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Vertical patterns of ichthyoplankton at the interface between a temperate estuary and adjacent coastal waters: seasonal relation to diel and tidal cycles

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ABSTRACT

Vertical distribution and migration pattern of ichthyoplankton assemblage in the Mondego estuary were investigated in relation to diel and tidal cycle. Summer and winter communities were sampled, at surface and bottom, over a diel cycle during spring and neap tides at a fixed station at the mouth of the estuary. Summer presented higher larvae density mainly of *Pomatoschistus* spp., *Gobius niger* and *Parablennius pilicornis*. Main species in winter assemblages were *Pomatoschistus* spp. and *Sardina pilchardus*. There were no differences between depth stratums across diel or tide cycle. Nevertheless, main species larval densities showed significant periodic variation associated with tide (M2) and diel (K1) cycles presenting generally, higher density at night and around low tide. Conversely, vertical patterns observed could not be related with diel or tidal cycle. Tough, main species presented some extent of vertical migration. Vertical patterns observed appear to be related to seasonal stratification and river flow, increasing amplitude during periods of less stratification and lower water currents. Present study provides a better understanding of ichthyoplankton vertical movement patterns and of small scale dynamics at the interface of two coastal European systems.

KEYWORDS: Diel migrations; ichthyoplankton; selective tidal-stream transport; vertical distribution; Mondego estuary; Portugal.

1. Introduction

For many marine species, recruitment success requires transport from open-ocean to estuarine nursery habitats during early life and larvae must therefore adopt strategies for successful ingress into estuarine nursery grounds (Islam et al., 2007). Estuary ingress strategies are often related with larvae vertical distribution patterns. These patterns are complex and can be actively altered depending biotic and abiotic factors, involving behaviours that can change from species to species and depending on factors such as endogenous rhythms or development stage (e.g. Irigoien et al., 2004; Barletta and Barletta-Bergan, 2009; Leis, 2010; Tamaki et al., 2010).

Recently, there has been a growing interest in vertical distribution and migration by decapods larvae (e.g. Queiroga et al., 2007; Tamaki et al., 2010), copepods (e.g. Ueda et al., 2010) and fish larvae (e.g. Auth et al., 2007; Voss et al., 2007; Barletta and Barletta-Bergan, 2009; Leis, 2010). Despite their planktonic nature, fish larvae can control their vertical position in the water column. This vertical migration seems to have a main role that allows the larvae to reach or stay in certain favourable areas and control passive drift (Aceves-Medina et al., 2008; Tamaki et al., 2010). Moreover, knowledge of the vertical distribution of larvae is critical to understand the structure and ecological interactions of ichthyoplankton communities (Gray, 1998). Larvae often use active upward or downward swimming and passive sinking coupled with both circadian and tidal rhythms (Tamaki et al., 2010).

Diel vertical migrations are common within larval phase of fish. The most common is movement into the upper water column during night and into deeper water during the day (e.g. Tsukamoto et al., 2001; Auth et al., 2007). However, the reverse pattern has also been observed (e.g. Brodeur and Rugen, 1994; Bradbury et al., 2006). Some species do not show diel variations but may form aggregations during the daytime and disperse at night (e.g. Jensen et al., 2003). Diel vertical migrations have been related to optimum light conditions, predator avoidance and suitable prey concentrations (e.g. Brodeur and Rugen, 1994; Irigoien et al., 2004).

Tidal vertical migration is also a common mechanism in estuarine species living in areas dominated by tidal currents. While diel patterns are widely associated with predation-prey relationships, tidal cycles of vertical migration are linked to horizontal transport, retention or dispersion mechanisms. The most common is selective tidal-stream transport (STST), during which larvae are supposed to ascend actively in the water column during flood and return to the bottom when the tide turns (e.g. Forward and Tankersley, 2001; Gibson, 2003). In this way, organisms can be advected to upstream parts of the estuary during floods and, on the other hand, getting out of seaward-moving water layers during ebb could facilitate larval retention within an estuary (e.g. Jager, 1999, DiBacco et al., 2001). STST have been imputed to several species of zooplankton (e.g. Rawlinson et al., 2005), larval benthic invertebrates (e.g. Queiroga et al., 2007; Jessopp and McAllen, 2008) and larval and juvenile fishes (e.g., Schultz et al., 2003; Miller and Shanks, 2004; Islam et al., 2007). For fish larvae, this behaviour can be critical to survival since changes in environmental conditions may affect recruitment success of both estuarine and non estuarine-dependent species (Parrish et al., 1981).

Fish larvae vertical migration studies are often performed in coastal/offshore habitats (e.g. Auth et al., 2007; Voss et al., 2007; Garrido et al., 2009) or focused on tidal transport (e.g. Jager, 1999) however integrated work on the seasonal effect of the three cycles (tidal, diel and semi-lunar) in estuarine icthyoplankton is uncommun (e.g. Schultz et al., 2003). This study aimed to investigate the influence of diel and tidal cycle on larval fish vertical distribution over one neap and one spring tide during two opposite seasons (winter and summer) in a temperate small European estuary. It describes how larval vertical distributions differed among species and which mechanisms of transport could result from these distributions. Also, it is the first examination of diel/tidal variation in the vertical distributions of ichthyoplankton in the Mondego estuary and intended to supplement previous spatial and temporal analyses (e.g. Primo et al., 2011).

2. Material and methods

2.1. Study site

The Mondego River estuary is a mesotidal system, located in the western coast of Portugal (40º08’N, 8º50’W) (Fig. 1). The hydrological basin of the Mondego, with an area of 6 670 km^2, provides an average freshwater flow rate of 79 m^3 s^{-1} (Dolbeth et al., 2010). The well-mixed estuary consists of two channels divided by the Murraceira Island converging again near the mouth. Here the influence of both the river flow and neritic waters is strong and the depth is around 6-13 m. The north arm is deeper, with 5–10 m depth at high tide while the south arm is shallower, with 2–4 m depth at high tide. Tides in this system are semi-diurnal, and at the inlet the tidal range is 0.35–3.3m.

2.2. Sample collection

Plankton samples were collected from a single station (M) located at the mouth of the estuary (Fig.1). Sampling cruises were performed both in summer (June 2005) and winter (December

2005). In each season, hourly samples were collected over two diel cycles coinciding with neap and spring tides. Samples were collected in the opposite direction of the current using horizontal tows (bongo net: mesh size 335µm, mouth diameter: 0.5 m; 3 min. tow; 2 knots) equipped with a Hydro-Bios flowmeter (average water filtered: $20m^3$) and preserved in a borax-buffered formalin seawater solution. Each hour samples were collected from both subsurface and near (<1m) bottom. Salinity and water temperature (ºC) were measured immediately after sampling using appropriate sensors. Samples were sorted in the laboratory and fish larvae were identified to the species level whenever possible under a stereoscopic microscope. Although restricted over the spatial scale, missing lateral gradients, this high frequency sampling allowed to detect changes in the main ichthyoplankton species density related to ebb-flood dynamics.

2.3. Data analysis

Larval density for each depth sampled was expressed as the number of larvae per 100 m^3. The samples were classified as day (from sunrise to sunset) and night periods. According to the expected moments of high- and low-water, samples were also classified as ebb or flood tides. Samples collected 1 hour before and after the expected moments of high or low tide were consider as collected during flood or ebb, respectively.

Diel and tidal fish larvae vertical distribution pattern were analysed separately. For that, main species mean density per depth was determined at each sampling moment. Also, weighted mean densities of dominant larval taxa at each sampling moment were calculated as $WMD=\sum(C_i d_i)/\sum(C_i$, where C_i stands for larval density at depth d_i (i, depth layer number) (Pearre, 2003). The amplitude of diel migration (DVM) was calculated as the difference between the WMD at day and night. A positive value indicated movement towards the surface during the night and a negative value reverse vertical migration.

