## Accepted Manuscript

Title: Impact of climate variability on ichthyoplankton communities: an example of a small temperate estuary

Authors: Ana Lígia Primo, Ulisses Miranda Azeiteiro, Sónia Cotrim Marques, Miguel Ângelo Pardal


PII: $\quad$ S0272-7714(10)00399-9
DOI: $\quad$ 10.1016/j.ecss.2010.11.009
Reference: YECSS 3419

To appear in: Estuarine, Coastal and Shelf Science

Received Date: 10 February 2010
Revised Date: 28 September 2010
Accepted Date: 30 November 2010

Please cite this article as: Primo, A.L., Azeiteiro, U.M., Marques, C., Pardal, Miguel Ângelo. Impact of climate variability on ichthyoplankton communities: an example of a small temperate estuary, Estuarine, Coastal and Shelf Science (2010), doi: 10.1016/j.ecss.2010.11.009

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

191 Ulisses Miranda Azeiteiro - ulisses@univ-ab.pt
example of a small temperate estuary

Pardal ${ }^{\text {a }}$ Apartado 3046, 3001-401 Coimbra, Portugal.

Sónia Cotrim Marques - scotrim@ci.uc.pt
Miguel Ângelo Pardal - mpardal@ci.uc.pt
*Corresponding author
Ana Lígia Primo
e-mail address: ana.primo@student.zoo.uc.pt

Apartado 3046, 3001-401 Coimbra, Portugal
Tel.: + 351239855780
Fax: + 351239823603

Impact of climate variability on ichthyoplankton communities: an

${ }^{\text {a }}$ CEF - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra,
${ }^{\text {b }}$ Department of Science and Technology, Universidade Aberta, 4200-055 Porto, Portugal

CEF - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra,

## ABSTRACT

Recent variations in the precipitation regime across southern Europe have led to changes in river fluxes and salinity gradients affecting biological communities in most rivers and estuaries. A sampling programme was developed in the Mondego estuary, Portugal, from January 2003 to December 2008 at five distinct sampling stations to evaluate spatial, seasonal and interannual distributions of fish larvae. Gobiidae was the most abundant family representing $80 \%$ of total catch and Pomatoschistus spp. was the most important taxon. The fish larval community presented a clear seasonality with higher abundances and diversities during spring and summer seasons. Multivariate analysis reinforced differences among seasons but not between years or sampling stations. The taxa Atherina presbyter, Solea solea, Syngnathus abaster, Crystallogobius linearis and Platichthys flesus were more abundant during spring/summer period while Ammodytes tobianus, Callionymus sp., Echiichthys vipera and Liza ramada were more abundant in autumn/winter. Temperature, chlorophyll $a$ and river flow were the main variation drivers observed although extreme drought events (year 2005) seemed not to affect ichthyoplankton community structure. Main changes were related to a spatial displacement of salinity gradient along the estuarine system which produced changes in marine species distribution.

Key words: Ichthyoplankton; seasonality; environmental factors; drought; Mondego estuary

## 1. Introduction

Nearshore estuarine and marine ecosystems serve many important functions in coastal waters. Often referred to as nurseries, estuaries play an important role in many species lifecycles, including fish (Beck et al., 2001; Elliott and McLusky, 2002; Martinho et al., 2007a) providing food abundance and shelter to marine fish larvae and juveniles and therefore maximizing their survival (Whitfield, 1999; Elliott and McLusky, 2002). Larval fish dynamics contribute significantly to understanding the ecology of fish populations (Doyle et al., 2002) as they can indicate the spawning-stock biomass and recruitment in adult fish stocks (Hsieh et al., 2005). Initial development stages of fishes are particularly vulnerable and are influenced by physical and biological processes. Indeed, several factors have already been related to survival and distribution of ichthyoplankton (e.g. hydrological conditions, transport processes, seasonal variability, spawning patterns of adults, food availability) (Franco-Gordo et al., 2002; Alemany et al., 2006; Sabatés et al., 2007; Isari et al., 2008). The effects of climate on fish populations can also be shown by long term trends in ichthyoplankton populations. Lower trophic level organisms should be more sensitive in reflecting environmental perturbations more quickly than higher trophic levels but early life stages may be environmentally sensitive prior to buffering through density-dependent mechanisms and community effects (Boeing and Duffy-Anderson, 2008). Thus a knowledge of the ichthyoplankton community dynamics are important in understanding changes in fish communities.

Recent studies indicate that the Mondego estuary ( $40^{\circ} 08^{\prime} \mathrm{N}, 8^{\circ} 50^{\prime} \mathrm{W}$ ), Portugal, is an important nursery ground for several commercial fish species (e.g. Dicentrarchus labrax, Platichthys flesus and Solea solea) (Leitão et al., 2007; Martinho et al., 2007a). Studies on ichthyoplankton started with Ribeiro (1991) but recently only Marques et al. (2006) referred to these communities. Previous works focused on community assemblages but information of the way environmental factors force community structure is still limited. In addition, Portugal recently has been under varying precipitation regimes with values of 45-60\% below average in the hydrological year 2004/2005 producing the biggest drought in a century (Portuguese Weather Institute: http://www.meteo.pt/en/index.html) and thus providing a unique opportunity to investigate ichthyoplankton responses to extreme events. Hence this study aimed to characterize ichthyoplankton assemblages, to evaluate environmental influence in its structure and establish the consequences of extreme events, such as droughts, on estuarine fish larvae communities. The hypothesis tested was that reduced river flow resulted from the decrease of precipitation mean levels lead to changes on community structure and longitudinal displacement of species according to salinity gradients.

