.

Figure 2. Monthly average water temperature and salinity in the Guadalquivir Estuary from May 1997 to January 2005. J, January; M, May; S, September.

Figure 3. Average salinity (upper panel) and tidal salinity change (lower panel) at the three sampling stations (32, 20 and 8 km upstream from the river mouth) of the Guadalquivir Estuary during the study period.

Figure 4. Spatial and salinity-related field distributions of the six most abundant crustacean decapods in the Guadalquivir estuary during the study period.

Figure 5. Salinity ($S_{cm;}$ upper panel) and distance (D_{cm}; lower panel) at which the centre of mass of different species in respect of water temperature was situated. Extremes of bars: 10 and 90 percentiles of the centre of mass distributions.

Figure 6. Hemolymph osmolality of each species studied in relation to water osmolality/salinity. Points: individual values; lines: regression lines.

Figure 7. Osmoregulation ranges and isosmotic point of each species (at 20 °C), and field distribution of the centre of mass (18°C < T < 22 °C). For *C. crangon,* dashed line indicates the salinity range of moderate osmorregulation.

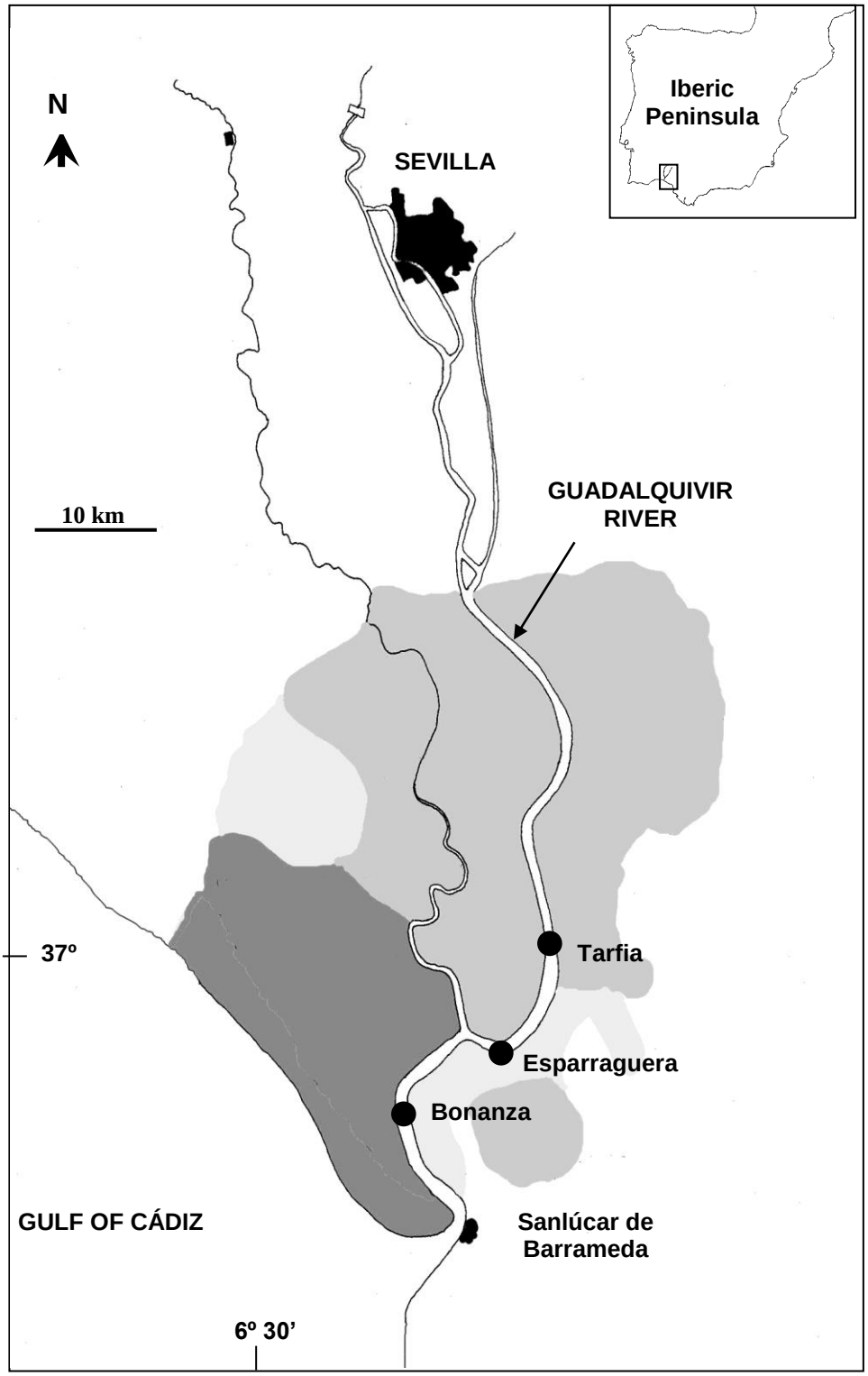
Table 1. Constancy of each species (% of samples in which it was present) in the different sampling sites during the studied period.

