Laboratorio de Biología Marina

Universidad de Sevilla

Spatio-temporal dynamic of the early life stages of fish assemblages in the Guadalquivir estuary. Responses to anthropic and natural disturbances.

Dinámica espacio-temporal de la comunidad de larvas y juveniles de peces del estuario del Guadalquivir. Respuestas ante perturbaciones antrópicas y naturales.

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Los directores **Dr. César Megina Martínez**, profesor titulas del Departamento de Zoología de la Universidad de Sevilla, y **Dr. José Carlos García Gómez**, catedrático del Departamento de Zoología de la Universidad de Sevilla,

INFORMAN:

Que la presente memoria de tesis doctoral, titulada “**Dinámica espacio-temporal de la comunidad de larvas y juveniles de peces del estuario del Guadalquivir. Respuestas ante perturbaciones antrópicas y naturales**” fue realizada por **Juan Miguel Miró Recio-Mensaque** bajo su dirección en el Departamento de Zoología de la Universidad de Sevilla. Asimismo, consideran que esta memoria de investigación reúne las condiciones necesarias para optar al Título de Doctor con Mención Internacional.

Sevilla, a 29 de enero de 2021

V°B° de los directores

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A mi familia

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GENERAL ABSTRACT

Estuarine environments are regions at the interface of riverine and marine systems and are critical habitats for many resident and migratory species. They may support high abundances of organisms due to their high productivity, playing an essential role in the nursery function of many species, especially for marine fishes. However, due to the increase of urban and agricultural development, they are among the most disturbed and threatened aquatic systems. Therefore, the aim of this thesis was to increase the knowledge of recruitment ecology processes for early life stages of fish in estuaries, in addition to observe the responses of these ecosystems to different natural and anthropic disturbances. For that, we used the Guadalquivir estuary as the main study area, and also it was compared to surrounding zones and other estuaries.

In this research, we monitored the composition, abundance and structure of its early life stages of fish assemblages and we related them with multiple environmental variables in its estuarine and nearshore zones during three consecutive years. Much higher abundances were found in the estuarine zone, where the poly and mesohaline waters showed more productivity and biodiversity. Temporal fluctuations in the freshwater inputs influenced in the water physiochemical characteristics and, consequently, in the shift of assemblages.

Also, we performed a study to determine the mechanisms for horizontal movements inside of the estuary in the most abundant fish species, *Engraulis encrasicolus* and *Pomatoschistus* spp. Selective tidal-stream transport, in combination with vertical migrations, is one of the mechanisms more used by invertebrates and small fishes in estuaries. However, no evidence of vertical migrations was found for these species. The benthic gobies were mostly found in the bottom, while the pelagic anchovies were in the surface layer, regardless to the tidal condition (ebb or flood). Hence, they applied alternative strategies.

During the monitoring program, three different freshet events that occurred after heavy rainfall were analysed in short-term. All of them compressed the salinity gradient downstream, increased the turbidity levels and caused river plumes, at the same time that reduced it nursery area. Depending on the species, they could be displaced downstream, flushed out the estuary or even predated in nearshore areas. In general, estuarine species (e.g. gobies) coped better with these disturbances than the marine organisms (e.g. anchovies).

Contrary effects were observed in the macrofauna during a maintenance dredging operation, where the gobies and decapods tended to decrease slightly, and most pelagic organisms did not show alterations. Still, scarce disturbances were detected in the

physiochemical variables of the water column as well as most of the macrofauna groups. In fact, the possible effects of this disturbance were of the same order or less than natural ones (e.g. freshet events registered), therefore, macrofauna organisms could be well-adapted to cope with them.

Finally, we compared the Guadalquivir estuary with other important estuaries in the Gulf of Cádiz. Estuaries with no transitional salinity gradients as Odiel-Tinto and Cádiz Bay showed similar biotic and abiotic characteristics than surrounding areas. Therefore, they cannot be considered to have an important nursery function. Instead, estuaries with well-developed salinity gradients, as Guadalquivir and Guadiana, presented different early fish assemblages and higher abundances than their nearshore zones, considering them important nursery grounds in the region. Still, some distinctive features arose between them, which made the Guadalquivir estuary much more productive.

RESUMEN GENERAL

Los ambientes estuarinos son regiones intermedias entre los sistemas fluviales y marinos, lo que los hace contener hábitats críticos para muchas especies residentes y migratorias. Estos pueden soportar una gran abundancia de organismos debido a su alta productividad, desempeñando un papel esencial en la función de cría de muchas especies, especialmente para los peces marinos. Sin embargo, debido al aumento del desarrollo urbano y agrícola, se encuentran entre los sistemas acuáticos más perturbados y amenazados. Por tanto, el objetivo de esta tesis fue incrementar el conocimiento de los procesos de ecología de reclutamiento para las etapas tempranas de vida de los peces en los estuarios, además de observar las respuestas de estos ecosistemas ante diferentes perturbaciones naturales y antrópicas. Para ello, utilizamos el estuario del Guadalquivir como principal área de estudio, y además se comparó con zonas costeras cercanas y otros estuarios de la región.

En esta investigación, monitoreamos la composición, abundancia y estructura de sus comunidades de larvas y juveniles de peces y los relacionamos con múltiples variables ambientales, tanto en su zona interna como cerca de su desembocadura durante tres años consecutivos. Se encontraron abundancias mucho mayores en la zona estuarina, donde las aguas poli y mesohalinas mostraron mayor productividad y biodiversidad. Las fluctuaciones temporales en los aportes de agua dulce influyeron en las características fisicoquímicas del agua y, en consecuencia, en el cambio de sus comunidades.

Asimismo, se realizó un estudio para determinar el mecanismo de movimientos horizontales dentro del estuario en las especies de peces más abundantes, *Engraulis encrasicolus* y *Pomatoschistus* spp. El transporte selectivo de corrientes de marea, en combinación con las migraciones verticales, es uno de los mecanismos más utilizados por los invertebrados y los pequeños peces en los estuarios. Sin embargo, no se encontró evidencia de migraciones verticales para estas especies. Los gobios bentónicos se encontraron principalmente en el fondo, mientras que los boquerones pelágicos se encontraron en la capa superficial, independientemente del estado de la marea (llenante o vaciante). Por lo tanto, tuvieron que aplicar otras estrategias alternativas.

Durante el programa de monitoreo, se analizaron a corto plazo tres eventos diferentes de elevada descarga de agua dulce, las cuales ocurrieron tras periodos de fuertes lluvias. Todos los eventos registrados comprimieron el gradiente de salinidad aguas abajo, aumentaron los niveles de turbidez y provocaron plumas en su desembocadura, al mismo tiempo que redujeron su área de cría. Dependiendo de la especie, estas pudieron ser desplazadas aguas abajo,

expulsadas del estuario o incluso depredadas en áreas cercanas a la costa. En general, las especies estuarinas (p. ej. gobios) lidiaron mejor con estas perturbaciones que los organismos marinos (p. ej. boquerones).

Por el contrario, los efectos observados en la macrofauna durante una operación de dragado de mantenimiento, afectó más a los gobios y decápodos, que tendieron a disminuir levemente, mientras que la mayoría de los organismos pelágicos no mostraron alteraciones. Aun así, se detectaron escasos cambios en las variables fisicoquímicas de la columna de agua, así como en la mayoría de los grupos de macrofauna presentes. De hecho, los posibles efectos de esta perturbación fueron del mismo orden o menores que los naturales (p. ej. los eventos de alta descarga registrados), por lo que los organismos de la macrofauna podrían estar bien adaptados para hacerles frente.

Finalmente, comparamos el estuario del Guadalquivir con otros estuarios importantes del Golfo de Cádiz. Los estuarios sin amplios gradientes de salinidad como el Odiel-Tinto y la Bahía de Cádiz mostraron características bióticas y abióticas similares a las de las áreas circundantes. Por lo tanto, no se puede considerar que tengan una función de cría importante. En cambio, los estuarios con gradientes de salinidad bien desarrollados, como el Guadalquivir y el Guadiana, presentaban diferentes comunidades de larvas y juveniles de peces, y sus abundancias fueron más altas que sus zonas costeras cercanas, considerándolos así importantes zonas de cría en la región. Aun así, entre ellos resaltaron algunas características distintivas que hicieron mucho más productivo al estuario del Guadalquivir.

GENERAL INTRODUCTION

Estuaries are regions at the interface of riverine and marine systems with a wide heterogeneity of hydrological, geological and physiochemical environments. Since Pritchard (1967) defined it as “a semi-enclosed coastal body of water which has a free connection with the open sea and within which the sea water is measurably diluted with fresh water derived from land drainage”, manifold modifications have been proposed to include a wider variety of transitional systems. Following the extensive review by Whitfield and Elliott (2011), a more encompassed definition of an estuary can be “a semi-enclosed coastal body of water which is connected to the sea either permanently or periodically, has a salinity that is different from that of the adjacent open ocean due to freshwater inputs, and includes a characteristic biota”. Regardless to the concept changes, salinity always has been a main factor for these ecosystems, which has been used also to divided them in zones. The zonation scheme applied is known as the Venice system (1958), which recognizes six distinct salinity ranges (‰): hyperhaline (>40), euhaline (40-30), polyhaline (30-18), mesohaline (18-5) oligohaline (5-0.5) and limnetic (<0.5).

As many factors affect estuaries, there have been multiple attempts to classify them according to single parameters. From a hydrological point of view, divisions were done by water circulation depending on vertical salinity structure [stratified, partially mixed or mixed, by Dyer (1972)] and tidal ranges [microtidal (0-2 m), mesotidal (2-4) or macrotidal (>4 m), by Hayes (1975)]. From a geological approach, estuaries have been classified into four main groups: coastal plain estuaries; lagoons (or bar-built estuaries); fjords, fjards and firths; and tectonically caused estuaries resulting from faulting, graben formation, landslide or volcanic eruption (Dyer, 1972). Thereafter, different multidisciplinary type of approaches to the classification of estuaries has been carried out based on their geomorphology, salinity distribution, water circulation, etc. [e.g., Ketchum (1983); Roy et al. (2001); Whitfield (1992)].

Generally, these ecosystems are subjected to high environmental fluctuations in short and long spatio-temporal dimensions (Elliott and Quintino, 2007). Environmental variables can change daily by tides such as salinity, turbidity, water current or bottom depth, and seasonally such as temperature, dissolved oxygen, freshwater inputs or photoperiod (Navarro et al., 2011). Moreover, physiochemical conditions inside estuaries can oscillate along years due to different anthropic influences (pollutants, water damming, etc.) and interannual hydrologic variations (rainfalls) (Rolls and Bond, 2017). However, despite of their apparently stressed environmental conditions (Elliott and Quintino, 2007), estuaries are considered among the world’s most productive ecosystems (Elliott et al., 2019). Their direct connectivity with the terrestrial system by the river inflow supplies them organic matter and nutrients continuously, supporting the bases of their complex trophic web (Hoffman et al., 2008).

There are several distinct groups of primary producers as phytoplankton, salt marsh grass, sea grasses, mangrove swamps (in tropical latitudes), macroalgae and benthic algae. They are key drivers of carbon, nutrient and oxygen cycling and play a central role in determining water quality (Leston et al., 2008). Among primary producers, phytoplankton often account for at least half of ecosystem primary production (Cloern, 2001; Harrison and Turpin, 1982), supporting a fundamental link in estuarine food webs. Nonetheless, depending on the estuary type, other groups can play a more important contribution. For example, in shallow estuarine environments, benthic microalgae (known as microphytobenthos) usually form brown or green mats on the surface photic layer of the sediment (1-3 mm), whose densities are often 100–1000 times higher than in the water column (Underwood, 2001; Underwood and Kromkamp, 1999). Thus, benthic microalgae may account higher contributions of the total primary production (Cadée and Hegeman, 1974; De Jonge and Van Beusekom, 1992), and even represent an important proportion of chlorophyll concentration in water column by wind or tidal resuspension (Brito et al., 2012; Irigoien and Castel, 1997; Statham, 2012).

Other important organisms which also carry out nutrient recycling, organic matter decomposition and conversion of small indigestible detrital materials to bigger structures for larger consumers are the microbes (Caraco et al., 1998). Suspended particles are able to provide habitats for bacteria, which attract flagellates and ciliates to feed on attached bacteria, forming aggregates of larger particles and flocculated materials. These “flocs” can be consumed directly by metazoan grazers, such as copepods and rotifers, passing materials and energy directly from detritus to higher trophic levels by microbial food web (Carpenter et al., 2005). In fact, in turbid estuaries were the photosynthesis activity in the water column can be limited, this heterotrophic energy pathway can be even more important for the base of the food web than the autotrophic way (Abrantes et al., 2013; Hitchcock et al., 2016; Hoffman et al., 2008).

In turn, this extensive autotrophic community in combination with abundant detrital material fuel a planktonic food web which produces abundant zooplankton. Zooplankton, which are small animals that inhabit in the water column but cannot swim against moderate water currents, range from single-celled heterotrophic flagellates to complex metazoans such as copepods, chaetognaths, mysids, ctenophores, cnidarians and early life stages of many fish species (Day et al., 2013). The species that comprise this high biomass vary according to the geographical location of the estuary, temperature, and the salinity regime within each system.

Among the most abundant zooplankters groups in number and biomass are the following crustaceans: copepods (common genera include *Acartia*, *Eurytemora* and *Pseudodiaptomus*) and mysids (common genera include *Neomysis*, *Mysis*, *Mesopodopsis*, and *Rhopalophthalmus*) (David et al., 2016; Wooldridge, 1999).

These small crustaceans have high secondary production and serve as prey for organisms such as zooplanktivorous fishes (Baldó and Drake, 2002), transferring carbon from phytoplankton and detrital pools into higher trophic levels (Vilas et al., 2008). The waters of most estuaries teem early life stages of fish, and often exceed to those found over adjacent continental shelves. For this reason, estuaries are considered as "nursery areas". Still, not all of them accomplish the conditions of supporting higher densities, enhancing the larval development and growth, increasing their survival rates and stocking juvenile to adult populations (Beck et al., 2001). Estuaries usually contain a high diversity of habitats (i.e. subtidal or intertidal soft substratum, saltmarshes, biogenic reefs, seagrass meadows, shallow zones, etc.) which offer refuge against predators (Elliot and Hemingway, 2002). In addition, most of them present turbid zones which hinder their visual detection by adult piscivorous fish, while these zones can benefit fish larvae (their shorter visual field leaves fewer particles between them and their prey, reducing the interference with detection; Utne-Palm (2002)). On the other hand, the salinity gradient limits the distribution of different species along the estuaries depending on their osmoregulatory abilities. Instead, it attract a wide variety of marine, estuarine, diadromous and freshwater fish species, which can be assigned to distinct guilds (straggler, opportunistic, dependent, migrant, etc.) by their functional use of estuaries (Potter et al., 2015).

A fundamental question in oceanography concerns those factors that control the input of larvae and juvenile of marine species to nursery grounds (Robins et al., 2013), as well as the mechanism that they use to remain into them (Forward and Tankersley, 2001). Processes such as pelagic larval delivery into estuaries depend on different biophysical interactions between regional hydrodynamic, offspring period, amount of larval supply and natural behaviour of every individual (Potter et al., 2015). Different hypotheses have been proposed for the recruitment of fish larvae, which can use multiple sensory cues (odor, sound, visual or geomagnetic) to detect estuarine environments and help them to navigate towards these areas (Teodósio et al., 2016). Once they reach estuarine habitats, they have to cope with strong water currents generated by freshwater input and periodical tidal flushing, which could be considered extreme environment for fish larvae (Teodósio and Garel, 2015). Distinct strategies have been reported, applying selective tidal stream transports with vertical and/or lateral migrations to move up or downstream, or to maintain the position along the estuary, in combination with environmental cues (Forward and Tankersley, 2001). Still, the knowledge of the mechanism used by many species is unknown, especially in well-mixed estuaries whose vertical differences in water physiochemical conditions are scarce.

Lots of marine early life stages of different fish species depend on estuaries to complete their life cycle, and many of them present an economical interest (Ray, 2005). The coastal and transitional areas are only a small fraction of the marine and brackish areas worldwide (~5%) but produce approximately half of the global fish catch per year (Palomares and Pauly, 2019). However, despite of the important ecosystem service that offer the estuaries, they are one of the environments most threatened by humans (Lotze et al., 2006). More than 60% of the Earth's population are living in coastal areas (Ray, 2006) and many anthropic activities in or near aquatic habitats generate alterations in their environmental conditions which could affect the organism that inhabit them (Halpern et al., 2008; Lotze et al., 2006). The rapid urban and agriculture development of the last decades (Lee et al., 2012; Wu et al., 2019) are affecting environmental conditions of rivers, wetlands, floodplains and estuaries by flow alteration and high sediment, nutrient or pollutant inputs (González-Ortegón and Drake, 2012; Rolls and Bond, 2017). Also, dredging of navigation channels in estuaries in order to reclaim land and to allow large ship access to inland waterways is another increasing source of disturbance since the container port industry has experienced an exponential growth transporting 80% of world's commodities currently (Tsolaki and Diamadopoulos, 2010; Yap and Lam, 2013).

Additionally, global climate change is imposing complementary modifications; recent studies, based on 30 years of historical data, have found a significant decrease in precipitations in south-central European and Mediterranean river basins (Xoplaki et al., 2004). This, together with anthropogenic water damming for diverse uses, has resulted in an increase in the number of days with low flow (Lobanova et al., 2018; Papadimitriou et al., 2016). These modifications have been reported to decrease the ecological status of affected ecosystems (Poff and Zimmerman, 2010), with fish being a taxonomic group of major concern (Schinegger et al., 2016). Moreover, climatic predictions in temperate regions indicate increasing temperatures and sea levels, subject to more erratic rainfall and freshwater discharges, and the likelihood of more frequent and severe droughts and storms (Day and Rybczyk, 2019).

Under these challenges, interpreting the link between freshwater flows, water physiochemical characteristics and plankton dynamic within estuaries is crucial to its management and conservation as they undergo change due to climate and anthropogenic development (Bates et al., 2008; Hughes, 2003). EU member states have legislated to manage and protect all running waters under the Water Framework Directive (European Commission, 2000), but the River Basin Management Plans from 2018 indicated that 60% of European water bodies failed to achieve good ecological status (European Environment Agency, 2018). Monitoring programs are necessary to assess and enhance the ecological status of aquatic ecosystems, and in the case of estuaries, which are continually flowing between unstable states, long-term or permanent studies are especially recommended (Henderson et al., 2011). Thus, it would generate a baseline knowledge about the variability of assemblages and the ecological preferences of its constituents which help us to predict effects of the modifications in the ecosystem, whether induced by humans or not (Soetaert and Van Rijswijk, 1993), and propose solutions to different kind of management problems.

Objectives and thesis structure

The main aim of this thesis was to increase the knowledge of recruitment ecology processes for early life stages of fish in estuaries, using the Guadalquivir as study area. For that general objective, different specifics approaches were performed. We determined the composition, abundance and structure of its assemblages in relation with multiple environmental variables in its estuarine and nearshore zones during three consecutive years. During that period, different short- and medium-term disturbances were observed and analysed, such as natural freseht events that occurred after heavy rainfall or anthropic disturbances due to a maintenance dredging operation. Also, we studied the mechanism for horizontal movements inside of the estuary in the most abundant fish species, *Engraulis encrasicolus* and *Pomatoschistus* spp. Finally, we compared the nursery function of the Guadalquivir estuary with other estuaries in the Gulf of Cádiz to contextualize its contribution for the fish populations in the region.

In addition to the introduction presented above, this thesis is composed by five chapters, in which the objectives are fully addressed, as well as one final section with a general discussion of the results obtained. Finally, the principal conclusions from this thesis are presented.

Specifically:

In **chapter 1**, we generated a baseline study of early life stages of fish assemblages in the Guadalquivir estuary using standard methodology to make the information comparable with other studies. We took samples monthly from October 2015 to September 2018 in its inner and nearshore zones to compare the spatio-temporal patterns of their larvae and juvenile fish assemblages. At the same time, multiple biotic and abiotic variables were sampled to analyse their relations with the environmental conditions and the interactions of its trophic web.

In **chapter 2**, we analysed the mechanisms for horizontal movements and the use of different zones across the transverse section of the estuary in two kind of fish species, the pelagic *Engraulis encrasicolus* and the benthic *Pomatoschistus* spp. Also, we determined the possible environmental cues which could lead the individuals of these species to follow their strategies.

In **chapter 3**, we analysed the effects of a maintenance dredging operation from multiple approaches (immediate, short and medium term) in the physiochemical variables, the early life stages of fish and other macrofauna groups present in the water column of two zones with different salinity ranges.

In **chapter 4**, we investigate the short-term effects of three different kinds (intensity, duration and period) of natural freshet events on the early life stages of fish species, other macrofauna groups and the water physiochemical conditions in the inner and outer zones of the estuary.

In **chapter 5**, we assessed the potential nursery function of the main estuaries in the Gulf of Cádiz (Guadiana, Odiel-Tinto, Guadalquivir and Cádiz Bay) and compared the structure and composition of their early life stages of fish assemblages. We also identified which variables affected assemblage distributions and quantified their influence in the nursery success of each estuary.

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1. MONITORING PROGRAM OF EARLY LIFE STAGES OF FISH ASSEMBLAGES IN THE GUADALQUIVIR ESTUARY

Abstract

The species composition, abundance and distribution of the early life stages of fishes in the Guadalquivir estuary and adjacent coastal area were studied. Sampling was carried out monthly during three consecutive years (October 2015 to September 2018) with 3 stations in the nearshore zone and 3 stations in the estuarine zone (poly-, meso- and oligohaline water masses). Multiple physiochemical and biological variables (distinct zooplankton groups) were measured to analyse their influences in the fish assemblage structure. Much higher abundances of early fish stages were found in the inner zone, showing a clear nursery function for many marine species of the region. Similar seasonal fluctuations were observed along the year in both zones, being the temperature the most influential factor in the temporal variation of assemblages and secondary production. Biological and physiochemical characteristics were spatially similar in the nearshore zone, while the inner zone showed different environments marked by the salinity, being the poly- and mesohaline waters the most diverse and productive. Hydrologic fluctuations in the region influenced the freshwater inputs into the estuary, which provoked changes between the dry and wet years in the recruitment time and abundance of the dominant fish species (*Pomatoschistus* spp. and *Engraulis encrasicolus*). Organic matter, supplied in high concentrations by the freshwater inputs, seemed to be the main food resource for the base of the trophic web in this estuary, which presented a high secondary production of mesozooplankton, macrozooplankton and early fish stages. Also, different trophic interactions were found along the temporal dynamic of these groups. The high biotic and abiotic variability observed in this ecosystem, in short (monthly) and long (annually) term, suggests performing different monitoring programs with multiple approaches to better understand the ecological responses to distinct situations or disturbances.

Resumen

Se estudió la composición, abundancia y distribución de las distintas especies de larvas y juveniles de peces en el estuario del Guadalquivir y su zona costera adyacente. El muestreo se realizó mensualmente durante tres años consecutivos (desde octubre de 2015 a septiembre de 2018) con 3 estaciones en la zona externa y 3 estaciones en la zona interna (masas de agua poli, meso y oligohalinas). Se midieron múltiples variables fisicoquímicas y biológicas (distintos grupos de zooplancton) para analizar sus influencias en la estructura de la comunidad de peces. En la zona interior se encontraron abundancias mucho más altas de etapas tempranas de peces, por lo que demostró tener una clara función de cría para muchas especies marinas de la región. Se observaron fluctuaciones estacionales similares a lo largo del año en ambas zonas, siendo la temperatura el factor más influyente en la variación temporal de la comunidad y la producción secundaria. Las características biológicas y fisicoquímicas fueron espacialmente similares en la zona externa, mientras que la zona interior mostró diferentes ambientes marcados por la salinidad, siendo las aguas poli- y mesohalinas las más diversas y productivas. Las fluctuaciones hidrológicas en la región influyeron en los aportes de agua dulce al estuario, lo que provocó cambios entre los años secos y húmedos en el tiempo de reclutamiento y abundancia de las especies de peces dominantes (*Pomatoschistus* spp. y *Engraulis encrasicolus*). La materia orgánica, suministrada en altas concentraciones por los insumos de agua dulce, pareció ser el principal recurso alimenticio para la base de la red trófica de este estuario, que presentaba una alta producción secundaria de mesozooplancton, macrozooplancton y larvas y juveniles de peces. Además, se encontraron diferentes interacciones tróficas a lo largo de la dinámica temporal de estos grupos. La alta variabilidad ambiental y biológica observada en este ecosistema, a corto (mensual) y largo (anualmente) plazo, sugiere realizar diferentes programas de monitoreo con múltiples enfoques para comprender mejor las respuestas ecológicas a distintas situaciones o perturbaciones.

1. Introduction

Estuaries are transition areas between land and sea, forming aquatic ecosystems that are characterized by a variety of inter-related biotic and abiotic structural components and intensive chemical, physical and biological processes (Day et al., 2013). They are sites of important connectivity and intense gradients that make them among the world's most productive ecosystems (Elliott et al., 2019). They may support high abundances of organisms, providing important nursery areas where multiple fish species in early life stages encounter suitable conditions for enhanced development (Elliot and Hemingway, 2002).

Generally, these ecosystems are subjected to high environmental fluctuations in short and long spatio-temporal dimensions (Elliott and Quintino, 2007). Environmental variables can change daily by tides such as salinity, turbidity, water current or level, and seasonally such as temperature, dissolved oxygen, freshwater inputs or photoperiod (Navarro et al., 2011). Moreover, physiochemical conditions inside estuaries can oscillate along years due to different anthropic influences (pollutants, water damming, etc.) and interannual hydrologic variations (rainfalls) (Rolls and Bond, 2017). On the other hand, larval supply coming from external adult stock are influenced by winds, water currents or fishing pressure (Ruiz et al., 2017; Schieler et al., 2014). For this, long temporal and spatial community studies of early life stages of fish are important to determine different aspects of species ecology like spawning zones, recruitment periods, habitats preferences, population dynamics, major influencing variables, etc. Good monitoring programmes generate a baseline study of the ecosystem, improving the knowledge of the variability of assemblages and the ecological preferences of its constituents to help us to predict effects of changes, whether induced by humans or not (Soetaert and Van Rijswijk, 1993).

The Guadalquivir estuary is one good example of a specific single location where numerous studies have been carried out to elucidate its nursery function for macrofauna and mesofauna separately [e.g. De Carvalho-Souza et al. (2018); Drake et al. (2002); González-Ortegón and Drake (2012; Taglialatela et al. (2014)]. Salinity has been considered the most influential factor in the community distribution, being mainly the part situated from seaward to the isohaline value of 5 PSU the estuarine zone used for macrofauna as nursery grounds (Fernández-Delgado et al., 2007). Also, a feeding study of small fishes in the Guadalquivir estuary showed that copepods (mesozooplankton) and mysids (macrozooplankton) were their most abundant preys (Baldó and Drake, 2002). However, no simultaneous sampling of all plankton groups have been carried out, which could show different interactions between top-down and bottom-up control in the estuarine food webs (Lynam et al., 2017). On the other hand, although the inner zone of this estuary has been widely studied, no research has been approached in its nearshore or inlet. Nearshore areas of estuaries can also be important rearing grounds (Able et al., 2013; Araújo et al., 2018), and some authors (Beck et al., 2001) proposed that a study of nursery function should also include a comparison with other surrounding habitats.

Therefore, the main goals of this study are: i) to generate a baseline study of early life stages of fish assemblages in the Guadalquivir estuary using standard methodology to make the information comparable with other studies; ii) to compare the temporal and spatial patterns of the larvae and juvenile fish assemblages between the nearshore and inner zones of the estuary to identify the major environmental parameters and stressors that may influence their distribution; iii) to analyse the interactions between the trophic web of the different plankton groups sampling the macrofauna and mesofauna simultaneously in the estuarine zone.

2. Material and methods

2.1. Study area

The Guadalquivir estuary is located in South-West of Iberian Peninsula, in a temperate North Atlantic region at the entrance of the Mediterranean Sea (Figure 1). It extends 110 km inland from its mouth and it presents a convergent morphology with widths of 800 m near the mouth and 150 m at the head (Díez-Minguito et al., 2012). The main channel is mostly isolated from surrounding natural areas, and includes a navigable channel of 7.1 m average depth (Ruiz et al., 2015). It is a well-mixed mesotidal system with 3.5 m amplitude range (spring tides) in the river mouth (Díez-Minguito et al., 2012), which presents a longitudinal salinity gradient with temporal displacement by tides, discharges and seasonal variations (González-Ortegón et al., 2014). Its waters flow to the Gulf of Cádiz, and the freshwater input into the estuary from the Guadalquivir River (680 km long and 57,527 km^2 basin area) is controlled by Alcalá del Río Dam.

2.2. Field sampling

Initial characterisation of the main physiochemical variables of the water column, along the longitudinal section of the estuary, was performed in September 2015 to design the monitoring program. For this, we used a multiprobe with sensors for depth, temperature, salinity, turbidity, dissolved oxygen concentration (DO), oxygen saturation (OS), pH and chlorophyll concentration (Chla) (Eureka™ Manta2)

Thereafter, sampling was carried out monthly during three consecutive years form October 2015 to September 2018. Samples were initially taken in 6 stations with 5 replicates of each one: 3 in the nearshore of the estuary inlet and 3 inside of the estuary (Figure 1). The last year of the monitoring program, nearshore stations were reduced to one, as well as the number of replicates to 3 in all stations. The outer stations were spatially distributed along the river mouth: Canal was located in the extension of the main river channel, Doñana and Faro on both sides, west and east respectively; the inner stations were spread along the salinity gradient, sampling always the water masses of 25 (polyhaline), 15 (mesohaline) and 5 (oligohaline) PSU (approx.) regardless of the geographical position it occupies by the tidal or water flow dynamic.