## 2. Material and Methods

### 2.1. Study area

The Mondego estuary, located on the Atlantic coast of Portugal ( $40^{\circ} 08^{\prime} \mathrm{N}, 8^{\circ} 50^{\prime} \mathrm{W}$ ), consists of two channels (northern and southern) with different hydrological characteristics separated by the Murraceira Island (Fig. 1). The north channel is deeper ( $4-8 \mathrm{~m}$ depth at high tide) has lower residence times ( $<1$ day) and constitutes the main navigation channel, while the south channel is shallower ( $2-4 \mathrm{~m}$ deep, at high tide), has higher residence times ( $2-8$ days) and is almost silted up in the upper areas. Most of the freshwater discharge is throughout the northern channel since it is directly connected with the Mondego River. In the southern channel, water circulation is mostly due to tides and the freshwater input from a small tributary, the Pranto River which is small and artificially regulated by a sluice. Previous studies demonstrated that distinct environmental factors provide a large variety of aquatic habitats for populations of marine, brackish and freshwater zooplankton species, mainly due to salinity and water temperature gradients (Azeiteiro et al., 1999; Marques et al., 2006; Primo et al., 2009).

### 2.2. Sample collection

Sampling was carried out monthly during daylight at high tide, from January 2003 to December 2008 at five stations distributed throughout both arms (Fig. 1). Samples were collected by horizontal subsurface tows (Bongo net: mesh size $335 \mu \mathrm{~m}$, mouth diameter: 0.5 m , tow speed: 2 knots), equipped with a Hydro-Bios flowmeter (the volume filtered averaged $45 \mathrm{~m}^{3}$ ) and preserved in a $4 \%$ buffered formaldehyde seawater solution. Additionally, at each site, salinity, water temperature $\left({ }^{\circ} \mathrm{C}\right)$, dissolved oxygen ( $\mathrm{mg} \mathrm{1} \mathrm{l}^{-1}$ ), pH and turbidity (Secchi disc depth, m ) were also recorded. Subsurface water samples were also collected for subsequent determination in the laboratory for chlorophyll $a\left(\mathrm{mg} \mathrm{m}^{-3}\right)$ and total suspended solids ( $\mathrm{mg} \mathrm{l}^{-1}$ ). In the laboratory, the ichthyoplankton was sorted, counted (number of individuals per $100 \mathrm{~m}^{3}$ ) and identified to the highest possible taxonomic separation (Petersen, 1919; Fives, 1970; Nichols, 1976; Demir, 1976; Russell, 1976; Ré, 1999; Ré and Meneses, 2008). Copepod densities (ind m ${ }^{-3}$ ) were also recorded. Monthly precipitation values were acquired from INAG - Instituto da Água (http://snirh.pt/) measured at the Soure 13 F/01G station and the freshwater discharge from the Mondego River was obtained from INAG station Açude Ponte Coimbra 12G/01AE.

### 2.3. Data analysis

Sampling months were combined into four conventional seasons: winter (W) included December, January and February; spring (S), March, April and May; summer (SM), June, July and August and autumn (A), September, October and November. Species were characterized in three main
ecological guilds (adapted from Elliott et al., 2007): marine stragglers (MS - Species that spawn at sea and typically enter estuaries in low numbers occurring frequently in the lower reaches), marine migrants (MM - Species that spawn at sea and often enter estuaries in large numbers) and estuarine species (ES - including estuarine species capable of completing their entire life cycle within the estuarine environment and those with stages of their life cycle completed outside the estuary).
Salinity anomalies were calculated by subtracting the mean seasonal value from the mean value of the given time period. The differences between seasons and years in each sampling station were tested by Analysis of Variance (ANOVA) for environmental factors. Log ( $\mathrm{x}+1$ ) transformation was performed and for pairwise multiple comparisons the Holm-Sidak method was applied. Temporal and spatial ichthyoplankton distribution maps were obtained by Sigmaplot software as well as diversity, expressed by Shannon-Wiener Index $\left(\log _{2}\right)$.
PERMANOVA+ for PRIMER software (PRIMER v6 \& PERMANOVA + v1, PRIMER-E Ltd.) was used to perform a non-parametric permutational multivariate analysis of variance (PERMANOVA) to test for differences in the assemblage structure between years, seasons and sampling stations. The analysis was based on Bray-Curtis similarities between samples, after a fourth root transformation of abundance data, considering all the factors (year, season, station) as fixed and unrestricted permutations of raw data. When necessary, a posteriori multiple comparisons were used to test for differences between/within groups for pairs of levels of factors.
The effects of environmental variables on the larval fish assemblage were analyzed with canonical correspondence analysis (CCA) using software CANOCO (version 4.5, Microcomputer Power). Environmental variables included salinity (Sal), water temperature (T) dissolved oxygen $\left(\mathrm{O}_{2}\right), \mathrm{pH}$, turbidity (Secchi), chlorophyll $a$ (Chl a), total suspended solids (TSS), copepod densities (Cop), freshwater discharge (Runoff) and precipitation (PP). Seasons were also included as nominal variables. All species were used and a new category coded as "no fish" was created to prevent CANOCO from eliminating samples containing no fish. "No fish" was assigned the minimum possible weight (density $=0.001$ ) to prevent an otherwise uniform concentration in samples from driving the ordination (Grothues and Cowen, 1999). Larval abundances were $\ln (2 x+1)$ transformed and environmental variables were standardized by subtracting the mean and dividing by the standard deviation. A forward stepwise selection procedure of explanatory variables was applied and a CCA triplot scaling with focus interspecies distances was performed.
3. Results