Stations	Species					
	M. kerathurus	*P. serratus*	*C. crangon*	*P. longirostris*	*P. macrodactylus*	*P.varians*
Tarfía	11.3	7.1	59.7	97.2	95.3	80.8
Esparraguera	23.9	18.5	81.2	93.1	89.6	74.2
Bonanza	37.2	34.1	92.3	96.2	79.8	30.1

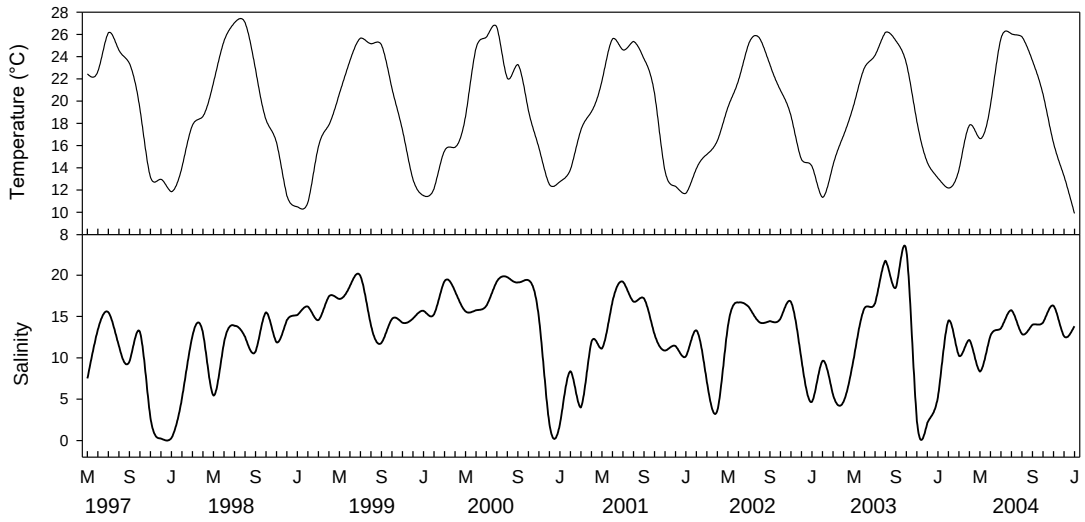
Table 2. Hemolymph concentration of each species in relation to water osmolality.

Species	Acclimation osmolality	Experimental osmolality	Regression equation	Correlation coefficient (R^2)	Isosmotic point	Lenght of Carapace (LC, in mm)		N
						Mean	±SE	
M. kerathurus	650	99 – 501	y = 0.711 x + 349.46	0.82		17.1	1.7	13
		501 – 946	y = 0.090 x + 684.04	0.21	751	15.1	1.5	14
P. serratus	650	47 – 419	y = 0.760 x + 325.68	0.69		9.7	0.2	26
		419 – 932	y = 0.089 x + 606.05	0.21	665	10.1	0.4	14
C. crangon	520	70 – 325	y = 0.464 x + 496.69	0.53		8.9	0.2	21
		325 – 820	y = 0.140 x + 613.27	0.25	713	9.5	0.1	35
		820 – 942	y = 0.893 x + 7.0142	0.75		9.1	0.1	17
P. longirostris	250	49 – 904	y = 0.101 x + 521.01	0.65	579	9.7	0.3	42
P. macrodactylus	350	44 – 962	y = 0.170 x + 482.89	0.62	584	8.3	0.3	43
P. varians	650	76 – 962	y = 0.132 x + 524.87	0.51	605	7.1	0.1	46