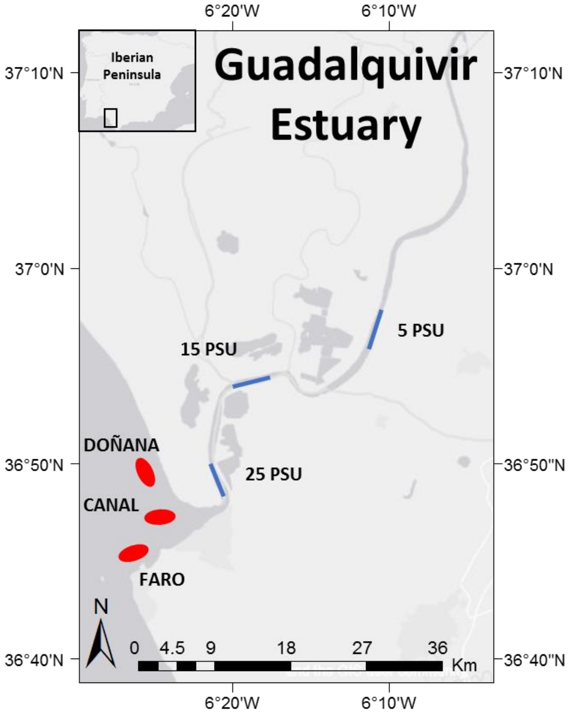

Figure 1. Study area of Guadalquivir estuary with sampling stations (nearshore zone in red and inner zone in red). The location of inner stations was approximated due to the fluctuation of salinity water masses along time.

Samples were always collected in the flood tide with a plankton net of 1 m diameter and 1 mm mesh size equipped with a flow-meter General Oceanics 2030R. Oblique tows of 10-12 min (345 ± 86m^3; mean ± SD) were done with a boat at a speed of 2–2.5 knots. Samples were fixed in 70% ethanol and the early fish stages were sorted from the rest of macrozooplankton organisms. Fishes were identified and quantified whenever possible, to species level, and macrozooplankton biomass was calculated in fresh weight. When gelatinous plankton was present in the samples, biovolume or density were determined. At the same time to early fish stages and macrozooplankton sampling, mesozooplankton samples were similarly taken in the inner stations during July 2016 to June 2018 with a Bongo net of 40 cm diameter and 200 µm mesh size. Samples were sub-sampled with a Motoda plankton splitter and processed using a digital camera (Nikon D810) for image acquisition, as well as the software ImageJ for analysing the images to calculate the total abundance.

Three replicated physicochemical profiles of the whole water column were recorded in every station with the multiprobe along the whole study. Water samples were taken at mid-depth with a Niskin bottle from June 2016 to measure total suspended solids (TSS) and nutrients (NO_2, NO_3, NH_4, PO_4, SiO_4). To measure total suspended solids, water was filtered through 0.7 µm pore precombusted (4 h, 500 °C) filters (Whatman GF/F); thereafter filters were dried (24 h, 60 °C) and weighted. Suspended organic (SOM) and inorganic matter (SIM) were obtained as weight loss by ignition (500 °C, 4 h). Concentrations of NO_2, NO_3, NH_4, PO_4 and SiO_4 were determined in filtered water samples, with an autoanalyzer (Skalar San^{plus} System) using colorimetric techniques.

2.3. Data analysis

Physiochemical profiles of the water column taken in the initial characterisation of the estuary were plotted in three dimensional figures with Ocean Data View software using “Weighted-average” as gridding method (Schlitzer, 2020).

A single value for physicochemical variables recorded with the multiprobe was obtained with a generalised additive mixed model fitted in every station, using the replicated individual profiles as random and obtaining the common smoother (Zuur et al., 2015). This allows an appropriate single mean value to represent the complete water column. Temporal series of depth averaged values of every physiochemical variable were plotted grouping by zones and stations.

Fish abundances were standardised by filtered volume and expressed as number of individuals/1000 m^3. Density data were organized in a species/sample abundance matrix, and a Bray–Curtis similarity matrix was calculated on fourth-root-transformed data with the addition of a dummy variable (value=1) (Clarke et al., 2006), as a distance measure among samples (Bray and Curtis, 1957). The distances between samples of every zone were represented by non-metric multidimensional scaling (nMDS) analysis. The differences in the multivariate structure of the early life stages of fish assemblages were analysed in a distance-based permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001; Mcardle and Anderson, 2001) for every zone. The experimental design included 3 crossed factors: Station (with 3 levels, “25 PSU, 15 PSU and 5 PSU” in inner zone and “Doñana, Canal and Faro” in nearshore zone), Year (with 3 levels, “1, 2 and 3” [every year was assigned for the period from October to September]) and Month (with 12 levels, from January to December), which was random and nested within Year. The sampling units were the 3–5 replicate tows taken at each Station. When appropriate, significant terms in the models were analysed individually using pair-wise comparison with the PERMANOVA test.

Total abundance of early life stages of fish and the biodiversity indices of Shannon-Wiener (H') and species richness (S) were calculated for every replicate and box-plotted per stations and months. We also examined significant differences in both parameters using PERMANOVA tests on Euclidean distance matrices for each zone, in an approach similar to parametric ANOVA (Anderson, 2001), using the same design as for assemblage structure. When appropriate, significant terms in the models were analysed individually using pair-wise comparison with the PERMANOVA test.

SIMPER (Clarke, 1993) was used to identify the contribution that each taxon made to the measures of similarity among the different levels of the zone and station factors. Temporal series of main contributor species were plotted.

To identify what environmental parameters were the most important in characterising differences between the assemblages of each zone and station, a distance-based redundancy analysis (dbRDA) was carried out. Physiochemical variables recorded with the multiprobe in addition to freshwater inputs and local rainfall were initially selected. As a measure of freshwater input and local rainfall, mean discharges from the dam to the estuary and mean daily accumulated rainfall during the week before sampling dates were calculated (data provided by Confederación Hidrográfica del Guadalquivir, http://www.chguadalquivir.es/saih/DatosHistoricos.aspx). One of the variables that were highly correlated ($r > 0.7$) were excluded (DO, negatively with temperature; Turbidity, positively with freshwater input) from the analysis. Multicollinearity of the selected variables was further analysed with a variance inflation factor (VIF) test using the "vif" function from the "car" package (Fox and Weisberg, 2011) in R software. All variables selected for the analysis had a VIF < 5 (Zuur et al., 2009). Additionally, trophic interactions were analysed by plotting temporal series of the total abundance of main plankton groups sampled (early fish stages, macrozooplankton, mesozooplankton and gelatinous plankton) for every zone and station.

All figures were performed with the package "ggplot2" of R 3.5.2 software (R Core Team, 2018), and univariate, multivariate and ordination analyses with PRIMERv6.1.11 and PERMANOVA+ v1.0.1 statistical package (Clarke and Gorley, 2006).

3. Results

3.1. Environmental data

Physiochemical condition of the water column along the whole estuary was plotted in the Figure 2. The estuary showed a horizontal salinity gradient, with a different spatial range of the distinct water masses. The polyhaline water mass (30-18 PSU) extended around 10 Km from Sanlúcar to Salinas; the mesohaline water mass (18-5 PSU) extended around 20 Km from Salinas to Tarfia; and the oligohaline water mass (5 – 0.5 PSU) extended 50 Km upstream, approximately, from Tarfia until the port entrance of Seville (Esclusa). Low vertical stratification was observed only in the polyhaline water mass close to the river mouth at the beginning of the flood tide.

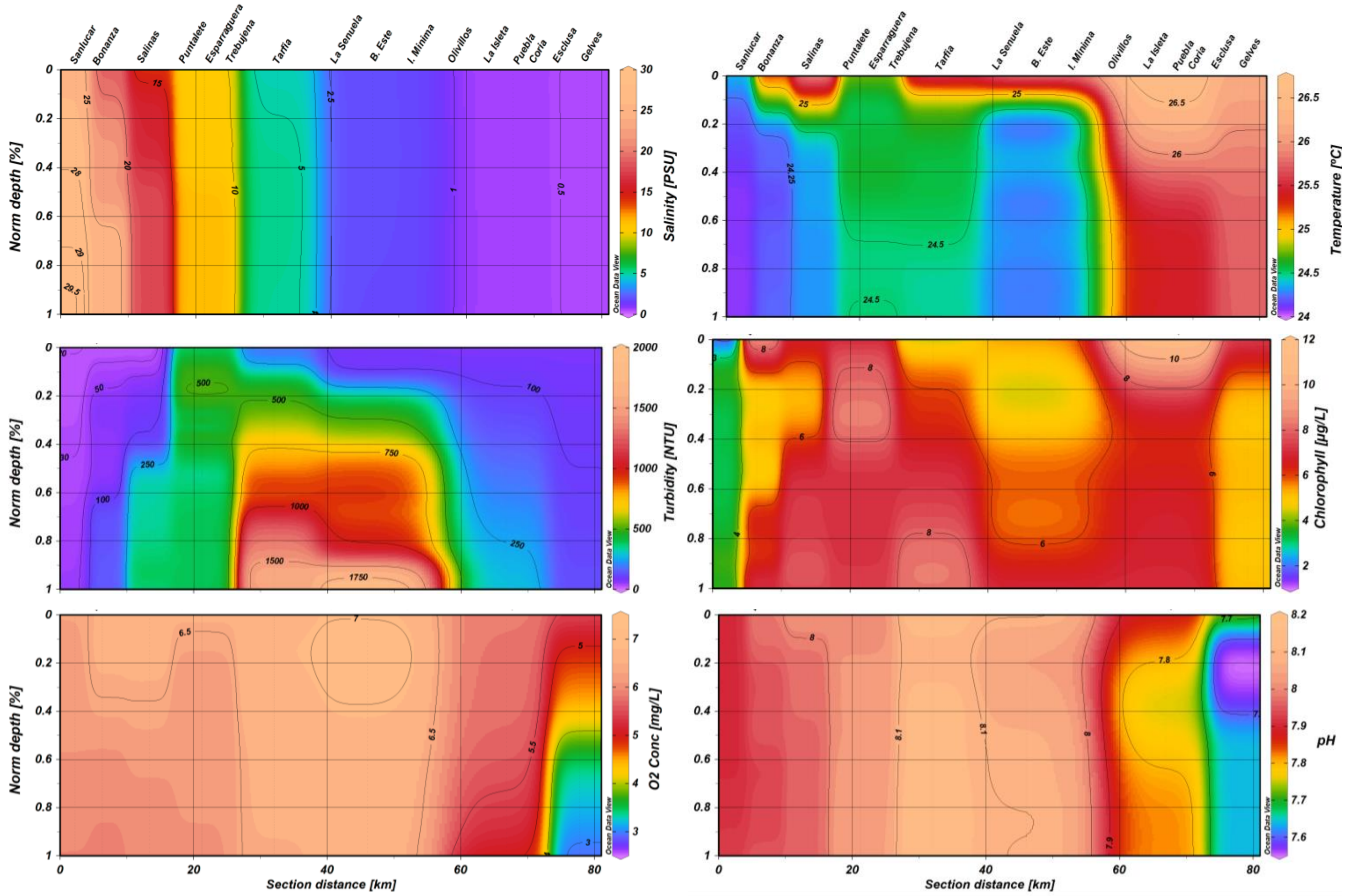

Figure 2. Physiochemical variables (salinity, (A), turbidity (B), dissolved oxygen (C), temperature (D), chlorophyll-a (E) and pH (F) recorded in the water column (standardized depth) along the whole estuary from the river mouth to Seville during the flood tide of 15-09-2015. Names on top are local names to different sections along the Guadalquivir estuary.

The turbidity showed a clear vertical stratification with higher values in the bottom layers. The maximum turbidity zone during this sampling was recorded between the kilometre 30 and 60 from the river mouth, with levels up to 1750 NTU. Dissolved oxygen concentration, which showed the same pattern than oxygen saturation, was stable along most of the estuary with levels around 6.5 mg/L, except for the last 10 Km upstream, which reached almost hypoxic conditions (<2 mg/L) in the bottom layers. The temperature showed a soft stratification with

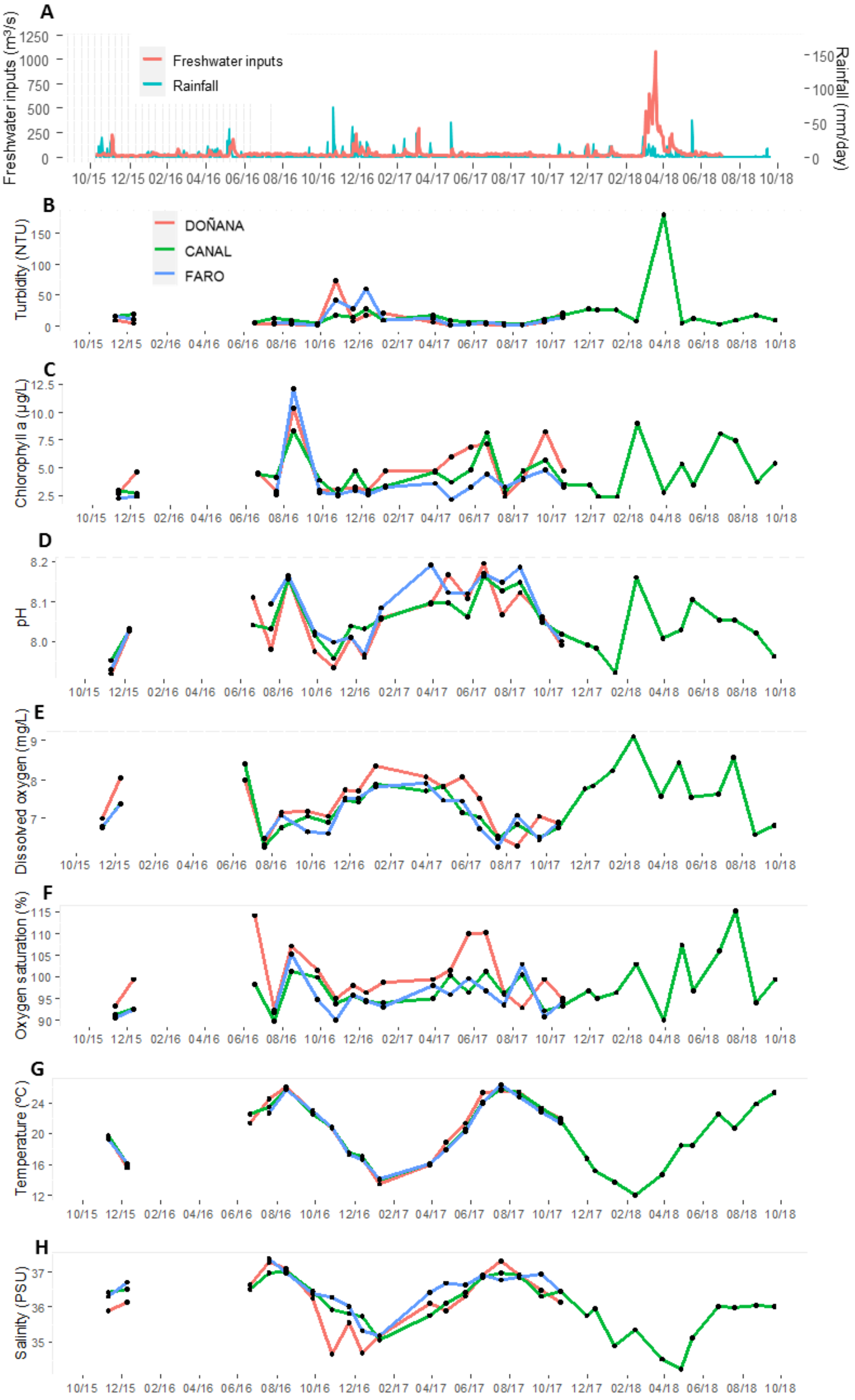

Figure 3. Temporal series of: daily mean of freshwater input from Alcalá del Río Dam and local daily accumulated rainfall (A); water turbidity (B), chlorophyll-a (C), pH (D), dissolved oxygen (E), oxygen saturation (F), temperature (G) and salinity (H) in every station (Doñana, Canal, Faro) of the nearshore zone of the Guadalquivir estuary.

higher levels close to the superficial layers, as well as a gradual increment upstream. Chlorophyll concentration was lower in the river mouth, especially in the surface. The inner parts of the estuary showed higher levels despite of high turbidity, which could limit the photosynthesis

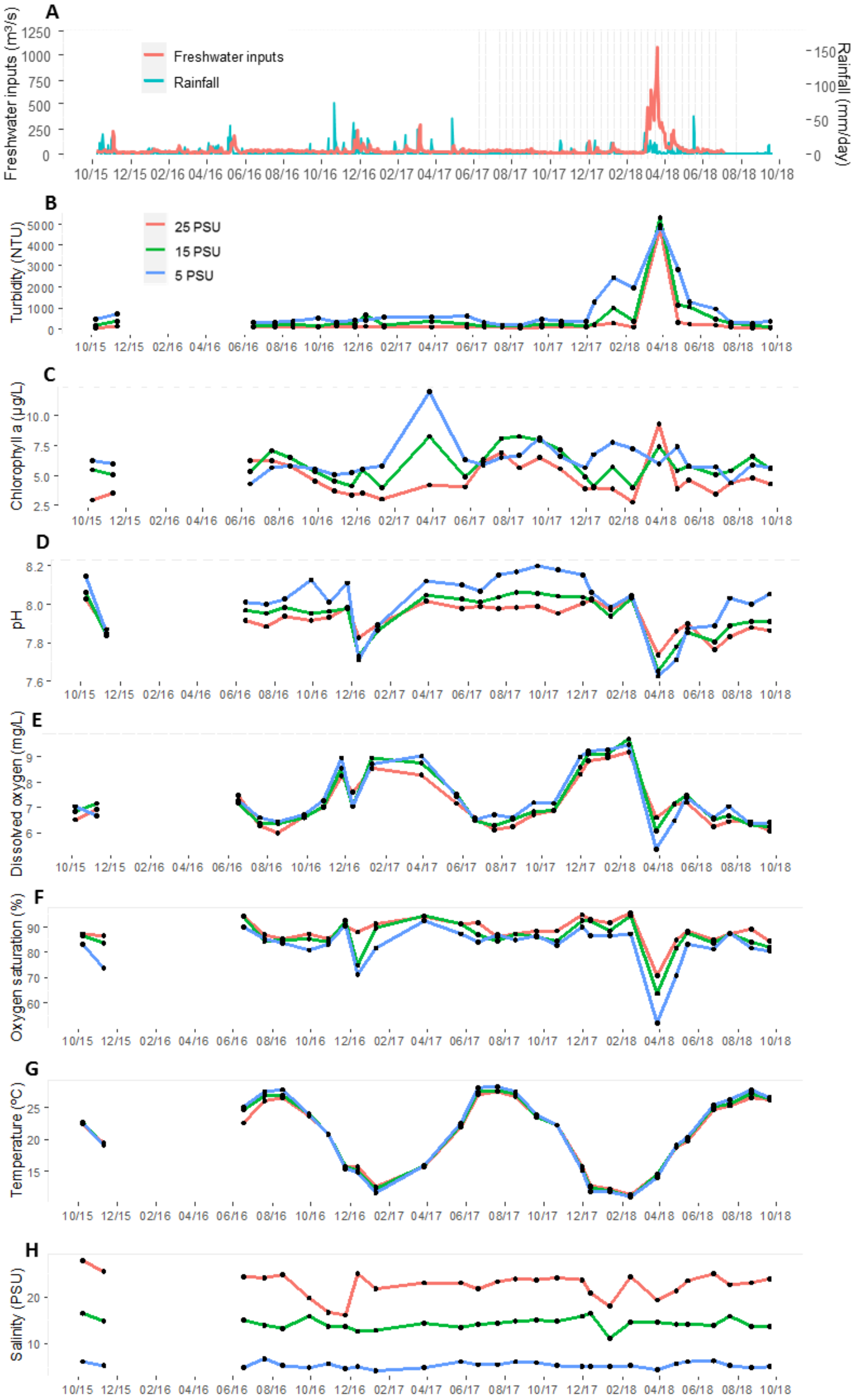

Figure 4. Temporal series of: daily mean of freshwater input from Alcalá del Río Dam and local daily accumulated rainfall (A); water turbidity (B), chlorophyll-a (C), pH (D), dissolved oxygen (E), oxygen saturation (F), temperature (G) and salinity (H) in every station (25, 15, 5 PSU) of the inner zone of the Guadalquivir estuary.

activity in most of the water column. The pH decreased gradually from the river mouth (8.2) toward upstream (7.6), with a more notable decline in the last 10 Km.

Freshwater input into the Guadalquivir estuary and local rainfall were plotted in the Figure 3A. The average of water discharged between 01-10-2015 and 30-09-2018 was 38.07 m^3/s. The freshwater input was continued and stable along years, although several high discharge events were observed in autumn and spring seasons due to the rainfalls. Most of the freshets were temporally shorts (1-3 days) and with discharges lower than 300 m^3/s (November 2015, May and November 2016 and March 2017). A longer (1 month approx.) and higher discharge (up to 1080 m^3/s) was registered in March 2018.

Figures 3 and 4 show the temporal series of depth averaged physiochemical profiles in every station, outside and inside the estuary, respectively. Technical problems and weather conditions precluded to collect the data in several months. Turbidity levels, directly correlated with total suspended solids, were different between zones, being the outside (Figure 3B) notably lower than inside (Figure 4B and Figure S.1). Similar levels among nearshore stations were observed, while the inner stations showed a gradual increment in upper water masses (25 < 15 < 5 PSU). Turbidity increase was associate to events of high freshwater inputs, even in the outer stations, being the highest levels recorded in March 2018 in inner stations. In fact, the levels registered in the three inner stations during this event were higher than the maximum quantification limit of the multiprobe (5400 NTU), therefore, real levels would be even higher. Instead, total suspended solids (Figure 5A and Figure S.2), which showed a similar pattern as turbidity, reached a maximum concentration higher than 6000 mg/L in all inner stations. Total suspended solids showed the same pattern as turbidity. Most of the suspended solids was inorganic matter, with more than 70% in all stations during the whole study (Figure 5C). No differences were observed between nearshore stations, but a trend was found in the inner stations, which showed a slight gradual decrease of suspended organic matter proportion from downstream to upstream water masses (Figure 5B). Additionally, the freshet of March 2018 notably decreased the proportion of suspended organic matter in the water column.

Chlorophyll concentration did not show a clear pattern in the nearshore stations due to high fluctuation inter months (Figure 3C). Still, a slight tendency of higher concentration in summer and lower in winter arise. This trend was more notable in the inner zone (Figure 4C). Higher concentrations were usually in 5 and 15 PSU stations. Also, a punctual increment was observed during the freshet of March 2018 in 25 and 15 PSU stations, while the Canal station showed the opposite tendency. Similar, temporal pattern was observed in pH levels of outer zone (Figure 3D). In fact, the increase of pH observed in August 2016 coincided with the chlorophyll increment. Instead, pH levels inside of the estuary were seasonally stable, but with differences between stations (Figure 4D). The pH values increased gradually toward upstream stations. Also, the freshets of November 2016 and March 2018 caused a high decrease of the pH levels.

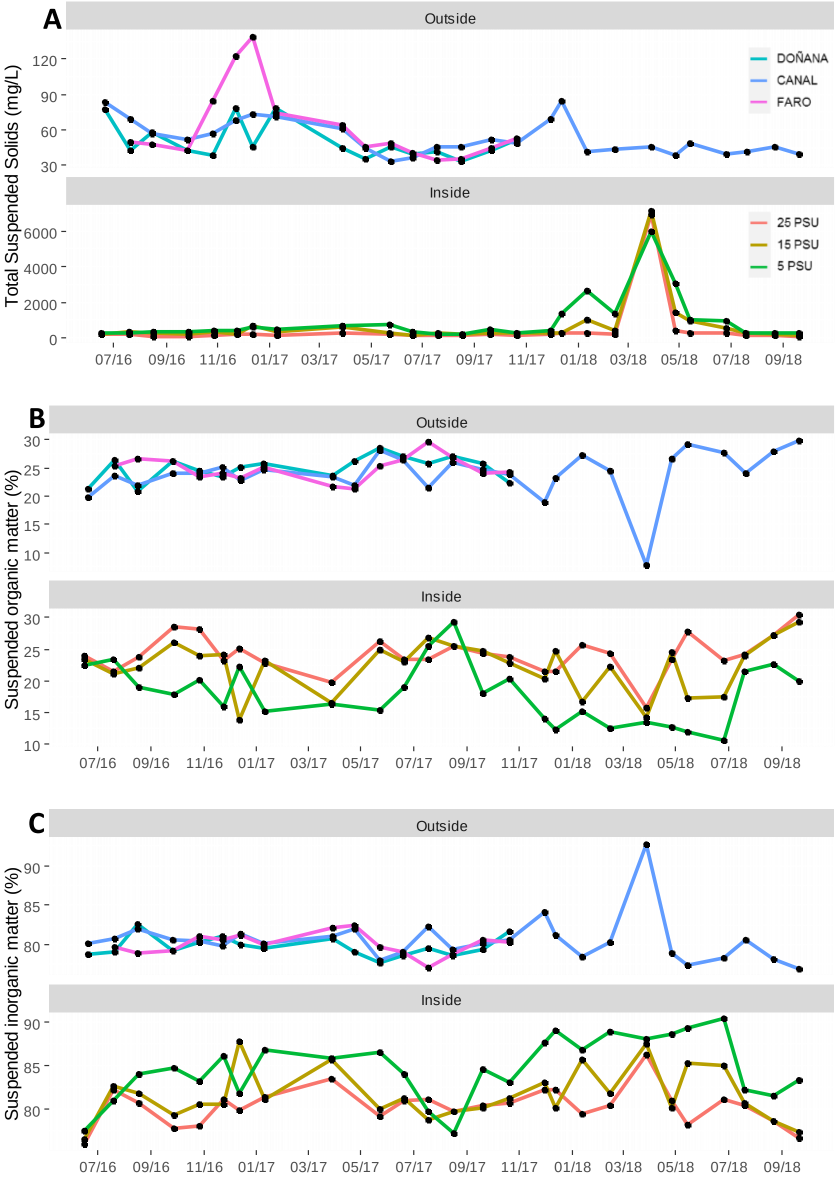

Figure 5. Temporal series of total suspended solids (A), suspended organic (B) and inorganic (C) matter in every station of both zones of the Guadalquivir estuary.

Oxygen saturation was higher in the outer zone with an intermonth fluctuation (Figure 3F), but always with more than 90%. Doñana station usually showed higher levels. Inner stations showed stable values along the whole study, with a gradual decrease toward upstream stations (Figure 4F). The freshets of November 2016 and March 2018 altered the saturation, with a notably decrease in both zones. The dissolved oxygen fluctuated seasonally, with higher concentration in winter and spring, and lower in summer and autumn. In nearshore stations, oxygen concentration (Figure 3E) followed the same pattern than oxygen saturation, while in inner stations, oxygen concentration (Figure 4E) increased upstream, contrary to oxygen saturation. Also, the freshets caused punctual decreases in its concentration in all stations. The temperature showed a seasonal variation with an expected opposite pattern than oxygen concentration, reaching the highest levels in summer and the lowest in winter. Different ranges of temperature were observed between the outer (12-26 ^{0}C; Figure 3G) and inner (10-28 ^{0}C; Figure 4G) zones, being more extreme in the latter, especially in upstream stations.

The nearshore zone presented a riverine influence in the salinity levels with a temporal variation (34-38 PSU), being higher in the summer and lower in the winter (Figure 3H). The inner

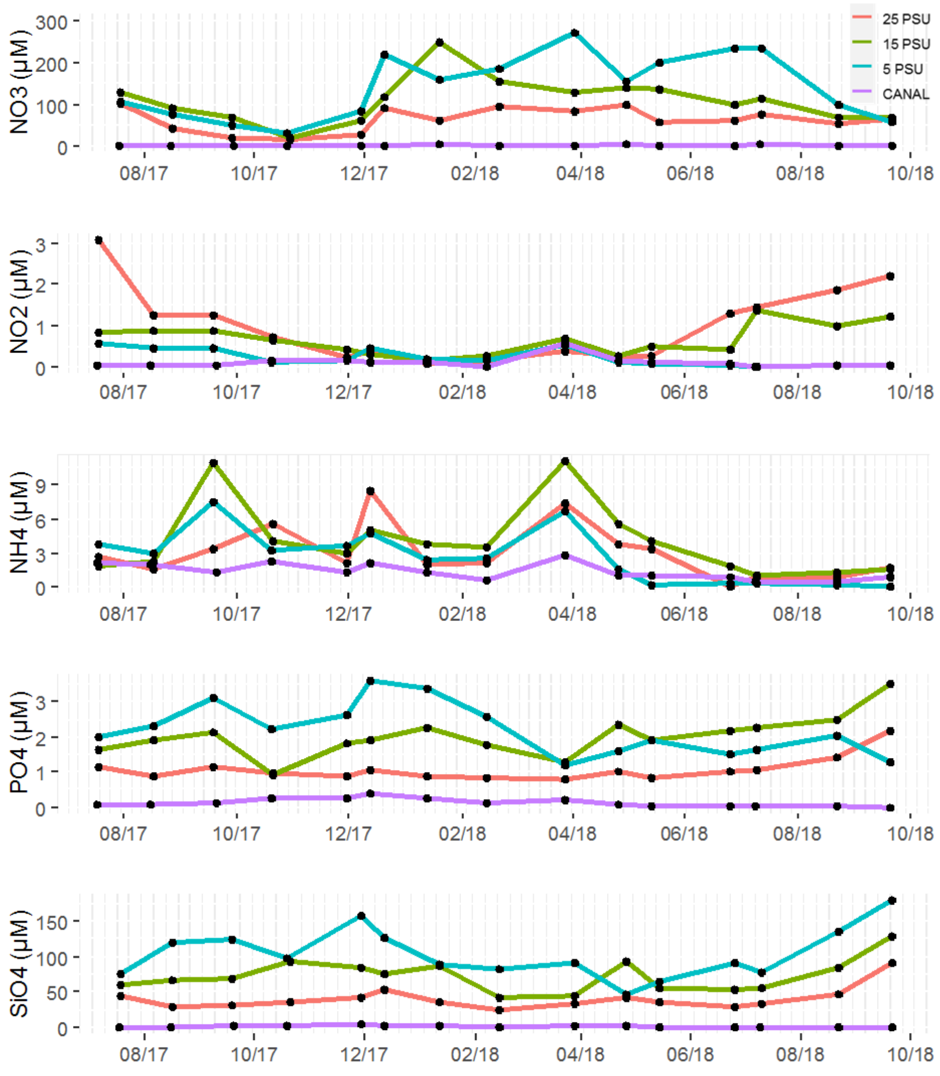

Figure 6. Temporal series of total NO_3 (A), NO_2 (B), NH_4 (C), PO_4 (D) and SiO_4 (E) in every station of the Guadalquivir estuary.

stations were selected because of their salinities, hence they do not show a significant variation in salinity, but seasonal variations were reflected in their position. The only station showing some variability was 25 PSU because it showed a higher vertical stratification in autumn and winter (Figure 4H).