### 3.1. Environmental conditions

The Mondego estuary has a typical seasonal pattern of precipitation and freshwater discharge throughout the six-year period with higher values during winter and lower during summer. However, 2004, 2005 and 2008 showed below-average precipitation and a low freshwater discharge, particularly in 2005 (Fig. 2). The salinity was highly variable between years with 2005 and 2008 presenting positive anomalies in all sampling sites during almost all seasons indicating higher salinity values than average (Fig. 3). Salinity values recorded at sampling stations M and S2 in 2005 were significantly higher than in 2006 (post hoc test $\mathrm{p}<0.05$ and $\mathrm{p}<0.01$, respectively). Also, the upstream sampling station N2 had significant higher salinity in 2005 and 2008 than in 2003 and 2006 (post hoc test p<0.01). Seasonally, only sampling stations M and N2 had significant differences with winter having lower salinity than summer, autumn and spring (post hoc test $\mathrm{p}<0.01$ ).

All sampling stations showed significant differences in water temperature (Fig. 3) between seasons with winter being lower values than summer, autumn and spring (post hoc test $\mathrm{p}<0.001$ ). No significant differences were detected between years at any sampling station ( $p>0.05$ ).
Chlorophyll $a$ reached higher values in the most upstream sampling stations (Fig. 3) although no significant differences were detected between years ( $\mathrm{p}>0.05$ ) and only sampling station N2 had significantly higher chlorophyll $a$ in summer than in autumn ( $\mathrm{F}=3.826$, $\mathrm{p}<0.05$; post hoc test $\mathrm{p}<0.01$ ).

### 3.2. Seasonal and spatial patterns of larval distribution

During the study period, a total of 7211 fish larvae were collected in Mondego estuary and identified to 31 different taxa (Table I). Unidentified larvae represented $8.75 \%$ of the total catch and were generally yolk-sac or damaged larvae. The most abundant family was Gobiidae (80\%) followed by Blenniidae (3\%), Soleiidae (3\%) and Engraulidae (2\%). Pomatoschistus spp. was the most abundant taxon accounting for $63.4 \%$ of all fish larvae caught, followed by Pomatoschistus microps (6.02\%), unidentified Gobiidae (4.65\%), Gobius niger (3.57\%), Engraulis encrasicolus (1.96\%) and Parablennius pilicornis ( $1.88 \%$ ). These species contributed for $81 \%$ of the total catch and are present in almost every seasons and sampling stations. Summer and spring had a higher species richness as well as sampling station S1 while autumn had a lower diversity. Species such as Atherina presbyter, Solea solea, Symphodus melops, Syngnathus abaster, Crystallogobius linearis, Platichthys flesus and Arnoglossus thori were exclusively captured during spring and summer.

Conversely, Solea senegalensis, Ammodytes tobianus, Callionymus spp., Echïchthys vipera and unidentified Ammodytidae were more abundant in winter.
Fish larvae density clearly showed seasonality with higher densities during spring and summer (Fig. 4). According to the ecological guilds, estuarine species showed greatest densities during the whole study period except for 2008 where marine straggler abundance reached maximum values. The estuarine taxa showed peaks of densities during summer except in 2004 and 2005 where higher values occurred early in spring (Fig. 4). In general, these species were well distributed along the estuary with highest densities in the downstream south arm station (S1) and in the upstream estuary (S2 and N2) (Fig.5A). The marine stragglers occurred mainly at station S1 reaching the upstream stations only in year 2008 (Fig. 5B). The same happened with marine migrant species which during 2005 and 2008 reached the upper estuary (N1, N2 and S2) (Fig. 5C).
Pomatoschistus spp. abundance and distribution mimic the total fish larvae pattern since this is the most important species in Mondego estuary (Fig. 6). Sampling station S1 had higher abundances across the years. Engraulis encrasicolus attained higher densities during 2008 and was found especially in the upper south arm (station S2) (Fig. 6). Solea senegalensis presented seasonal peaks mainly in summer and spring but occasionally also in winter and autumn (Fig. 6). Its spatial distribution was generally restricted to the downstream sampling stations M and S1 but in 2005 and 2008 it reached high densities in the upstream sampling stations S2 and N2. Seasonality was also shown by the Shannon-Wiener diversity index with higher values in spring/summer (Fig.6).