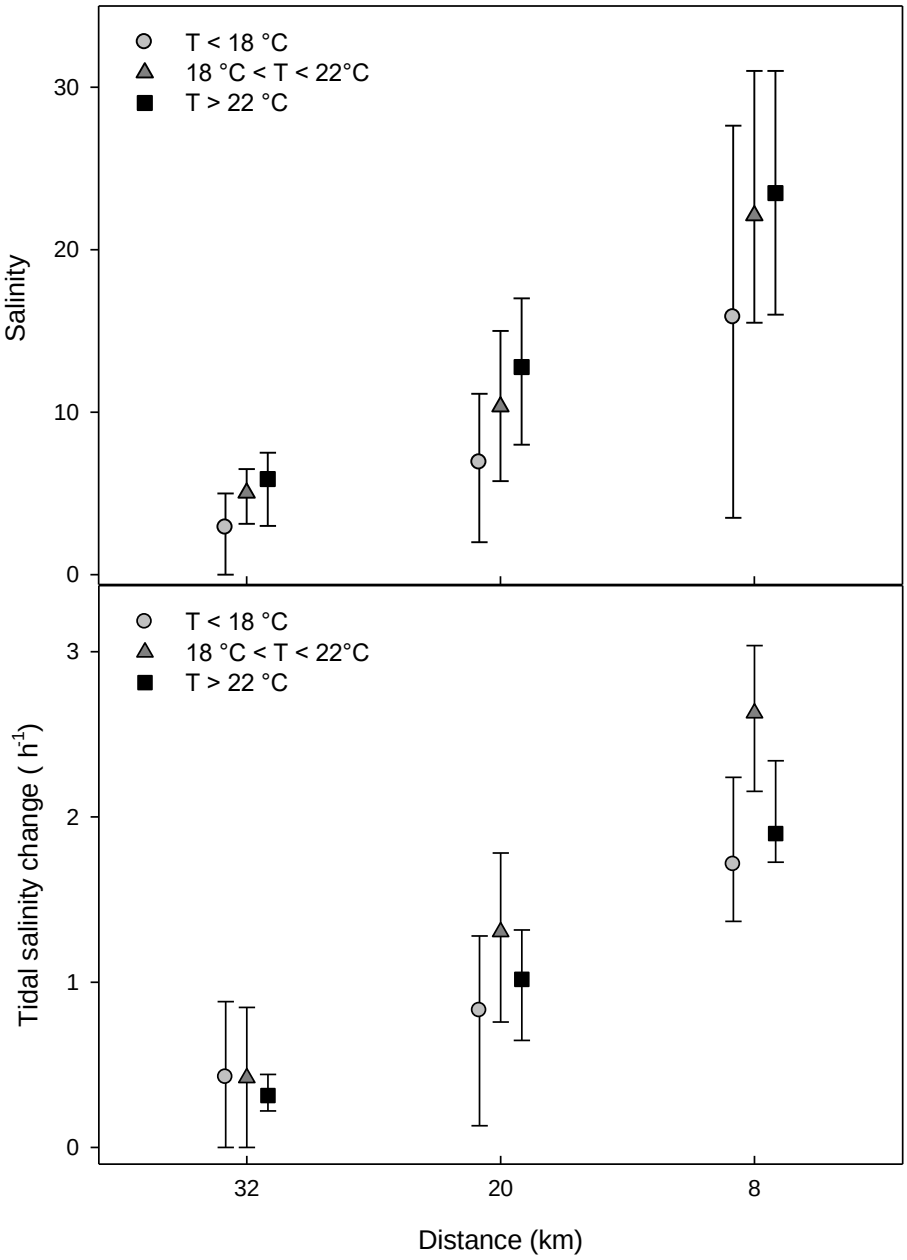
Y = hemolymph concentration and x = medium osmolality. Hemolymph and water osmolalities expressed in mmol Kg^{-1} .