Temporal series of the nutrients measured in the water samples were plotted in the Figure 6 (technical problems in the analysis methodology caused the data from June 2016 to June 2017 to be rejected). Different temporal and spatial patterns were observed for each nutrient, although the Canal station showed the lower levels in all of them. NO_3 showed seasonal oscillations, with lower concentration in autumn, and spatial differences, generally with higher levels in upstream stations (Figure 6A). Similar spatial trend was found for NO_2, but the temporal tendency was the opposite, with lower concentration in winter and spring seasons (Figure 6B). No clear spatial pattern was found for NH_4 concentration, except for the lowest levels in Canal station, and it showed several punctual increments (Figure 6C). PO_4 and SiO_4 showed similar spatio-temporal patterns with stable values along the study and a gradual increment of concentrations towards upstream stations (Figure 6D and 6F).

3.2. Biological data

In total, 121277 fish individuals in early life stages belonging to 41 species, 32 genera and 23 families were caught in the Guadalquivir estuary during the whole study (Table S.1). Total fish abundance was much higher in the estuarine zone than the nearshore, collecting 112528 individuals of 37 species inside and 8749 individuals of 30 species outside. The nMDS ordination of all samples indicated clear differences in the structure of early fish assemblages between the inner and nearshore zones (Figure 7).

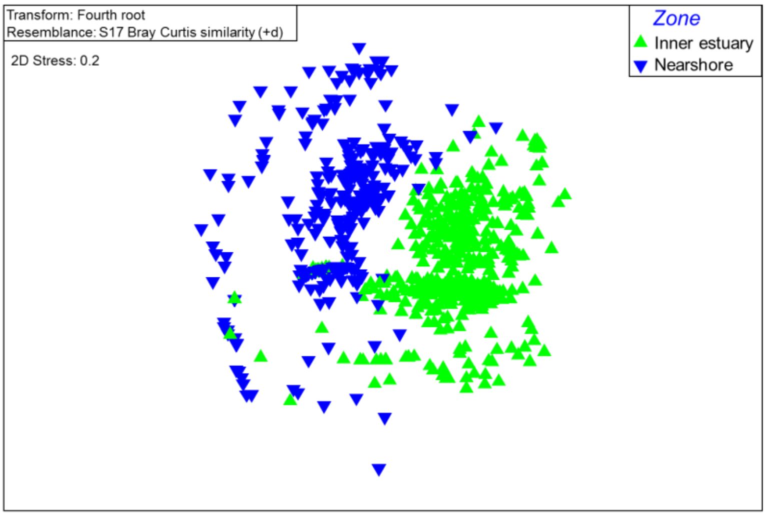

Figure 7. nMDS ordination of early life stages of fish samples. Samples coded by zones.

Table 1. PERMANOVA results of the Bray-Curtis similarity matrix based on the fourth-root-transformed assemblage data in every zone.

	NEARSHORE ZONE						INNER ZONE					
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**perms**	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**perms**
Station	2	4588	2294	0.99924	0.428	9952	2	59185	29592	14.364	**0.0001**	9927
Year	2	19617	9808.7	1.179	0.2923	9950	2	32622	16311	1.9892	**0.0363**	9918
Month (Year)	29	325300	11217	19.74	**0.0001**	9824	31	257020	8291	27.294	**0.0001**	9728
Station x Year	4	7058.9	1764.7	0.73471	0.7244	9917	4	7459.8	1864.9	0.89232	0.5743	9907
Station x Month (Year)	33	83957	2544.2	4.4771	**0.0001**	9783	62	131120	2114.9	6.9622	**0.0001**	9688
Res	259	147180	568.26				340	103280	303.77			
Total	329	614550					441	592340				

Table 2. Pair-wise analysis of significant terms in PERMANOVA results of assemblages in inner zone.

Station	t	P(perm)	perms
25 PSU - 15 PSU	1.7241	**0.0197**	9942
25 PSU - 5 PSU	3.8419	**0.0001**	9941
15 PSU - 5 PSU	4.2585	**0.0001**	9940
Year	t	P(perm)	perms
1 - 2	1.173	0.2121	9945
1 - 3	1.7595	**0.01**	9944
2 - 3	1.2887	0.1418	9948

Table 3. PERMANOVA results of the Euclidian similarity matrix based on species richness and Shannon index and total fish abundance data in every zone.

	Nearshore zone						Inner zone					
Richness	df	SS	MS	Pseudo-F	P(perm)	perms	df	SS	MS	Pseudo-F	P(perm)	perms
Station	2	1.7837	0.89186	0.21751	0.7883	9956	2	283.38	141.69	35.844	**0.0001**	9949
Year	2	14.168	7.0838	0.58955	0.4725	9922	2	43.16	21.58	0.68667	0.515	9956
Month (Year)	29	468.33	16.149	16.846	**0.0001**	9913	31	985.07	31.777	28.18	**0.0001**	9896
Station x Year	4	8.3557	2.0889	0.48654	0.7383	9963	4	32.533	8.1332	2.0328	0.109	9952
Station x Month (Year)	33	150.22	4.5521	4.7486	**0.0001**	9878	62	250.54	4.041	3.5836	**0.0001**	9868
Res	259	248.28	0.95862				340	383.4	1.1276			
Total	329	920.62					441	1977.1				
Shannon index	df	SS	MS	Pseudo-F	P(perm)	perms	df	SS	MS	Pseudo-F	P(perm)	perms
Station	2	0.18478	0.0924	0.27851	0.7487	9957	2	5.0147	2.5073	9.1581	**0.0005**	9933
Year	2	0.50654	0.25327	0.31439	0.6083	9926	2	3.4817	1.7408	1.9653	0.1553	9938
Month (Year)	29	31.14	1.0738	12.176	**0.0001**	9892	31	27.757	0.89539	17.298	**0.0001**	9883
Station x Year	4	0.79473	0.19868	0.57307	0.6857	9954	4	0.16285	0.0407	0.14668	0.9626	9956
Station x Month (Year)	33	12.103	0.36675	4.1585	**0.0001**	9886	62	17.404	0.28071	5.423	**0.0001**	9874
Res	259	22.842	0.0882				340	17.599	0.0518			
Total	329	68.745					441	72.363				
Total abundance	df	SS	MS	Pseudo-F	P(perm)	perms	df	SS	MS	Pseudo-F	P(perm)	perms
Station	2	49572	24786	0.4224	0.4151	9896	2	57463000	28732000	15.026	**0.0001**	9954
Year	2	200260	100130	1.0653	0.3439	9946	2	20276000	10138000	1.4244	0.266	9955
Month (Year)	29	3301900	113860	2.7873	**0.0438**	9893	31	223070000	7195900	22.272	**0.0001**	9874
Station x Year	4	81391	20348	0.34041	0.7245	9945	4	7904500	1976100	1.0191	0.4019	9954
Station x Month (Year)	33	2021000	61243	1.4993	**0.0359**	9880	62	121620000	1961700	6.0715	**0.0001**	9879
Res	259	10580000	40849				340	109850000	323090			
Total	329	16497000					441	542910000				

Table 4. Pair-wise analysis of significant terms in PERMANOVA results of diversity indices and total fish abundance in inner zone.

Richness	t	P(perm)	perms
25 PSU - 15 PSU	0.40297	0.6918	9839
25 PSU - 5 PSU	6.6771	**0.0001**	9858
15 PSU - 5 PSU	6.8372	**0.0001**	9835
Shannon index	t	P(perm)	perms
25 PSU - 15 PSU	1.948	0.068	9836
25 PSU - 5 PSU	3.9967	**0.0008**	9818
15 PSU - 5 PSU	2.4682	**0.0238**	9844
Total abundance	t	P(perm)	perms
25 PSU - 15 PSU	0.24448	0.8066	9841
25 PSU - 5 PSU	4.0679	**0.0004**	9850
15 PSU - 5 PSU	4.986	**0.0001**	9814

PERMANOVA analysis, separating inner and nearshore zones, revealed different patterns for each one, although the structure varied inter-monthly in all stations of both zones (Table 1). The assemblage was annually and spatially constant in nearshore zone, with no differences between stations and years. Conversely, the inner assemblage showed differences between stations and years. Pair-wise comparison showed that all the assemblages of inner stations were different from each other, and only the first year was different with the third one (Table 2).

Total fish abundance, species richness and Shannon index showed similar patterns (Table 3). No diversity and total fish abundance differences were found between years in both zones, although it varied inter-monthly in all stations (Figure 8). Generally, lower diversity was observed in summer season, due to the increment of total fish abundance, which was dominated by a few species. Only the inner zone showed differences between stations, being 5 PSU significantly lower in abundance and diversity than the others (Table 4).

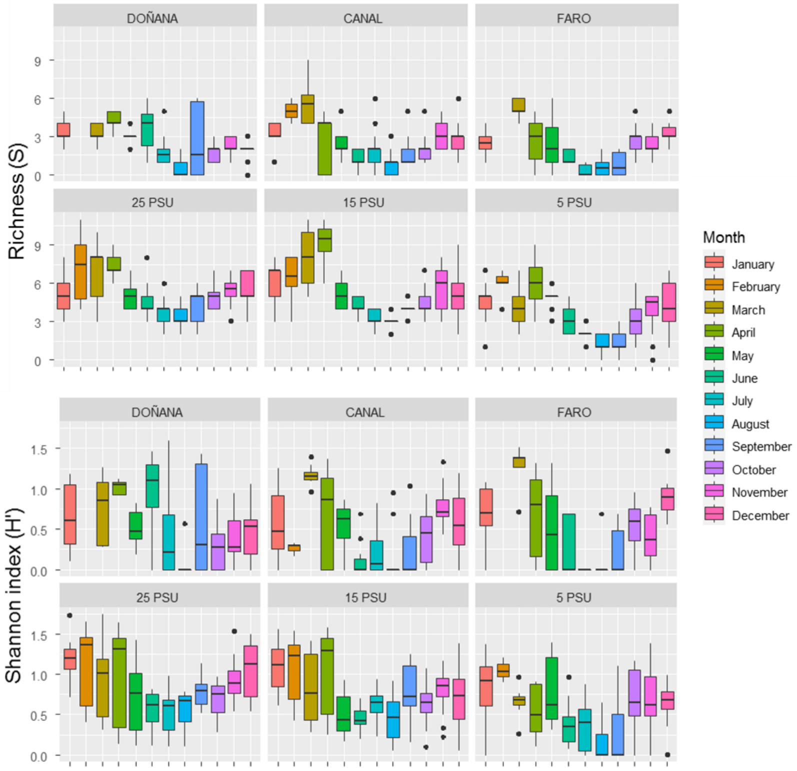

Figure 8. Boxplot of species richness (A) and Shannon index (B) per month in every station of both zones during the study period.

SIMPER analysis of zones and stations combined (Table 5) showed that *Engraulis encrasicolus,* and *Sardina pilchardus* in a lesser extent, were characteristics species in most stations of both zones. Additionally, *Pomatoschistus* spp. (*Pomatoschistus minutus* and

Pomatoschistus microps) and *Argyrosomus regius* contributed to the similarity of the inner zone, while *Aphia minuta* contributed to the outer zone.

Table 5. Results of SIMPER analysis on the abundance of all larval and juvenile fish species from every zone and their stations.

NEARSHORE ZONE

Average similarity: 26.69

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Engraulis encrasicolus	1.39	17.18	0.77	64.38	64.38
Aphia minuta	0.94	5.35	0.44	20.06	84.44
Sardina pilchardus	0.56	2	0.3	7.5	91.94

DOÑANA

Average similarity: 24.96

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Engraulis encrasicolus	1.44	17.45	0.83	69.9	69.9
Aphia minuta	0.63	3.06	0.34	12.26	82.16
Sardina pilchardus	0.55	2.21	0.3	8.84	90.99

CANAL

Average similarity: 32.26

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Engraulis encrasicolus	1.71	22.07	0.93	68.42	68.42
Aphia minuta	1.22	6.74	0.51	20.88	89.3
Dicologoglossa cuneata	0.56	1.49	0.26	4.63	93.93

FARO

Average similarity: 16.87

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Engraulis encrasicolus	0.9	6.99	0.45	41.42	41.42
Aphia minuta	0.84	4.57	0.4	27.09	68.51
Sardina pilchardus	0.64	3.07	0.37	18.19	86.7
Diplodus bellottii	0.34	0.76	0.15	4.48	91.18

INNER ZONE

Average similarity: 48.06

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Pomatoschistus sp	3.47	23.58	2.03	49.07	49.07
Engraulis encrasicolus	2.82	16.79	1.28	34.94	84.01
Argyrosomus regius	0.71	1.99	0.42	4.13	88.14
Sardina pilchardus	0.75	1.64	0.39	3.41	91.56

25 PSU

Average similarity: 51.99

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Pomatoschistus sp	3.8	23.05	3.3	44.33	44.33
Engraulis encrasicolus	3.45	19.48	1.75	37.47	81.8
Argyrosomus regius	0.8	2.31	0.49	4.45	86.25
Sardina pilchardus	0.86	2.19	0.45	4.21	90.46

15 PSU

Average similarity: 54.05

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Pomatoschistus sp	3.98	25.66	3.45	47.48	47.48
Engraulis encrasicolus	3.47	19.14	1.72	35.41	82.88
Argyrosomus regius	0.92	2.97	0.53	5.5	88.39
Sardina pilchardus	1	2.22	0.47	4.1	92.49

5 PSU

Average similarity: 38.42

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Pomatoschistus sp	2.63	22.04	1.3	57.36	57.36
Engraulis encrasicolus	1.55	11.93	0.78	31.04	88.41
Anguilla anguilla	0.44	1.39	0.29	3.62	92.02

Temporal series of fish species that most contributed to the similarity of every zone were plotted in the Figure 9. *Pomatoschistus* spp. was abundant only inside of the estuary, mainly in spring and summer seasons, being the 25 and 15 PSU stations the densest (Figure 9A). Particular increment was observed in all stations, even in Canal, during and after the freshet of March 2018. *Engraulis encrasicolus* showed similar tendencies in both zones, although inner stations contained much higher densities, except for 5 PSU (Figure 9B). The recruitment period of anchovy was mainly along the late spring and summer seasons, but the months of its maximum density peaks changed between years (2016: August; 2017: May; 2018: July), as well as the temporal range of the period. In fact, during the last year, its abundance was lower in the inner zone, reducing its recruitment spatial and temporally to only polyhaline water mass in July. *Argyrosomus regius* was characteristic inside the estuary, although some individuals were caught outside too (Figure 9C). Temporal pattern was found with higher abundances during the summer and early autumn, but no clear differences were observed between stations. *Sardina pilchardus* started its recruitment period during the end of winter, reaching the highest densities in the spring season (Figure 9D). No spatial differences were observed in nearshore stations,

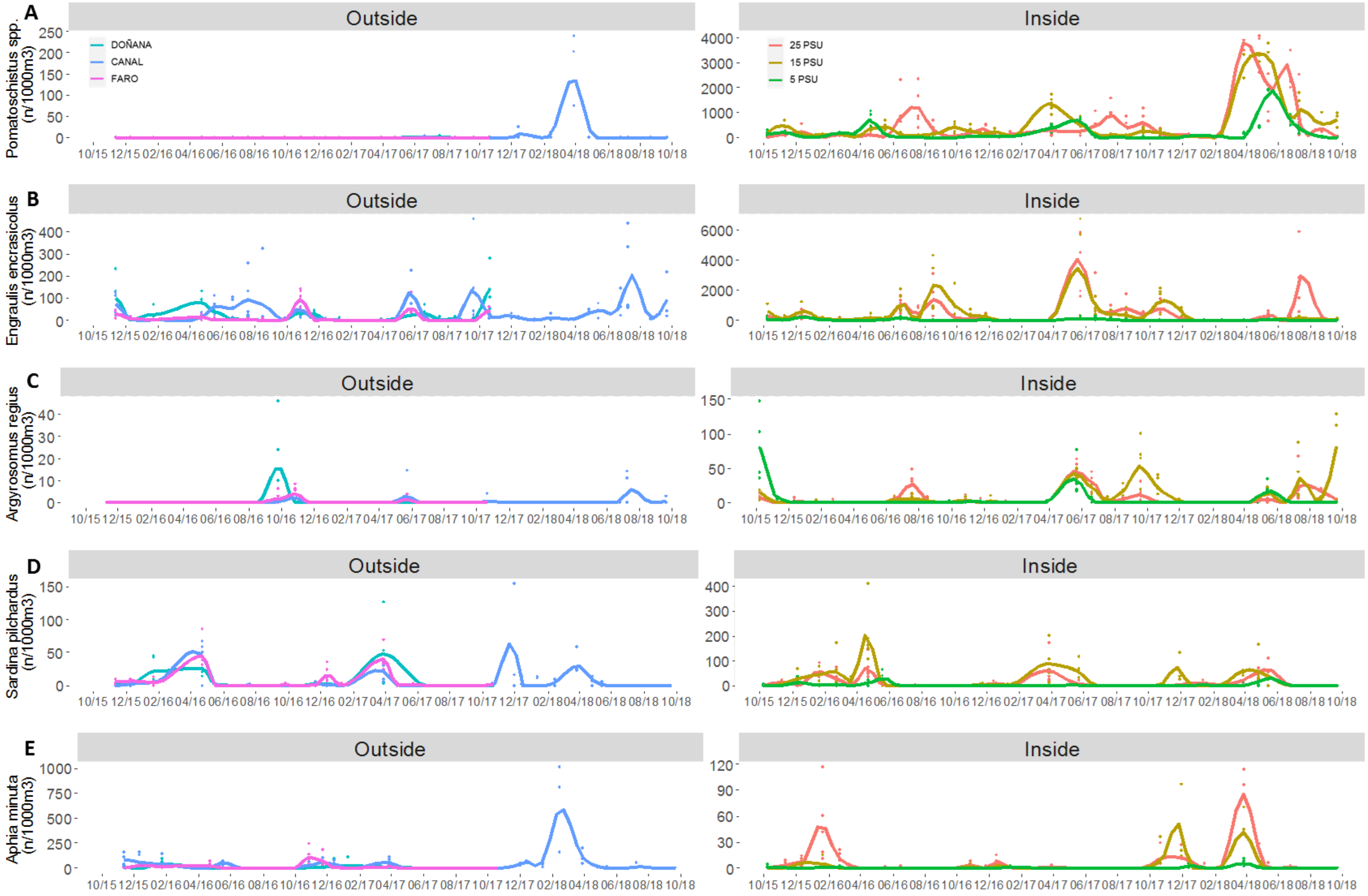

Figure 9. Temporal series of the density of the main species characteristics of every zone. A: *Pomatoschistus* spp.; B: *Engraulis encrasicolus*; C: *Argyrosomus regius*; D: *Sardina pilchardus*; E: *Aphia minuta*.

while the 25 and 15 PSU were denser than the most upstream station. *Aphia minuta* was one of the few species which usually were more abundant outside of the estuary (Figure 9E). Its density increment occurred along the winters.

The structure of fish assemblages of every zone was related to multiple environmental variables. The ordination analysis obtained a correlation of 40.3% for the outer zone and 47.8% for the inner zone; their first two dbRDA axes accounted for 32.6% (Figure 10A) and 38.7% (Figure 10B) of the total variation, respectively. Temperature (and inversely correlated the DO), marked a temporal gradient in both zones along the first axis. Also, high freshwater inputs and rainfalls were associated to autumn and spring months. Nearshore stations were not related to any variable specifically, instead, salinity, and inversely associated the pH, separated the inner stations in the second axis. Chlorophyll and oxygen saturation showed a low influence in the assemblage structure.

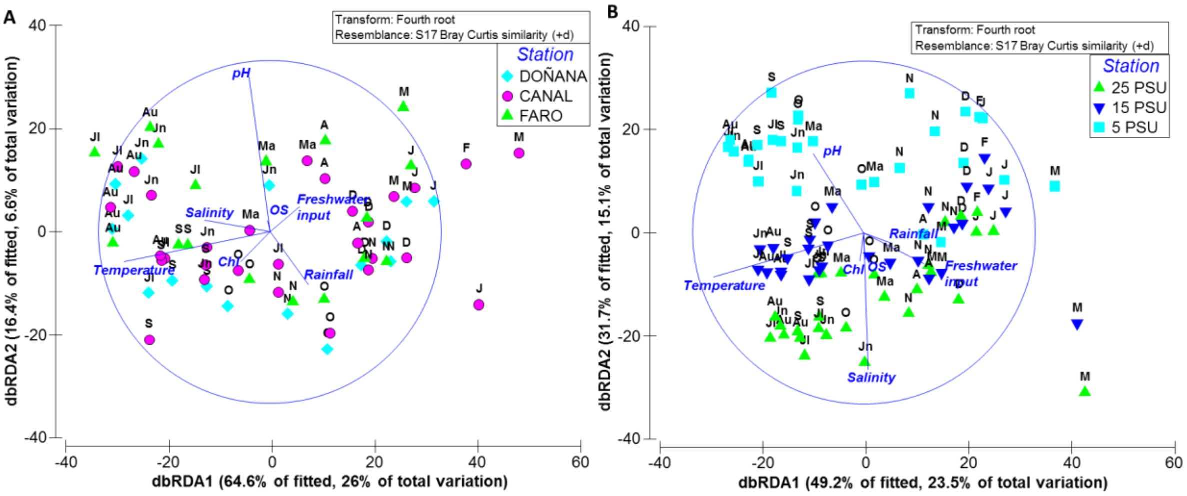

Figure 10. Ordination of the sampling stations along the first two axis of the distance-base redundancy analysis (dbRDA) relating all the species with environmental variables (blue arrows) in the nearshore (A) and inner (B) zones. Chl: chlorophyll-a; OS: oxygen saturation; J: January; F: February; M: March; A: April; Ma: May; Jn: June; Jl: July; Au: August; S: September; O: October; N: November; D: December.

In addition to the effect of environmental variables in assemblages, trophic interactions were analysed by plotting temporal series of total abundance of early life stages of fish, macrozooplankton, mesozooplankton and gelatinous plankton for each station of every zone in the Figure 11. Mesozooplankton abundance was mainly compound by the copepod *Acartia tonsa*, and *Calanipeda aquaedulcis* in lesser extent in the most upstream station. Among macrozooplankton species caught, most of biomass were mainly mysids (e.g. *Rhopalophthalmus tartessicus, Mesopopdosis slabbery, Neomysis integer*), followed of decapods (e.g. *Palaemon* spp. and *Crangon crangon*) and isopods (e.g. *Synidotea laticauda* and *Lekanesphaera rugicauda*).

Two kind of gelatinous plankton were found: the ctenophore *Bolinopsis* sp. in the nearshore zone, and the jellyfish *Maeotias marginata* in the inner zone. The density of macrozooplankton in outer zone was quite low along the whole study. Instead, *Bolinopsis* sp. was found from spring to autumn seasons of 2017 in high abundance. Its density fluctuated between nearshore stations along these months, with an extreme abundance case in Doñana during April and May that made impossible to take samples. When *Bolinopsis* sp. was present in high densities, the abundance of total fish decreased notably. On the other hand, the jellyfish *Maeotias marginata* was found mainly in the 5 PSU station of the inner zone. The occurrence of this species was frequent every year from the end of summer to the beginning of the winter. These increments coincided with a gradual reduction of the other plankton groups. The stations of 25 and 15 PSU usually showed higher abundances of zooplankton than 5 PSU. Generally, abundance increased in spring and summer, with different peaks during this period, and decrease in autumn. Still, different oscillations were found for every year and station. In most cases, macrozooplankton density increased before or at the same time that early fish stages. However, the smaller size group, mesozooplankton, tended to increase when the other groups started to decrease.

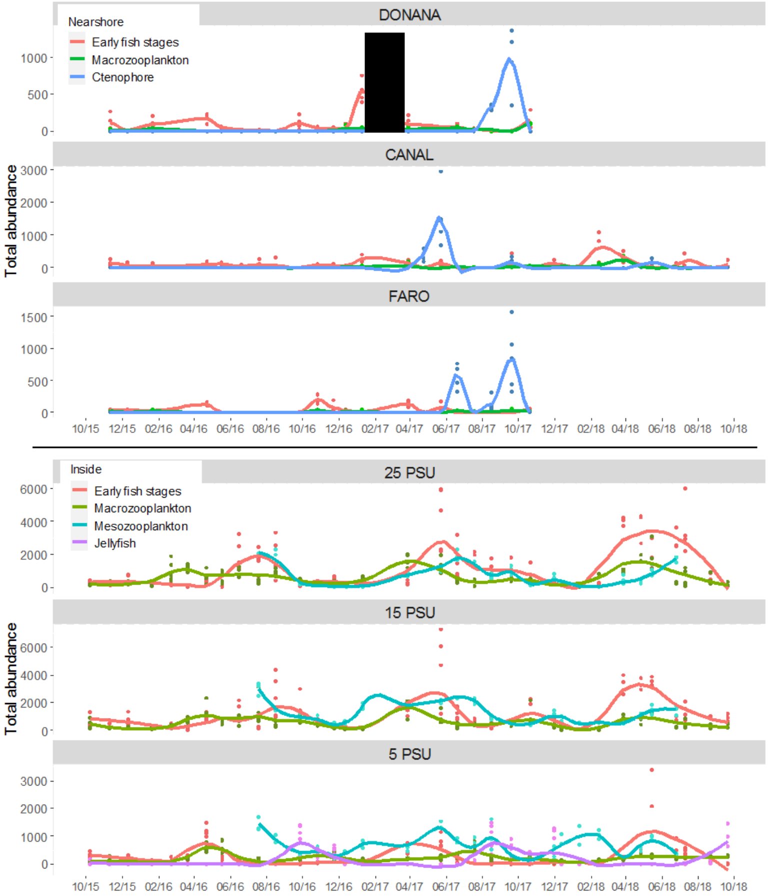

Figure 11. Temporal series of different plankton groups (Early fish stages (n/1000 m^3); Macrozooplankton (gr fresh weight/1000 m^3); Mesozooplankton (n x 10^{-1}/m^3); Ctenophora (mL/100 m^3); Jellyfish (n/1000 m^3) along the study period in each station of every zone (A: outside; B: inside). Black square in Doñana station during April and May 2017 means no possible sampling due to extremely high density of ctenophora.

4. Discussion

The Guadalquivir estuary presents typical characteristics of warm-temperate regions with seasonal fluctuations of temperature and different hydrologic years that influence in the rest of physiochemical variables of water column, and consequently, in the spatio-temporal distribution and abundance of plankton communities. Following the classification of hydrological periods in Guadalquivir described by (González-Ortegón and Drake, 2012), we could consider from 2015 to 2017 as a dry hydrological period, while 2018 was a wet hydrological period. The hydrological regime registered in the Guadalquivir was mainly tidally-dominated, except for the month of March 2018, which was fluvially-dominated with discharges higher than 400 m^3/s (Díez-Minguito et al., 2012). Despite of the hydrological oscillation, general patterns could be found for abiotic and biotic variables measured in both zones. In fact, the biological and physiochemical trends observed in the nearshore zone were very similar between stations, which made to consider the reduction of stations to only one during the third monitoring year.

A previous study indicated that the sea water intrusion into the estuary could be determined by the freshwater inputs and tidal conditions (Fernández-Delgado et al., 2007). These factors could displace the salinity gradient up or downstream and change the salinity levels, as well as the associated pH observed in the nearshore zone. However, contrary trends were observed during autumn-winter (salinity decreased) and spring-summer (salinity increased) of the dry hydrological period despite of the similar averaged freshwater inputs and tidal conditions. In fact, during the summer, the position of the inner sampling water masses was usually displaced upstream, reaching the isohaline of 5 PSU up to 50 Km from the estuary mouth (in contrast with the 30 Km approx. during autumn). This displacement of the salinity gradient coincided with the irrigation campaign in surrounding areas, which intakes high volumes of freshwater from upper estuarine zones for rice crops. Therefore, the water abstraction seemed to play an important role in the distribution of the nursery area in this estuary, in addition to freshwater input and tidal conditions.

Nutrient increment showed different temporal patterns. In the case of ammonium, it seems to be associated by freshets events, showing higher values during elevated freshwater discharges. Nitrate, instead, increased during the winter and spring, which coincided with agricultural fertilization practices in surrounding crops. Additionally, a spatial dilution effect was observed, with a gradual concentration decrease from upstream stations, which were closer to pollutants sources as agriculture crops and sewage effluents (Mendiguchía et al., 2007). Similar spatial trend found for chlorophyll concentration could indicate that the estuarine zone could be working as a nutrient removal area which avoid eutrophication phenomena in the nearshore (Barbier et al., 2011). However, the most turbid stations were landward, as registered in other estuaries (Bate et al., 2002; Irigoien and Castel, 1997), where photosynthetic activity by phytoplankton could be limited for the scarce light penetration in most of the water column (Ruiz et al., 2017). Instead, other primary producers as microphytobenthos can develop in the sediment of shallower zones and intertidal mud flats (Underwood, 2001; Underwood and Kromkamp, 1999), playing a more important role in the primary production than phytoplankton (Cadée and Hegeman, 1974; De Jonge and Van Beusekom, 1992). Therefore, other biogeochemical process as sorption, precipitation, flocculation or biological uptake by bacteria and/or other primary producers should be working to reduce nutrient levels (Eyre and Balls, 1999).

The different environmental characteristics found between zones also marked the structure of the early life stage of fish assemblages. Both zones showed temporal influence by temperature, but only the inner zone was spatially different among stations, which were governed by the salinity. Similar relationships have been reported in other temperate estuaries [e.g. Faria et al. (2006); Marques et al. (2006)] as well as in the inner zone of the Guadalquivir (Baldo et al., 2005; Drake et al., 2002). Biodiversity also showed the same pattern as the assemblages, changing over time, due to the temporal dominance of a few species in summer and early autumn (*Engraulis encrasicolus* and *Pomatoschistus* spp.), and among inner stations. Whitfield et al. (2012) proposed a conceptual model for fish distribution within salinity gradient in estuaries and it appears that the early stages of these fish species follow a similar pattern. Both model and trends emerging from this larval and juvenile fish study, confirm that species diversity increases notably from oligohaline to mesohaline waters, keeping steady in polyhaline conditions and followed by slight decline once seawater is reached.