### 3.3. Community structure and relation with environmental variables

The PERMANOVA results showed significant differences in community structure between years and seasons, as well as significant interactions between the factors "year" and "season" (Pseudo F= $1.429, \mathrm{p}(\mathrm{perm})<0.05)$. A pairwise a posteriori comparison revealed that in the summer, the 2003 community differed from the other years ( $\mathrm{t}<2.060, \mathrm{p}($ perm $)<0.05$ ). Autumn and winter showed no differences between years ( $\mathrm{p}(\mathrm{perm}$ ) $>0.05$ ) and in spring only pairwise comparison for 2003/2008 presented significant differences $(\mathrm{t}=1.582$, $\mathrm{p}($ perm $)<0.05)$. In addition 2003, 2006 and 2007 showed differences between seasons with summer having a community differing from autumn and winter ( $\mathrm{p}(\mathrm{perm}$ ) <0.05). In 2008, the summer presented differences only from the winter ( $\mathrm{p}(\mathrm{perm})<0.01$ ) and in 2004 and 2005 no seasonal differences were detected ( $\mathrm{p}(\mathrm{perm}$ ) >0.05).
Community differences between sampling stations were also detected (Pseudo $\mathrm{F}=2.003$, $\mathrm{p}($ perm $)<0.01$ ) but only between M and S 1 (Pairwise a posteriori comparison $\mathrm{t}=2.186$, $\mathrm{p}($ perm $)<0,001)$.
A Monte Carlo test of F-ratio showed that only six environmental variables contributed significantly to explaining the species distribution ( $\mathrm{p}<0.05$ ) (winter, salinity, temperature,
chlorophyll $a$, summer and runoff). Taken together, the environmental variables considered in the final CCA explained $8 \%$ of the total variation in fish larvae assemblages. The first two CCA axes accounted for $57 \%$ of the variability explained. The first axis is correlated with winter while the second axis is positively correlated with summer, temperature, chlorophyll $a$ and salinity and negatively with runoff. The right hand side of ordination diagram of the first two axes grouped winter samples characterized by low temperatures and high river flow (Fig. 7). Species such as Echiichthys vipera, Callionymus spp. and Liza ramada were more prevalent in winter whereas summer samples clustered in the upper left side and are more related to Atherina presbyter, Crystallogobius linearis, Syngnathus abaster, Syngnathus acus or Solea solea (Fig. 7).

## 4. Discussion

The larval fish assemblages of the Mondego estuary supported 31 taxa dominated by Pomatoschistus spp. Dominance by few species and presence of a high number of rare species is a common feature observed in estuaries around the world either in larval or juvenile fish populations (e.g. Barletta-Bergan et al., 2002; Strydom et al., 2003; Selleslagh et al., 2009). Pomatoschistus spp. larvae dominance has been encountered in other Portuguese estuaries (e.g. Faria et al., 2006; Ramos et al., 2006) and the success of gobies in estuarine environment may be related to their benthic reproductive strategy ensuring that eggs are not flushed out from the estuary and are less exposed to salinity and temperature fluctuations, which are more pronounced in surface waters (Ribeiro et al., 1996). Mazzoldi and Rasotto (2001) also suggested that in highly productive habitats with warm summers, the long breeding season of short-lived species (such as $P$. microps) can give rise to more than one spawning peak in the breeding period, which may be the case of $P$. microps in the Mondego estuary as already been noticed by Dolbeth et al. (2007). In the Mondego estuary, Pomatoschistus microps and P, minutus are amongst the most abundant species in estuarine fish assemblages (Leitão et al., 2007; Martinho et al., 2007a) but, unfortunately, during early life stages these species cannot be easily differentiated.

Ribeiro and Gonçalves (1993) found that Engraulis encrasicolus captures in the Mondego estuary accounted for $44 \%$ of total fish larvae while in the present study the species only represents $2 \%$ of the total capture. This reduction was also recently recorded in the Guadiana estuary (Faria et al., 2006) and in the Lima estuary where this species was present occasionally and in low numbers ( $<1 \%$ ) (Ramos et al., 2006). The abundance and distribution of anchovy is closely related with environmental factors as temperature, turbidity, salinity or prey availability (Ribeiro et al., 1996, Chícharo et al., 2001; Drake et al., 2007) and salinity gradient changes due to dry periods may have stimulated anchovy to spawn in inner parts since E. encrasicolus can modify its spatial position in
order to remain within limited salinity bands (Drake et al., 2007). Anchovy abundances increased in coastal areas adjacent to the Guadiana estuary during high river flow periods and it is suggested that a reduction of inflow may have more negative consequences for eggs and larval stages that are more susceptible (Chícharo et al., 2001).
Most taxa displayed a seasonal pattern presenting higher abundances during spring and summer and the multivariate analysis confirmed distinct seasonal communities. However, this seasonal sign decreases during years with low freshwater discharges and consequent higher salinity anomalies. Indeed differences among seasons were higher than across years or sampling stations. Several studies have already shown that temporal changes in composition and abundance are mostly related to spawning patterns of adult fishes (e.g. Barletta-Bergan et al., 2002; Ramos et al., 2006; Sabatés et al., 2007). Hence, Atherina presbyter, Solea solea, Syngnathus abaster, Crystallogobius linearis and Platichthys flesus were more abundant during spring/summer period while Ammodytes tobianus, Callionymus sp., Echiichthys vipera and Liza ramada were more abundant in autumn/winter. The spatial distribution indicated that fish larvae predominated in inner areas of estuary, mainly in the south arm probably due to the higher residence time thus avoiding being washed out by river flux. Conversely, station M, at the mouth of the estuary, had a different fish larval assemblage with fewer species and abundances.
Seasonal changes in temperature, chlorophyll $a$ and runoff were found to be the main factors forcing larval fish assemblage distributions leading to a decrease in abundance and diversity in fish larval communities during colder months. Seasonal variations in environmental parameters seemed to influence the assemblage structure but there was not a similar set of inter-annual changes in the last six years. The Mondego estuary has recently experienced periods of low precipitation resulting in reduced freshwater runoff and consequently changes in salinity gradients; this has influenced estuarine communities at different trophic levels (e.g. Marques et al., 2007; Martinho et al., 2007b; Cardoso et al., 2008). In the 2004/2005 dry years there was an increase in zooplankton density, a higher abundance and prevalence of marine species throughout the year and a replacement of the freshwater community by one predominantly dominated by estuarine organisms in the most upstream areas (Marques et al., 2007; Primo et al., 2009). However, the main drought-induced effects detected on juvenile fish assemblages were related to a depletion of freshwater species and an increase in marine straggler species (Martinho et al., 2007b). The absence of a close correlation between fish populations and environmental signals is a sign that species might show nonlinear responses to external forcing (Hsieh et al., 2005) and the incidence of droughts may have a similar impact on larval fish assemblages. Despite the community structure remaining relatively unchanged, as indicated by multivariate analysis, the main effects detected were observed in the species distribution. During years with positive salinity anomalies (mainly 2005 and 2008), marine
species (both stragglers and migrants) were able to reach the upper estuary in higher densities. Short term fluctuations in larval abundances are mostly related to reproductive output or geographic shifts (Hsieh et al., 2005) and Fernández-Delgado et al. (2007) suggest that temporal changes in freshwater discharge cause longitudinal displacement of the estuarine salinity gradient leading to related changes in marine species distribution. Also, during 2004 and 2005 the seasonal peak occurred in spring regardless of summer conditions, as recorded during all the other years. The early timing of seasonal peaks is an important response to climate change since it can influence trophic interactions eventually leading to ecosystem-level changes (Edwards and Richardson, 2004). Short-term drought events seem to have a little influence on fish communities probably because fish species are characterized by a slow response time to disturbance (Cabral et al., 2001). Also, the number of influencing factors is too large and individual species may differ very widely in their response. Nevertheless longer time-series are necessary to detect more significant impacts and long term effects of climate change on larval fish assemblages in contrast to other trophic levels.