Due to the large variability of data and to unbalanced design, a univariae PERMANOVA test was applied to assess variation in environmental factors, fish larvae density and weighted mean depths of larvae. The PERMANOVA tests hypotheses for multi-factors in a great variety of designs using permutation methods. Analysis was run for each season/lunar cycle combination separately and was based on Euclidian distances between samples, considering all the factors as fixed and unrestricted permutation of raw data.

Salinity and temperature was tested for differences between depth (surface, bottom) and tidal cycle (flood, ebb) with a two-way design (Tide/Depth). Fish larvae density was tested to assess separate and interactive effects of depth (surface, bottom), diel (day, night) and tidal cycle (flood, ebb) with a two-way design (Diel/Depth or Tide/Depth). Both analysis were made on ln(x+1) transformed data matrix. Weighted mean depth was tested to find differences in median depth positions between diel (day/night) and tidal (ebb/flood) cycles. For that a two-way design (Diel/Tidal) was applied on un-transformed data matrix. PERMANOVA tests were applied with PERMANOVA+ for PRIMER software (PRIMER v6 & PERMANOVA+ v1, PRIMER-E Ltd.). Significant results were investigated using a *post-hoc* test and Bonferroni correction for multiple comparisons was applied to final significance value.

To identify periodic changes on larval vertical distribution, a harmonic regression (Batschelet, 1979) was performed. Harmonic regression is a simple adaptation of ordinary regression to situations where *x*-variables are cyclic or periodic. In practice it is a multiple regression carried out on trigonometric functions of angular transforms of periodic *x*-variables (Bell et al., 2001). The model was tested for 3 tidal constituents K1 (diurnal; time period = 23.9h), M2 (semi-diurnal; time period = 12.4h) and M4 (quarter-diurnal; time period = 6.2h) in order to determine diel and tidal variations of species mean density in each sampling moment. Analyses were performed in SAS® software and regression models were constructed according to Schultz et al. (2003). Analysis was carried out on larval density at both depths and on weighted mean depth of main species.

3. Results

3.1. Hydrological conditions

During summer sampling, salinity values ranged from 28 to 33 at surface and 32 to 34 at bottom (Fig. 2). Neap tide presented differences in salinity values between surface and bottom samples while during spring tides no significant differences were detected (Table 1). During winter, these differences were more evident with surface waters presenting salinity values ranging from 4 to 11 and bottom from 31 to 35 (Fig. 2, Table 1). At winter spring tide, a significant interaction between depth and tide occurred with ebb bottom samples presenting higher salinity values than during flood (*post hoc* t=3.19, corrected p=0.002).

Regarding temperature, summer samples showed significantly higher values at the surface while at winter temperature was higher at bottom (Fig. 2, Table 1). Ebb and flood tide presented no significant differences both for salinity or temperature values (except for salinity winter spring tide) (Fig. 2, Table 1).

3.2. Tidal and diel vertical pattern of larval distribution

A total of 8476 fish larvae were collected during the study period. In summer, densities reached 1753 ind. 100 m^{-3} during neap tide and 485 ind. 100 m^{-3} during spring tides. In winter density varied from 20 and 28 ind. 100 m^{-3} between neap and spring tides. *Pomatoschistus* spp., *Parablennius pilicornis* and *Gobius niger* the most abundant during summer and *Sardina pilchardus* and *Pomatoschistus* spp. during winter.

Species presented an even vertical distribution throughout the study period with exception for *Pomatoschistus* spp. which showed significantly higher densities in bottom, mainly during winter spring tide (Table 2). At summer neap tide both *G. niger* and *P. pilicornis* showed higher density during ebb with no distinction between depths (Table 2). Also, the majority of the species showed increased mean density standard deviation during ebb tides (Table 2).

All the species were collected both at surface and bottom and during day and night period with the exception of *P. pilicornis* at summer spring tide and *Sardina pilchardus* at winter neap tide (Table 2). Regarding diel cycle, species distribution was also uniform across the water column and, again, depth stratum was a significant factor only for *Pomatoschistus* spp. at winter spring tides (Table 2). Regardless of season, the majority of species presented significantly higher density during night period. Also, mean density standard deviation is consistently higher during night periods (Table 2). No significant interactions between depth and tide or diel cycle were detected (Table 2).

At summer neap tide species weighted mean depth (WMD) ranged from 2.6 to 6.2m. *Parablennius pilicornis* presented, generally, higher WMD while *Gobius niger* showed a shallower distribution (Fig. 3). At summer spring tide, WMD ranged from 4.4 to 6m indicating a species distribution generally deeper than at summer neap tide. Main species analysed presented similar weighted mean depth (Fig. 3). During winter neap tide *Pomatoschistus* spp. WMD ranged from 4.7 to 5.4m while at spring tide was around 6m. *Sardina pilchardus* WMD ranged between 4.9 and 6 in neap tide and 4 and 5.1 in winter spring tide, reaching shallower layers of water column during flood (Fig. 3). *Pomatoschistus* spp. and *G. niger* were the only species presenting significant differences between flood and ebb WMD ($PseudoF_{1,1}=5.13$, $p<0.05$ and $PseudoF_{1,1}=6.57$, $p<0.05$, respectively) in summer neap tides. Both species presented higher WMD during floods (Fig. 3).

Diel vertical migration amplitude varied from 0.72 to 5.34m with *S. pilchardus* reaching the highest value during winter neap tide (Fig. 4). In summer and winter spring tide, species showed a positive DVM indicating higher WMD during the day than at the night. At summer neap tide, *Pomatoschistus* spp. and *G. niger* presented a reverse pattern with higher WMD at night (Fig. 4). Significant differences between day and night WMD were detected for *G. niger*

(-1.6m) and *Pomatoschistus* spp. (-1.20m) at summer neap tide ($\text{PseudoF}_{1,1}$=6.57, $p<0.05$ and $\text{PseudoF}_{1,1}$=5.13, $p<0.05$, respectively), for *P. pilicornis* (2.81m) at summer spring tide ($\text{PseudoF}_{1,1}$=6.13; $p<0.05$) and for *S. pilchardus* (-5.34m) at winter neap tide ($\text{PseudoF}_{1,1}$=5.13; $p<0.05$).

3.3. Harmonic analysis of tidal and diel vertical pattern of larval distribution

Harmonic regression revealed periodic variability on most larval species analysed (Table 3; Fig. 5). *Pomatoschistus* spp. mean density at surface and at bottom varied periodically according to K1 (day-night) and M2 tidal constituents during all the analysed periods except for winter neap tides. *Pomatoschistus* spp. density was generally higher at night period and around low tide (Fig. 5) and variability explained by the model was higher during summer neap tides at the surface (Table 3). *Parablennius pilicornis* mean density presented periodic variability only during summer neap tide at bottom samples with predicted model explaining 54% of the variability. Again mean density varied according to K1 and M2 periodicity (Table 3; Fig. 5). *P. pilicornis* density at bottom was higher during night around high tide (Fig. 5). Also at summer neap tide, the effect of K1 and M2 on *Gobius niger* larval mean density were significant and, together with M4 constituent explained 85% of the variability observed at the bottom samples. Higher densities were mainly collected during night at high tide (surface) and low tide (bottom). In summer spring tides *G. niger* variability explained by the model was lower, either for bottom or surface larval mean density (Table 3; Fig. 5). K1 and M2 periodicity were significant also for *Sardina pilchardus* larvae density during winter neap and spring tides. However during winter spring tide effect was only significant in the bottom samples (Table 3; Fig. 5). In winter neap tide, *S. pilchardus* density was higher at night around high tide while in spring tide larvae density maximum was observed at dusk high tide (Fig. 5).