Density trends for early fish stages follow the same pattern to species diversity, where maximum abundance usually occurred in the mesohaline and polyhaline stations. The high concentrations of total suspended solids that forms a frontal zone between fresh and saline water trigger and form the basis of biological functioning in estuaries (Snow et al., 2000). Brackish waters are generally associated of high primary and secondary production areas, which in turn support the highest densities of early life stages of fishes (Pihl et al., 2002). Also, oligohaline waters are suggested as an important nursery regions to contain high amounts of detritus which support the base of the food web (Islam and Tanaka, 2006). However, in our case, despite of containing maximum organic matter, nutrients and chlorophyll, the plankton abundance was lower than meso and polyhaline waters. Mesozooplankton species as copepods are the main prey in larval fishes in these systems, but many developing late stage larvae shift diet to include macrozooplankton species as mysids (Baldó and Drake, 2002), which in turns also share preys as copepods and smaller mysids (Vilas et al., 2008). Peak productivity in temperate estuaries is usually linked to spring and early summer when primary and secondary production increases and ensures more food resources are available for developing larvae (Strydom, 2014). Similar trends were found in this research, although the increment of the different trophic levels was not synchronic. The patterns observed were not uniform for every station and year, but some food web interactions could arise. Macrozooplankton usually increased before the early life stages of fish, and declined when this reached its maximum density peak, showing a bottom-up or resource control. However, mesozooplankton generally achieved its peak when both higher trophic levels were decreasing, showing a top-down or predator control. On the other hand, suspended organic matter in form of detritus has been reported to contribute in the diet of these meso and macrozooplankton species (Donázar-Aramendía et al., 2019; Vilas et al., 2008). As its concentration is usually high and constant in the inner zone, these species can diversify its diet to more complex feeding pathways (Donázar-Aramendía et al., 2019). Therefore, its proliferation would not be limited by only one lower trophic level, which explain the variability found in these patterns.

Furthermore, demographic blooms of gelatinous plankton could impact in the plankton assemblage by predation and competition with mesozooplanktivorous consumers such as macrozooplankton and early life stages of fishes (Boero, 2013; Purcell and Arai, 2001; Robinson et al., 2014). That is the case of jellyfish *Maeotias marginata* found in the oligohaline station, which seems to influence in the rest of zooplankton groups, although its increment coincided with the natural decline of zooplankton abundance over the autumn season hindering the evaluation of its possible impact. This species was previously recorded in the Guadalquivir (Diz et al., 2015), as well as in other estuaries all around the world in low salinity waters too (Mills and Rees, 2000; Nowaczyk et al., 2016). Although some studies have found fish larvae in their guts, no direct predation impact have been reported (Schroeter, 2008). Instead, potentially prey competition between *M. marginata* and early life stage of fishes were found (Wintzer et al., 2011). Similar influences have been described for the ctenophora *Bolinopsis* spp., which are also widely distributed in seawaters (Öztürk et al., 2011; Purcell et al., 2010; Shiganova et al., 2014). The demographic blooms found in the nearshore zone during spring and summer of 2017 reduced the early life stages of fish density in its stations. The high density persistent in the estuary mouth during the main recruitment period could work as a barrier for the input and output of marine migrant fish species. However, no effects were detected in the inner zone, which maintained similar density levels as other years. Still, the high invasive character and the recurrent demographic blooms of both species should be monitored to assess potential ecological interactions in the Guadalquivir estuary.

Most of dominant estuary-dependent fish species showed generally similar catch trends in this study when compared to past investigations in the inner zone of Guadalquivir estuary (Drake et al., 2002; González-Ortegón et al., 2012). *Pomatoschistu*s spp. were one of the most abundant fish species in all inner stations, while their presence in the nearshore zone was negligible. Several studies have documented seaward migration of these gobies during the early spring for reproduction in other areas (Guelinckx et al., 2008; Pampoulie et al., 1999), however no evidences were found in this research. Still, different development stages were found along the whole year. Individuals dominated mainly by early stages were caught during the spring, which grew up to adult stages along the summer and autumn, showing the highest densities, and later, declined in winter. Also, *Pomatoschistus* spp. showed a high recruitment response after the freshet event of March 2018, whose abundances were the highest of the whole study. Therefore, in the Guadalquivir estuary, it is possible, that this species could migrate to outer and deeper zones than nearshore to reproduce in winter, and the estuarine signals released further by the freshet during the recruitment season provoked a higher attraction of early stages into the estuary. Contrary behaviour has been proposed for the meagre *Argyrosomus regius* in the Gulf of Cádiz, where adults individuals could migrate into the estuaries to reproduce between March to August, and early stages could remain inside during the 2-3 first years of life (González-Quirós et al., 2011). Our catches were higher in inner stations, mainly in summer and early autumn, which coincide in space and time with the spawning and hatchery process suggested. However, developing larvae were also found, at the same period in the nearshore zone, which could indicate that estuary mouth could be also a spawning zone, or larvae do not stay so long periods into the estuaries. Further research would be necessary to elucidate the early development process of *A. regius*.

The anchovy *Engraulis encrasicolus* maintained a high contribution to catches along the whole year, but with higher densities during the summer, especially in poly and mesohaline stations. Its spatio-temporal trend remained similar as the previous work performed by Drake et al. (2007) from 1997 to 2005. Nonetheless, the high freshet event of March 2018 seemed to influence on the high recruitment period with an opposite pattern of *Pomatoschistus* spp., being shorter and with lower abundance, especially in the mesohaline water mass. The similar abundance (or even higher) as previous years in the nearshore zone suggests that external factors did not influence the approach of early stages toward the estuary. Hence, it indicates that some internal factors could influence on its recruitment. Among the distinct environmental variables measured, turbidity showed a different pattern in comparison with previous years. Its levels, even though declined quickly after the freshet event, maintained elevated in the following months with a slow recovery until the summer in meso and oligohaline water masses, covering most of the high recruitment period of this species. In fact, De Carvalho-Souza et al. (2018) showed that turbidity levels higher than 100 NTU was negative for the early anchovy in the Guadalquivir estuary, being all the averaged values registered after the freshet higher than this reference in the meso and oligohaline water masses. Anchovy was also the most abundant species in the outside zone, followed by *Aphia minuta*, which seemed to prefer more saline stations as also reported González-Ortegón et al. (2015). In addition, other species as *Sardina pilchardus, Dicologoglossa cuneata* and several sparids contributed, in lesser extent, to the assemblage of the nearshore zone, showing a composition comparable to that of other coastal waters in the Gulf of Cádiz (Baldó et al., 2006). Conspicuously, *Sardina pilchardus*, despite of being also characteristic in poly and mesohaline stations, showed much lower densities in the estuarine zone in comparison with previous records during 1997-1999 by Drake et al. (2002), suggesting a dramatic temporal decline. Similar trend has been reported around the Iberian Peninsula waters for adult stocks during the last decade (ICES, 2019), where the authorities have recently developed a restoration plan to manage the fishery pressure over this species (MAPAMA, 2018). In addition, different studies revealed a possible shift of sardine populations to northwards Atlantic waters during the last years, being the increment of the sea surface temperature in the region the main driver (Alheit et al., 2012; Beare et al., 2004; Montero-Serra et al., 2015).

When large dam discharges occur in the estuary, they can cause multiple alterations in its physiochemical and biological conditions, even in long-term. Compression of the longitudinal salinity gradient or extreme turbidity levels have been registered in the Guadalquivir estuary previously (Díez-Minguito et al., 2013; Ruiz et al., 2017). In addition, we also observed an increment of chlorophyll concentration in the inner zone at the same time that total suspended solids and organic matter, which could indicate an import of phytoplankton from the upstream water reservoirs (González-Ortegón and Drake, 2012) and/or a higher resuspension of microphytobenthos from the riversides (Díez-Minguito and de Swart, 2020). On the other hand, depending on the kind of discharge, plankton organisms could be pushed downstream or even flushed out of the estuary (Chícharo et al., 2006), as showed *Pomatoschistus* spp. in March 2018, but also they could be attracted by the higher spread of estuarine cues (Kingsford and Suthers, 1994). González-Ortegón et al. (2012) also showed that the nekton of the Guadalquivir estuary could responded to high freshwater inputs with immediate changes in its structure, but the recovery of environmental conditions within the estuary allowed its reestablishment. Estuarine biota is well-adapted to cope with different types of stress and so the areas may be regarded as resilient because of the inherent variability of these ecosystems (Elliott and Quintino, 2007). Notwithstanding, the short-term process of how plankton organisms restore to normal conditions in the outside and inside zones of the estuary is still scarcely known.

In conclusion, the Guadalquivir estuary showed a clear nursery function, with much higher abundance of early life stages of fishes and other zooplankton groups in its estuarine zone than the surrounding area. The nearshore zone showed similar environmental conditions, while the inner zone was more diverse, being the poly and mesohaline water masses the most productive. The freshwater inputs, in addition to generate a longitudinal salinity gradient, supplied high amounts of organic matter, which seems to be the main food resource of its complex trophic web. Different hydrologic years, caused by heavy rainfall, modified its physiochemical conditions, which in its turn, changed its assemblages. The high variability observed in this ecosystem, in short (monthly) and long (annually) term, suggests performing different monitoring programs with multiple approaches to better understand the ecological responses to distinct situations or disturbances.

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Supplementary data

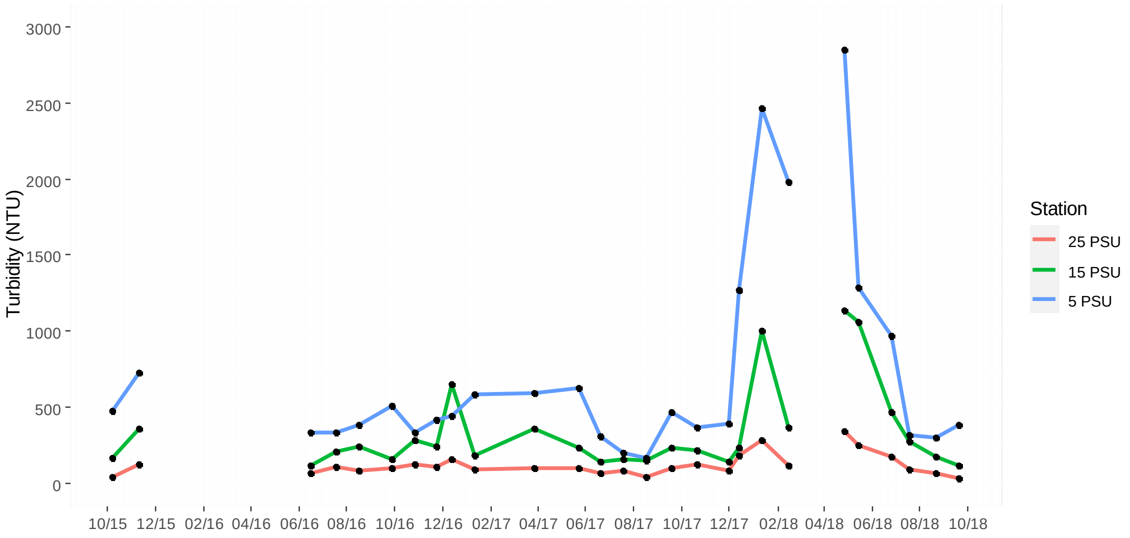

Figure S.1. Zoomed facet of the Figure 4B without extreme values of March 2018. Temporal series of water turbidity in every station (25, 15, 5 PSU) of the inner zone of the Guadalquivir estuary.

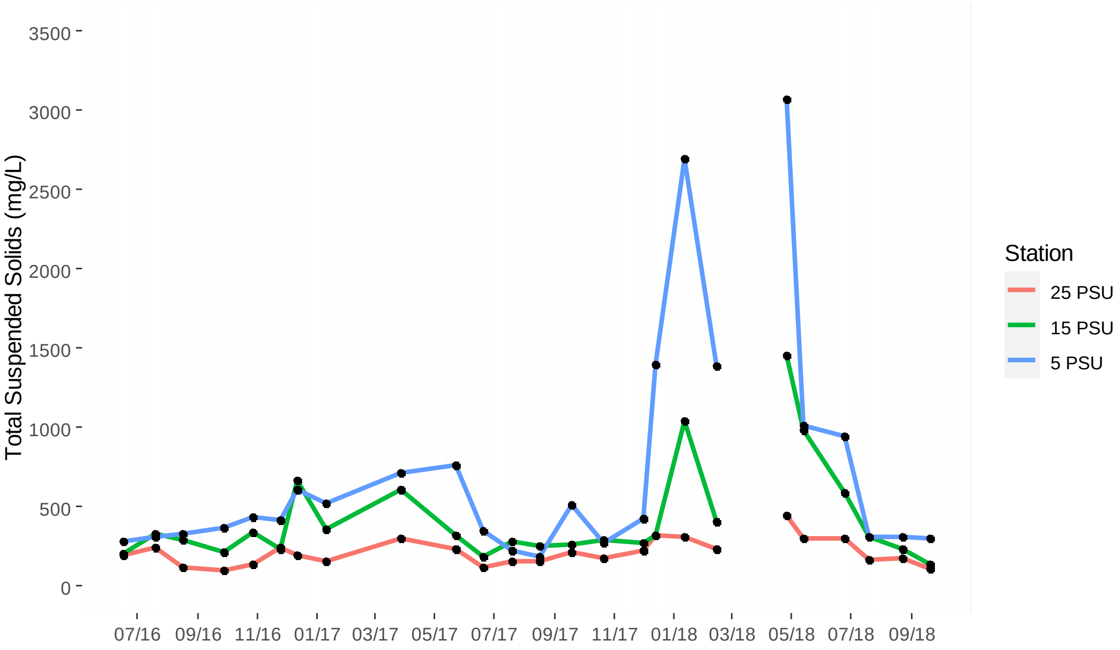

Figure S.2. Zoomed facet of the Figure 5A without extreme values of March 2018. Temporal series of total suspended solids in every station (25, 15, 5 PSU) of the inner zone of the Guadalquivir estuary.

Table S.1.1. Monthly average (mean±SE) densities (ind./1000m^3) of early life stages of fish in the nearshore zone of the Guadalquivir estuary during October 2015 to September 2018.

	NEARSHORE ZONE											
	January	February	March	April	May	June	July	August	September	October	November	December
Ammodytidae	0±0	0±0	0.16±0.16	0±0	0±0	0.11±0.11	0±0	0±0	0±0	0±0	0±0	0±0
Aphia minuta	23.49±5.93	658.99±257.6	40.75±12.53	6.72±2.79	11.76±4.28	0.11±0.11	1.82±1.54	0±0	0±0	25.65±10.08	39.44±8.47	29.97±7
Argyrosomus regius	0±0	0±0	0±0	0±0	0.79±0.54	0±0	0.73±0.51	0±0	2.87±1.56	1.29±0.49	0±0	0±0
Callionymidae	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	3.75±1.5	0±0	0±0	0±0
Chelon ramada	0±0	0±0	0.16±0.16	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.15±0.15
Dicentrarchus labrax	1.45±0.6	6.17±1.87	0.34±0.24	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Dicentrarchus punctatus	0±0	0±0	0.15±0.15	0±0	0±0	0±0	0±0	0±0	0±0	0.53±0.44	0±0	0±0
Dicologoglossa cuneata	114.03±32.44	0±0	23.61±8.01	1.3±0.63	1.2±0.54	0.62±0.35	1.2±0.8	0.09±0.09	0.09±0.09	0.1±0.1	2.45±0.69	0.35±0.15
Diplodus annularis	0.07±0.07	0±0	0.17±0.17	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Diplodus bellottii	0±0	0±0	23.36±8.49	2.88±1.05	5.65±2.3	1.72±0.82	0±0	0±0	0±0	0±0	0±0	0±0
Diplodus sargus	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.1±0.1	0±0	0±0	0±0
Diplodus spp.	0±0	0±0	1.86±1.17	0±0	0±0	0±0	0.41±0.23	0±0	0.1±0.1	0±0	0±0	0±0
Diplodus vulgaris	0.08±0.08	0±0	2.29±1.21	23.58±6.46	0.61±0.29	0±0	0±0	0±0	0±0	0.73±0.37	0.11±0.11	0.15±0.1
Engraulis encrasicolus	5.58±2.28	11.53±10.06	0.16±0.16	23.55±6.4	54.25±9.55	25.86±7.02	42±16.75	12.3±9.74	29.94±15.48	59.73±10.35	35.11±9.25	4.05±0.68
Gobius paganellus	0±0	0±0	1.13±0.98	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Halobatrachus didactylus	0±0	0±0	0.19±0.19	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Hippocampus hippocampus	0±0	0±0	0±0	0±0	0±0	0.18±0.18	0.1±0.1	0.2±0.14	0.26±0.15	0.29±0.2	0±0	0±0
Labridae	0±0	0±0	0.32±0.22	0±0	0±0	0.32±0.22	0±0	0±0	0.14±0.14	0.49±0.38	0±0	0±0
Lipophrys pholis	0±0	0±0	0±0	0±0	0.4±0.3	0.63±0.32	2.09±0.94	0.53±0.25	0.18±0.13	0.1±0.1	0±0	0±0
Pegusa nasuta	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Pomadasys incisus	0.15±0.1	0±0	0±0	0±0	0±0	1.5±0.6	0.09±0.09	0±0	7.99±4.11	0±0	0.09±0.09	0.14±0.1
Pomatoschistus spp.	0±0	6.14±3.5	28.7±17.06	0±0	0±0	0.29±0.2	0.14±0.14	0±0	0±0	0±0	0±0	0.76±0.76
Sardina pilchardus	5.61±1.85	3.16±3.16	36.07±6.69	21.74±4.7	0.86±0.31	0±0	0.07±0.07	0±0	0.09±0.09	0.12±0.12	7.36±5.09	5.39±1.3
Solea senegalensis	5.11±1.96	3.06±0.05	1.6±0.96	0±0	0.13±0.13	0±0	0.1±0.1	0±0	0.8±0.45	0±0	0.09±0.09	0.08±0.08
Sparus aurata	0±0	1.99±1.99	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Symphodus sp.	0±0	0±0	0±0	0.24±0.13	0.48±0.23	0.1±0.1	0.27±0.15	0±0	0.09±0.09	0.2±0.14	0±0	0±0
Syngnathus acus	0±0	0±0	0±0	0.08±0.08	0±0	0±0	0±0	0.09±0.09	0±0	0±0	0±0	0±0
Syngnathus typhle	0±0	0±0	0±0	0±0	0.13±0.13	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Trachurus trachurus	0±0	0±0	0±0	0±0	0±0	0.41±0.23	0.17±0.12	0.13±0.13	0.19±0.13	0±0	0±0	0±0
Umbrina cirrosa	0±0	0±0	0±0	0±0	0±0	0.75±0.32	0±0	0.09±0.09	0.09±0.09	0±0	0±0	0±0
Unknown	0±0	0±0	0±0	0±0	0±0	0±0	0.16±0.11	0±0	1.21±0.67	0±0	0±0	0±0
Total	**155.57±33.89**	**691.03±269.9**	**161.03±29.17**	**80.08±13.7**	**76.27±11.32**	**32.59±7.24**	**49.36±18.63**	**13.42±9.71**	**47.88±16.75**	**89.22±15.41**	**84.66±13.37**	**41.04±7.19**

Table S.1.2. Monthly average (mean±SE) densities (ind./1000m^3) of early life stages of fish in the inner zone of the Guadalquivir estuary during October 2015 to September 2018.

	INNER ZONE											
	January	**February**	**March**	**April**	**May**	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Ammodytidae	0±0	0±0	0.36±0.15	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Anguilla anguilla	0.89±0.29	0.6±0.3	0.63±0.21	1.54±0.56	0.69±0.29	0.59±0.19	0.07±0.07	0.07±0.07	0±0	2.66±0.87	3.75±1.17	1.28±0.36
Aphia minuta	7.63±3.44	2.44±1.2	12.6±4.77	1.43±1.13	0.17±0.17	0.22±0.12	0±0	0±0	0±0	2.65±1.11	5.6±2.8	3.56±0.65
Argyrosomus regius	0.18±0.13	0.29±0.21	0.22±0.13	0.22±0.22	20.33±3.47	9.09±1.88	10.27±2.81	5.23±1.12	15.09±5.27	17.56±4.85	1.42±0.39	0.66±0.32
Atherina boyeri	0±0	0±0	0.07±0.07	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Belone belone	0±0	0±0	0.07±0.07	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Carassius auratus	0±0	0±0	0±0	0±0	0±0	0±0	0.33±0.33	0±0	0±0	0±0	0±0	0±0
Chelon aurata	0.07±0.07	0.21±0.15	0.06±0.06	0.15±0.15	0±0	0.14±0.1	0.12±0.09	0±0	0±0	1.75±1.5	0±0	0.72±0.25
Chelon ramada	1.97±0.73	2.02±1.07	0.19±0.11	0.52±0.36	0.23±0.17	0±0	0.07±0.07	0±0	0±0	0.09±0.09	3.23±0.88	3±0.85
Chelon saliens	0.18±0.1	0±0	0±0	0±0	0±0	0±0	0±0	0.2±0.2	0.24±0.18	0±0	0±0	0.05±0.05
Dicentrarchus labrax	0.26±0.13	0.47±0.22	48.74±15.6	96.56±41.19	3.95±1.61	2.36±0.87	0.53±0.33	1.22±0.84	1.89±0.66	0±0	0±0	0.07±0.07
Dicentrarchus punctatus	0.17±0.09	0±0	1.65±0.59	21.76±10.25	9.57±3.45	1.91±0.66	0.24±0.14	0.08±0.08	7.4±2.37	8.36±2.26	6.05±1.62	0.48±0.24
Dicologoglossa cuneata	1.3±0.49	0.49±0.31	0.07±0.07	0.79±0.49	0.64±0.39	0±0	0±0	0±0	0.07±0.07	0±0	0±0	0±0
Diplodus annularis	0±0	0.1±0.1	0.15±0.11	0.99±0.4	0±0	0.11±0.11	0.35±0.15	0±0	0±0	0±0	0±0	0±0
Diplodus bellottii	0±0	0±0	5.05±1.57	6.96±2.29	1.21±0.78	0±0	0.21±0.15	0±0	0±0	0±0	0±0	0±0
Diplodus spp.	0±0	0.21±0.15	2.37±1.3	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Diplodus vulgaris	0.14±0.1	3.36±0.82	1.17±0.31	17.37±4.1	4.12±1.03	0.07±0.07	0±0	0±0	0±0	0.09±0.09	0±0	0.12±0.08
Engraulis encrasicolus	24.11±6	18.62±7.06	22.88±4.94	48.46±19.17	1057.55±327.83	557.9±109.04	474.38±151.13	631.79±165.33	222.38±67.8	348.78±83.87	142.09±31.05	144.3±38.94
Gambusia holbrooki	0±0	0±0	0±0	0±0	0±0	0.07±0.07	0.08±0.08	0±0	0±0	0.4±0.2	0±0	0±0
Gobius paganellus	0.14±0.09	0±0	17.17±7.12	3.64±1.78	0±0	0±0	0±0	0±0	1.49±0.54	2.31±1.06	0±0	0.06±0.06
Halobatrachus didactylus	0±0	0±0	0±0	0±0	0±0	0.1±0.1	0.06±0.06	0±0	0±0	0±0	0±0	0.14±0.14
Hippocampus hippocampus	0±0	0±0	0±0	0.08±0.08	0.09±0.09	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Labridae	0±0	0±0	0±0	0±0	0.15±0.15	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Lipophrys pholis	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.07±0.07	0±0	0±0	0±0
Pegusa nasuta	0.35±0.21	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Pomadasys incisus	0.27±0.14	11.23±4.03	0±0	0.09±0.09	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Pomatomus saltatrix	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.12±0.12	0±0	0±0
Pomatoschistus spp.	59.07±8.58	130.6±10.59	821.98±194.95	1071.1±265.69	845.38±172.96	497.59±124.29	502.5±96.28	186.62±39.59	228.71±49.51	130.7±18.71	179.04±27.26	101.12±16.54
Sardina pilchardus	14.69±3.89	18.71±7.98	26.84±7.23	71.51±18.49	24.65±5.06	0.07±0.07	0.06±0.06	0±0	0±0	0.2±0.14	8.45±3.79	8.11±2.03
Scophthalmus rhombus	0±0	0±0	0.07±0.07	0.09±0.09	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Solea senegalensis	30.15±12.63	6.28±1.26	7.2±2.69	13.31±3.44	0.14±0.14	0.14±0.14	0±0	0±0	0±0	0.34±0.18	4.22±1.5	0.99±0.31
Solea vulgaris	0.09±0.09	0±0	1.26±0.51	1.27±0.45	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Sparus aurata	2.53±0.55	21.95±3.7	1.97±0.52	0±0	0±0	0.07±0.07	0±0	0±0	0±0	0±0	0±0	0.06±0.06
Symphodus sp.	0±0	0±0	0±0	0±0	0±0	0±0	0.06±0.06	0±0	0.07±0.07	0±0	0±0	0±0
Syngnathus acus	0.05±0.05	0.36±0.26	0.46±0.22	1.74±0.64	0.06±0.06	0.41±0.21	0.12±0.09	0±0	0.07±0.07	0±0	0±0	0.11±0.08
Syngnathus typhle	0±0	0±0	0.23±0.17	0±0	0.44±0.23	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Umbrina cirrosa	0.07±0.07	0±0	0±0	0±0	0±0	0.1±0.1	0.06±0.06	0.2±0.15	0.7±0.34	0.75±0.42	0±0	0.32±0.15
Total	**144.32±20.45**	**217.94±20.54**	**973.47±206.11**	**1359.59±267.06**	**1969.38±334.36**	**1070.93±154.22**	**989.53±176.49**	**825.41±171.11**	**478.18±99.29**	**516.75±88.94**	**353.85±41.98**	**265.16±43.89**

2. MECHANISMS FOR HORIZONTAL MOVEMENTS IN A WELL-MIXED ESTUARY FOR BENTHIC AND PELAGIC FISH SPECIES

Abstract

The mechanisms that control the ingress and maintenance of larvae and juvenile into nursery grounds as estuaries is still a fundamental question in oceanography. In this study, we analysed the strategies that some species can use for horizontal movements inside of the estuary. For that, we tested the selective tidal-stream transport hypothesis in combination with vertical and lateral migrations in the fish species more abundant in the Guadalquivir: the gobies (*Pomatoschistus* spp.) and the anchovy (*Engraulis encrasicolus*). We took plankton samples in surface and bottom simultaneously during the ebb and flood tides in three different zones of a section (both sides and the middle channel) of the Guadalquivir estuary along three different cruises with spring tide condition in the summer season. In addition, multiple physiochemical variables (temperature, turbidity, dissolved oxygen, pH, salinity, chlorophyll, wind and water current velocities) were related to determine the different cues that they use in their strategies. The results showed benthic distribution for the gobies, which used the bottom and the flood tide for both sides to move upstream in the estuary, being the temperature and/or dissolved oxygen their main cues. The anchovy distribution was the opposite, with higher abundance in the surface layer and especially during the ebb tide, showing a downstream advection. They were influenced by the salinity variable. Still, the presence of bigger individuals of anchovy in the lateral/shallow zones suggested a behavioural ontogeny. This, together with the push of surface water layers upstream by the wind observed could help its retention. This comparation also enhanced the knowledge in the distribution of two common and abundant species that use estuaries as anchovies and gobies.

Resumen

Los mecanismos que controlan la entrada y el mantenimiento de larvas y juveniles en los criaderos como estuarios siguen siendo una cuestión fundamental en la oceanografía. En este estudio analizamos las estrategias que pueden usar algunas especies para moverse horizontalmente dentro del estuario. Para ello, testamos la hipótesis del transporte selectivo de corrientes de marea en combinación con migraciones verticales y laterales en las especies de peces más abundantes del Guadalquivir: los gobios (*Pomatoschistus* spp.) y el boquerón *(Engraulis encrasicolus*). Se cogieron muestras de plancton en superficie y fondo simultáneamente durante las mareas de llenado y vaciado en tres zonas diferentes de una sección transversal (ambos lados y el canal central) del estuario del Guadalquivir a lo largo de tres cruceros diferentes en condición de marea viva durante la estación de verano. Además, se relacionaron múltiples variables fisicoquímicas (temperatura, turbidez, oxígeno disuelto, pH, salinidad, clorofila, velocidades del viento y de las corrientes de agua) para determinar las diferentes señales que utilizan en sus estrategias. Los resultados mostraron una distribución bentónica para los gobios, que utilizaron el fondo y la marea llenante por ambos laterales para moverse río arriba en el estuario, siendo la temperatura y / o el oxígeno disuelto sus principales señales. La distribución del boquerón fue la opuesta, con mayor abundancia en la superficie y durante la marea vaciante, mostrando una advección hacia aguas abajo. La salinidad pareció ser la variable que más influyó en su estrategia. Aun así, la presencia de individuos de boquerón de mayor tamaño en las zonas laterales/poco profundas sugirieron un comportamiento influido por el desarrollo ontogénico. Esto, junto con el empuje de las capas superficiales hacia río arriba por el viento observado, podría ayudar a su retención en del estuario. Esta comparación también mejoró el conocimiento en la distribución de dos especies comunes y abundantes que utilizan los estuarios como son los boquerones/anchoas y los gobios.

1. Introduction

A fundamental question in oceanography concerns those factors that control the input of eggs, larvae and juvenile to nursery grounds (Robins et al., 2013). The better understanding of connectivity mechanisms between spawning areas and nurseries is valuable because the supply of early stages of life is often an essential determinant in adult population sizes (Cowen and Sponaugle, 2009). The meroplankton drift along the water currents is a critical phase in the development of many species to get a successful recruitment (Cowen et al., 2006). Many species of meroplankton, especially larvae of marine migrant fishes, concentrate in estuaries (Pineda et al., 2007). These ecosystems generally provide high food availability and good predator refuge for ichthyoplankton (Elliot and Hemingway, 2002). Processes such as pelagic larval delivery into estuaries depend on different biophysical interactions between regional hydrodynamic, offspring period, amount of larval supply and natural behaviour of every individual (Potter et al., 2015).