## 5. Conclusions

Mondego estuary fish larvae assemblages displayed a clear seasonal pattern presenting higher abundances and diversities during warmer months. The main effects of dry events apparently did not affect fish larval community structure but changes in estuarine salinity gradient appear to lead to related changes in marine species distribution. Therefore, river flow played a key role in structuring the ichthyoplankton assemblage thus representing also an important retention mechanism responsible for a successful larval development and recruitment.

## Acknowledgments

The present work was supported by FCT (Portuguese Foundation for Science and Technology) through a PhD grant awarded to AL Primo (SFRH/BD/42351/2007). The authors are indebted to all the colleagues that assisted in the field and lab work. The authors would also like to acknowledge to Dr Rita Borges for her help with fish larvae identification and Susana Mendes for her suggestions in statistical analysis.

## References

Alemany, F., Deudero, S., Morales-Nin, B., López-Jurado, J.L., Jansà, J., Palmer, M., Palomera, I., 2006. Influence of physical environmental factors on the composition and horizontal distribution of summer larval fish assemblages off Mallorca Island (Balearic archipelago, western Mediterranean). Journal of Plankton Research 28(5), 473-487.

Azeiteiro, U.M., Marques, J.C., Ré, P., 1999. Zooplankton annual cycle in the Mondego river estuary (Portugal). Arquivos do Museu Bocage 3, 239-263.

Barletta-Bergan, A., Barletta, M., Saint-Paula, U., 2002. Structure and seasonal dynamics of larval fish in the Caeté river estuary in North Brazil. Estuarine, Coastal and Shelf Science 54, 193-206.
Beck, M.W., Heck, K.L., Able, K.W., Childers, D.L., Eggleston, D. B., Gillanders, B. M., Halpern, B., Hays, C. G., Hoshino, K., Minello, T. J., Orth, R. J., Sheridan, P. F., Weinstein, M. R., 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. BioScience 51, 633-641.

Blaber, S.J.M., 1987. Factors affecting recruitment and survival of mugilids in estuaries and coastal waters of Southeastern Africa. American Fisheries Society Symposium 1, 507-518.
Boeing, W.J., Duffy-Anderson, J.T., 2008. Ichthyoplankton dynamics and biodiversity in the Gulf of Alaska: Responses to environmental change. Ecological Indicators 8, 292-302.
Cabral, H.N., Costa, M.J., Salgado, P., 2001. Does the Tagus estuary fish community reflect environmental changes? Climate Research 18, 119-126.

Cardoso, P.G., Raffaelli, D., Pardal, M.A., 2008. The impact of extreme weather events on the seagrass Zostera noltii and related Hydrobia ulvae population. Marine Pollution Bulletin 56, 483492.

Chícharo, L., Chícharo, M.A., Esteves, E., Andrade, J.P., Morais, P., 2001. Effects of alterations in freshwater supply on the abundance and distribution of Engraulis encrasicolus in the Guadiana Estuary and adjacent coastal areas of south Portugal. Ecohydrology Hydrobiology 1, 341-345.
Clarke, K.R. and Warwick, R.M., 2001. Changes in Marine Communities: An Approach to Statistical Analysis and Interpretation. Plymouth Marine Laboratory, Plymouth, 172 pp.
Demir, N., 1976. Callionymidae of the northeastern Atlantic. Fiches d'Identification du Zooplankton 148, 1-5.
Dolbeth, M., Martinho, F., Leitão, R., Cabral, H., Pardal, M.A., 2007. Strategies of Pomatoschistus minutus and Pomatoschistus microps to cope with environmental instability. Estuarine, Coastal and Shelf Science 74, 263-273.

Doyle, M.J., Mier, K.L., Busby, M.S., Brodeur, R.D., 2002. Regional variation in springtime ichthyoplankton assemblages in the northeast Pacific Ocean. Progress in Oceanography 53, 247281.