Regarding larvae mean depth, periodic variability was only significant for *Pomatoschistus* spp. during summer spring tides with tidal predictor M2 explaining 63% of the variability observed (Table 3; Fig. 5). Position in the water column was generally shallower around low tide (Fig. 5).

Pomatoschistus spp. presented higher amplitude estimates than the other species reaching maximum values for bottom samples in summer neap tide (Table 4). Bottom larval densities showed generally higher amplitude estimates than at surface (Table 4). Also, species presented amplitude estimates generally higher during neap tides. Only *Sardina pilchardus* showed higher M2 amplitude during winter spring tide (Table 4).

4. Discussion

Hydrological conditions experienced during both studied periods indicated that ebb and flood tides presented similar conditions (salinity and temperature). During summer, the entire water column presented salinities over 30 while in winter, surface water presented visibly lower salinity leading to water column stratification. These patterns reflect the strong influence of river flow in small estuaries like Mondego estuary. In dry months, low river flow lead to an increase of tidal influence on the estuary resulting in high sea water incursion. Marine influence is expected to increase also during higher amplitude tides so, as expected, both spring tides showed generally higher salinities and lower temperatures, mainly at surface. It is expected that these different environmental conditions influence fish larvae vertical distribution.

Seasonal pattern of high fish larvae density in summer and lower in winter was already reported for Mondego estuary and is a common feature in several estuaries being probably related to spawning period of species (Primo et al., 2011). For this reason, it was chosen to analyse vertical distribution in each season separately.

Larval fish density showed no clearly preference in terms of vertical distribution, since differences between surface and bottom samples were not significant for the majority of the species. Also, no evidence of vertical stratification of larvae relative to diel cycle was found however species showed significant periodic variability in larval density related with K1 (day/night effect). Diel cycle seemed to have a strong influence on fish larvae density which presented, generally, a marked reduction in number during the day, both in summer and winter conditions. Diel changes in estuarine ichthyoplankton have been documented by several authors (e.g. Brodeur and Rugen, 1994; Islam et al., 2007; Auth et al., 2007; Aceves-Medina et al., 2008) and the differences between night and day periods were often attributed to factors as gear avoidance or diel vertical migration (Rodríguez et al., 2006; Auth et al., 2007). Gear avoidance is a common concern when sampling larval fishes, particularly the older developmental stages, as it is usually lower during night resulting in the misleading idea of higher larvae densities during nocturnal sampling. Also, rhythms of swim bladder inflation/deflation were often related with larvae vertical distribution (e.g. Ré, 1996; Santos et al., 2006). Swim bladder inflation/deflation rhythms seem to be synchronized with light cycle and vary according to larvae development stage (Santos et al., 2006).

Migration amplitude and vertical distribution patterns may vary with size, development stage and from species to species (Hays et al., 1994; De Robertis, 2002; Rodríguez et al., 2006). Although present study lacks ontogenetic stage determination, larvae collected were small larvae (pre-flexion or flexion stage) and thus analyzed together. Diel vertical migration is a common behaviour in estuarine fish and invertebrate larvae and seems to be related with light-dependent predation mortality (e.g. De Robertis, 2002; Hays, 2003; Irigoien et al., 2004). Though, low amplitude migrations observed are not expected to be light-mediated. The highest DVM amplitude was recorded for *Sardina pilchardus* (-5.34m) during winter neap tide which could be consistent with reverse diel vertical migration however, periodic analysis revealed no correlation between *S. pilchardus* WMD and day-night effect, similarly to all the remaining species. Reverse vertical migration is common and was previously found in copepods (e.g. Rawlinson et al., 2004) decapods (e.g. Tamaki et al., 2010) and fish larvae (e.g. Rodríguez et al., 2006). In the Catalan Sea, most of the mesopelagic larvae fish species were closer to the surface during the day than at night (Sabatés, 2004). Moreover Olivar et al. (2001) found the reverse DVM for *S. pilchardus*. One interpretation about adaptive significance for reverse DVM is avoidance of larger predatory invertebrates that perform normal DVM to escape from visual predators (Hays, 2003). Furthermore, as visual predators fish larvae need an adequate level of light in the water column to detect and capture prey (Batty, 1987) and have a preferred light level that determines their diurnal distributions and diel changes in the vertical distribution result from the dispersal of larvae once the light stimulus disappears (Leis, 1991).

Similarly to DVM, tidal vertical migration has also been widely verified in several zooplankton and fish larvae species (e.g. Jager, 1999; Ueda et al., 2010). Again, larvae densities showed no evidence of vertical stratification relative to tide. Nevertheless, species showed significant periodic variability in larval density related with tide presenting generally higher densities around low tide. The increasing larval density during ebb may result in larval export from the estuary since this behaviour represents a typical ebb-tide transport. *Pomatoschistus* spp. preference for shallower waters during ebb confirmed at summer spring tide reinforces this idea. However, for estuarine resident species like *Pomatoschistus* spp. or *G. niger*, it would be disadvantageous. Though, during this season no water vertical stratification occurred neither in ebb or flood, as a result of the low river inflow experienced, resulting in an increased landward transport. In this way, deeper position in the water column during floods may be related with species exploiting stronger landward currents. Preference for deeper layers of water column was also found in Chesapeake Bay (Hare et al., 2005), Ariake Bay (Yamaguchi and Kume, 2007) and for gobies larvae in Hudson River estuary (Schultz et al., 2003). Residual currents are often referred as important mechanisms promoting upstream transport and the retention of planktonic larvae (Schultz et al., 2003; Hare et al., 2005; Islam et al., 2007). In estuaries and bays, flood-tide transport may depend on current flow at the sampling location since it can prevent larvae from successfully undertaking vertical migrations (Forward and Tankersley, 2001).

Tidal transport can also undergo diurnal changes and several authors refer an increased in flood transport during the night (e.g. Forward and Tankersley, 2001; Islam et al., 2007). Increased larvae density during ebb night time observed during this study may reflect an inverse tendency with higher downstream transport at night, particularly at winter which showed stronger seaward transport. This pattern was already mentioned for several invertebrate larvae (e.g. Queiroga et al., 1994; Forward and Tankersley, 2001).

The efficiency of larval transport on shallow depths seemed to depend on hydrodynamics condition of the estuary and inputs of river flow can be the main driven-force responsible for differences in larval distribution between the studied seasons. During summer a stronger landward transport occurred, both during ebb and flood tides while in winter, water column stratification lead to two different transport patterns, seaward on the surface and landward at the bottom. Therefore, *Pomatoschistus* spp. preference for bottom waters in winter was probably associated with retention and upstream transport in the estuary. Differences in physical transport processes within partially mixed and low-inflow estuaries may influence the dispersal and exchange of planktonic larvae (DiBacco et al., 2001). For instance, Lagadeuc et al. (1997) observed that the copepod *Temora longicornis* exhibited more significant vertical migrations in stratified water, but when water was mixed showed less significant migrations and a deeper distribution where vertical mixing was lowest. On the contrary, in Lough Hyne, the same species only undertook vertical migration under weakly stratified water, stopping when stratification was stronger (Rawlinson et al., 2005).