On the other hand, the distribution of these organisms in estuaries is shaped by multiple environmental conditions, being the hydrodynamic regime a main factor (González-Ortegón et al., 2012). The freshwater input, together with periodic tidal flushing, generate strong water currents which could be considered extreme environment for fish larvae (Teodósio and Garel, 2015). Thus, horizontal transport into and up-estuaries is problematic for fish larvae because estuaries usually have a net flow of water to the ocean and current velocities frequently exceed larval swimming speeds (Teodósio et al., 2016). Attention has particularly focused on how larval behaviors, especially depth preferences and periodic vertical movements, may promote up-estuary advection or at least retention in the estuary despite mean seaward flow (Forward and Tankersley, 2001).

According to De Wolf (1973), larvae could be retained without requiring an active swimming by mechanical transport in combination with asymmetrical tides. In that sense, Creutzberg (1961) suggested the Selective Tidal Stream Transport (STST) hypothesis, in which larvae move up in the water column during flood tides and down in the water column during ebb tides. A combination of physical variables characterized by directional gradients, for example water temperature, salinity, turbidity and hydrostatic pressure, could act as synchronizing cues in inducing tidal rhythms in the larvae movements (Boehlert and Mundy, 1988). Studies have discussed this behavior for different species, such as fish (Burke et al., 1998; Hench et al., 2004) and invertebrate (De Vries et al., 1994; Hench et al., 2004) larvae, and even how ontogeny influence the perception of these cues (Teodósio et al., 2016). Most researches have tested the STST in stratified or partially stratified estuaries, where there are notably differences between the upper and lower water layers. However, in mixed estuaries, there are weak differences between water layers (Fortier and Leggett, 1982). Thus, fish larvae may need to adopt alternative strategies for successful ingress into estuarine nursery grounds. Other mechanisms have been proposed: wind forcing, residual bottom inflow and passive ingress via tidal processes (Morgan et al., 2011; Roman and Boicourt, 1999; Simons et al., 2006). Also, lateral movements have been considered as another strategy (Forward Jr et al., 1999) because along river flow can have pronounced lateral variability, depending on bathymetry and water mixing (Valle-Levinson and Lwiza, 1995).

The Guadalquivir is a well-mixed mesotidal estuary (Vanney, 1970) in the Gulf of Cadiz (Southwestern Iberian Peninsula). It has suffered an extensive anthropic alteration during the recent history with construction of numerous dams along the river basin, by reducing the natural course with multiple river cuts to facilitate vessel navigation, decreasing of marshes for agriculture fields, isolating of its natural marshes from the main channel and the consequent reduction of the tidal flooding, etc. (Llope, 2017; Ruiz et al., 2015). Hence, the estuary is currently composed of the main river channel with a few tidal creeks, but with a significant reduction of the original intertidal area. Still, this estuary has been considered the most productive in the region (Miró et al., 2020).

The early life stages of fish community in Guadalquivir estuary have been widely studied, being the dry-warm season the period with higher densities (Drake et al., 2007). The main species captured are the anchovy *Engraulis encrasicolus* (Linnaeus, 1758) and the gobies *Pomatoschistus* spp. [mainly Pomatoschistus *minutus* (Pallas, 1770) and *Pomatoschistus microps* (Kroyer, 1838)] (Baldó and Drake, 2002). The anchovy is a marine migrant fish with its larval maximum recruitment period from May to November in Guadalquivir estuary (Drake et al., 2007), and their spawning area have been reported offshore (Baldó et al., 2006). It is a commercial species important in the fishery sector of the region (Ruiz et al., 2017). On the other hand, the gobies are resident estuarine species typical in many estuaries in temperate zone. Their seaward migration during early spring for reproduction has been documented by several authors (Guelinckx et al., 2008; Pampoulie et al., 1999), but not in this estuary.

Although STST is widely accepted as the mechanism by which larvae move into estuaries, other mechanisms have been proposed, but are rarely evaluated in combination with other factors. This study aims to analyse the movement strategies and the use of different zones across the transverse section of the estuary of two kind of fish species, the pelagic *Engraulis* *encrasicolus* and the benthic *Pomatoschistus* spp., as well as to elucidate the possible environmental cues which could lead the individuals of these species to follow their strategy. For that, we have tested the STST hypothesis combined with lateral migrations, water physiochemical characteristics and wind effects from an Eulerian approach (e. g. over time at a fixed estuary section in space).

2. Material and Methods

2.1. Study area

The Guadalquivir estuary is located in South-West of Iberian Peninsula, a warm temperate region with Mediterranean climate conditions, and its waters flow to the Gulf of Cadiz (Atlantic Ocean; Figure 1). The estuary extends 110 km inland from its mouth. It is well-mixed mesotidal system with 3.5 m amplitude range (spring tides) in the river mouth (Díez-Minguito et al., 2012). The estuary is convergent with widths of 800 m near the mouth and 150 m at the head (Díez-Minguito et al., 2012). The morphology of the estuary is a single channel isolated from surrounding natural areas in most of its length, with a main navigable channel of 7.1 m average depth (Ruiz et al., 2015).

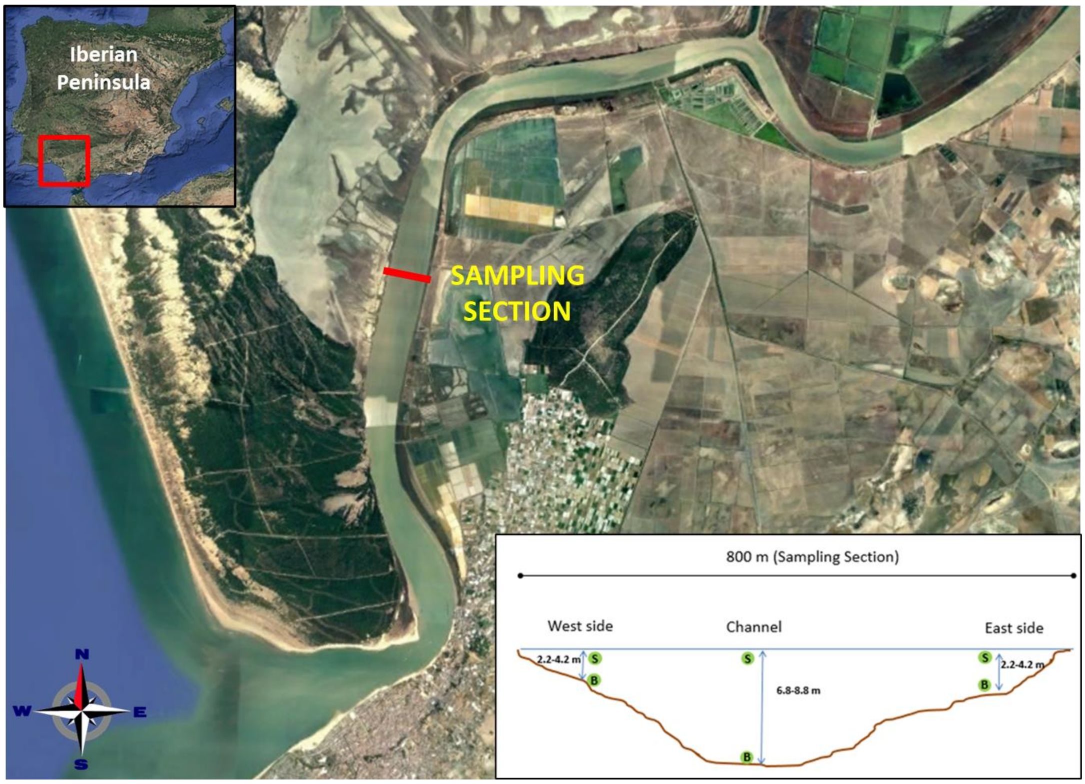

Figure 1. Sampling station locality and scheme of cross section with the zones and depths (S: surface; B: bottom). Bottom depth and across distance are plotted with a proportion of 1:20 approximately.

2.2. Biological sampling

The sampling was carried out during the summer of 2017, in three different cruises during spring tides. Every sampling cruise was done in two consecutive days during the daylight (12th – 13th June, 11th – 12th July and 9th – 10th August 2017). Samples were collected against main flow direction in a passive haul with an anchored boat along the ebb and flood tides. Three different zones were selected across the section of the estuary, 13 km upstream the river mouth: one in every side of the estuary section with a depth range of 2.2 – 4.2 m, over the shoals, and a third zone in the middle of the channel with 6.8 – 8.8 m (Figure 1). The surface and the bottom were simultaneously sampled to determine whether there were any vertical migration patterns. A plankton net of 60 cm of diameter was used for subsurface sampling, and an epibenthic sledge trawl of 43 x 60 cm for the bottom, both nets with 500 µm of mesh size. Each net was equipped with a flowmeter (2030R General Oceanics) and the volume filtered per tow was 90 ± 27 m^3. The trawl deposited in the bottom had a strangulation mechanism, which was released once reaching the bottom, to keep the net closed during the up and down movement of the tow. At these two depths (surface-bottom), four samples were collected in every zone at different times along the flood tide and four along the ebb tide; this was replicated in every cruise. Samples were fixed in ethanol 70% and early stages of *E. encrasicolus* and *Pomatoschistus* spp. were sorted. Also, in the case of *E. encrasicolus*, due to the presence of individuals in different life stages, their total length was measured using an image-analysis system, where individuals were scanned and measured with the software Image J. We did not find differences in sizes and life stages of *Pomatoschistus* spp., and we did not perform size analysis with these species.

2.3. Physiochemical data collection

Simultaneously to every biological sampling, physiochemical and current velocity profiles of the whole water column were recorded in every zone. Physiochemical data were taken with a multiprobe with depth, temperature, salinity, turbidity, dissolved oxygen (DO), chlorophyll-a (Chla) and pH sensors (Eureka Manta2). Current velocity was measured with an Acoustic Doppler Current Profiler (ADCP 1 MHz – Aquadopp Profiler Nortek) from the surface. The ADCP was used in surface-tracking mode, and velocity profiles, per triplicate, were obtained as ensembles averaged over 120 s, in cells of 0.5 m thickness from surface to bottom, during every fishing tow.

Wind speed and direction data were recorded every five minutes in the meteorological station Vetalengua (6°22' 55.96381"W – 36°55'21.93697"N) and supported by the Spanish National Research Council (CSIC-EBD-ICTS).

2.4. Data analysis

Physiochemical variables of every profile (temperature, salinity, turbidity, DO, Chla, pH and magnitude velocity current) in each sample tow were averaged in a single value over the first meter, for surface samples, and the last meter, for the bottom samples. Results of all environmental variables were plotted with boxplot grouped by depth, tide and zone using the package 'ggplot2' of R 3.5.2 software (R Development Core Team, 2018).

Physiochemical variables were organized in a variable/sample matrix and a Euclidian distance similarity matrix was calculated on normalised data. The differences in the multivariate structure of environmental variables were analysed in a distance-based permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001; Mcardle and Anderson, 2001). When the number of total possible permutations to obtain the p-values were low, we used the estimate obtained by Monte Carlo sampling (Anderson and Robinson, 2003). The experimental design included 3 crossed fixed factors: depth (2 levels, "Surface and Bottom"), tide (2 levels, "Flood and Ebb") and zone (3 levels, "West side, Channel and East side"); and 2 random factors: cruise (3 levels, "June, July and August"), orthogonal to the three previous factors, and sampling time (4 levels, "1-4") nested within tide, zone and cruise. This last factor is a random block used to consider the simultaneous samples taken in surface and bottom; in this sense, this is a "randomised block design" and it was analysed following the general consensus of excluding the interaction term between "Depth" and "Sampling time" from the analysis (Anderson et al., 2008). When appropriate, significant terms with more than two levels were analysed using pairwise comparison with the PERMANOVA test. In addition, patterns on environmental variables were graphically represented using a plot of the two first principal axes of a Principal Component Analysis (PCA). Multivariate analyses were performed using the software PRIMER v6.1.11 and PERMANOVA+ v1.0.1 statistical package (Clarke and Gorley, 2006).

Data on wind speed and direction of every sampling day were summarized during each tide period using a wind rose plot with the package 'openair' in R software (R Core Team, 2018).

To investigate what factors drive the strategy used by both species to move across the estuary section, two generalised linear mixed-effects models (GLMM) were run with counts of *Engraulis encrasicolus* and *Pomatoschistus* spp. as response variables. The distribution most adequate for these data was the negative binomial, and the model was fitted with the function 'glmer.nb' of the package 'Lme4' (Bates et al., 2015). Design of categorical factors used was the same as multivariate environmental analysis (fixed factors: depth, tide, zone; random factors: cruise, sample time). Water physiochemical factors (temperature, salinity, turbidity, DO, Chla, pH and current velocity) were used as explanatory variables as well, in addition to the filtered volume as an offset variable.

The model selection process for determining the best fitting GLMM was to first create a global model with all predictor variables included (Bates et al., 2015). Previously, one of the variables which showed a similar or complementary pattern in PCA were excluded [dissolved oxygen (negatively correlated with temperature) and chlorophyll (positively correlated with turbidity)] from the analysis to avoid collinearity. Multicollinearity of the selected variables was further analysed using variance inflation factors (VIF) with the function 'vif' from the package 'car' (Fox and Weisberg, 2011); all variables from the global model had a VIF < 3. We performed information theoretic model selection based on Akaike's information criterion (AIC; Akaike 1974) and Akaike weights (Burnham and Anderson, 2002) using the function 'dredge' of the 'MuMIn' package (Barton, 2018). The model with the highest adjusted Akaike weight was considered the best-fit model used for the analysis (Burnham and Anderson, 2002). Finally, significant categorical terms in the best-fit model with more than 2 levels, were analysed using the Tukey correction for pairwise comparisons comparison with the package 'emmeans' (Lenth, 2018).

Total lengths of anchovies were compared to test if individuals with different sizes used distinct strategies. For this analysis, linear mixed-effects models (LMM) were run using the function 'lmer' of the package 'Lme4' with a Gaussian distribution. In this case, global model had three crossed fixed factors (depth, tide, zone) and one random factor (cruise). The model selection process and *post hoc* analysis of the best-fit model was similar as explained above.

3. Results

3.1. Environmental analysis

PERMANOVA showed different physicochemical conditions of samples in the estuary section between depths and tides as well as zones and tides along cruises (Table 1). Pair-wise analysis of significant interactions showed differences between surface and bottom in both tidal conditions, and also between zones during the flood, being the East and West side distinct to the Channel, although only the West side was significant (Table 2).

PCA analysis showed a clear pattern between tide cycles: higher water current velocities and DO concentration during the ebb tides; and higher temperature and pH during the flood tides (Figure 2). Also, high levels of turbidity and chlorophyll were associated to both sides. On the other hand, comparisons between depths showed a slight vertical stratification in some environmental variables (Figure S.1) with higher relative values of temperature and velocity current in the surface layers; while the bottom depth showed higher values in turbidity, salinity and chlorophyll.

Table 1. PERMANOVA results of the Euclidian similarity matrix based on environmental variables.

Source	df	SS	MS	Pseudo-F	P(perm)	Perms
Depth	1	60.041	60.041	21.206	0.0876	192
Tide	1	108.87	108.87	5.4735	**0.0189**	9952
Zone	2	22.847	11.424	1.2642	0.3214	9962
Cruise	2	209.98	104.99	19.559	**0.0001**	9930
DepthxTide	1	22.221	22.221	11.275	**0.0301**	9305
DepthxZone	2	1.8788	0.93939	0.73702	0.5997	9963
DepthxCruise	2	5.6739	2.837	2.5216	**0.0208**	9927
TidexZone	2	44.411	22.206	4.7071	**0.0086**	9943
TidexCruise	2	39.877	19.938	3.7144	**0.0027**	9932
ZonexCruise	4	36.2	9.05	1.686	0.0729	9920
DepthxTidexZone	2	7.7155	3.8578	2.5679	0.1281	9953
DepthxTidexCruise	2	3.9475	1.9738	1.7544	0.1018	9934
DepthxZonexCruise	4	5.1005	1.2751	1.1334	0.3214	9932
TidexZonexCruise	4	18.861	4.7151	0.8784	0.5533	9904
Station(ZonexTidexCruise)	48	257.66	5.3678	4.7711	**0.0001**	9824
DepthxTidexZonexCruise	4	6.0147	1.5037	1.3365	0.2	9921
Res	48	54.003	1.1251			
Total	131	917				

Table 2. Pair-wise analysis of significant interactions in PERMANOVA results of environmental variables. * p estimation obtained by Monte Carlo.

Term 'TidexDepth'	**Ebb**			**Flood**		
Groups	t	P(perm)	perms	t	P(perm)	perms
Surface-Bottom	7.0251	**0.0002**	9942	3.3084	**0.0023**	9957

Term 'TidexZone'	**Ebb**			**Flood**		
Groups	t	P(perm)	perms	t	P(perm)	perms
East, West side	1.5361	0.1245	9952	1.1316	0.3481	9958
East side, Channel	1.308	0.2555	9957	2.151	0.0533	9961
West side, Channel	0.87768	0.5824	9950	2.182	**0.0441***	38

In addition, wind data (Figure S.2) showed a dominant direction from West-Southwest to East-Northeast in most of the flood tides, except for the last sampling day. However, no clear patterns were found during the ebb tides, where some sampling days had a direction from East-Northeast to West-Southwest and other days from South-Southwest to North-Northeast.

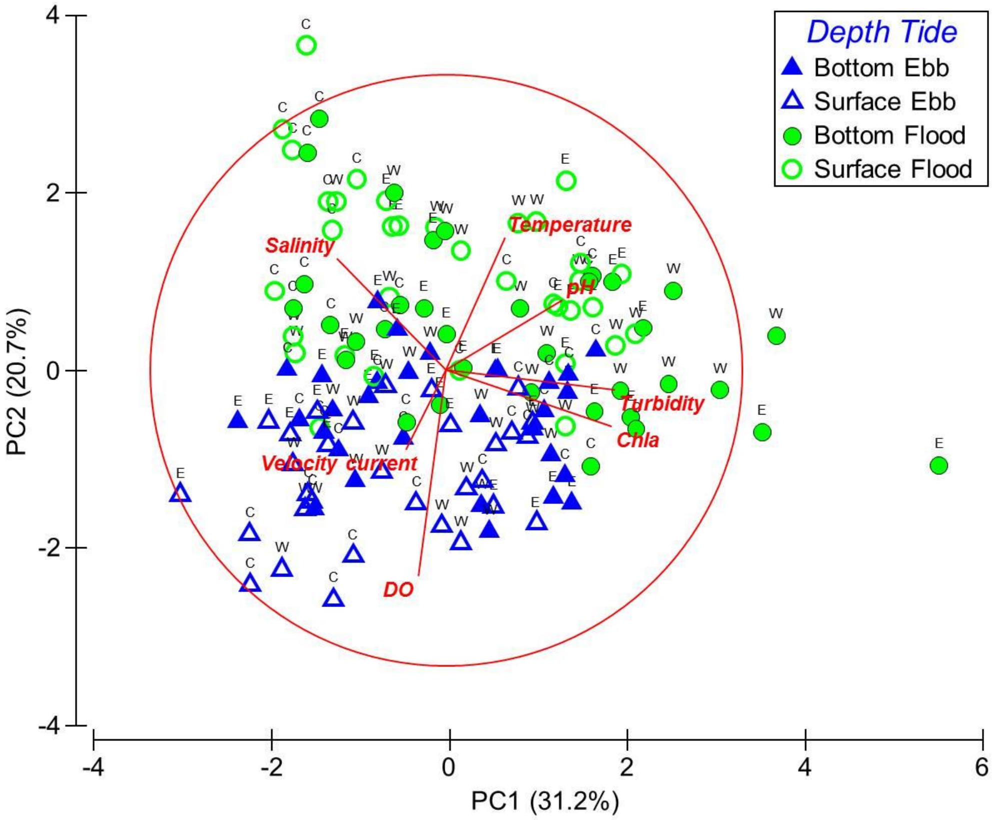

Figure 2. Plot of the 2 first principal components of a PCA with environmental variables (DO: dissolved oxygen; Chla: chlorophyll-a; W: west side; C: channel; E: east side).

3.2. Biological analysis

A total of 144 tows were conducted, 48 every cruise. Several early life stages of fishes were caught, in addition to *Engraulis encrasicolus* and *Pomatoschistus* spp. (*P. minutus* and *P. microps*), like *Umbrina cirrosa, Argirosomus regius, Dicentrarchus punctatus, Solea solea, Halobatrachus didactylus, Hippocampus hippocampus, Diplodus sp., Stromateus fiatola, Mullus surmuletus* and *Cyprinus carpio*. Anchovy and gobies were the most abundant and consistently present with a total of 11.676 (79%) and 2.695 (18%) individuals sampled respectively.

Pomatoschistus spp.

Figure 3A shows the densities (ind/1000 m^3; mean ± SE) of gobies *Pomatoschistus* complex by tide, depth and zone averaged along all cruises. The best-fit model (Table S.1) showed significant differences with depth: gobies were ever mainly in the bottom, being very scarce in the surface. In fact, in the surface, due to the low general abundances, there were not any clear pattern and not any significant differences between tides or zones. In the bottom, instead, density differences were found between ebb and flood tide in both sides. Also, a different global pattern was found between flood and ebb: during floods, abundances were significantly higher in both sides compared with the Channel; during ebbs, there was a gradient of densities, increasing from West to East, being the density in the West side significantly lower than the rest of zones. The analysis also showed a slightly positive relationship between temperature and the density of gobies (Table S.1).

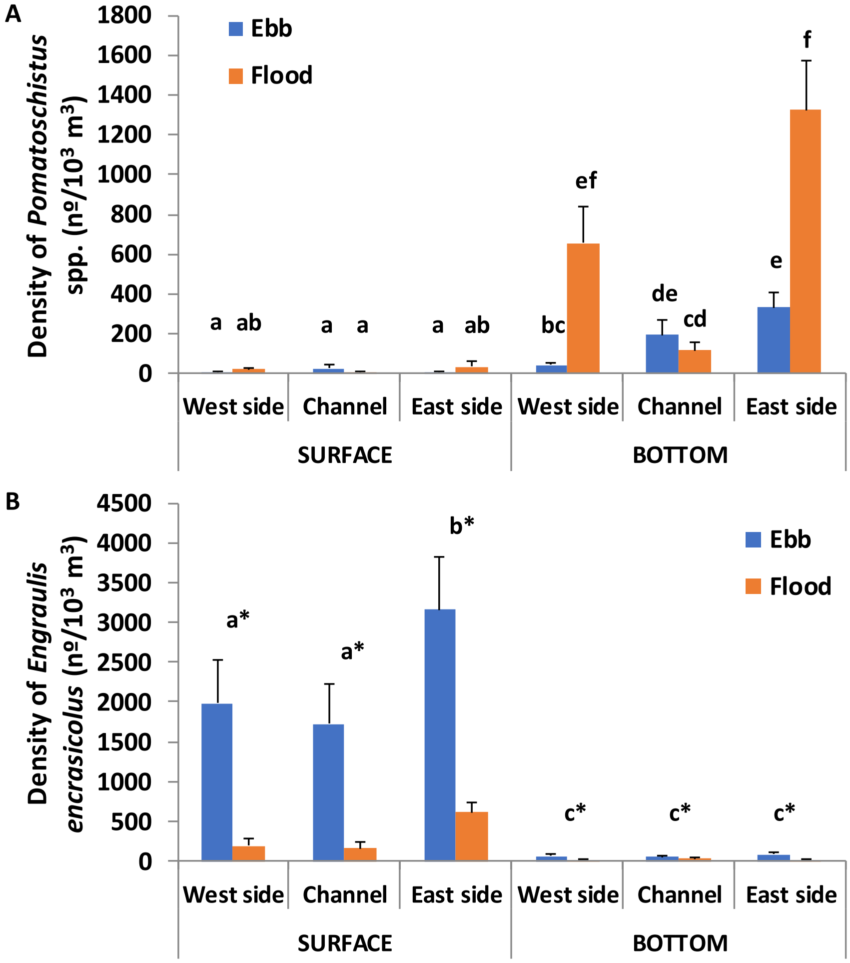

Figure 3. Density (mean ± SE) of *Pomatoschistus* spp. (A) and *Engraulis encrasicolus* (B) in different stations along all cruises. Different letters indicate significant differences ($p < 0.05$) among stations from *post hoc* analysis of GLMM. Asterisks (*) indicate significant differences ($p < 0.05$) among tide factor in the same zone and depth of *Engraulis encrasicolus*.

Engraulis encrasicolus

Figure 3B shows the densities (ind/1000 m^3; mean ± SE) of anchovy *Engraulis encrasicolus* by depth, tide and zone averaged along all cruises. The best-fit model (Table S.2) showed significant differences with depth: anchovies were mainly in the surface, being very scarce in the bottom, showing a pattern of use of the space virtually inverse to the gobies. In fact, similar to what we found in the gobies but in the opposite depth (in the bottom), there were not any clear pattern and not significant differences were found between zones, although small but significant differences were found between tides. In the surface, instead, significant differences were found with tides and zones: there were higher abundances during the ebb compared with flood and there were higher densities in the East side than in the rest of the zones.

The analysis also showed that salinity was the only environmental variable with significant influence in anchovy distribution along samples (Table S.2). A negative relationship was found, being the low salinity range (19-27 PSU) where higher anchovies were caught, mostly in the surface samples.

Best-fit LMM (Table S.3) showed that mean total length of anchovies was different between tides, zones and depths (Figure 4). Flood condition showed bigger sizes compared with ebb tide; during the flood, bigger sizes were found in both sides (East and West) compared with the Channel; during the ebb tide in the surface, East side and Channel mean length were slightly but significantly higher than West side.

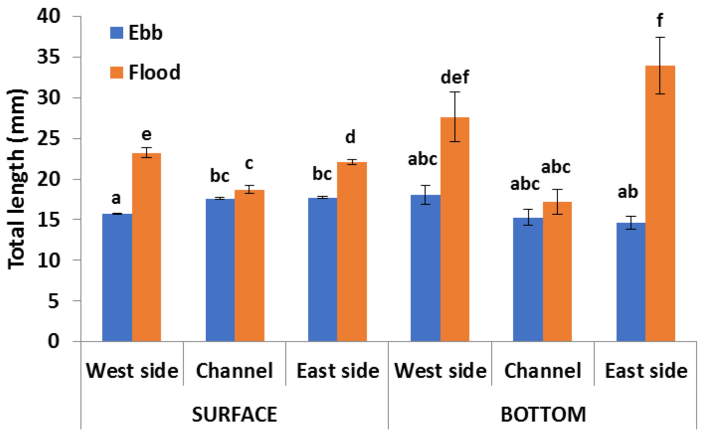

Figure 4. Total length (mean ± SE) of early life stages of *Engraulis encrasicolus* in different stations along all cruises. Different letters indicate significant differences (p<0.05) among stations from *post hoc* analysis of LMM.

4. Discussion

Different strategies were found in the anchovy and the gobies to move within the estuary, and the differences found in physicochemical variables across the estuary section and along the tidal cycle could work as cues to lead these strategies.

4.1. Environmental conditions

Although, since a hydrological point of view, the Guadalquivir estuary is defined as a well-mixed one (Vanney, 1970), since a biological point of view, the differences found in physicochemical variables between surface and bottom or among different zones, could be detected by fish larvae guiding them through different pathways into the estuary. The estuary section sampled was located in the diffusion part of the tide propagation process with a strong tidal dominated condition (Díez-Minguito et al., 2012), which could provoke periodic and slight stratifications (Díez-Minguito et al., 2013). Tidal asymmetry has been previously showed in the middle channel, being a flood dominated estuary (Díez-Minguito et al., 2012). However, in our study, higher maximum current velocities were measured during the ebb tide in the surface than the flood tide samples, mainly in the channel zone, probably for the proximity of the samples section to the estuary inlet. DO and temperature were, as expected, negatively related, and played a major role in the differences found, especially between tides. In addition to the tidal asymmetry, irregularities in the section bathymetry could affect the water circulation (Valle-Levinson and Lwiza, 1995). According to Scully and Friedrichs (2007), the reduced friction in the channel at the end of ebb delays the beginning of the flood tide, increasing the duration of ebb. Conversely, over the shoal zones where stratification is more inhibited by tidal mixing, there is greater friction and the transition from ebb to flood occurs more quickly. This phenomenon generates a residual current seaward over the channel and landward over the shoals (Scully and Friedrichs, 2007). Another effect of the different current velocities was a wider salinity range in the channel than in the sides due to advection of the salinity front that was not driven at the same speed at different lateral locations, creating lateral salinity gradients (Nunes and Simpson, 1985). On the other hand, the greater friction in the shoals would enhance both sediment and microphytobenthos resuspension, in agreement with the higher turbidity and chlorophyll concentration observed in these zones (Díez-Minguito and de Swart, 2020; Miró et al., 2020).

4.2. Pomatoschistus spp.

The gobies *Pomatoschistus* spp. (*P. minutus* and *P. microps*) are benthic species which showed a clear bottom behaviour, remaining deep in the water column at all times of the tide cycle as other species described [e.g. *Micropogonias undulates* by Boehlert and Mundy (1988)]. A strategy of an alternative use of surface and bottom layers to compensate the net downstream transport or to reach upper zones of the estuary, as proposed by the STST hypothesis, seems not to be applicable here. They must be using an alternative mechanism. The current velocities measures in the bottom were lower enough to facilitate the movements against the current or any lateral migrations. Experimental studies of critical swimming speed in other species of gobies, but with similar total length, showed values around 0.4 m/s (Donaldson et al., 2013), which are higher than the mean current velocity recorded with the ADCP in the bottom. However, the critical swimming speed cannot be maintained for long periods as whole tide phase, even though in the slack water periods the current velocities decrease notably. The higher densities found in flood tide may indicate a strategy to take advantage of flood-tide transport for this purpose. Related to its benthic life-style, their locomotion is described as short hops and darts, remaining close to the bottom and frequently resting on it between darts, being propelled by combined adduction of the pectoral fins and tail beating (Asriaens et al., 1993). In addition, Magnhagen and Forsgren (1991) described in these species the burrowing behaviour as method to avoid different kind of risky situations, which could be used to avoid water currents unwished too. The combination of these behaviours with their swimming capacities could make these species to select the environments more suitable (i.e. flood tide and bottom depth) for the ingress and maintenance into the estuary. This would need a confirmation.