Drake, P., Borlán, A., González-Ortegón, E., Baldó, F., Vilas, C., Férnandez-Delgado, C., 2007. Spatio-temporal distribution of early life stages of the European anchovy Engraulis encrasicolus L. within a European temperate estuary with regulated freshwater inflow: effects of environmental variables. Journal of Fish Biology 70, 1689-1709.

Edwards, M., Richardson, A.J., 2004. Impact of climate change on marine pelagic phenology and trophic mismatch. Nature 430, 881-884.

Elliott, M., McLusky, D.S., 2002. The need for definitions in understanding estuaries. Estuarine, Coastal and Shelf Science 55, 815-827.

Elliott, M., Whitfield, A.K., Potter, I.C., Blaber, S.J.M., Cyrus, D.P., Nordlie, F.G., Harrison, T.D., 2007. The guild approach to categorizing estuarine fish assemblages: a global review. Fish and Fisheries 8, 241-268.

Faria, A., Morais, P., Chícharo, M.A., 2006. Ichthyoplankton dynamics in the Guadiana estuary and adjacent coastal area, South-East Portugal. Estuarine, Coastal and Shelf Science 70, 85-97.
Fernandez-Delgado, C., Baldo, F., Vilas, C., Garcia-Gonzalez, D., Cuesta, J.A., Gonzalez-Ortegon, E., Drake, P., 2007. Effects of the river discharge management on the nursery function of the Guadalquivir river estuary (SW Spain). Hydrobiologia 587, 125-136.
Fives, J.M., 1970. Blenniidae of the North Atlantic. Fiches d'Identification du Zooplancton, 3, 1-4. Franco-Gordo, C., Godinez-Dominguez, E., Suarez-Morales, E., 2002. Larval fish assemblages in waters off the central Pacific coast of Mexico. Journal of Plankton Research 24, 775-784.

Hsieh, C., Reiss, C., Watson, W., Allen, M.J., Hunter, J. R., Lea, R. N., Rosenblatt, R. H., Smith, P. E., Sugihara, G., 2005. A comparison of long-term trends and variability in populations of larvae of exploited and unexploited fishes in the Southern California region: a community approach. Progress in Oceanography 67, 160-185.
INAG - Instituto Nacional da Água. Portuguese Water Institute. http://snirh.pt/. (Accessed 08.01.10)

Isari, S., Fragopoulu, N., Somarakis, S., 2008. Interranual variability in horizontal patterns of larval fish assemblages in the northeastern Aegean Sea (eastern Mediterranean) during early summer. Estuarine, Coastal and Shelf Science 79, 607-619.

Leitão, R., Martinho, F., Cabral, H., Jorge, I., Marques, J.C., Pardal, M.A., 2007. The fish assemblage of the Mondego estuary: Composition, structure and trends over the past two decades. Hydrobiologia 587, 269-279.

Marques, S.C., Azeiteiro, U.M., Marques, J.C., Neto, J.M., Pardal, M.A., 2006. Zooplankton and ichthyoplankton communities in a temperate estuary: spatial and temporal patterns. Journal of Plankton Research 28, 297-312.

Marques, S.C., Azeiteiro, U.M., Martinho, F., Pardal, M.A., 2007. Climate variability and planktonic communities: the effect of an extreme event (severe drought) in a southern European estuary. Estuarine, Coastal and Shelf Science 73, 725-734.
Martinho, F., Leitão, R., Neto, J.M., Cabral, H., Marques, J.C., Pardal, M.A., 2007a. The use of nursery areas by juvenile fish in a temperate estuary, Portugal. Hydrobiologia 587, 281-290.

Martinho, F., Leitão, R., Viegas, I., Dolbeth, M., Neto, J.M., Cabral, H.N., Pardal M.A., 2007b. The influence of an extreme drought event in the fish community of a southern Europe temperate estuary. Estuarine, Coastal and Shelf Science 75, 537-546.
Mazzoldi, C., Rasotto, M.B., 2001. Extended breeding season in the marbled goby, Pomatoschistus marmoratus (Teleostei: Gobiidae), in the Venetian Lagoon. Environmental Biology of Fishes 61, 175-183.
Nichols, J.H., 1976. Soleidae of the Eastern North Atlantic. Fiches d'Identification du Zooplankton 150-151, 1-10.

Petersen, C.G.J., 1919. Our gobies (Gobiidae). From the egg to the adult stages. Rep. Dan. biol. Stn., 26, 45-66.

Portuguese Weather Institute. http://www.meteo.pt/en/index.html (Accessed 08.01.07).
Primo, A.L., Azeiteiro, U.M., Marques, S.C., Martinho, F., Pardal, M.A., 2009. Changes in zooplankton diversity and distribution pattern under varying precipitation regimes in a southern temperate estuary. Estuarine, Coastal and Shelf Science 82, 341-347.

Ramos, S., Cowen, R.K., Ré, P., Bordalo, A.A., 2006. Temporal and spatial distribution of larval fish assemblages in the Lima estuary (Portugal). Estuarine, Coastal and Shelf Science 66, 303-313. Ré, P., 1999. Ictioplâncton estuarino da Península Ibérica. Guia de identificação dos ovos e estados larvares planctónicos. Câmara Municipal de Cascais, Cascais, 163 pp.
Ré, P., Meneses, I., 2009. Early stages of marine fishes occurring in the Iberian Peninsula. IPIMAR/IMAR, Lisboa, 282 pp .

Ribeiro, R., 1991. Ictioplâncton do estuário do Mondego e Resultados. Revista de Biologia: Actas do I Encontro de Planctologistas Portugueses 4, 233-244.