Despite vertical patterns observed could not be related with diel or tidal cycle, main species presented some extent of vertical migration. Also, spring-neap tidal cycle seemed to influence vertical distribution of fish larvae species. During summer, neap tide seemed to favour this behaviour with species presenting higher amplitude movements. At summer, spring tide conditions seemed to present a more uniform water column, concerning to environmental condition, which can be preventing species vertical migration. Often, during spring tides, faster currents and increased water turbidity occur, limiting vertical migrations (Schultz et al., 2003). In winter, stratification is probably higher during spring tides due to high river flow and increased tidal influence leading to a significant vertical density distribution, mainly for *Pomatoschistus* spp. The reverse pattern occurred in Hudson River estuary, where spring tide resulted in reduced stratification of the water column and more uniform distribution of larvae (Schultz et al., 2003). Species migration was, however, higher during neap tide, probably because of weaker currents. Some species seem to present an endogenous rhythm associated with spring-neap cycle in order to synchronise to specific tidal amplitude (e.g. Hough and Naylor, 1991; Queiroga et al., 1994).

Periodic regression advantages' over analysis of variance techniques have been previously reported (e.g. Bell et al., 2001, Schultz et al., 2003). In the present study, harmonic regression revealed patterns that analysis of variance approach could not identify reinforcing the merit of this analysis in this kind of studies.

Vertical distribution of larvae influences their transport to and across the estuary being crucial to larvae development. Seaward transport could result in exportation of to the adjacent coastal area while landward reflect larvae import from ocean or retention mechanisms within the estuary and transport to the upstream areas. For many species, reaching estuarine nursery areas is essential to successfully complete their life-cycle and understanding this process is fundamental to a more general knowledge of the population dynamics of the species involved.

5. Conclusions

Fish larvae seem to be evenly distributed throughout the water column in Mondego estuary. Also, tide and diel cycles seemed to be strongly related with fish larvae density which may influence their entrance in the estuary. Despite vertical migration observed could not be related with diel or tidal cycle, species showed variation in their position in the water column. The main feature associated with vertical patterns observed appear to be related with hydrological regime (inputs of freshwater flow) and consequently seasonal stratification, increasing amplitude during periods of less stratification and weaker water currents.

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References

Aceves-Medina, G., Saldierna-Martínez, R., Hinojosa-Medina, A., Jiménez-Rosenberg, S.P. A., Hernández-Rivas, M.E., Morales-Ávila, R., 2008. Vertical structure of larval fish assemblages during diel cycles in summer and winter in the southern part of Bahía de La Paz, México. Estuar. Coast. Shelf Sci. 76, 889-901.

Auth, T.D., Brodeur, R.D., Fisher, K.M., 2007. Diel variation in vertical distribution of an offshore ichthyoplankton community off the Oregon coast. Fish. Bull. 105, 313-326.

Barletta, M., Barletta-Bergan, A., 2009. Endogenous Activity Rhythms of Larval Fish Assemblages in a Mangrove fringed Estuary in North Brazil. Open Fish Sci. J. 2, 15-24.

Batschelet, E., 1979. Introduction to mathematics for life scientists. Springer-Verlag, Berlin.

Batty, R.S., 1987. Effect of light intensity on activity and food searching of larval herring, *Clupea harengus*: a laboratory study. Mar. Biol. 94, 323-327.

Bell, K.N., Cowley, P.D., Whitfield, A.K., 2001. Seasonality in frequency of marine access to an intermittently open estuary: implications for recruitment strategies. Estuar. Coast. Shelf Sci. 52, 327–337.

Bradbury, I.R., Gardiner, K., Snelgrove, P.V.R., Campana, S.E., Bentzen, P., Guan, L., 2006. Larval transport, vertical distribution, and localized recruitment in anadromous rainbow smelt (*Osmerus mordax*). Can. J. Fish. Aquat. Sci. 63, 2822-2836.

Brodeur, R.D., Rugen, W.C., 1994 Diel vertical distribution of ichthyoplankton in the northern Gulf of Alaska. Fish. Bull. 92, 223-235.

De Robertis, A., 2002. Size-dependent visual predation risk and the timing of vertical migration: An optimization model. Limnol. Oceanogr. 47, 925-933.

DiBacco, C., Sutton, D., McConnico, L., 2001. Vertical migration behavior and horizontal distribution of brachyuran larvae in a low-inflow estuary: implications for bay-ocean exchange. Mar. Ecol. Prog. Ser. 217, 191-206.

Dolbeth, M., Martinho, F., Freitas, V., Costa-Dias, S., Campos, J., Pardal, M.A., 2010. Multi-year comparisons of fish recruitment, growth and production in two drought-affected Iberian estuaries. Mar. Freshwater Res. 61, 1399–1415.

Forward Jr., R.B., Tankersley, R.A., 2001. Selective tidal-stream transport of marine animals. Oceanogr. Mar. Biol., Annu. Rev. 39, 305–353.

Garrido, S., Santos, A.M.P., Santos, A. Dos, Ré, P. 2009 Spatial distribution and vertical migrations of fish larvae communities off Northwestern Iberia sampled with LHPR and Bongo nets. Estuar. Coast. Shelf Sci. 84, 463-475.

Gibson, R.N., 2003. Go with the flow: tidal migration in marine animals. Hydrobiologia 503, 153-161.

Gray, C.A., 1998. Diel changes in vertical distributions of larval fishes in unstratified coastal waters off southeastern Australia. J. Plankton Res. 20, 1539−1552.

Hare, J.A., Thorrold, S., Walsh, H., Reiss, C., Valle-Levinson, A., Jones, C., 2005. Biophysical mechanisms of larval fish ingress into Chesapeake Bay. Mar. Ecol. Prog. Ser. 303, 295-310.

Hays, G.C., 2003 A review of the adaptive significance and ecosystem consequences of zooplankton diel vertical migrations. Hydrobiologia 503, 163-170.

Hays, G.C., Proctor, C.A., John, A.W.G., Warner, A.J., 1994. Interspecific differences in the diel vertical migration of marine copepods: The implications of size, color, and morphology. Limnol. Oceanogr. 39, 1621-1629.

Hough, A.R., Naylor, E., 1991. Field studies on retention of the planktonic copepod *Eurytemora affinis* in a mixed estuary. Mar. Ecol. Prog. Ser. 76, 115-122.

Irigoien, X., Conway, D., Harris, R., 2004. Flexible diel vertical migration behaviour of zooplankton in the Irish Sea. Mar. Ecol. Prog. Ser. 267, 85-97.

Islam, M.S., Hibino, M., Tanaka, M., 2007. Tidal and diurnal variations in larval fish abundance in an estuarine inlet in Ariake Bay, Japan: implication for selective tidal stream transport. Ecol. Res. 22, 165-171.

Jager, Z., 1999. Selective Tidal Stream Transport of Flounder Larvae (*Platichthys flesus* L.) in the Dollard (Ems Estuary). Estuar. Coast. Shelf Sci. 49, 347-362.

Jensen, H., Wright, P.J., Munk, P., 2003. Vertical distribution of pre-settled sandeel (*Ammodytes marinus*) in the North Sea in relation to size and environmental variables. ICES J. Mar. Sci. 60, 1342-1351.

Jessopp, M.J., McAllen, R.J., 2008. Go with the flow: tidal import and export of larvae from semi-enclosed bays. Hydrobiologia 606, 81-92.

Lagadeuc, Y., Bouté, M., Dodson, J.J., 1997. Effect of vertical mixing on the vertical distribution of copepods in coastal waters. J. Plankton Res. 19, 1183-1204.

Leis, J.M., 1991. Vertical distribution of fish larvae in the Great Barrier Reef Lagoon, Australia. Mar. Biol. 109, 157-166.

Leis, J.M., 2010. Ontogeny of behaviour in larvae of marine demersal fishes. Ichthyol. Res. 57, 325-342.