N
SEVILLA
Iberic
Peninsula
10 km
GUADALQUIVIR
RIVER
37º
Tarfia
Esparraguera
Bonanza
GULF OF CÁDIZ
Sanlúcar de
Barrameda
6º 30'

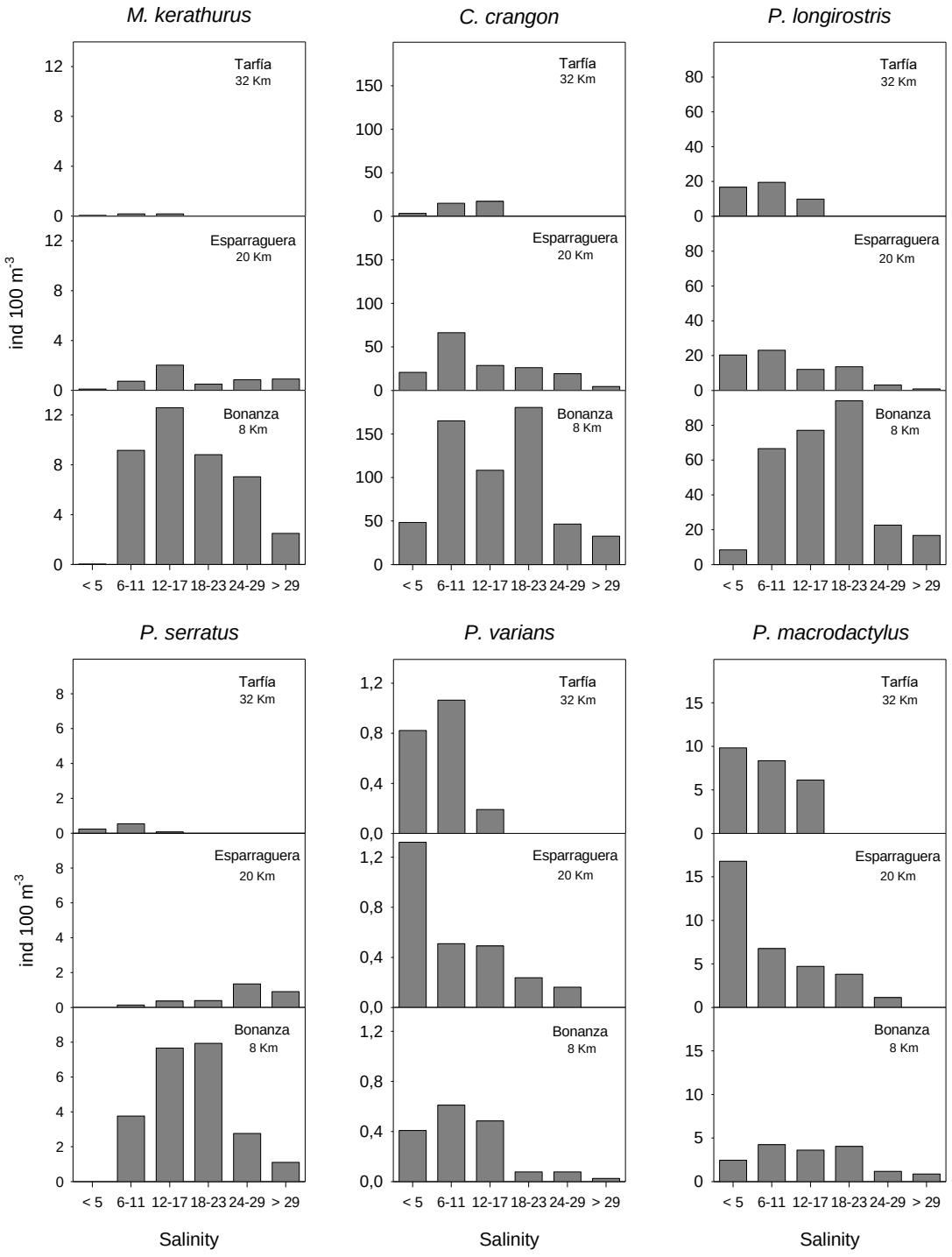
(Fig. 1)


Temperature (°C)
28
26
24
22
20
18
16
14
12
10
8
Salinity
20
15
10
5
0
M S J M S J M S J M S J M S J M S J M S J M S J
1997 1998 1999 2000 2001 2002 2003 2004