Furthermore, some authors [e.g. Pampoulie et al. (1999); Guelinckx et al. (2008)] have described in other regions seaward migrations during the early spring to reproduce. In that case, in the summer season, when the study was carried out, individuals could enter upwards in the estuary through the bottom layers to reach upper zones using a flood-tide transport (Forward and Tankersley, 2001), which could partially explain the density differences found between tides. Additionally, their higher presence in the shoals would make them to start the upstream transport more quickly and during more time than in the channel (Scully and Friedrichs, 2007), where the longer ebbs delay the beginning of flood tide and increase the time of flushing out (Díez-Minguito et al., 2012). Some authors observed a pattern of circulation in a similar estuary with outflow in the deep channel and inflow over the shoals (Garel and Ferreira, 2013), which could facilitate their transport upstream, if they move to sides, or downstream, if they migrate to the channel.

Different studies have observed the influence of the temperature on the recruitment phenology and coastal migration of *Pomatoschistus* spp. (Dolbeth et al., 2007; Pampoulie et al., 1999). Our study, despite of limited range of temperature, showed a significant and positive relationship between this variable and the abundance of gobies. On the other hand, due to the opposed pattern of temperature and DO, without an experimental study with controlled environmental conditions, it is not possible to elucidate whether the cue potentially leading the behaviour of the gobies could be one or the other variable. Also DO has been showed to affect the *P. minutus* behaviour, increasing their swimming activity to avoid concentrations lower than 3.5 mg O_2/L (Petersen and Petersen, 1990). However, we did not register concentrations lower than 5 mg O_2/L, therefore, it is likely that temperature could act as a main cue in this case. Notwithstanding, during this study, higher mean temperatures and lower DO values were recorded during the flood together with higher densities of gobies. Both variables, separated or combined, could work as cues for these species to know when they have to go with the flow up or downstream.

4.3. Engraulis encrasicolus

The anchovy *Engraulis encrasicolus* is a pelagic species which remains in the surface layers at all times of the tide cycle. Similar to the gobies, STST hypothesis would not be applicable in this case, as it also has been reported by previous studies on another anchovy species (Schultz et al., 2003, 2000). It must be using an alternative mechanism. On the other hand, the different densities found between flood and ebb tides, consistently higher during ebb, have been previously observed in this estuary (Drake et al., 2007). This was tentatively interpreted as an indication of a tide related lateral migration to shallower more productive areas during high tide for feeding, as it was previously observed in other nursery areas in this region (Drake and Arias, 1991). As argued by Drake et al. (2007) for their sampling, the boat was anchored in a fixed point during a 24 h cycle and, consequently, its position with regard to the lateral water edge as well as bottom depth was changing with the tide: during high tide, the samples would be collected further away from the water edge than during ebb. In fact, if anchovies remained in shallower zones during the high tide, closer to the water edge than the anchored boat, they could capture the anchovies during low tide without a need for any lateral migration, simply because the boat is closer to the water edge in low water. The present study, however, was specifically designed to detect migrations, either vertical or lateral, completing this previous knowledge. Thus, samples in lateral zones were collected with the boat anchored the closest possible to the water edge both in high and in low tide. A clear evidence for a lateral migration would imply a significant interaction between “tide” and “zone” (a different relative density in lateral zones with regard to the channel between ebb and flood), but not a global reduction of density during flood both in the lateral zones and in the central channel, as it was observed.

Larvae and juveniles not collected during flood must be using a different zone in the river section [a certain dilution effect, because of the higher volume in high tide, can explain a small part of these differences (Strydom and Wooldridge, 2005), but it would not explain the notable differences found]. The possibility of a lateral migration still exists, as anchovies could be using the shallowest zones, not accessible with the boat, during the high tide (a different approach would be necessary to explore this possibility, such as the use of fixed traps). In fact, when the water spread over the shoals during the flood, larvae and juveniles would be transported over this zones as Jager (1999) suggested for flounder. An indication for this process was described in another nursery area in this region (e.g. Cádiz Bay), where juveniles and larvae ingress small intertidal channels every flood (Arias and Drake, 1990). In the Guadalquivir estuary, provided that intertidal marshes were mostly transformed and isolated from the main river, small fish would accumulate in the shallowest shore. The higher densities found in the sides (mainly in east side) would suggests that, other than the passive transport by water spreading over the intertidal flats, larvae would tend to remain in these areas. Indeed, the larger sizes found in the sides indicate that animals with higher swimming capacity, that can better swim against the ebb tide returning into the channel, would also tend to select the sides.

In this regard, the highest densities found where always in the East side, coinciding with the pattern of the wind direction during most cruises in flood tide condition (South-Western to North-Eastern), which could push the surface water layers, and consequently the organism inside, to this side of the estuary. Schieler et al. (2014) showed that wind can induce transport of larvae, in their case from the nearshores into the estuary.

Alternatively, Schultz et al. (2003) suggested a slight net transport upstream using vertical migrations to mid-depth of the water column. Whether anchovies made a different use of mid water in the main channel (in lateral samplings, the distance between the surface net and the epibenthic sledge was negligible) remains to be checked. However, we did not find a significant decrease of current velocity nor any other environmental variable that could justify a very different pattern in this zone.

Overall, considering both this study and the information already available for this estuary, a clear strategy for the anchovies to ingress upper into the estuary has not been observed. Lateral shallowest areas, where higher densities and larger sizes have been found, present low water velocities (Garel and Ferreira, 2013). This would allow the anchovies for a more efficient swim against the current and a better control of their position. This would be particularly applicable in neap tides. This study and Drake et al. (2007) were carried out in spring tides, and the tidal dynamic in Guadalquivir estuary showed that current velocities in spring tides almost doubles the velocities of neap tides (Díez-Minguito et al., 2012). Several studies have found distinct abundances in the exchange between the spring and neap tides (Pollock et al., 1983; Strydom and Wooldridge, 2005; Tanaka et al., 1989) and the importance of neaps for the ingress of some species into the estuary should be checked: anchovies could have a net downstream transport during the spring that could be compensated during neaps, using shallower areas as proposed by Teodósio and Garel (2015). As well, shallow lateral areas present a faster transition from ebb to flood than the middle channel, resulting a residual circulation landward over the shoal in some cases (Scully and Friedrichs, 2007). This kind of current circulation and the lower velocities could facilitate the intrusion of anchovies, which are attracted upstream for lower salinity levels (as observed in this study), or even swimming against slow currents as described Pattrick and Strydom (2014) for other fish larvae and juveniles. In fact, the physiochemical changes between zones during the flood, as the shift in water current velocity and its consequently salinity variations, could work as cues in this species. Thus, an ontogenetic transition in behavioural capability (Teodósio and Garel, 2015) comparable to sense acuity and behavioural hypothesized by Teodósio et al. (2016) could be happening but inside of the estuary.

4.4. General patterns

Anchovies and gobies are common species that usually domain the early life stage of fish assemblages in temperate estuaries all around the world (Bouchereau and Guelorget, 1998; James et al., 2007). The knowledge of their distribution in estuaries have been widely studied, being the anchovies pelagic species and most of the gobies benthic ones. The results obtained show that these species may have opposite mechanism to move across the estuary section. This antagonistic behaviour avoids the overlap of their physical habitats, enhancing the use of resources offered by the ecosystem and making these species particularly compatible. In addition, different physiochemical variables were found to influence their distributions across the estuary section. For a marine estuarine-opportunistic species, as *E. encrasicouls*, salinity generally presents an essential signal to detect estuaries (Elliott et al., 2007). Estuarine resident species, as the *Pomatoschistus* spp., are euryhaline organism with a high range of salinity tolerance (Souza et al., 2014), and values within the range recorded in this study (19-32 PSU) seems not to be particularly important for their distribution across the estuary section. In contrast, variables such as temperature and/or dissolved oxygen, which also exhibit a wide range of variation in estuaries, seem to be more relevant. Nevertheless, the influence of these signals should be tested with experimental studies under controlled conditions to confirm the effects of their variations in the behaviour of early stages of fishes.

Notwithstanding, both species presented the same transversal zonal pattern with higher abundances in the East side, which coincided with the pattern of the wind direction during most cruises in flood (South-Western to North-Eastern). Some authors described a residual axial landward current (or a lower net seaward velocity) in this side of the section, in other estuaries in this region (Garel and Ferreira, 2013; Teodósio and Garel, 2015). This is originated by the tidal, flows, wind and density-driven (Hare et al., 2005; Yamaguchi and Kume, 2008). This would facilitate the passive ingress and maintenance of individuals into the Guadalquivir estuary.

Also, the different densities found between depths, zones and tides for both species helps to design proper methodologies to sample the early life stage of fish assemblages. To avoid vertical distribution or migration, the whole water column should be sampled in plankton studies, even in estuaries oceanographically defined as well-mixed. Bathymetry plays an important role in the velocity current and modify physiochemical characteristics between zones, which in its turns, could produce a heterogeneous distribution of plankton organisms across the estuary section. Also, tidal comparisons (flood and ebb) are important to detect individuals which are entering or leaving the estuary. Therefore, although other estuaries could not show the same patterns, it would be recommended to analysis these spatial and tidal differences to not over- or underestimate the abundance and diversity of assemblages in estuaries.

The results obtained in this study shows that STST hypothesis does not hold for these species, at least in a well-mixed estuary. Pelagic and benthonic behaviour, of anchovies and the gobies respectively, seems to be rather fixed and did not exhibited a plasticity under the conditions of this study. The ability to detect pressure changes is known in mollusk, crab, barnacle and fish larvae (Kingsford et al., 2002), and hydrostatic pressure changes associated with tides may guide larvae to maintain their position in the water column despite of the current velocities. In fact, in a well-mixed and tidally dominated estuary such as Guadalquivir, where the physiochemical conditions are relatively similar in the water column, small organism may be using other alternative strategies to ingress and maintain their position, such as the use of current dynamics of shallower sides.

In conclusion, gobies showed an upstream flood transport, especially by both sides in the bottom. Contrary, the anchovy showed an advection transport downstream by the surface, although lateral uses were observed during the flood tide by bigger individuals, showing an influence of ontogenetic stages. Distinct physicochemical factors were associated to every species distribution in the estuary section. Although further research is necessary to better understand the recruitment strategy of these fish species (for instance, neap tide or the shallowest riverside shores), a first view of distributions was found for each one. Furthermore, the present analysis of across-river distribution helps to design appropriate sampling protocols for future plankton studies in estuaries.

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Supplementary data

Table S.1. GLMM results of *Pomatoschistus* spp. for comparison between depth, tide and zone along cruises in relation to salinity, turbidity, temperature, pH and current velocity: a) global model; b) best-fit model.

a) Global model

Pomatoschistus spp. ~ (Depth+Tide+Zone)^3 + Salinity + Turbidity + Temperature + pH + Current velocity + (1|Cruises) + (1|Sampling time) + offset(log(filtered volume))

b) Best-fit model

Pomatoschistus spp. ~ (Depth+Tide+Zone)^3 + Temperature + (1|Cruises) + (1|Sampling time) + offset(log(filtered volume))

	Estimate	SE	z	P
(Intercept)	-1.8114	0.3095	-5.852	**>0.0001**
Depth (Surface)	-3.9076	0.3674	-10.637	**>0.0001**
Tide (Flood)	-0.9544	0.4153	-2.298	**0.0215**
Zone(East)	0.4588	0.4113	1.115	0.2646
Zone(West)	-1.5662	0.4472	-3.503	**0.0004**
Temperature	0.2476	0.1244	1.991	**0.0464**
Depth(Surface)*Tide(Flood)	0.8263	0.533	1.55	0.121
Depth(Surface)*Zone(East)	-0.154	0.5506	-0.28	0.7796
Depth(Surface)*Zone(West)	1.7743	0.5632	3.15	**0.0016**
Tide(Flood)*Zone(East)	2.2722	0.5764	3.942	**>0.0001**
Tide(Flood)*Zone(West)	3.422	0.5972	5.73	**>0.0001**
Depth(Surface)*Tide(Flood)*Zone(East)	-1.3142	0.7288	-1.803	0.0713
Depth(Surface)*Tide(Flood)*Zone(West)	-2.2623	0.7198	-3.143	**0.0016**

Table S.2. GLMM results of *Engraulis encrasicolus* for comparison between depth, tide and zone along cruises in relation to salinity, turbidity, temperature, pH and current velocity: a) global model; b) best-fit model.

a) Global model

E. encrasicolus ~ (Depth+Tide+Zone)^3 + Salinity + Turbidity + Temperature + pH + Current velocity + (1|Cruises) + (1|Sampling time) + offset(log(filtered volume))

b) Best-fit model

E. encrasicolus ~ (Depth+Tide)^2 + (Depth+Zone)^2 + Salinity + (1|Cruises) + (1|Sampling time) + offset(log(filtered volume))

	Estimate	SE	z	P
(Intercept)	-2.0043	0.3907	-5.13	**>0.0001**
Depth (Surface)	1.9673	0.5318	3.699	**0.0002**
Tide (Flood)	-1.2792	0.3471	-3.685	**0.0002**
Zone(East side)	-0.6224	0.4297	-1.448	0.1475
Zone(West side)	-0.951	0.4418	-2.153	**0.0313**
Salinity	-0.4155	0.1371	-3.031	**0.0024**
Depth(Surface)*Tide(Flood)	-0.955	0.4567	-2.091	**0.0365**
Depth(Surface)*Zone(East side)	2.0926	0.5876	3.561	**0.0003**
Depth(Surface)*Zone(West side)	1.3747	0.5821	2.361	**0.0182**

Table S.3. LMM results of total length of *Engraulis encrasicolus* for comparison between depth, tide and zone along cruises: a) global model; b) best-fit model.

a) Global model

Total length ~ (Depth+Tide+Zone)^3 + (1|Cruises)

b) Best-fit model

Total length ~ (Depth+Tide+Zone)^3 + (1|Cruises)

	Estimate	SE	t	P
(Intercept)	15.628	1.1069	14.119	**>0.0001**
Depth (Surface)	1.5227	0.9074	1.678	0.0933
Tide (Flood)	1.7112	1.338	1.279	0.2010
Zone (East)	-0.4793	1.1308	-0.424	0.6720
Zone (West)	2.4397	1.1524	2.117	**0.0342**
Depth(Surface)*Tide(Flood)	-0.909	1.3972	-0.651	0.5150
Depth(Surface)*Zone(East)	0.5264	1.1376	0.463	0.6440
Depth(Surface)*Zone(West)	-3.7096	1.162	-3.192	**0.0014**
Tide(Flood)*Zone(East)	16.8244	2.3475	7.167	**>0.0001**
Tide(Flood)*Zone(West)	7.6671	2.4424	3.139	**0.0017**
Depth(Surface)*Tide(Flood)*Zone(East)	-12.8	2.3928	-5.349	**>0.0001**
Depth(Surface)*Tide(Flood)*Zone(West)	-0.5908	2.519	-0.235	0.8150

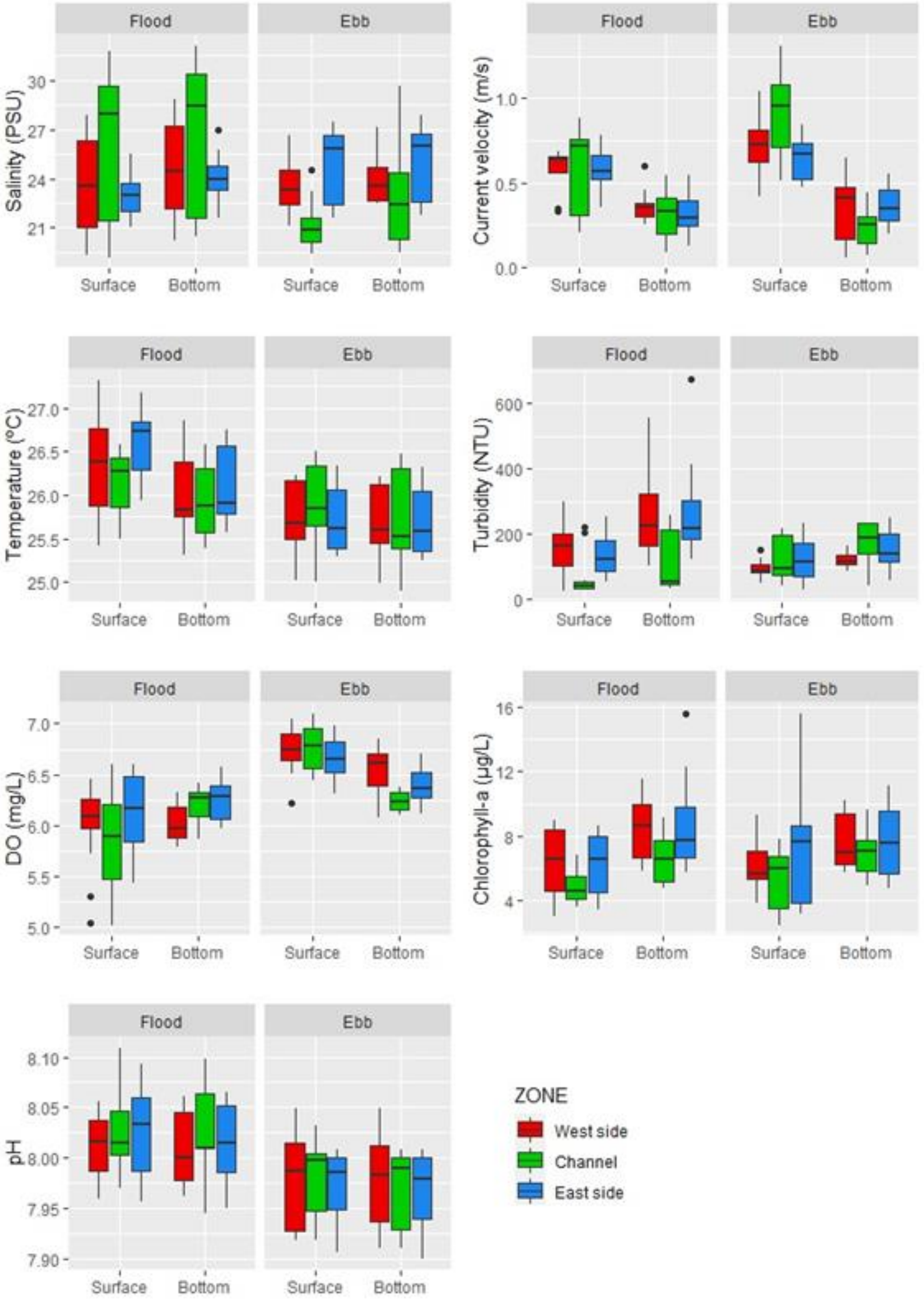

Figure S.1. Boxplots of all water physiochemical variables per zone, depth and tide during the three cruises.

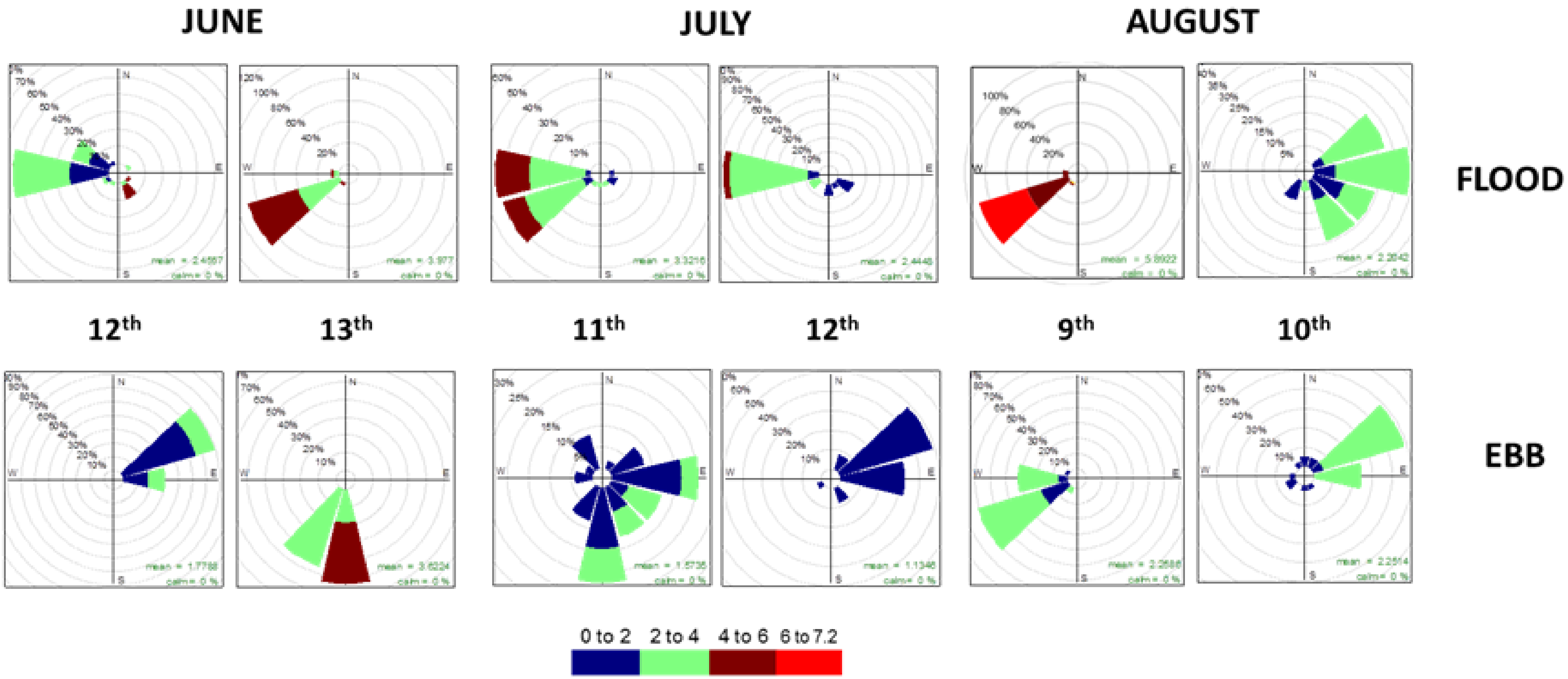

Figure S.2. Wind rose plots with speed and direction data of Vetalengua Station during every sampling day in each tide condition.

3. MAINTENANCE DREDGING EFFECTS ON THE MACROFAUNA OF THE WATER COLUMN IN THE GUADALQUIVIR ESTUARY

Abstract

Many human activities in or near aquatic habitats generate alterations in their environmental conditions which could affect the organisms that inhabit in them. Maintenance dredging of navigation channels in order to allow large ship access to inland ports is one such source of disturbance. In this study, we analysed the effects of a maintenance dredging operation from multiple approaches (immediate, short and medium term) in the physiochemical variables, the early life stages of fish and other macrofauna groups present in two zones of the Guadalquivir estuary with different salinity ranges (poly- and mesohaline). Most physiochemical variables were homogenized in the water column immediately after the water mass passed by the dredger, including sediment resuspension. However, this process seemed to be temporarily short as no significant increments in the depth-averaged levels of turbidity were observed in short- and medium-term. Instead, metals concentration of Cr, Fe y Zn increased in the polyhaline station. Even so, these perturbations did not appear to be severe enough to influence the macrofauna. Still, organisms can suffer direct mechanical impacts by the trailer suction. Epibenthic species, like *Pomatoshcistus* spp. or decapods, tended to decrease slightly, while pelagic species as *Engraulis encrasicolus* or mysids did not, indicating that benthic organisms usually are more prone to a high entrainment. Nonetheless, the possible effects of this disturbance were of the same order or less than natural ones, therefore, macrofauna organisms could be well-adapted to cope with them.

Resumen

Muchas actividades humanas en o cerca de hábitats acuáticos generan alteraciones en sus condiciones ambientales que podrían afectar a los organismos que habita en ellos. El dragado de mantenimiento de los canales de navegación para permitir el acceso de grandes barcos a los puertos interiores es una de esas fuentes de perturbación. En este estudio analizamos los efectos de una operación de dragado de mantenimiento desde múltiples enfoques (inmediato, corto y mediano plazo) en las variables fisicoquímicas, las larvas y juveniles de peces y otros grupos de macrofauna presentes en dos zonas del estuario del Guadalquivir con diferente salinidad (poli- y mesohalina). La mayoría de las variables fisicoquímicas se homogeneizaron en la columna de agua inmediatamente después de que la masa de agua pasara por la draga, incluyendo la resuspensión de sedimentos. Sin embargo, este proceso pareció ser temporalmente corto, ya que no se observaron incrementos significativos en los niveles de turbidez promediados en profundidad a corto y medio plazo. En cambio, la concentración de metales de Cr, Fe y Zn aumentó en la estación polihalina. Aun así, estas perturbaciones no parecieron ser lo suficientemente graves como para influir en la macrofauna. Por otro lado, los organismos pueden sufrir impactos mecánicos directos por la succión de la draga. Las especies epibentónicas, como *Pomatoshcistus* spp. o los decápodos, tendieron a disminuir ligeramente, mientras que las especies pelágicas como *Engraulis encrasicolus* o los misidáceos no lo hicieron, lo que indica que los organismos bentónicos suelen ser más propensos a ser succionados. No obstante, los posibles efectos de esta alteración fueron del mismo orden o menores que los naturales, por lo que los organismos de la macrofauna podrían estar bien adaptados para hacerles frente.

1. Introduction

Many human activities in or near aquatic habitats generate alterations in their environmental conditions which could affect the organism that inhabit in them (Halpern et al., 2008; Lotze et al., 2006). Capital dredging of navigation channels in order to reclaim land and to allow ever larger ship access to inland waterways is one such source of disturbance. Adverse effects of dredging operations in coastal systems have generally included habitat degradation, increased turbidity and suspended sediment, tidal amplification, altered current dynamics, changes in salinity and water quality, etc. [e.g. Torres et al. (2009); Wilber and Clarke (2001); Winterwerp and Wang (2013)]. On the other hand, periodic maintenance dredging operations are necessary to keep the appropriate bottom depth of the navigable channels. These recurrent dredging activities may have serious repercussions on the coastal environment, although to a lesser extent than capital dredging, since they also may alter the bottom topography, resuspend sediments, release pollutants, modify the water column and lead to the removal of a stable substrate (Donázar-Aramendía et al., 2018; Jones et al., 2015; Rehitha et al., 2017). The container port industry has experienced phenomenal growth along the past decades since the era of containerization, where currently shipping moves over the 80% of world’s commodities (Tsolaki and Diamadopoulos, 2010; Yap and Lam, 2013). The growing of world trade will increase the number of ships and their capacities, which consequently require extensive dredging service in coastal areas to reach ports (Yap and Lam, 2013).

Coastal ecosystem are among the most ecologically and economically important worldwide (Barbier et al., 2011). In particular, estuaries are sites of important connectivity and intense gradients that make them high productive ecosystems with an essential nursery function for many species (Elliott et al., 2019). At the same time, they are dynamic and complex systems where high variability of the physical-chemical gradients makes them one of the most stressful aquatic environments for aquatic fauna (González-Ortegón et al., 2015, 2010). The constant fluctuation of environmental characteristics such as temperature, turbidity, oxygen and salinity due to tidal dynamics and freshwater inputs generate that singular communities inhabit these ecosystems (Day et al., 2013). Therefore, the alterations in these ecosystems due to anthropic disturbances could be difficult from discriminate of natural changes (Elliott and Quintino, 2007). Achieving an accurate assessment of the anthropic impacts is necessary to improve the management of coastal development while maintaining a balance with a ‘good ecological status’ of coastal environment (Borja and Elliott, 2007).

Impacts on benthic communities as a consequence of dredging have been documented in numerous studies [e.g. Bemvenuti et al. (2005); Donázar-Aramendía et al. (2018); Ponti et al. (2009)]. However, organisms which inhabits in the water column as plankton or fishes remain largely unquantified. Although dredging often has more repercussions on benthic communities due to the relative immobility of organisms (Simonini et al., 2005), extensive literature have demonstrated that dredging can directly impact fishes (Kjelland et al., 2015; Wenger et al., 2017), and their associated habitats (Erftemeijer and Lewis, 2006; Jones et al., 2016). Many studies analysed the different effects of suspended sediment increment in behaviour (Collin and Hart, 2015), predation (Ohata et al., 2011) and physiology (Au et al., 2004); other investigations observe the effect of released contaminants as metals or hydrophobic organic pollutants (Haynes and Johnson, 2000); some of them research the entrainment of fish in different history stages (Reine et al., 1998); and a few studies assessment the dredging sounds (Reine et al., 2014). Notwithstanding, most of these studies are carried out in laboratory under controlled environments, being the investigations *in situ* very scarce. The methodology to assess the real impacts over the organisms which inhabit in the water column usually are difficult to apply in the field due to the continues changes of multiple variables such as current dynamics, tidal condition, salinity gradient, mobility of the organisms, etc.

Recent studies show that adult fish are more likely to undergo sublethal stress from dredging operations rather than lethality because of their ability to move away from or out of an area of higher impact to one of lower impact (Wenger et al., 2018). However, larvae and eggs are subject to lethal impacts more frequently due to their lower mobile capacities (Wenger et al., 2018) as well as small individuals of macrozooplankton or hyperbenthos (Hoffmann and Dolmer, 2000). For this reason, early life stages of fishes or plankton organisms could be more sensitive and may show clearer the impacts of dredging in species that inhabits in the water column.

Our *in situ* study pretends to determine different effects on small organisms which inhabits in the water column as early life stages of fish, macrozooplankton and hyperbenthos species during a maintenance dredging operation in different zones of an estuary with horizontal salinity gradient, which is considered one of the most important coastal areas of the region for its nursery function (Miró et al., 2020). Two temporal aims were proposed: i) analyse the immediate effects of a trailer suction dredger working in the physiochemical variables and macrofauna species present in the water column; ii) analyse the accumulated short- and medium-term effects of a maintenance dredging operation in the physiochemical variables and main macrofauna species present in the water column of two zones with different salinity ranges.