Miller, J.A., Shanks, A.L., 2004. Ocean-estuary coupling in the Oregon upwelling region: abundance and transport of juvenile fish and of crab megalopae. Mar. Ecol. Prog. Ser. 271, 267-279.

Olivar, M P., Salat, J., Palomera, I., 2001. Comparative study of spatial distribution patterns of the early stages of anchovy and pilchard in the NW Mediterranean Sea. Mar. Ecol. Prog. Ser. 217, 111-120.

Parrish, R.H., Nelson, C.S., Bakun, A., 1981. Transport mechanisms and reproductive success of fishes in the California Current. Biol. Oceanogr. 1, 175-203.

Pearre Jr., S., 2003. Eat and run? The hunger/satiation hypothesis in vertical migration: history, evidence and consequences. Biol. Rev. 78, 1–79.

Primo, A.L., Azeiteiro, U.M., Marques, S.C., Pardal, M.A., 2011. Impact of climate variability on ichthyoplankton communities: An example of a small temperate estuary. Estuar. Coast. Shelf Sci. 91, 484-491.

Queiroga, H., Costlow, J.D., Moreira, M.H., 1994 Larval abundance patterns of *Carcinus maenas* (Decapoda, Brachyura) in Canal de Mira (Ria de Aveiro, Portugal). Mar. Ecol. Prog. Ser. 111, 63-72.

Queiroga, H., Cruz, T., dos Santos, A., Dubert, J., González-Gordillo, J.I., Paula, J., Peliz, A., Santos, A.M.P., 2007. Oceanographic and behavioural processes affecting invertebrate larval dispersal and supply in the western Iberia upwelling ecosystem. Prog. Oceanogr., 74 174-191.

Rawlinson, K.A., Davenport, J., Barnes, D.K.A., 2004. Vertical migration strategies with respect to advection and stratification in a semi-enclosed lough: a comparison of mero- and holozooplankton. Mar. Biol. 144, 935-946.

Rawlinson, K.A., Davenport, J., Barnes, D.K.A., 2005. Tidal exchange of zooplankton between Lough Hyne and the adjacent coast. Estuar. Coast. Shelf Sci. 62, 205-215.

Ré, P., 1996. Anchovy spawning in the Mira estuary (southwestern Portugal). Sci. Mar. 60, 141-153.

Rodríguez, J.M., Hernández-León, S., Barton, E.D., 2006. Vertical distribution of fish larvae in the Canaries-African coastal transition zone in summer. Mar. Biol. 149, 885-897.

Sabatés, A., 2004. Diel vertical distribution of fish larvae during the winter-mixing period in the Northwestern Mediterranean. ICES J. Mar. Sci. 61, 1243-1252.

Santos, A.M.P., Ré, P., Santos, A. dos, Peliz, A., 2006. Vertical distribution of the European sardine (*Sardina pilchardus*) larvae and its implications for their survival. J. Plankton Res., 28, 523-532.

Schultz, E.T., Lwiza, K.M. M., Fencil, M.C., Martin, J.M., 2003. Mechanisms promoting upriver transport of larvae of two fish species in the Hudson River estuary. Mar. Ecol. Prog. Ser. 251, 263-277.

Tamaki, A., Mandal, S., Agata, Y., Aoki, I., Suzuki, T., Kanehara, H., Aoshima, T., Fukuda, Y., Tsukamoto, H., Yanagi, T., 2010. Complex vertical migration of larvae of the ghost shrimp, *Nihonotrypaea harmandi*, in inner shelf waters of western Kyushu, Japan. Estuar. Coast. Shelf Sci. 86, 125-136.

Tsukamoto, Y., Zenitani, H., Kimura, R., Watanabe, Y., Oozeki, Y., 2001. Vertical distribution of fish larvae in the Kuroshio and Kuroshio-Oyashio transition region in early summer. Bull. Nat. Res. Inst. Fish. Sci. 16, 39–56.

Ueda, H., Kuwatani, M., Suzuki, K.W., 2010. Tidal vertical migration of two estuarine copepods: naupliar migration and position-dependent migration. J. Plankton Res. 32, 1557-1572.

Voss, R., Schmidt, J.O., Schnack, D., 2007. Vertical distribution of Baltic sprat larvae: changes in patterns of diel migration? ICES J. Mar. Sci. 64, 956-962.

Yamaguchi, A., Kume, G., 2007. Evidence for up-estuary transport of puffer Takifugu larvae (Tetraodontidae) in Ariake Bay, Japan. J. Appl. Ichthyol. 24, 60-62.

FIGURE CAPTIONS

Fig. 1. Map of the Mondego estuary and location of sampling site (M).

Fig. 2. Salinity and temperature (ºC) (mean+SD) recorded during each sampling period according to tide (ebb and flood).

Fig. 3. Tidal variation of weighted mean depth (m) of the main species at each sampling moment. Significantly differences between ebb and flood weighted mean depths are marked with (*).

Fig. 4. Amplitude of diel vertical migration (m) for the main species at each sampling moment. Significantly differences between day and night weighted mean depths are marked with (*). Negative values indicate reverse migration (deeper at night time than during the day).

Fig. 5. Variation in fish larvae density (ind. $100m^{-3}$) and weighted mean depth (m) at each season and lunar phase according to tidal cycle (LT, low tide; HT, high tide) and diel phase at each sampled depth. Symbols represent observed values and lines the fitted prediction based on harmonic regression. Only data relative to significant ($p \leq 0.05$) predicted models are presented.

Table 1

Summary results of PERMANOVA analysis on salinity and temperature. p≤0.05*, **p≤0.01, ***p≤0.001.

		Summer Neap (n=50)	Summer Spring (n=50)	Winter Neap (n=44)	Winter Spring (n=50)
		$F_{1,1}$	$F_{1,1}$	$F_{1,1}$	$F_{1,1}$
Salinity	Tide (TI)	0.34	0.03	0.46	7.91^{**}
	Depth (DE)	22.93^{***}	0.02	653.51^{***}	340.76^{***}
	TIxDE	2.13	0.07	0.57	11.87^{**}
Temperature	Tide (TI)	0.11	1.60	0.92	1.11
	Depth (DE)	43.34^{***}	9.39^{**}	4.68^{***}	171.70^{***}
	TIxDE	0.48	0.08	0.78	1.37

Table 2

Vertical tidal and diel pattern of mean total fish larvae density (ind. 100 m^{-3}) in each sampling period. Significant pairwise comparisons are listed from highest mean (left) to lowest mean (right). B, bottom; S, surface. E, Ebb; F, Flood; D, Day; N, Night. p≤0.05*, **p≤0.01, ***p≤0.001.