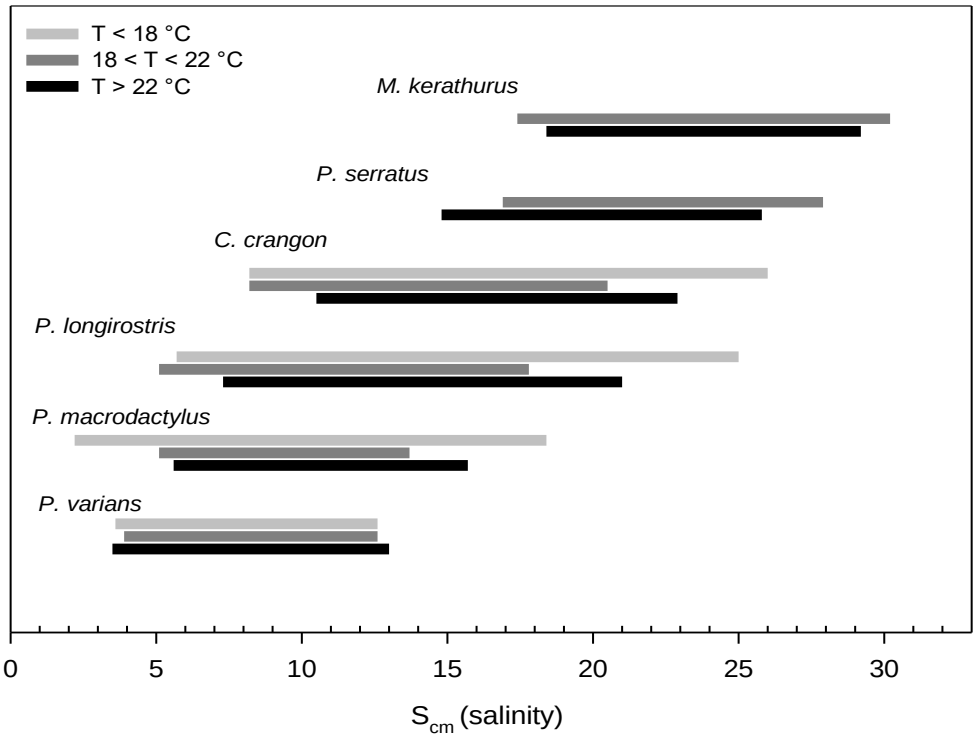
(Fig. 2)

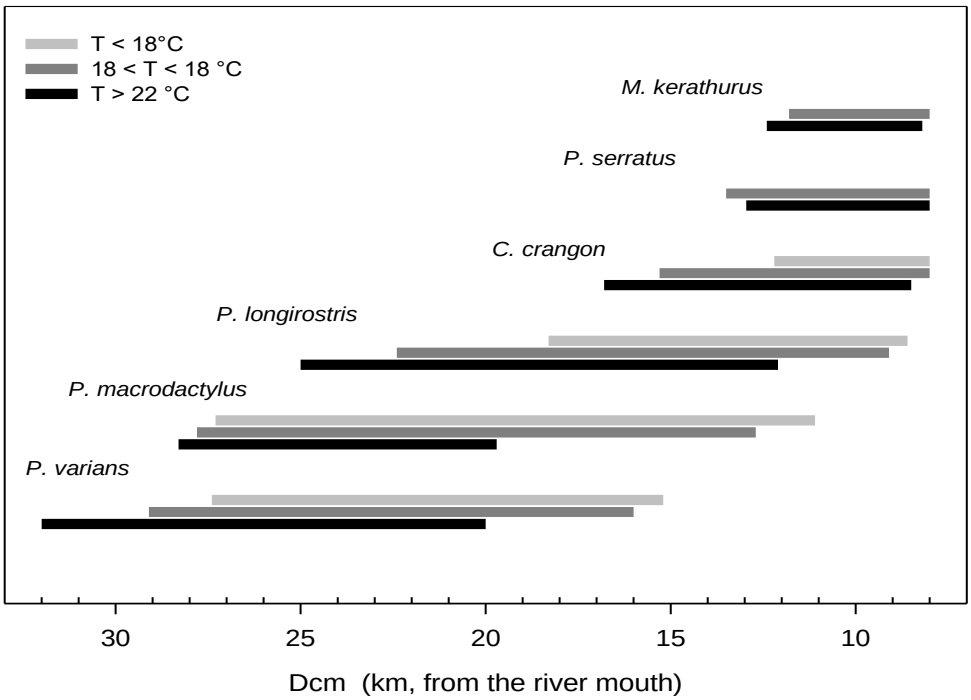

T < 18 °C
18 °C < T < 22°C
T > 22 °C
Salinity
0
10
20
30
T < 18 °C
18 °C < T < 22°C
T > 22 °C
Tidal salinity change (h-1)
0
1
2
3
32
20
8
Distance (km)

(Fig. 3)