2. Material and methods

2.1. Study area

The Guadalquivir estuary is located in South-West of Iberian Peninsula, a warm temperate region, and its waters flow to the Gulf of Cadiz (Atlantic Ocean). The estuary extends 110 km inland from its mouth. It is well-mixed mesotidal system with 3.5 m amplitude range (spring tides) in the river mouth (Díez-Minguito et al., 2012), which presents a longitudinal salinity gradient with temporal displacement by tides, discharges and seasonal variations (González-Ortegón et al., 2014). The morphology of the estuary is a single channel mostly isolated of surrounding natural areas, with a main navigable channel of 7.1 m average depth, which is dredged every one or two years to guarantee the navigation depth (Ruiz et al., 2015). In autumn 2017, a maintenance dredging operation was carried out in several zones of the estuary. The dredging work was performed by a trailer suction dredge. Our study was focused on two dredging zones (Figure 1), one in the polyhaline water mass and the other in the mesohaline water mass. Approximately 19600 and 20500 m^3 of dredged material were extracted in each range, respectively, and it was carried out for 15 days (18-11-2017 to 3-12-2017).

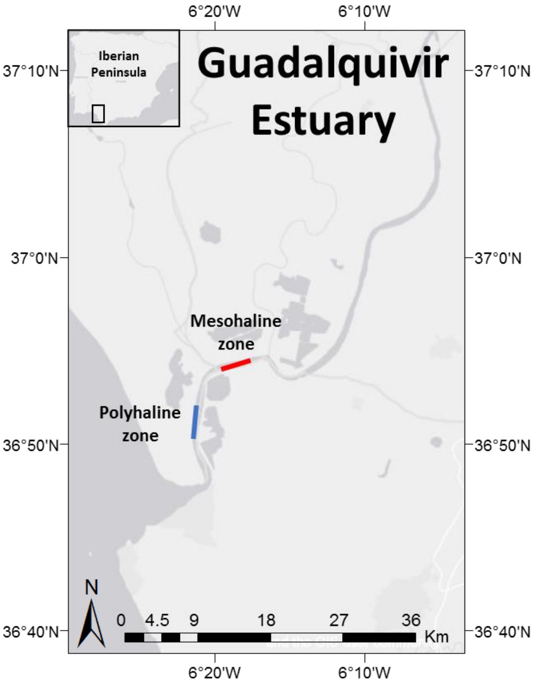

Figure 1. Study area of Guadalquivir estuary with dredging zones.

2.2. Field sampling

To analyze the immediate effect in the whole water column, biological samples were collected against the main water current, before (in front of the bow) and after (behind the stern) the water mass would have passed the dredging vessel while it was working, in three different moments (Figure 2). Samples of physiochemical variables were also collected. To analysis the short- and medium-term cumulative effect, biological and physiochemical samples were collected in three cruises before, five cruises during and three cruises after the dredging with four samples in every zone. In order to analyze the intra-seasonal trends on abundances in the main organisms found in the estuary, comparisons of a monthly monitoring sampling were done at the same zones using the same periods of the two years prior to 2017. No dredging operations were carried out in 2015, which was used as the natural trend, while a similar dredging was performed in 2016, which was used to compare the effects with 2017.

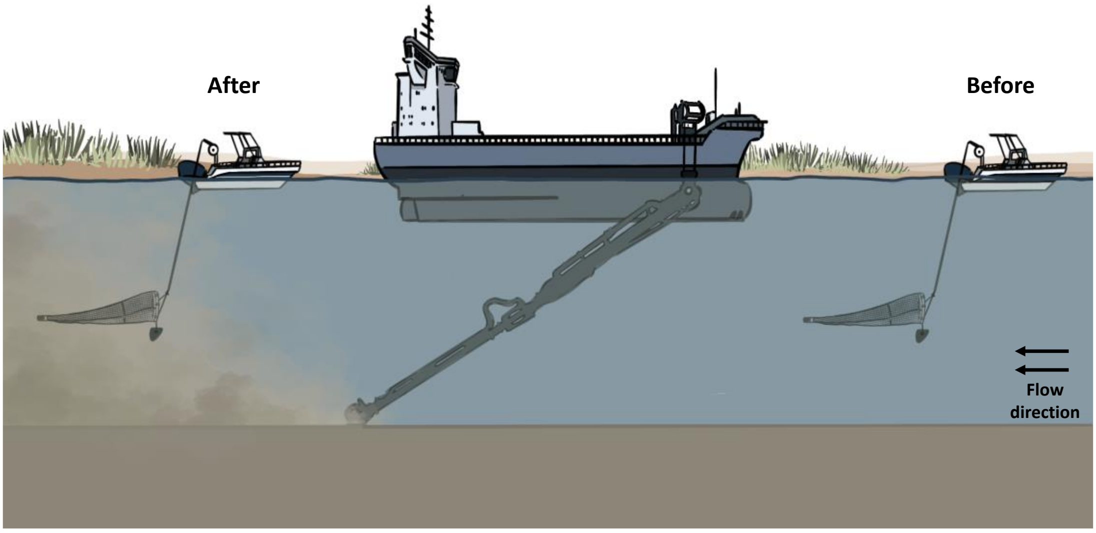

Figure 2. Experimental design to analyse the immediate effects in the water column before and after the water mass passed by the dredger while it was working.

Biological samples were collected with a plankton net of 1 m diameter and 1 mm mesh size equipped with a flow-meter General Oceanics 2030R. Oblique tows of 10 min (305±46m^3; mean±SD) were done with a boat against water current at a speed of 2–2.5 knots. Samples were fixed in 70% ethanol. The early fish stages and the rest of macrofauna groups were sorted. Fishes were counted and, whenever possible, identified to species level. The rest of macrofauna were quantified as biomass in fresh weight per group (mysids, decapods and isopods).

Physiochemical profiles of the whole water column were recorded before of every plankton tow with a multiprobe (depth, temperature, salinity, turbidity, dissolved oxygen concentration (DO), pH and chlorophyll concentration (Chla); Eureka™ Manta2).

Water samples were taken at mid-depth with a Niskin bottle to measure total suspended solids (TSS), inorganic nutrients (NO_2, NO_3, NH_4, PO_4, SiO_4) and metals (As, Cd, Co, Cr, Cu, Fe, Ni, Pb, Zn) concentration. To measure total suspended solids (TSS), water was filtered through 0.7 µm pore pre-combusted (4 h, 500 °C) filters (Whatman GF/F); thereafter filters were dried (24 h, 60 °C) and weighted. Suspended organic (SOM) and inorganic matter (SIM) were obtained as weight loss by ignition (500 °C, 4 h). Filtered water samples for trace metals were acidified with high-purity HNO_3 (Suprapur, Merck) and analyzed by inductively coupled plasma optical emission spectrometry (Varian ICP 720-ES) after being stored one month. Concentrations of NO_2, NO_3, NH_4, PO_4 and SiO_4 were determined in filtered water samples, with an autoanalyzer (Skalar San^{plus} System) using colorimetric techniques.

2.3. Data analysis

To investigate the effects of the dredging operation in the different variables measured, generalized linear mixed models (GLMMs) were applied using ‘Lme4’ (Bates et al., 2015). Different experimental designs were run for every approach.

1) In the immediate approach, models were applied to most abundant (>2% of total) fish species and main macrofauna groups caught. Normal distribution was the most adequate for biomass of macrofauna groups, and the Poisson distribution for count data of fish species with the log of filtered volume as an offset variable. The experimental design included 2 factors: one fixed factor “Moment” (with 2 levels, “Before and After”) and 1 random factor, “Cruise” (with 3 levels, “1, 2 and 3”), nested within Moment.

2) In the short- and medium-term approach, models were applied to the most abundant fish species and macrofauna groups, as well as all environmental variables measured for every zone (polyhaline and mesohaline) separately. Normal distribution was the most adequate for most of response variables, except for counts of fish species abundances, for which we used a negative binomial distribution and the log of the filtered volume as an offset variable. The experimental design included 2 factors: one fixed factor “Period” (with 3 levels, “Before, During and After”) and 1 random factor nested within Period “Cruise” (within 3 levels Before, 5 levels During and 3 levels After). If Period factor was significant, *Post hoc* pairwise comparison between levels was analysed using the package ‘emmeans’ (Lenth, 2018).

Generalized additive models (GAMs) were fitted for depth profiles of physiochemical variables recorded with the multiprobe. As GLMMs uses a single value of every predictor variable for every value of the response variable, predicted values of GAMs were depth averaged obtaining a single value representative of the complete water column. Results of all variables were plotted by zone using the package ‘ggplot2’. Analysis were performed using the R 3.5.2 software (R Core Team, 2018).

Additionally, daily mean discharge from Alacalá del Río Dam during the whole study period was observed to analyse the influence of freshwater input (data provided by Confederación Hidrográfica del Guadalquivir, http://www.chguadalquivir.es/saih/DatosHistoricos.aspx) on the environmental variables measured.

3. Results

3.1. Immediate effects

3.1.1. Environmental analysis

Profiles of the different environmental variables recorded with the multiprobe were plotted in the Figure 3. A general pattern was found after dredging for chlorophyll, turbidity and

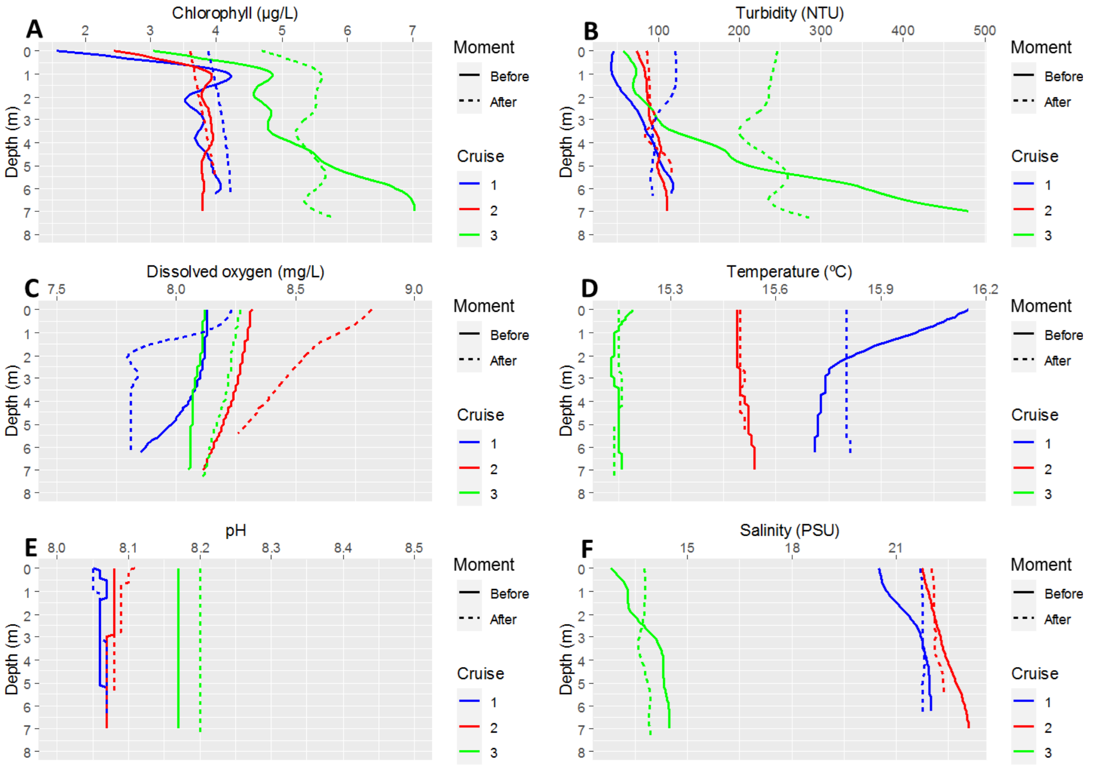

Figure 3. Vertical profiles of the environmental variables (A: chlorophyll; B: turbidity; C: Dissolved oxygen; D: Temperature; E: pH; F: Salinity) before (solid line) and after (dash line) the water mass pass through the dredger while it is working in the three different cruises.

salinity variables, which consisted in a homogenization of the whole water column with similar values in the surface than in the bottom. Only the dissolved oxygen showed the inverse tendency, with higher values in the surface after dredging. The pH maintained homogeneous values in both moments, similar as temperature except for the cruise 1.

3.1.1. Biological analysis

The fish species found were *Engraulis encrasicouls* (45.6%), *Pomatoschistus* spp. (44.9%), *Aphia minuta* (2.9%), *Pomadasys incisus* (2.3%), *Solea senegalensis* (2.2%), *Sardina pilchardus* (1.1%), *Argyrosmus regius* (0.5%), *Anguilla anguilla* (0.3%) and *Gobius paganellus* (0.2%). Among the rest of macrofauna groups, mysids were the most abundant (77.9%; e.g. *Rhopalophthalmus tartessicus, Mesopopdosis slabberi and Neomysis integer*), followed by decapods (17.4%; e.g. *Palaemon* spp. and *Crangon crangon*) and isopods (4.6%; e.g. *Synidotea laticauda* and *Lekanesphaera rugicauda*).

Species showed different responses after water mass passed by the dredger, although the variations between moments hindered to find clear patterns (Figure S.1). Only the mysids and *Solea senegalensis* showed significant differences, increasing in all cruises (Table 1). Also, the anchovy *Engraulis encrasicolus* tended to increase. Contrary, decapods, *Pomatoschistus* spp. *Aphia minuta* and *Pomadasys incisus* tended to decrease. Isopods maintained stable densities in front and behind the dredge.

Table 1. Results of the fixed effect "Moment" for GLMM on main early fish species (count data) and macrofauna groups (biomass data) of the immediate approach. Level "Before" was used as intercept to calculate estimates.

	Estimate	SE	z value	p value
Engraulis encrasicolus	0.328	0.213	1.537	0.124
***Pomatoschistus* spp.**	-0.426	0.411	-1.034	0.301
Aphia minuta	-0.369	0.492	-0.75	0.453
Pomadasys incisus	-0.36	1.195	-0.302	0.763
Solea senegalensis	1.778	0.763	2.329	**0.019**
	Estimate	**SE**	**t value**	**p value**
Mysids	41.67	10.14	4.111	**>0.001**
Decapods	-10.01	27.6	-0.362	0.717
Isopodos	0.333	0.981	0.34	0.734

3.2. Short- and medium-term effects

3.2.1. Environmental analysis

The duration of the study was 81 days between the first and the last cruise, showing different temporal patterns between the different physiochemical variables. Statistical differences of the fixed effect "Period" from GLMM on all variables were summarized in the plots by letter codes and extended in Table S.1. Daily mean freshwater input into the estuary (Figure 4A) increased (discharges higher than 50 m^3/s) at the end of the dredging period and thereafter. Turbidity did not show any change during dredging, while their values increased during the post-dredging period, being significant in polyhaline zone (Figure 4B). Chlorophyll

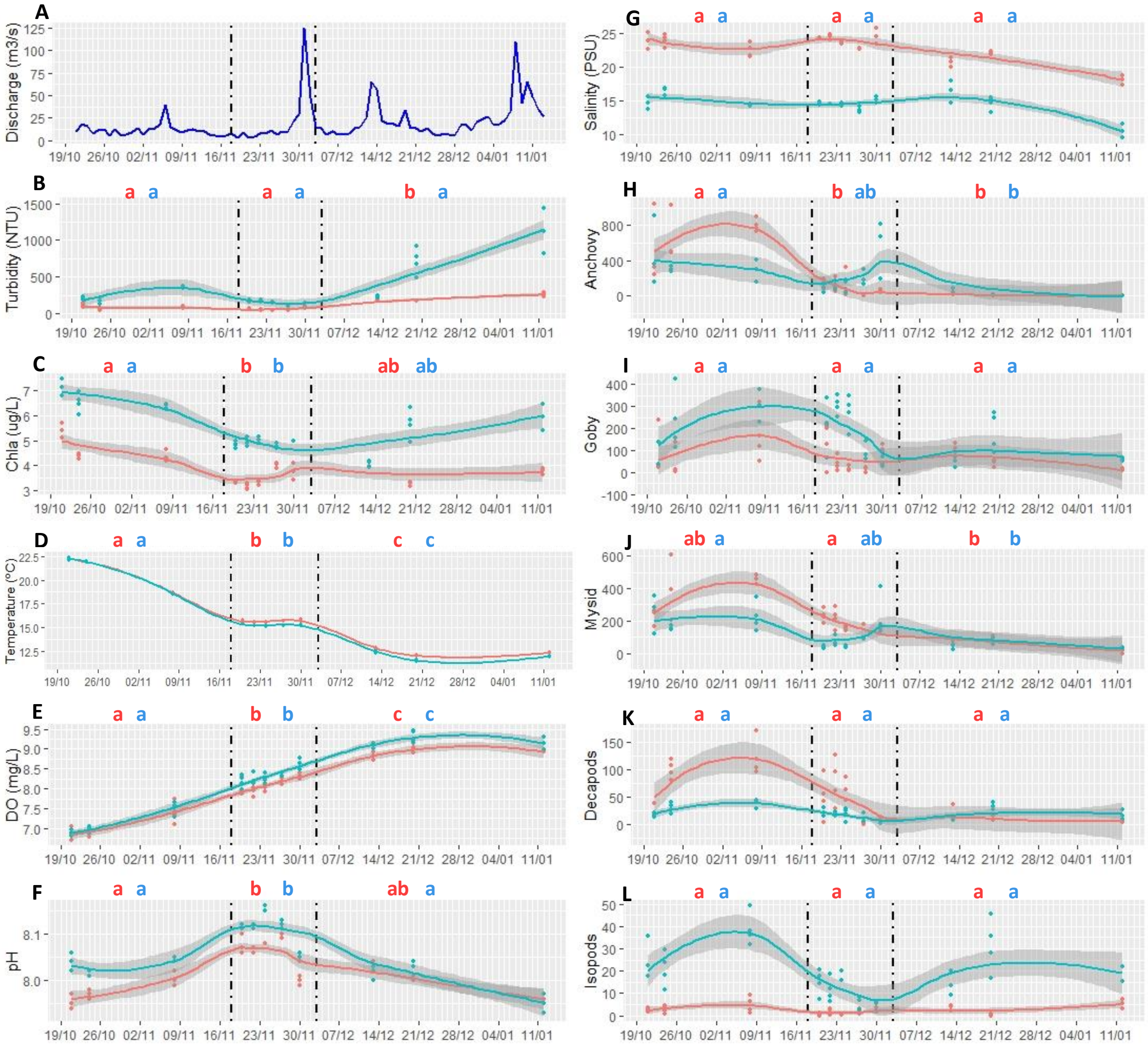

Figure 4. Daily mean of freshwater inputs in the Guadalquivir estuary (A) and temporal values of physiochemical variables measured in the water column (turbidity (B), chlorophyll (C), temperature (D), dissolved oxygen (E), pH (F), salinity (G)) and biological (*Engraulis encrasicolus* (H), *Pomatoschistus spp*. (I), mysids (J), decapods (K), isopods (L)). Dush lines point out the dredging period. Solid lines are smoother models with loess method for polyhaline (Red) and mesohaline (Blue) zones. Grey shades mean standard errors. Different letters indicate significant differences (p<0.05) between levels of Period factor (before, during and after) from GLMM in every zone (Red letters: polyhaline; Blue letters: mesohaline).

concentration showed a decrease during the dredging period, and thereafter a soft recovery (Figure 4C). Water temperature decreased gradually from 22.5°C to 12.5°C, typical in autumn-winter transition of temperate regions in the north hemisphere, although this decline ceased during the dredging (Figure 4D). Dissolved oxygen showed the inverse trend during the study (Figure 4E), although their values increased more during dredging period despite the fact that temperature was stable during these dates. The pH increased in both zones during dredging, decreasing to pre-dredging values afterwards. Salinity was stable in both zones during the whole study (Figure 4F), although it tended to decrease after dredging associated to the increment of freshwater input in this period.

TSS, directly correlated with SIM and SOM (Figures 5A-C), showed the same pattern as turbidity, being the post-dredging period when higher concentrations were found in every zone, although the wide range found in the mesohaline zone did not make this zone significantly different. Silicate and nitrite concentrations did not show differences (Figures 5D-E). Nitrates showed a gradual increment in both zones, with significant differences in the polyhaline zone between all periods (Figure 5F). Similar trend showed the ammonium, but without significant differences (Figure 5G). Phosphates did not show any difference in the mesohaline zone, while values measured in polyhaline zone were higher during dredging, with a partial recovery thereafter (Figure 5H).

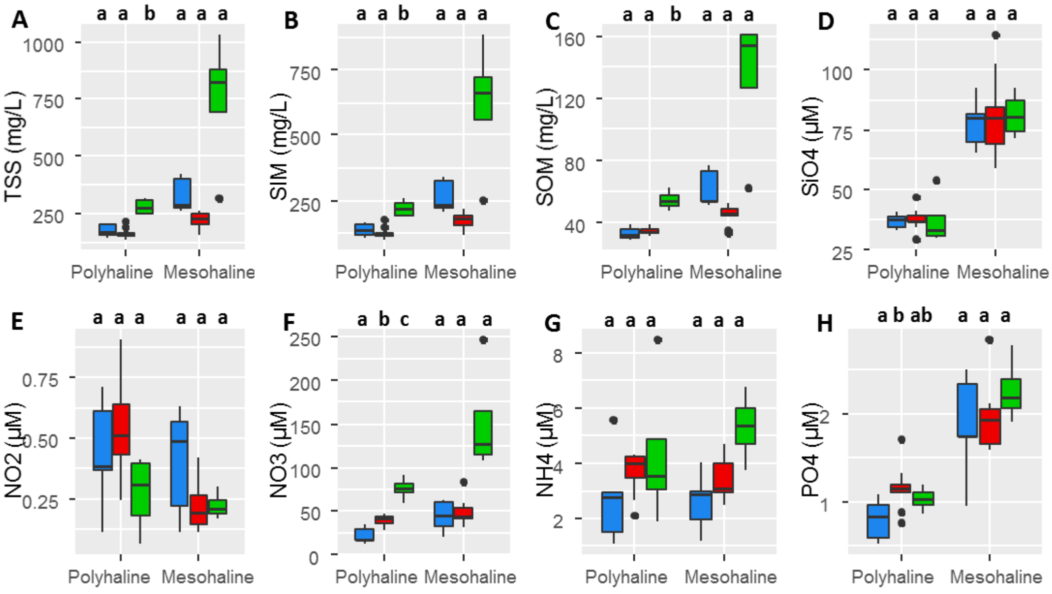

Figure 5. Boxplot of total suspended solids (A), suspended inorganic matter (B), suspended organic matter (C), SiO_4 (D), NO_2 (E), NO_3 (F), NH_4 (G) and PO4 (H) in every zone. Blue: before dredging; red: during dredging; green: after dredging. Different letters indicate significant differences ($p<0.05$) between levels of Period factor from GLMM in every zone.

Similar trends between zones were found in the concentration of most metals analyzed, although the mesohaline zone did not show significant differences between periods for any of them (Figure 6 and Table S.2). As, Cd, Co, Cu and Pb did not show any clear patterns due to variations between replicates (Figure 6A-D, H). Ni, despite not being significantly different between periods, showed a cumulative trend during the dredging period (Figure 6G). Cr and Fe started to increment gradually during the dredging and continued in post-dredging period (Figure 6D, F). Zn showed the highest relative increment during the dredging, with different trends after dredging for the polyhaline zone, in which it decreased slightly, and the mesohaline zone, in which it was maintained high but with oscillations (Figure 6I).

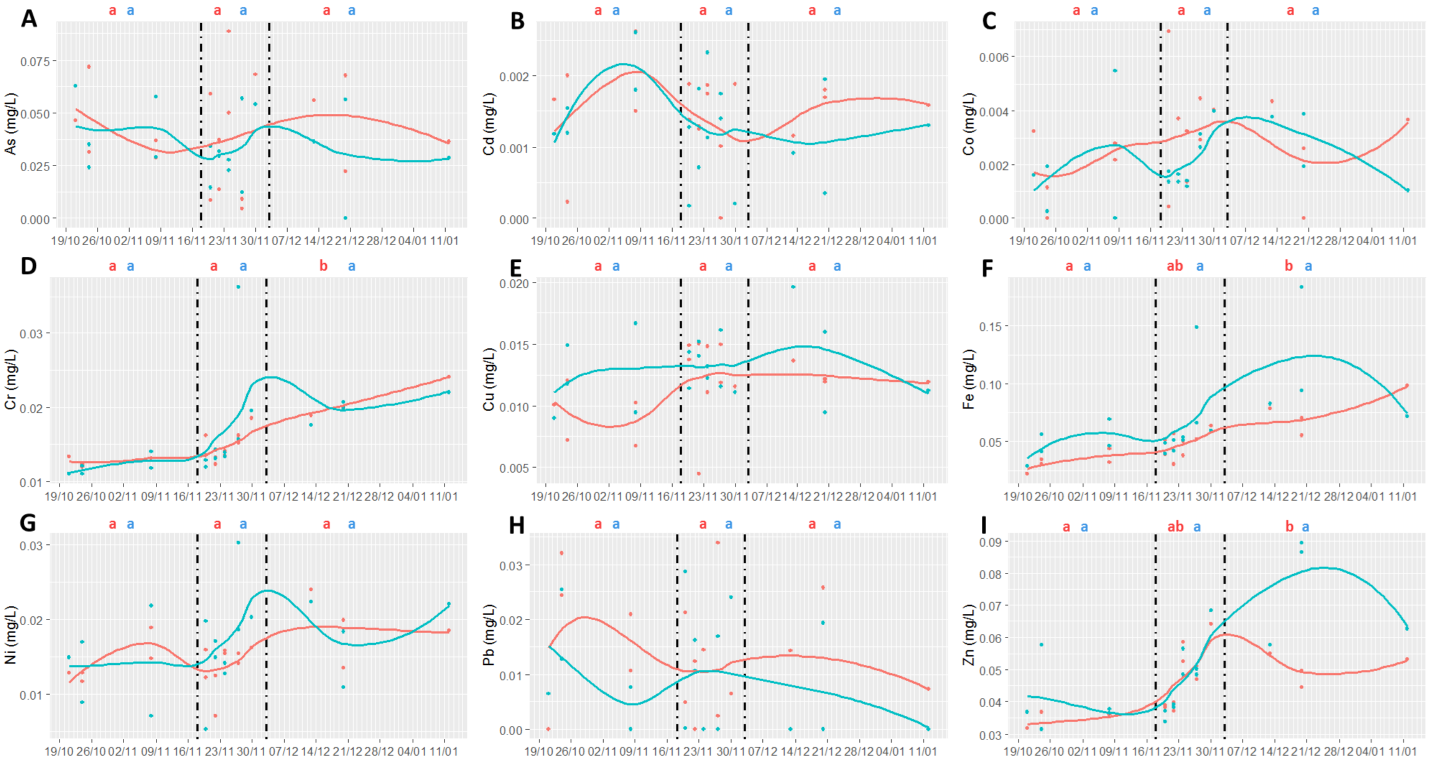

Figure 6. Temporal series of metal concentrations (As, Cd, Co, Cr, Cu, Fe, Ni, Pb, Zn; A-I respectively) in the water column. Dush lines point out the dredging period. Solid lines are smoother models with loess method for polyhaline (Red) and mesohaline (Blue) zones. Different letters indicate significant differences ($p<0.05$) between levels of Period factor from GLMM in every zone (Red letters: polyhaline; Blue letters: mesohaline).

3.2.2. Biological analysis

A total of 16 fish species in early life stages were found with two dominant species that compounded more than 90% of the total abundance of the fish assemblage, the anchovy *Engraulis encrasicolus* (58.3%) and the goby *Pomatoschistus* spp. (32.8%). In relation with the rest of macrozooplankton and hyperbenthos groups, similar species as described in the immediate approach were found, with mysids showing the highest biomass (76.6%), followed of decapods (17.6%) and isopods (5.3%).

Temporal series of the densities of most abundant fish species and the rest of macrofauna were plotted by zones in the figure 4. A general pattern was observed for most taxa, which showed a wider density variation before and during dredging cruises, as well as a temporal decreasing along the whole study. Notwithstanding, distinct responses and significant differences were found between periods for every species (Table S.3). Anchovy and mysids showed similar patterns in both zones, with a marked decrease from the beginning of dredging, especially in polyhaline waters, that continued until the last cruise (Figures 4H, J). Isopods, goby and decapods did not show differences between periods due to high cruise variability intra-periods, although different tendencies were observed (Figures 4 I, K, L). Isopod densities were too low in the polyhaline zone to detect any change along periods, while in the mesohaline zone, its biomass showed a notably descend during the dredging in comparison with the last cruise of the period before; also, it showed a partially recovery in the second cruise after dredging, although it did not continue in the next one. Goby and decapods showed a similar trend, but in opposite zones, with a decrease at the end of the dredging which continued also in the period after dredging.