		Tidal cycle					**Diel cycle**				
		Flood		**Ebb**			**Day**		**Night**		
Sampling period	**Species**	**B**	**S**	**B**	**S**	**Stat.**	**B**	**S**	**B**	**S**	**Stat.**
Summer Neap	n	12	12	13	13		16	16	9	9	
	Pomatoschistus spp.	242.96 (352.47)	145.40 (283.34)	992.54 (1241.35)	630.16 (640.11)		201.67 (352.14)	143.84 (269.28)	1130.88 (1237.41)	686.11 (653.12)	ND***
	P. pilicornis	11.30 (13.96)	26.08 (25.14)	60.89 (94.75)	293.53 (499.57)	EF*	6.33 (2.58)	11.83 (6.82)	63.09 (93.72)	253.70 (457.35)	ND***
	Gobius niger	18.98 (20.37)	30.53 (40.43)	55.30 (27.98)	195.89 (285.64)	EF**	25.02 (19.24)	28.39 (32.36)	55.31 (31.99)	317.68 (328.31)	ND***
Summer Spring	n	13	13	12	12		15	15	10	10	
	Pomatoschistus spp.	168.23 (309.32)	211.70 (303.95)	157.36 (296.92)	184.81 394.94)		48.60 (52.93)	45.39 (91.38)	353.13 (427.50)	348.13 (443.64)	ND***
	P. pilicornis	10.59 (5.04)	10.71 (9.03)	9.90 (7.15)	57.84 (0)		9.97 (5.75)	0.00 (0)	10.34 (6.80)	22.49 (24.69)	ND**
	Gobius niger	30.95 (30.18)	24.43 (18.28)	37.15 (30.22)	31.12 (24.73)		26.09 (30.24)	30.63 (19.66)	41.12 (28.26)	26.91 (23.44)	ND*
Winter Neap	n	10	10	12	12		6	6	16	16	
	Pomatoschistus spp.	2.59 (0.83)	3.82 (1.67)	5.50 (3.41)	4.99 (3.51)		3.00 (1.03)	1.23 (0)	4.44 (3.15)	4.86 (2.47)	
	S. pilchardus	14.55 (16.55)	10.03 (9.84)	11.55 (11.31)	13.11 (13.69)		0.00 (0)	3.56 (0)	13.26 (14.11)	12.46 (11.67)	ND***
Winter Spring	n	13	13	12	12		10	10	14	14	
	Pomatoschistus spp.	10.18 (6.20)	4.34 (2.49)	7.51 (5.27)	4.49 (3.19)	BS**	5.47 (2.79)	2.20 (0.43)	10.09 (6.14)	5.03 (2.88)	ND*** BS**
	S. pilchardus	12.17 (13.62)	19.76 (30.24)	10.62 (12.88)	6.60 (4.57)		17.70 (15.97)	20.23 (34.31)	5.28 (3.50)	9.10 (6.15)	

Table 3

Coefficient of determination (R^2) of harmonic regression adjusted to surface and bottom larvae densities and to weighted mean depth (WMD). Significant regression estimates are marked with p≤0.05*, **p≤0.01, ***p≤0.001.

		Summer Neap		**Summer Spring**		**Winter Neap**		**Winter Spring**	
Pomatoschistus spp.	Surface	**0.77*****	K1, M2	**0.50***	K1, M2	0.36		**0.63****	K1, M2
	Bottom	**0.73*****	K1, M2	**0.54***	K1, M2	0.27		**0.58****	K1, M2
	WMD	0.39		**0.48***	M2	0.68		0.21	
Parablennius pilicornis	Surface	0.32		0.26					
	Bottom	**0.54***	K1, M2	0.17					
	WMD	0.40		0.69					
Gobius niger	Surface	**0.58****	K1, M2	**0.50***	M2				
	Bottom	**0.85*****	K1, M2, M4	**0.50***	K1, M2				
	WMD	0.23		0.63					
Sardina pilchardus	Surface					**0.61****	K1, M2	0.40	
	Bottom					**0.71***	K1, M2	**0.63****	K1, M2
	WMD					0.43		0.49	

Table 4

Amplitude estimates for each tidal constituent (K1, M2, M4). Estimates are presented only for significant (p≤0.05) fitted models.

		Summer Neap			**Summer Spring**			**Winter Neap**			**Winter Spring**		
		K1	**M2**	**M4**	**K1**	**M2**	**M4**	**K1**	**M2**	**M4**	**K1**	**M2**	**M4**
Pomatoschistus spp.	Surface	501.0	344.9		219.3	206.0					2.8	1.9	
	Bottom	838.9	576.1		199.7	213.4					4.89	4.61	
	WMD					1.2							
Parablennius pilicornis	Surface												
	Bottom	43.1	37.0										
	WMD												
Gobius niger	Surface	168.5	134.1			15.9							
	Bottom	30.0	24.1	9.7	20.7	15.7							
	WMD												
Sardina pilchardus	Surface							7.65	5.92				
	Bottom							12.2	8.19		7.8	9.61	
	WMD												

HIGHLIGHTS

- Vertical distribution and migration pattern of ichthyoplankton assemblage.
- Night samples presented higher density than during day.
- No differences in depth stratums' densities across diel or tide cycle.
- Larval densities varied according to diel and tidal cycles.
- Vertical migration was related to hydrological regime and seasonal stratification.

Figure 1

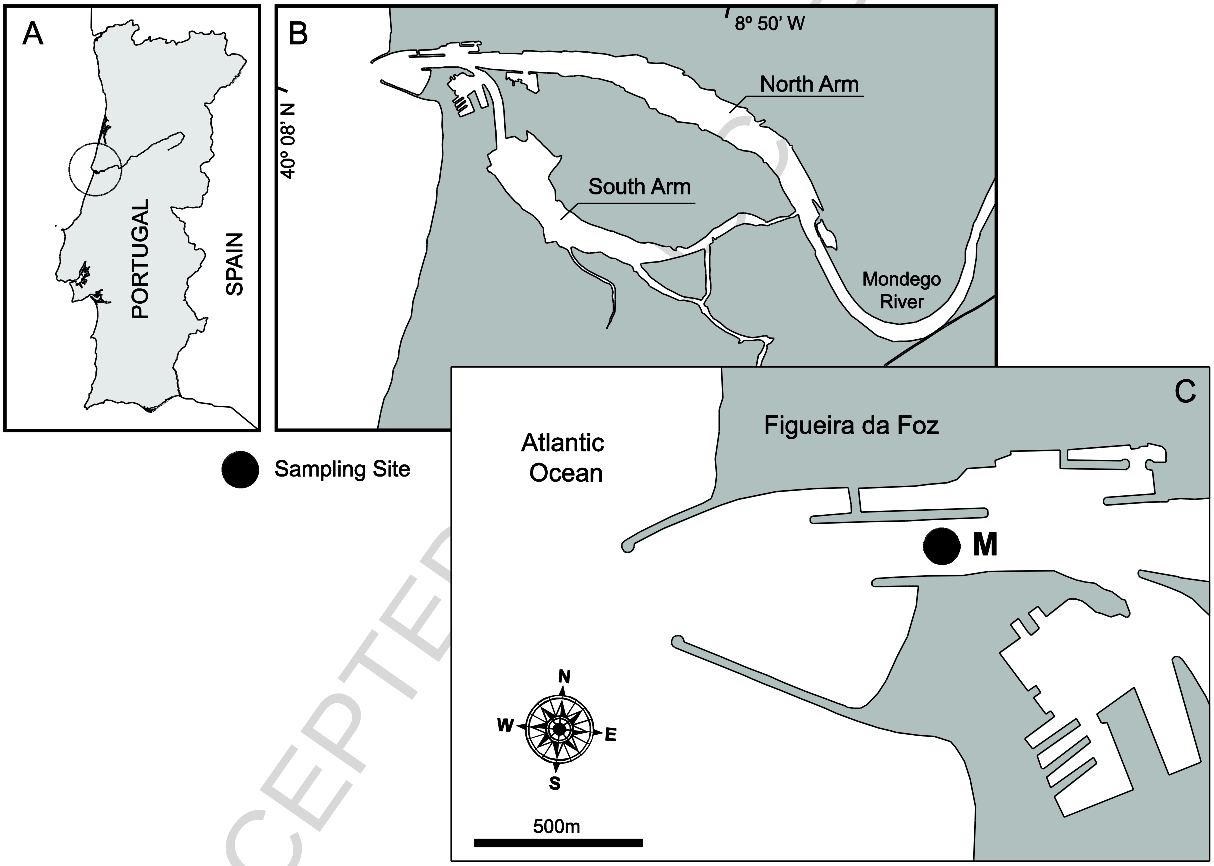

A
PORTUGAL
SPAIN
B
8º 50' W
40º 08' N
North Arm
South Arm
Mondego
River
C
Atlantic
Ocean
Figueira da Foz
M
N
W
E
S
500m
Sampling Site