Ribeiro, R., Gonçalves, F., 1993. Padrões espacio-temporais na diversidade da comunidade larvar de teleósteos no estuário do Mondego. Boletim Uca 1, 490-506.
Ribeiro, R., Reis, J., Santos, C., Gonçalves, F., Soares, A.M.V.M., 1996. Spawning of anchovy Engraulis encrasicolus in the Mondego estuary, Portugal. Estuarine, Coastal and Shelf Science 42, 467-482.

Russell, F.S., 1976. The Eggs and Planktonic Stages of British Marine Fishes. Academic Press, London, 539 pp .
Sabatés, A., Olivar, M.P., Salat, J., Palomera, I., Alemany, F., 2007. Physical and biological processes controlling the distribution of fish larvae in the NW Mediterranean. Progress in Oceanography 74, 355-376.
Selleslagh, J., Amara, R., Laffargue, P., Lesourd, S., Lepage, M., Girardin, M., 2009. Fish composition and assemblage structure in three Eastern English Channel macrotidal estuaries: A comparison with other French estuaries. Estuarine, Coastal and Shelf Science 81, 149-159.

Strydom, N.A., Whitfield, A.K., Wooldridge, T.H., 2003. The role of estuarine type in characterizing early stage fish assemblages in warm temperature estuaries, South Africa. African Zoology 38, 29-43.
Whitfield , A.K., 1999. Ichthyofaunal assemblages in estuaries: a South African case study. Reviews in Fish Biology and Fisheries 9, 151-186.

FIGURE CAPTIONS

Fig. 1 - Map of the Mondego estuary, located on the western coast of Portugal. Sampling stations surveyed in this study are indicated (M, mouth station; S1 and S2, southern arm stations; N1 and N 2 , northern arm stations).
Fig. 2 - Seasonal water runoff $\left(\mathrm{m}^{3}\right)$ and average of precipitation (mm) in Mondego estuary during the study period.
Fig. 3 - Seasonal average water temperature $\left({ }^{\circ} \mathrm{C}\right)$, chlorophyll $a\left(\mathrm{mg} / \mathrm{m}^{-3}\right)$ and salinity anomalies in Mondego estuary during the study period.
Fig. 4 - Seasonal density (larvae $100 \mathrm{~m}^{-3}$ ) of total fish larvae and of each main ecological guild. ES - Estuarine species; MS - Marine Stragglers; MM - Marine Migrants.

Fig. 5 - Interannual and spatial density (larvae $100 \mathrm{~m}^{-3}$ ) distribution of the three main ecological guilds. (A) Estuarine Species; (B) Marine Stragglers; (C) Marine Migrants.
Fig. 6 - Temporal and spatial density (larvae $100 \mathrm{~m}^{-3}$ ) distributions of Pomatoschistus spp., Engraulis encrasicolus, Solea senegalensis and Shannon Wiener index $(\log 2)$ in the Mondego estuary.

Fig. 7 - Triplot ordination diagram of the larval fish assemblages in Mondego estuary using the first two canonical correspondence axes. Samples were classified in winter, spring, summer and autumn. Significant environmental variables are plotted as arrows (T - temperature; Chl a - chlorophyll $a$; Sal - salinity; Runoff - river discharge) or nominal variables ( W - winter; SM - summer). Species codes are presented in Table I.







Salinity anomalies - Chlorophyll a - - Temperature


(A) ESTUARINE SPECIES
(B) MARINE STRAGGLERS
(C) MARINE MIGRANTS



Figure 6

## res

ACCEPTED MANUSCRIPT






80
100


0.2
0.7
1.2
1.7
2.2
2.7
$\square$ 0.0
0.5
1.0
1.5
2.0


| $\triangle$ | SUMMER |
| :---: | :--- |
| $\triangle$ | AUTUMN |
| $\bigcirc$ | $\bigcirc$ |
| - | WINTER |

Table I: Mean Density (larvae $100 \mathrm{~m}^{-3}$ ) of species caught during the sampling period and relative contribution (\%) to the total catch in whole estuary, in each season and sampling station. MD -

Mean Density; W - winter; S - spring; SM - summer; A - autumn.