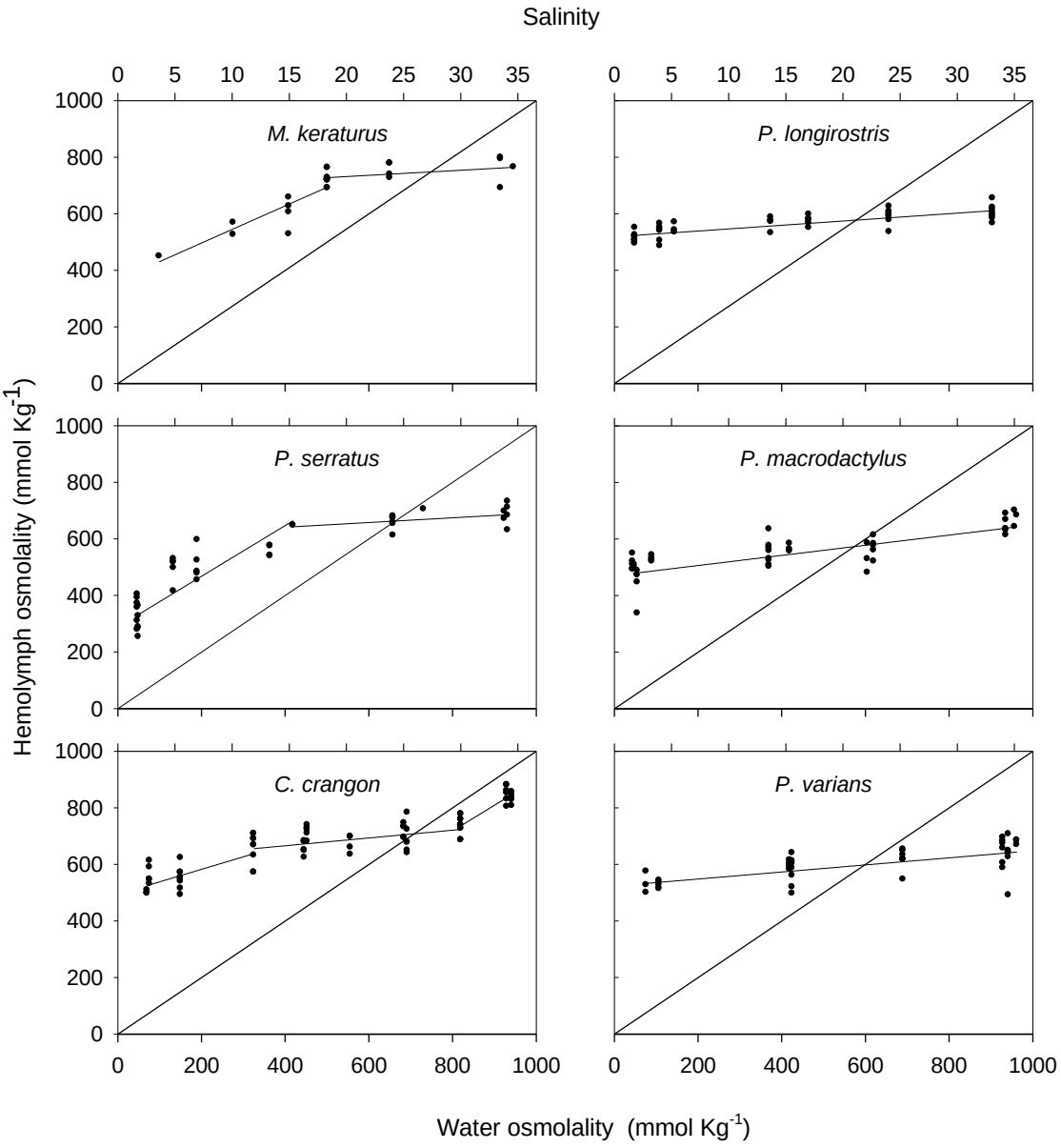

M. kerathurus
C. crangon
P. longirostris
P. serratus
P. varians
P. macrodactylus
Tarfía
32 Km
Esparraguera
20 Km
Bonanza
8 Km
ind 100 m-3
< 5
6-11
12-17
18-23
24-29
> 29
Salinity

(Fig. 4)