Figure 2

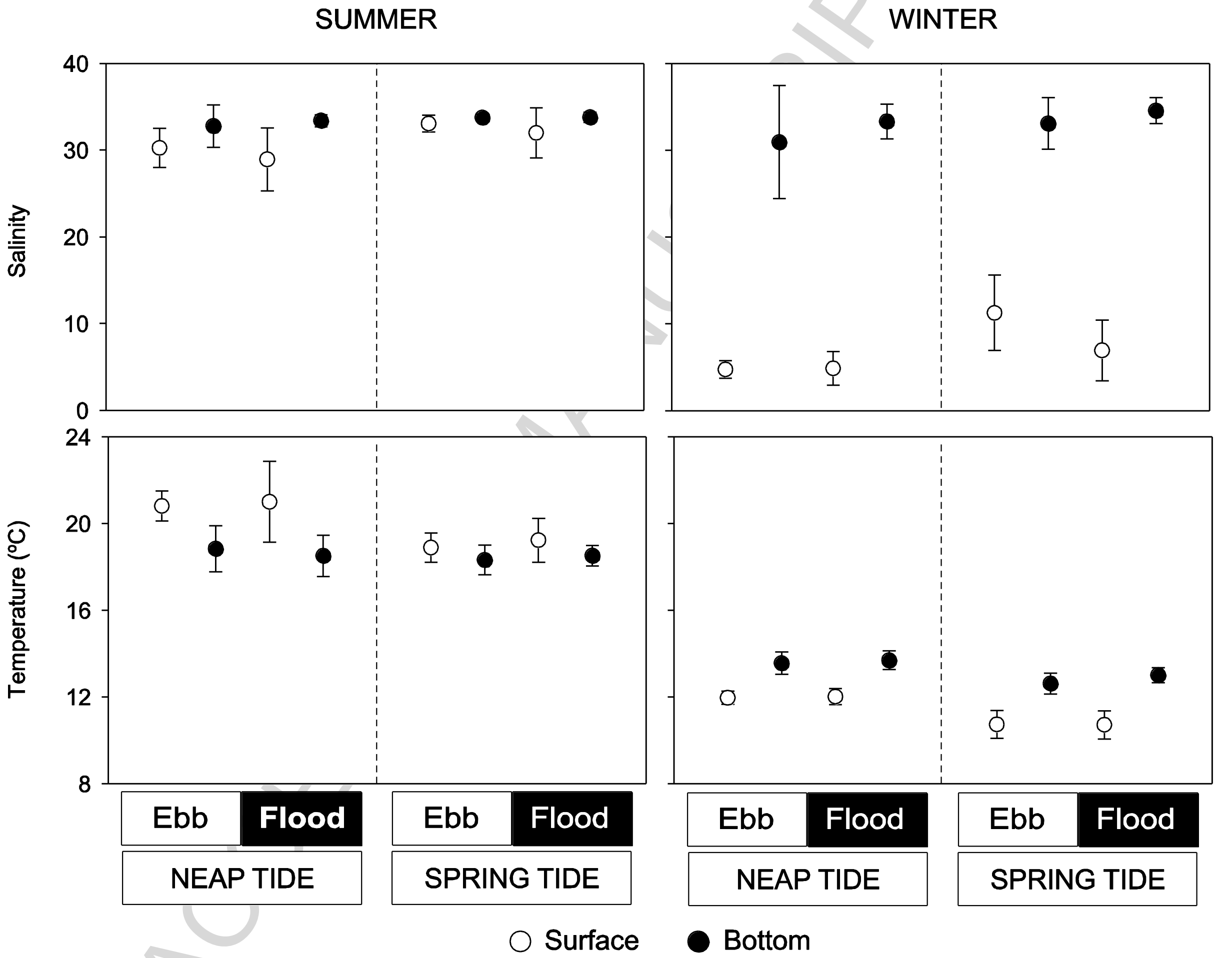

SUMMER
WINTER
Salinity
40
30
20
10
0
Temperature (ºC)
24
20
16
12
8
Ebb
Flood
NEAP TIDE
Ebb
Flood
SPRING TIDE
Ebb
Flood
NEAP TIDE
Ebb
Flood
SPRING TIDE
○ Surface
● Bottom

Figure 3

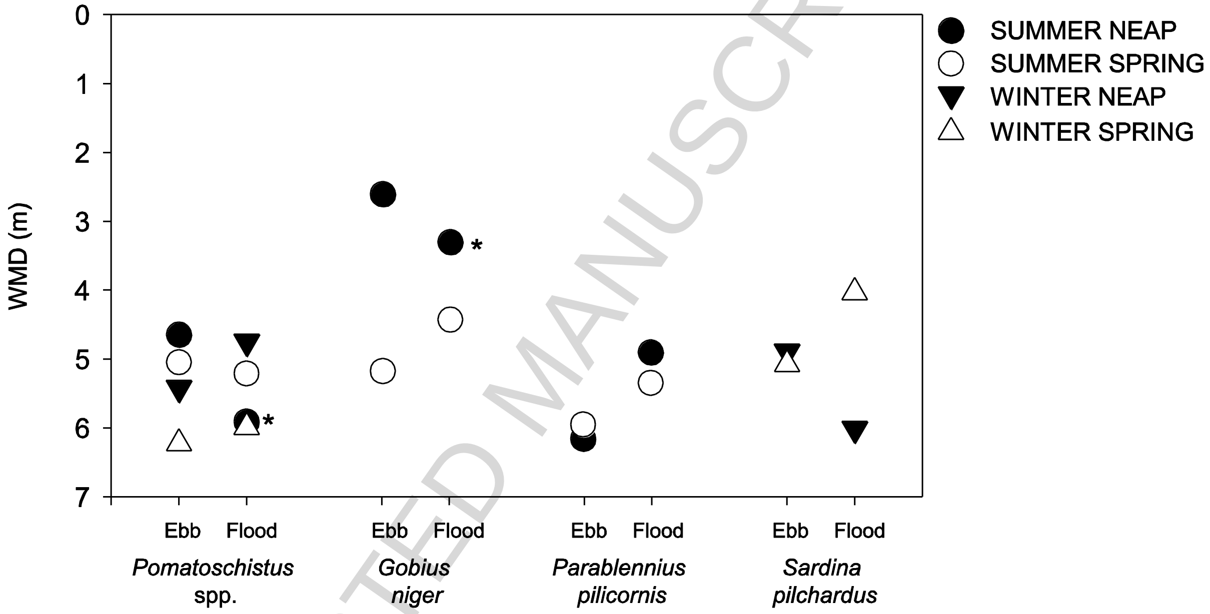

WMD (m)
0
1
2
3
4
5
6
7
Ebb
Flood
Ebb
Flood
Ebb
Flood
Ebb
Flood
Pomatoschistus spp.
Gobius niger
Parablennius pilicornis
Sardina pilchardus
*
*
SUMMER NEAP
SUMMER SPRING
WINTER NEAP
WINTER SPRING