| Family | Species | $\begin{gathered} \hline \text { CCA } \\ \text { CODE } \end{gathered}$ | Ecological guild | Total catch |  | Season (\%) |  |  |  | Sampling station (\%) |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | MD | \% | W | S | SM | A | M | S1 | S2 | N1 | N2 |
| Gobiidae | Pomatoschistus spp. | Pspp | ES | 70.65 | 63.40 | 54.42 | 58.26 | 68.52 | 65.39 | 3.19 | 18.52 | 16.35 | 7.74 | 17.59 |
| Not identified | Not identified | - |  | 9.52 | 8.75 | 6.69 | 13.54 | 4.20 | 12.31 | 3.82 | 2.20 | 1.01 | 1.50 | 0.22 |
| Gobiidae | Pomatoschistus microps | Pmic | ES | 6.63 | 6.02 | 3.44 | 9.92 | 3.20 | 6.76 | 0.05 | 0.98 | 2.61 | 1.39 | 0.98 |
| Gobiidae | Gobiidae not identified | Gobi | ES | 5.37 | 4.65 | 15.29 | 4.49 | 3.23 | 3.09 | 0.74 | 0.40 | 2.56 | 0.50 | 0.46 |
| Gobiidae | Gobius niger | Gnig | ES | 3.79 | 3.57 | 1.22 | 2.85 | 5.59 | 0.62 | 0.06 | 1.62 | 1.24 | 0.29 | 0.36 |
| Engraulidae | Engraulis encrasicolus | Eenc | MS | 2.01 | 1.96 | 0.49 | - | 4.16 | 0.83 |  | 0.12 | 1.78 | - | 0.06 |
| Blenniidae | Parablennius pilicornis | Ppil | MS | 2.02 | 1.88 | - | 1.36 | 3.08 | 0.60 | 0.13 | 0.93 | 0.10 | 0.24 | 0.47 |
| Gobiidae | Gobius spp. | Gspp | ES | 2.49 | 1.86 | 1.17 | 2.05 | 1.63 | 2.52 | 0.07 | 0.90 | 0.73 | 0.11 | 0.04 |
| Soleidae | Solea senegalensis | Ssen | MM | 1.51 | 1.33 | 3.19 | 0.55 | 1.37 | 1.97 | 0.32 | 0.44 | 0.04 | 0.39 | 0.14 |
| Soleidae | Soleidae not identified | Solei |  | 1.32 | 1.06 | 1.65 | 0.86 | 0.26 | 3.60 | 0.09 | 0.48 | 0.16 | 0.28 | 0.06 |
| Syngnathidae | Syngnathus acus | Sacu | ES | 0.96 | 0.85 | - | 0.37 | 1.55 | 0.40 | 0.04 | 0.17 | 0.16 | 0.24 | 0.24 |
| Gobiidae | Aphia minuta | Amin | MS | 0.61 | 0.59 | - | 1.73 | - | - | - | 0.08 | 0.51 | - | - |
| Blenniidae | Coryphoblennius galerita | Cgal | MS | 0.55 | 0.49 | - | 0.75 | 0.42 | 0.42 | 0.27 | 0.08 | - | 0.10 | 0.05 |
| Clupeidae | Sardina pilchardus | Spil | MM | 0.50 | 0.42 | 0.40 | 0.50 | 0.18 | 0.97 | - | 0.17 | 0.04 | 0.17 | 0.04 |
| Blenniidae | Lipophrys pholis | Lpho | MS | 0.38 | 0.38 | 2.38 | 0.44 |  | 0.22 | 0.12 | 0.15 | - | 0.08 | 0.03 |
| Ammodytidae | Ammodytes tobianus | Atob | MS | 0.35 | 0.32 | 3.86 |  |  | - | - | 0.27 | - | 0.06 | - |
| Atherinidae | Atherina presbyter | Apres | ES | 0.32 | 0.28 | - | 0.30 | 0.40 | - | 0.19 | 0.08 | - | - | - |
| Atherinidae | Atherina spp. | Aspp | ES | 0.44 | 0.27 | - | 0.12 | 0.52 | - | - | 0.15 | - | 0.12 | - |
| Soleidae | Solea solea | Ssol | MM | 0.26 | 0.26 |  | 0.15 | 0.48 | - | - | 0.26 | - | - | - |
| Gobiidae | Pomatoschistus minutus | Pmin | ES | 0.23 | 0.23 |  | 0.68 | - | - | - | 0.10 | 0.09 | 0.04 | - |
| Syngnathidae | Syngnathus spp. | Sygn | ES | 0.22 | 0.21 |  | 0.44 | 0.14 | - | - | 0.19 | - | - | 0.02 |
| Blenniidae | Blenniidae n . id | Blenn |  | 0.23 | 0.17 | 1.35 | 0.17 | - | - | - | - | 0.06 | - | 0.11 |
| Mugilidae | Liza ramada | Lram | CA | 0.20 | 0.16 | 1.07 | - | 0.17 | - | 0.07 | - | 0.09 | - | - |
| Labriidae | Symphodus melops | Smel | MS | 0.15 | 0.15 | - | 0.17 | 0.22 | - | - | 0.15 | - | - | - |
| Syngnathidae | Syngnathus abaster | Saba | ES | 0.26 | 0.13 | - | - | 0.30 | - | - | 0.07 | - | - | 0.06 |
| Callionynidae | Callionymus spp. | Cspp | ES | 0.17 | 0.11 | 1.35 | - | - | - | 0.06 | - | - | 0.06 | - |
| Trachinidae | Echiichthys vipera | Evip | MS | 0.17 | 0.11 | 1.35 | - | - | - | 0.06 | - | - | 0.06 | - |
| Gobiidae | Crystallogobius linearis | Clin | MS | 0.24 | 0.11 | - | - | 0.25 | - | - | 0.07 | 0.04 | - | - |
| Pleuronectidae | Platichthys flesus | Pfle | MM | 0.10 | 0.10 | - | 0.30 | - | - | - | - | - | - | 0.10 |
| Ammodytidae | Ammodytidae n. id. | Amm |  | 0.09 | 0.06 | 0.68 | - | - | - | - | - | - | - | 0.06 |
| Bothidae | Arnoglossus thori | Atho | MS | 0.05 | 0.05 | - | - | 0.12 | - | - | - | 0.05 | - | - |
| Blenniidae | Lipophrys spp. | Lspp | MS | 0.04 | 0.04 | - | - | - | 0.29 | - | - | 0.04 | - | - |
| Total Number of Larvae |  |  |  | 7211 |  | 608 | 2449 | 3112 | 1042 | 669 | 2060 | 1995 | 965 | 1522 |
| Number of Species |  |  |  | 31 |  |  |  |  | 15 |  | 25 | 19 |  | 19 |