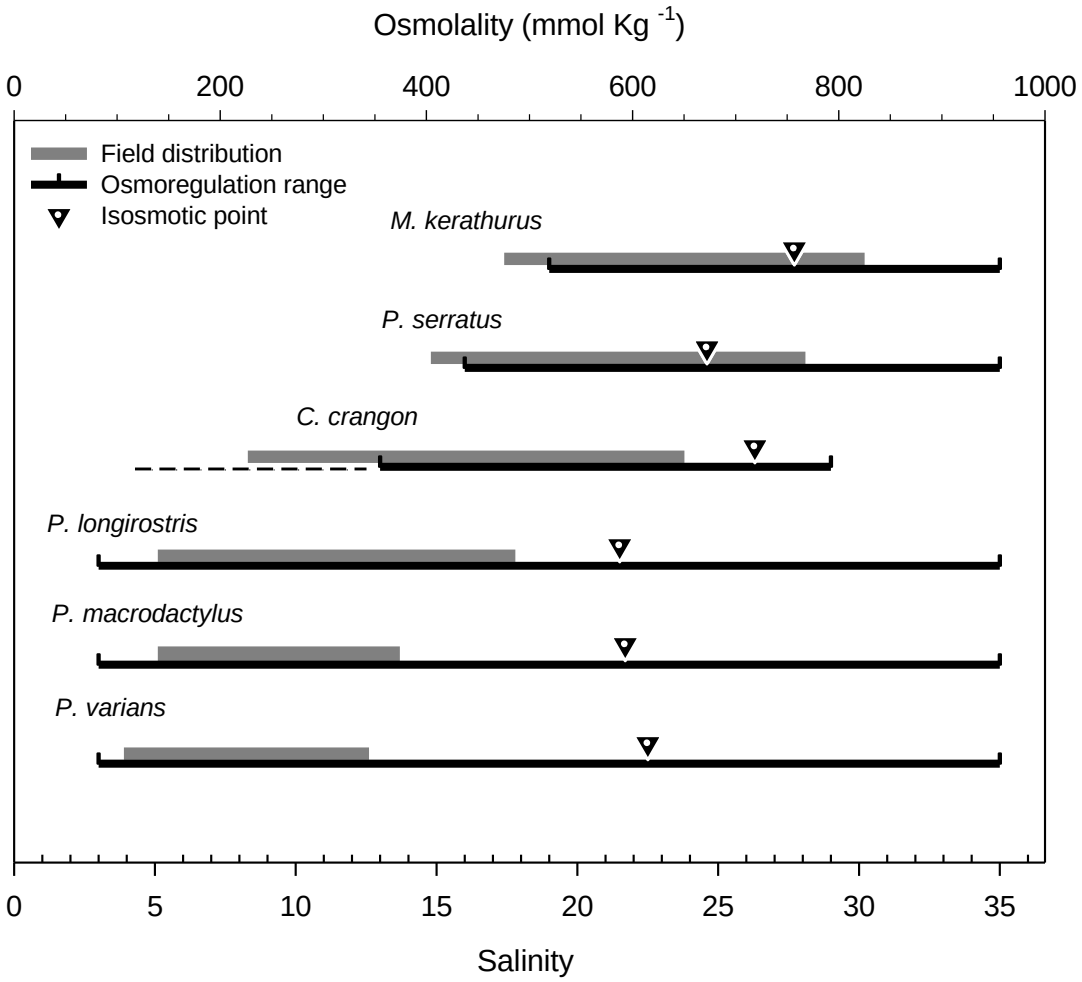

T < 18 °C
18 < T < 22 °C
T > 22 °C
M. kerathurus
P. serratus
C. crangon
P. longirostris
P. macrodactylus
P. varians
0
5
10
15
20
25
30
S$_{cm}$ (salinity)


T < 18°C
18 < T < 18 °C
T > 22 °C
M. kerathurus
P. serratus
C. crangon
P. longirostris
P. macrodactylus
P. varians
30
25
20
15
10
Dcm (km, from the river mouth)

(Fig. 5)


Salinity
M. keraturus
P. longirostris
P. serratus
P. macrodactylus
C. crangon
P. varians
Hemolymph osmolality (mmol Kg-1)
Water osmolality (mmol Kg-1)
0
200
400
600
800
1000
0
5
10
15
20
25
30
35

(Fig. 6)


Osmolality (mmol Kg -1)
0
200
400
600
800
1000
Field distribution
Osmoregulation range
Isosmotic point
M. kerathurus
P. serratus
C. crangon
P. longirostris
P. macrodactylus
P. varians
0
5
10
15
20
25
30
35
Salinity

(Fig. 7)