Figure 4

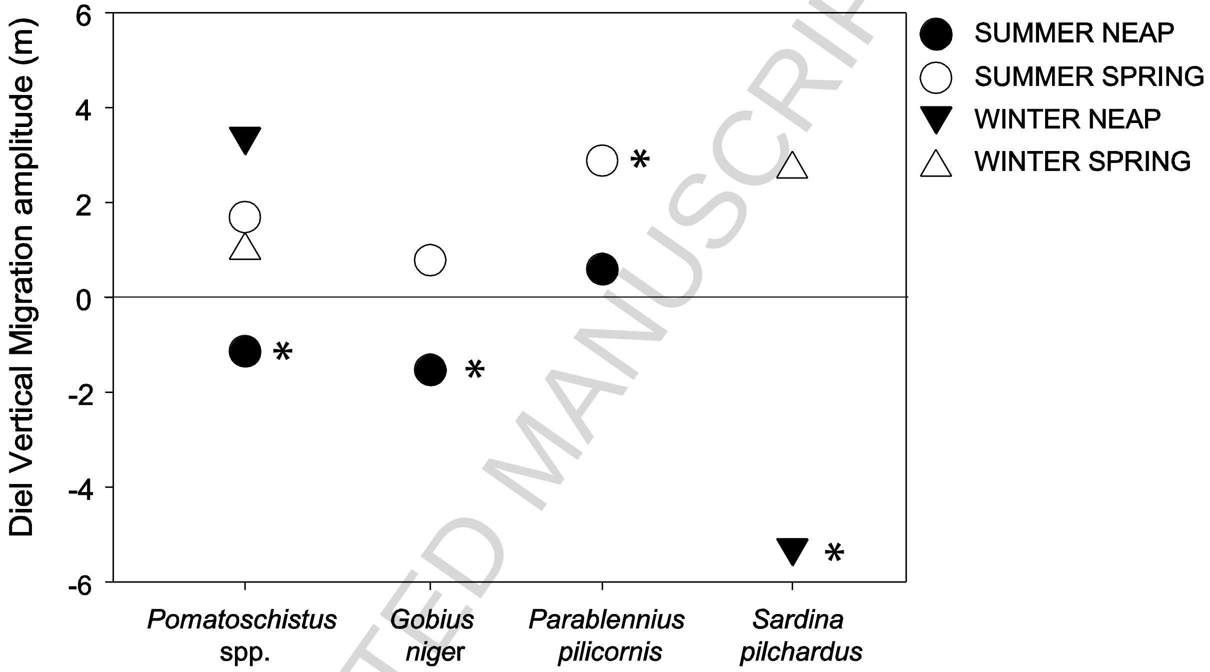
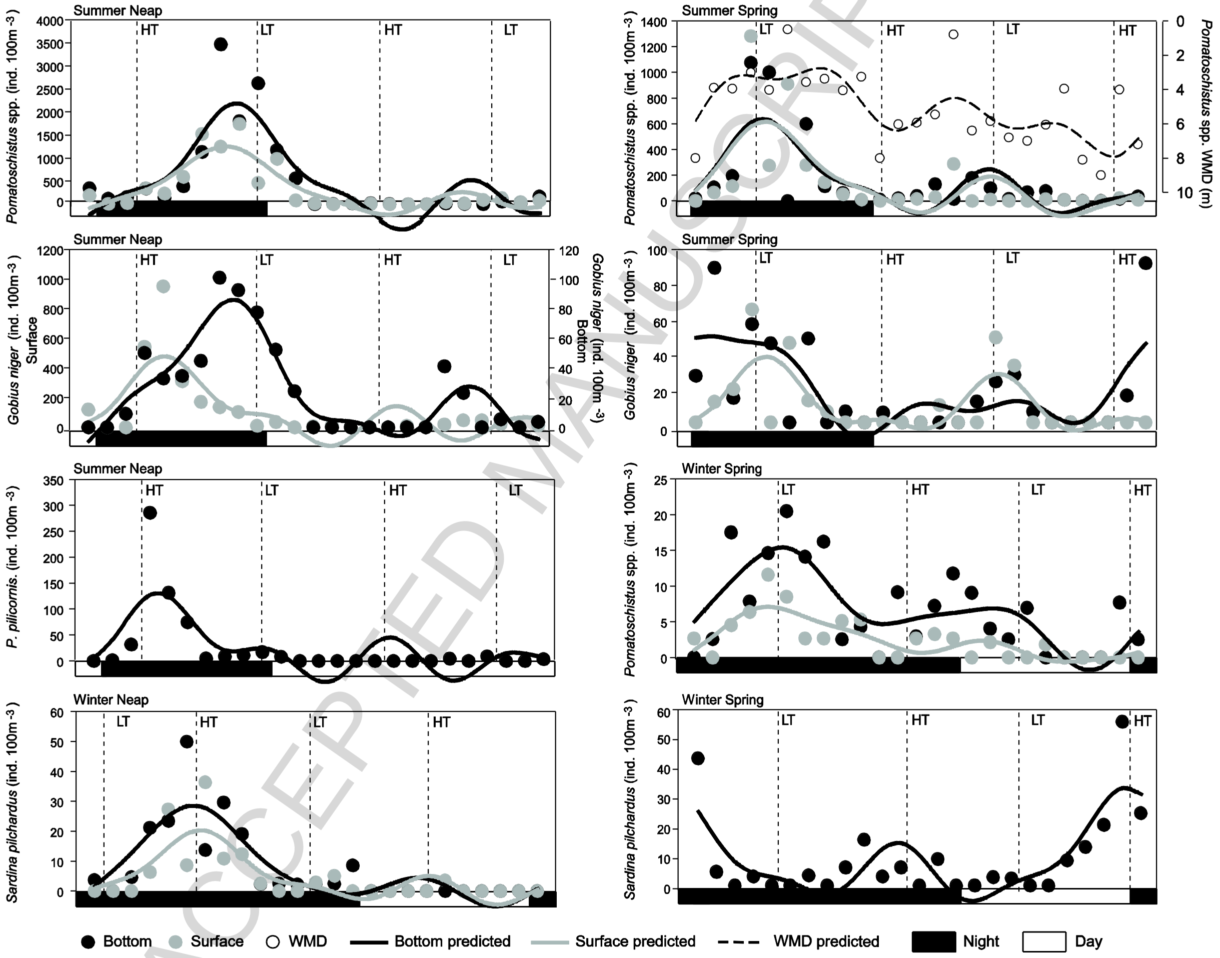

Diel Vertical Migration amplitude (m)
6
4
2
0
-2
-4
-6
*
*
*
*
Pomatoschistus spp.
Gobius niger
Parablennius pilicornis
Sardina pilchardus
● SUMMER NEAP
○ SUMMER SPRING
▼ WINTER NEAP
△ WINTER SPRING

Figure 5


Summer Neap
Pomatoschistus spp. (ind. 100m -3)
4000
3500
3000
2500
2000
1500
1000
500
0
HT
LT
HT
LT
Summer Spring
Pomatoschistus spp. (ind. 100m -3)
1400
1200
1000
800
600
400
200
0
LT
HT
LT
HT
Pomatoschistus spp. WMD (m)
0
2
4
6
8
10
Summer Neap
Gobius niger (ind. 100m -3) Surface
1200
1000
800
600
400
200
0
HT
LT
HT
LT
Gobius niger (ind. 100m -3) Bottom
120
100
80
60
40
20
0
Summer Spring
Gobius niger (ind. 100m -3)
100
80
60
40
20
0
LT
HT
LT
HT
Summer Neap
P. pilicornis. (ind. 100m -3)
350
300
250
200
150
100
50
0
HT
LT
HT
LT
Winter Spring
Pomatoschistus spp. (ind. 100m -3)
25
20
15
10
5
0
LT
HT
LT
HT
Winter Neap
Sardina pilchardus (ind. 100m -3)
60
50
40
30
20
10
0
LT
HT
LT
HT
Winter Spring
Sardina pilchardus (ind. 100m -3)
60
50
40
30
20
10
0
LT
HT
LT
HT
Bottom
Surface
WMD
Bottom predicted
Surface predicted
WMD predicted
Night
Day