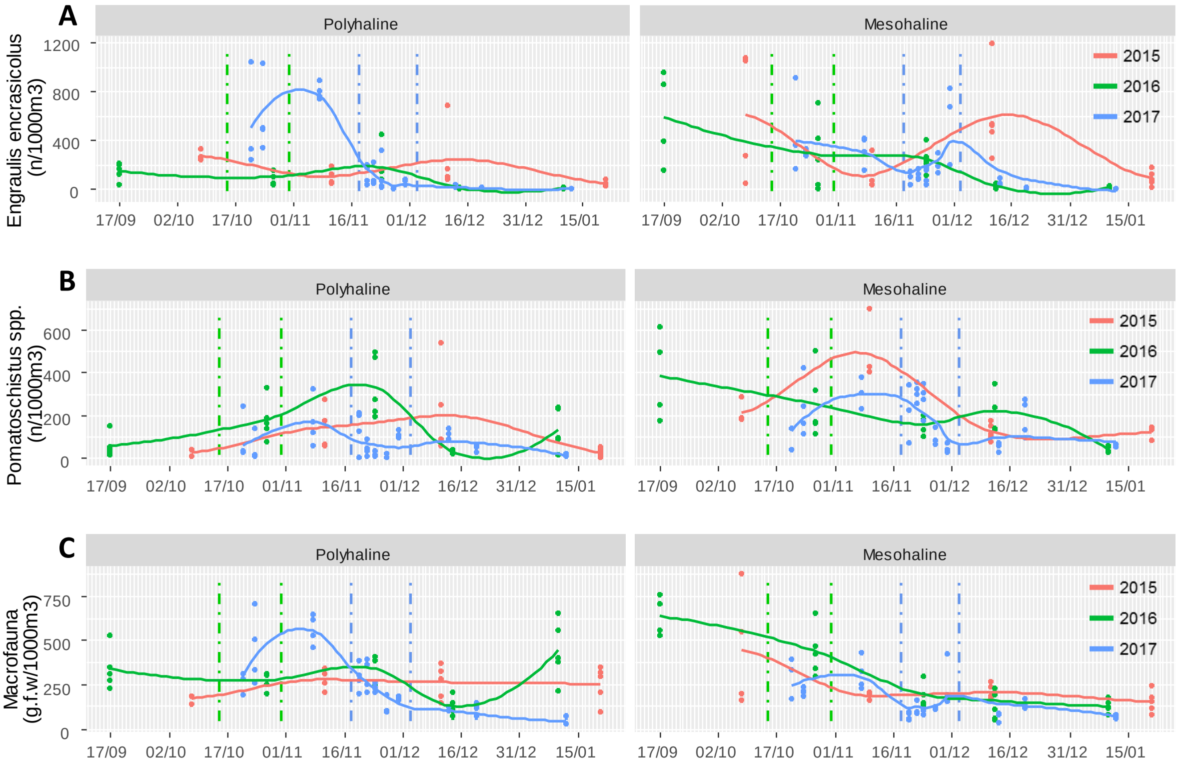

Figure 7. Density of *Engraulis encrasicolus* (A) and *Pomatoschistus* spp. (B), and biomass of the rest of macrofauna (C) during the dredging study (2017) and on similar dates in previous years (2015 and 2016) in every zone. Dush lines point out the dredging periods: colour green for 2016 and blue for 2017. Solid lines are smoother models with loess method for every year.

Interannual comparison (2015, 2016 and 2017) of *E. encrasicolus, Pomatoschistus* spp. and the rest of macrofauna groups together (the main component was mysids) in every zone were plotted in Figure 7. Anchovy (Figure 7A) showed stable densities in the polyhaline zone during the whole period in 2015 and 2016, despite the dredging operation carried out in the latter year. Instead, 2017 presented higher anchovy abundances before dredging, but it decreased during dredging until similar levels as previous years in the same dates. The densities of anchovies in the mesohaline zone showed a different trend, with a gradual decrease but with oscillations depending on the year. In fact, the natural inter-month variations found in 2015 where higher than those observed in years with dredging. The trend of the rest of macrofauna (Figure 7C) was similar than anchovy in the polyhaline zone, and a gradual decrease was repeated for all years in the mesohaline zone. The goby (Figure 7B) showed a distinct pattern in both zones all years, except for the mesohaline zone in 2016, with a progressive increment at the beginning of studied period that varied between dates, to finally decrease with different slopes.

4. Discussion

Estuaries are ecosystem with wide environmental variations that make difficult to detect anthropic stress from natural changes, unless the human impact is severe, leading to the 'Estuarine Quality Paradox' (Elliott and Quintino, 2007). In addition, the impossibility to establish controls replicates per zone, due to the absent of polyhaline or mesohaline waters without dredging in the same estuary, makes it necessary to interpret these results with caution. Notwithstanding, the immediate-, short- and medium-term design applied in this study, in addition with interannual comparisons, helped to discriminate different effects of a dredging operation in biological and physiochemical variables of a high fluctuating estuary as Guadalquivir.

Among the physiochemical variables, only DO and pH showed a clear influence by the dredging, increasing their levels. These observations contrast with the general assumption that sediment resuspension releases chemical substances which react with DO, reducing temporally its concentration and acidifying the water (Jones-Lee and Lee, 2005). In this case, observing the stratification of DO in the immediate approach, it is possible that mechanical perturbation as ship-propeller and cavitation, in addition to the action of trailer arm, could mix the water column with atmospheric air (Bowie et al., 1985), balancing the chemical demanding of DO, and even increasing its values. Nonetheless, DO concentration never reached levels lower than 6 mg/L during the whole study at any depth in both zones and the pH increased only 0.1, which did not seem to have a relevant negative effect in well oxygenated waters with low temperatures as reported by Jabusch et al. (2008).

On the other hand, DO depletion is also associated with persistent high turbidity levels which reduce light penetration in the water column and limit photosynthesis activity (Desmit et al., 2005). Still, Guadalquivir estuary is considered a turbid system where the primary production in the water column is scarce and constrained to the surface layer (Ruiz et al., 2015). Hence, oxygen production by phytoplankton would be little influenced in a temporal increment of turbidity. Turbidity increase, directly correlated with TSS, has been widely studied as common effect of dredging operations in coastal areas which may affect to marine biodiversity (Magris and Ban, 2019; Wenger et al., 2017), being able to cause lethal and sublethal impacts in 10–20% of fish species respectively (Wenger et al., 2018). An analysis of surface satellite images showed increments of total suspended solids (1000 mg/L approx.) in plumes during dredging operation in upper zones of the same estuary (Caballero et al., 2018). These observations coincided with the immediate profiles recorded after the water mass passed by the dredger working, when the homogenization of the water column incremented the turbidity levels on the surface. But surprisingly, we did not detect significant changes in the depth-averaged values of turbidity. Dredging operation must have necessarily increased the suspended sediment and, consequently, turbidity; but the effect seems to be spatially very local and its persistence temporally short, and we could not detect significant changes either in the short- and in the medium-term approaches. Nonetheless, turbidity, TSS, SIM and SOM concentration increased after dredging, which seems to be rather associated to higher freshwater inputs in this period. (González-Ortegón et al., 2010) reported similar observations during other freshets in the same estuary (up to 700 NTU approx.), describing adverse effects on the estuarine food web at different levels. Increasing exposure to suspended sediment may causes damage to gill tissue and structure, being more easily to clog the gills and reduce their efficiency in smaller fish and larvae (Au et al., 2004). The Guadalquivir estuary has been considered the most productive and important nursery areas of the region in spite of being the most turbid (Miró et al., 2020). The high flows originated by tides and the high chronic turbidity in the Guadalquivir estuary (Losada et al., 2017) could have overshadow the sediment resuspension effect caused by dredging in our sampling zones, and consequently, we could not observe a clear increase, nor any direct effect on species found.

The dynamics of chlorophyll concentration appear to follow a seasonal pattern related with temperature reduction, provided that dredging did not significantly modify turbidity, and consequently light penetration. However, higher values were observed in the latter cruises while temperature continued decreasing and turbidity increased. These observations could be explained by imports from upstream waters with higher primary production caused by rising freshwater inputs (González-Ortegón and Drake, 2012) and/or a higher resuspension of microphytobenthos from riversides (Díez-Minguito and de Swart, 2020; Miró et al., 2020). Also, nutrients as nitrate and ammonium, which presented similar tendencies as chlorophyll, could be imported due to sewage effluents and nitrification processes from upstream waters close to urban and agriculture areas (Mendiguchía et al., 2007). Different stages of ammonium could be found depending on pH levels, with higher un-ionized [ammonia (NH_3)] proportion associated to high pH, which it has been considered toxic for fishes (Brinkman et al., 2009). In our case, the changes of pH observed during the dredging could increase the NH_3 proportion around 0.1-0.2% (poly- and mesohaline zones respectively), which means a total concentration found of 0.1 µM NH_3 during that period. Therefore, un-ionized ammonia showed levels far from toxicity references [1.16 µM NH_3 (Eddy, 2005)]. The concentration of phosphate, whose increment is usually attributed to fertilizers via river flow (Mainstone and Parr, 2002), showed changes during the dredging in polyhaline waters, although concentrations reached were lower than levels found in mesohaline zone. Globally, we could not clearly assign an increase in inorganic nutrients due to dredging operations. In contrast, dredging has been primarily related to remobilize metals associated with sediment particles into the water column, which change its environmental conditions and promotes the shift of metals from the particulate into the dissolved state (Van Den Berg et al., 2001). This phenomena could be even more noticeable in the Guadalquivir estuary given that it received a toxic spill from Aznalcóllar mine in 1998 (Riba et al., 2002) [although ten years afterwards, studies showed that the decline in metal contamination in the area was evident (Tornero et al., 2014, 2011)]. It is known that oxidation of sulfides liberates different heavy metals because the precipitates are degraded (Caille et al., 2003). This phenomenon was observed in our case for Cr, Fe, Ni and Zn, with a cumulative trend during dredging. However, the oxidation of Fe also causes precipitation of iron-(oxo)hydroxides which could form a very strong adsorptive layer in the surface of the new dredged bottom and decrease the release of metals (Goossens and Zwolsman, 1996). On the other hand, a previous study in the Guadalquivir estuary of heavy metals concentration in the sediment of the same zones observed an increment of As, Co and Ni after dredging operations, especially in the polyhaline site (Donázar-Aramendía et al., 2018). In our water samples, higher effects were found in the same zone, where slight differences could be due to the salinity of water, the oxidation-reduction potential of the sediment and the pH of the sediment pore water and overlying water on site (Eggleton and Thomas, 2004).

Maximum values recorded in the field were lower than minimal concentration used in controlled experiments in the laboratory that test lethal effects of trace metals in early life stages of fishes (Jezierska et al., 2009). Experiments in crustacea also show that ranges measured usually do not cause significant effects on individuals (Lavolpe et al., 2004; Martin and Holdich, 1986). Thus, the metal uptake for fish and the rest macrofauna present in both zones is expected to be low in this study. Other than the effect of dredging, also the daily tidal currents, wind energies or storms in estuarine systems can cause periodical remobilization of surface sediments, releasing metals naturally (Eggleton and Thomas, 2004). The higher freshwater inputs observed after dredging, and the associated increment in TSS, could also contribute to maintain the increased values of some metals. On the other hand, some metals can be released and/or re-absorbed more readily than others (Maddock et al., 2007). Faster release and re-absorption could be happening for Ni and Cr, which increased quickly only during the dredging, and slower release and re-absorption for Zn and Fe, which reached higher levels after dredging. Also, fine sediments could remain longer in suspension and consequently liberate more metals after dredging (Maddock et al., 2007). The Guadalquivir estuary have showed high persistent turbidity events in wet years (González-Ortegón et al., 2010), and also is considered one of the estuaries in the region with higher metal fluxes (González-Ortegón et al., 2019) due to urban and agriculture supports (Mendiguchía et al., 2007). As organisms take up and accumulate trace metals during their whole life cycle, which have the potential to cause toxic effects over time (Rainbow, 2007), especial attention should be taken in metal release after higher bottom disturbance situations such as longer dredging periods or torrential freshets. Still, this is more probably a concern with longer living estuarine organisms, such as adult resident fishes, than short living ones, as most species included in this study.

Physiochemical alterations of dredging operation appear to be minor in comparison with the natural changes observed, however, other possible effects have been observed in planktonic organisms. The limited swimming capacity of small individuals could make it impossible for them to avoid the water mass affected by dredging. Therefore, the main and direct cause that could decrease densities after water mass passed through the dredger working is the hydraulic entrainment, leading to their death by the mechanical action of the suction arm (Reine and Clarke, 1998).

Different tendencies were found for epibenthic and pelagic species in the immediate approach. Epibenthic species, like *Pomatoshcistus spp.* or decapods, tended to decrease, probably due to the direct impact of physical removal of bottom sediments where epibenthic organisms inhabit (Hoffmann and Dolmer, 2000). In fact, a previous study showed that

Pomatoschistus spp. is prone to a high entrainment by a trailer suction with estimated rates between 0.0018 and 0.009 ind./m^3 (Drabble, 2012a). Also, Armstrong et al. (1982) reported that sand shrimp (*Crangon* sp.) showed the highest rates of entrainment by dredges in Pacific northwest estuaries, with a range between 0.08 and 4.44 ind./m^3, and estimating a loss of population during a dredging project around 1.2 to 6.5%. In contrast, pelagic species like *E. encrasicolus, S. pilchardus, P. incisus* or mysids tended to maintain stable their densities, or even to increase in some cases. Their behavior of inhabiting in the water column could minimize the entrainment risk. Most of studies report demersal organisms entrained (Barletta et al., 2016; Reine and Clarke, 1998), however, adult stages of pelagic species have been collected as well, including anchovy (0.001 ind./m^3), herring (0.01 ind./m^3) and smelt (0.01 ind./m^3) (Armstrong et al., 1982). In fish larvae, some authors estimated the entrainment of striped bass (*Morone saxatilis*), herring (*Alosa* spp.) and white perch (*Morone americana*), involving the simultaneous operation of four hydraulic dredges in the Delaware River, and concluded that less than 1% of the total larval population would be entrained by the dredges (Burton et al., 1992). In our case, the results obtained with not significant differences for any species in this approach suggest that entrainment caused a low incident over epibenthic species and no effects in pelagic ones.

On the other hand, the pelagic species exhibited different trends between immediate and short-medium approaches. A high decrease in density was found just after the beginning of dredging in *E. encrasicolus* and mysids in the polyhaline zone, suggesting that this disturbance could affect to these species. However, interannual comparisons found that their densities can fluctuate similarly without dredging (anchovy in mesohaline zone of 2015) or long after the dredging activity (macrofauna in polyhaline zone of 2016). Also, these organisms showed low and stable values (anchovy in polyhaline zone of 2015-16) or decreased gradually (anchovy and macrofauna in mesohaline zone of 2015-16) along this season, either in years with or without dredging operations. The goby showed different trends, decreasing its density notably in the mesohaline zone of 2015, when there was not any dredging operation, and even increasing during and after the dredging in the polyhaline zone of 2016. These patterns make difficult to elucidate whether these changes correspond to natural variability and reduction, typical for this period (Drake et al., 2002), or they are an impact of dredging. A long monitoring study of several years in Eastern English Channel (UK), which is dredged annually, observed a temporal and gradually reduction in several species, including *Pomatoschistus* spp. (Drabble, 2012b). In our case, no clear differences were found between the seasonal densities of previous years. Some of these species inhabits across the whole estuary section, with higher densities in the shallower banks of the Guadalquivir estuary (Miró et al., unpublished). Further, most of species found were marine migrant, as *E. encrasicolus, S. pilchardus, S. senegalensis*, etc., which locate their spawning zone offshore (Baldó et al., 2006). These behaviors could minimize the mechanical impact of the dredging, which was carried out into the estuary and only in the middle channel, constraining the impact in nursery function.

In summary, the observed modifications of water physiochemical variables in comparison with natural changes as freshets, the no significant differences found in the immediate approach and the similar temporal fluctuations of density as previous years with or without dredging suggest that this dredging operation did not cause a severe impact in the Guadalquivir estuary. Still, this does not mean that there is not any effect. This ecosystem showed high natural fluctuations which did not make possible to clearly associate the variations observed to the dredging effects, leading to the 'Estuarine Quality Paradox' (Elliott and Quintino, 2007). Nonetheless, as the possible effects of this disturbance were of the same order or less than natural ones, planktonic organisms could be well-adapted to cope with them. Still, polyhaline water mass showed more notorious effects than mesohaline waters. In addition, the dredging operation was undertaken during the natural decline period of recruitment, which could minimize the effects over the nursery function. However, a dredging activity during the main recruitment period and larval development [March to November in the case of Guadalquivir estuary; (Drake et al., 2007, 2002)], could directly constrain larval supply by contributing to higher mortality rates of larvae or lowering recruitment success (Wenger et al., 2017). The evidence found here and the changes detected made advisable to implement systematic monitoring programs to any dredging project. The accumulated experience and the use of new approaches may allow to detect the nature of the effect of these operations more clearly, allowing the design of specific control strategies to mitigate impacts, as well as a thorough evaluation of the effectiveness of these strategies (Wenger et al., 2018), promoting sustainable fishery management.

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Supplementary Data

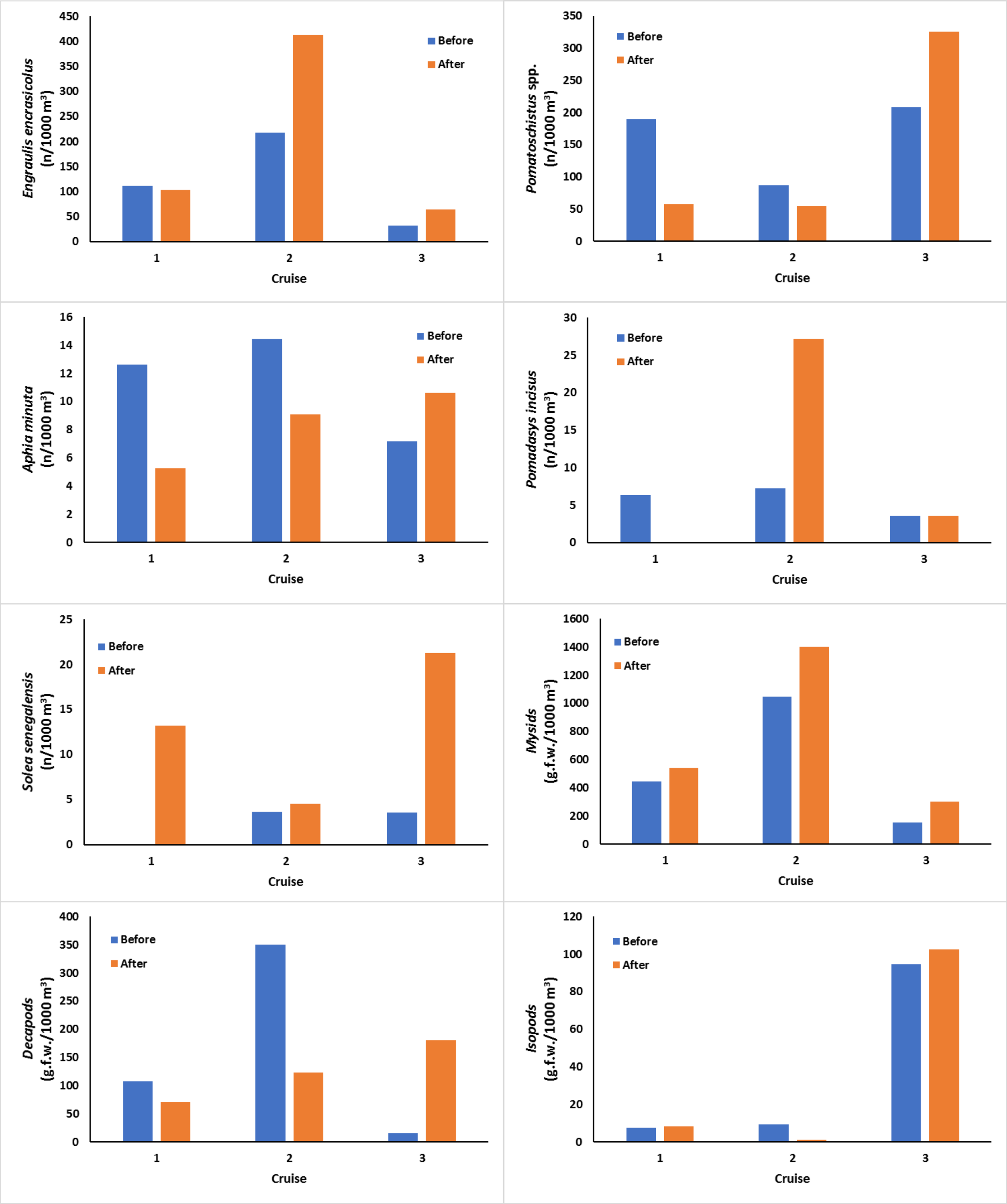

Figure S.1. Abundance of main early fish stages and the rest of macrofauna groups captured before and after the water mass passed by the dredger working in every cruise.

Table S.1.1. Pairwise comparisons to evaluate the water physiochemical variables among the different periods of the dredging (Before, During and After) in every zone (polyhaline and mesohaline). Significant terms p ≤ 0.05 are highlighted in bold.

POLYHALINE ZONE						**MESOHALINE ZONE**					
Turbity	estimate	SE	df	t.ratio	p.value	**Turbity**	estimate	SE	df	t.ratio	p.value
Before-During	23	21.4	6.31	1.078	0.5591	Before-During	97.2	59.9	6.66	1.623	0.301
Before-After	-124	32.9	8.37	-3.777	**0.0124**	Before-After	-439	265.1	4.93	-1.656	0.3088
During-After	-147	27.5	5.51	-5.356	**0.0053**	During-After	-536.2	260.2	4.62	-2.061	0.1999
pH	estimate	SE	df	t.ratio	p.value	**pH**	estimate	SE	df	t.ratio	p.value
Before-During	-0.0869	0.0217	10.75	-4.013	**0.0055**	Before-During	-0.0783	0.0192	9.22	-4.085	**0.0066**
Before-After	-0.0221	0.0249	8.09	-0.886	0.6636	Before-After	0.0303	0.0281	5.66	1.079	0.5617
During-After	0.0649	0.0261	9.9	2.482	0.0766	During-After	0.1086	0.0312	8.43	3.483	**0.0188**
Temperature	estimate	SE	df	t.ratio	p.value	**Temperature**	estimate	SE	df	t.ratio	p.value
Before-During	5.28	1.147	1.42E+05	4.604	**<.0001**	Before-During	5.68	1.18	4.52	4.801	**0.0142**
Before-After	8.56	1.166	2.90E+03	7.344	**<.0001**	Before-After	8.96	1.21	4.95	7.393	**0.0017**
During-After	3.28	0.222	5.12E+00	14.753	**0.0001**	During-After	3.28	0.27	4.89	12.129	**0.0002**
Chla (Multi)	estimate	SE	df	t.ratio	p.value	**Chla (Multi)**	estimate	SE	df	t.ratio	p.value
Before-During	1.192	0.386	6.64	3.084	**0.0439**	Before-During	1.759	0.247	5.41	7.11	**0.0015**
Before-After	1.045	0.405	7.11	2.579	0.0817	Before-After	1.446	0.628	6	2.303	0.1314
During-After	-0.147	0.253	9.73	-0.58	0.8339	During-After	-0.313	0.585	4.68	-0.535	0.8583
DO	estimate	SE	df	t.ratio	p.value	**DO**	estimate	SE	df	t.ratio	p.value
Before-During	-1.052	0.1889	6.04	-5.569	**0.0034**	Before-During	-1.203	0.213	6.28	-5.633	**0.0028**
Before-After	-1.886	0.1859	5.45	-10.144	**0.0002**	Before-After	-2.076	0.21	5.62	-9.902	**0.0002**
During-After	-0.833	0.0894	10.63	-9.321	**<.0001**	During-After	-0.874	0.106	10.62	-8.217	**<.0001**
Salinity	estimate	SE	df	t.ratio	p.value	**Salinity**	estimate	SE	df	t.ratio	p.value
Before-During	-0.544	0.599	8.47	-0.908	0.6499	Before-During	0.731	0.679	5.48	1.077	0.5636
Before-After	3.089	1.297	6.03	2.381	0.1184	Before-After	1.379	1.848	5.74	0.746	0.7472
During-After	3.632	1.24	5.29	2.928	0.0674	During-After	0.648	1.742	4.64	0.372	0.9276

Table S.1.2. Pairwise comparisons to evaluate the water physiochemical variables among the different periods of the dredging (Before, During and After) in every zone (polyhaline and mesohaline). Significant terms p ≤ 0.05 are highlighted in bold.

POLYHALINE ZONE						MESOHALINE ZONE					
TSS	estimate	SE	df	t.ratio	p.value	**TSS**	estimate	SE	df	t.ratio	p.value
Before-During	4.39	20.7	9.43	0.212	0.9757	Before-During	100	47.4	6.11	2.12	0.1644
Before-After	-114.54	26	8.41	-4.398	**0.0051**	Before-After	-401	218	4.89	-1.839	0.2516
During-After	-118.93	23.9	8.19	-4.979	**0.0026**	During-After	-501	214.2	4.57	-2.34	0.1472
SIM	estimate	SE	df	t.ratio	p.value	**SIM**	estimate	SE	df	t.ratio	p.value
Before-During	0.01752	0.01044	9.45	1.678	0.263	Before-During	0.01463	0.00969	8.48	1.51	0.3341
Before-After	0.00101	0.00987	7.98	0.102	0.9943	Before-After	-0.00965	0.01838	5.01	-0.525	0.8629
During-After	-0.01651	0.01225	11.14	-1.348	0.3994	During-After	-0.02428	0.01995	7.08	-1.217	0.4805
SOM	estimate	SE	df	t.ratio	p.value	**SOM**	estimate	SE	df	t.ratio	p.value
Before-During	-0.01752	0.01044	9.45	-1.678	0.263	Before-During	-0.01463	0.00969	8.48	-1.51	0.3341
Before-After	-0.00101	0.00987	7.98	-0.102	0.9943	Before-After	0.00965	0.01838	5	0.525	0.8629
During-After	0.01651	0.01225	11.14	1.348	0.3994	During-After	0.02428	0.01994	7.07	1.217	0.4805
NO2	estimate	SE	df	t.ratio	p.value	**NO2**	estimate	SE	df	t.ratio	p.value
Before-During	-0.0565	0.158	9.07	-0.357	0.9328	Before-During	0.2068	0.1496	5.74	1.383	0.4088
Before-After	0.1903	0.163	7.84	1.165	0.505	Before-After	0.2193	0.1461	5.01	1.501	0.366
During-After	0.2468	0.127	8.99	1.938	0.1839	During-After	0.0124	0.0607	9.99	0.205	0.9772
NO3	estimate	SE	df	t.ratio	p.value	**NO3**	estimate	SE	df	t.ratio	p.value
Before-During	-17.5	4.44	4.69	-3.942	**0.0276**	Before-During	-4.68	9.95	5.37	-0.47	0.8876
Before-After	-53.6	9.92	6.19	-5.405	**0.0036**	Before-After	-118.04	43.25	4.88	-2.729	0.091
During-After	-36.1	9.59	5.7	-3.767	**0.0237**	During-After	-113.36	42.83	4.73	-2.646	0.1025
NH4	estimate	SE	df	t.ratio	p.value	**NH4**	estimate	SE	df	t.ratio	p.value
Before-During	-0.339	1.29	5.35	-0.262	0.9631	Before-During	-0.781	0.657	12.69	-1.189	0.4805
Before-After	-1.44	2.33	7.65	-0.617	0.8154	Before-After	-2.484	0.936	7.82	-2.654	0.0686
During-After	-1.1	2	4.83	-0.55	0.8513	During-After	-1.703	0.818	6.26	-2.082	0.1708
PO4	estimate	SE	df	t.ratio	p.value	**PO4**	estimate	SE	df	t.ratio	p.value
Before-During	-0.347	0.147	7.85	-2.359	0.105	Before-During	-0.185	0.381	7.11	-0.486	0.8802
Before-After	-0.234	0.151	4.18	-1.547	0.361	Before-After	-0.503	0.427	6.9	-1.176	0.5029
During-After	0.113	0.162	6.55	0.699	0.7723	During-After	-0.317	0.273	3.62	-1.163	0.5366
SiO4	estimate	SE	df	t.ratio	p.value	**SiO4**	estimate	SE	df	t.ratio	p.value
Before-During	-0.968	2.46	6.99	-0.393	0.9192	Before-During	-3.569	9.33	10.32	-0.382	0.9231
Before-After	-2.392	7.29	5.04	-0.328	0.9431	Before-After	-3.316	10.12	4.57	-0.328	0.9433
During-After	-1.424	7.34	5.24	-0.194	0.9796	During-After	0.252	9.62	5.07	0.026	0.9996

Table S.2.1. Pairwise comparisons to evaluate the trace metal concentration among the different periods of the dredging (Before, During and After) in the polyhaline zone. Significant terms p ≤ 0.05 are highlighted in bold.

POLYHALINE ZONE

As	estimate	SE	df	t.ratio	p.value
Before-During	0.00368	0.0157	8.05	0.234	0.9704
Before-After	-0.00262	0.0157	4.19	-0.167	0.9849
During-After	-0.0063	0.0173	6.83	-0.365	0.9299

Cd	estimate	SE	df	t.ratio	p.value
Before-During	2.23E-04	0.000423	8.69	0.529	0.8596
Before-After	5.25E-05	0.000473	4.88	0.111	0.9932
During-After	-1.71E-04	0.000425	4.7	-0.402	0.9163

Cr	estimate	SE	df	t.ratio	p.value
Before-During	-0.00231	0.00104	8.12	-2.221	0.1261
Before-After	-0.00829	0.00159	5.39	-5.228	**0.0064**
During-After	-0.00598	0.0018	8.58	-3.318	**0.0234**

Co	estimate	SE	df	t.ratio	p.value
Before-During	-0.00133	0.00103	5.01	-1.287	0.4602
Before-After	-0.000817	0.0014	6.25	-0.582	0.8344
During-After	0.000513	0.00127	6.99	0.405	0.9147

Cu	estimate	SE	df	t.ratio	p.value
Before-During	-3.25E-03	0.00163	5.13	-1.995	0.2064
Before-After	-3.18E-03	0.0021	4.32	-1.511	0.3721
During-After	7.03E-05	0.00188	4.01	0.037	0.9992

Fe	estimate	SE	df	t.ratio	p.value
Before-During	-0.0154	0.00565	4.75	-2.729	0.0932
Before-After	-0.0459	0.01156	5.96	-3.971	**0.0175**
During-After	-0.0305	0.01109	5.45	-2.748	0.081

Ni	estimate	SE	df	t.ratio	p.value
Before-During	0.000288	0.00201	8.13	0.143	0.9888
Before-After	-0.005139	0.00276	8.06	-1.862	0.2109
During-After	-0.005427	0.00251	7.54	-2.159	0.1425

Pb	estimate	SE	df	t.ratio	p.value
Before-During	0.0059	0.00861	7.68	0.686	0.7785
Before-After	0.00475	0.01002	6.28	0.474	0.8857
During-After	-0.00115	0.00725	3.63	-0.159	0.9862

Zn	estimate	SE	df	t.ratio	p.value
Before-During	-0.01465	0.00507	7.06	-2.889	0.0535
Before-After	-0.01689	0.00288	6.01	-5.861	**0.0026**
During-After	-0.00223	0.00554	8.79	-0.403	0.9152

Table S.2.2. Pairwise comparisons to evaluate the trace metal concentration among the different periods of the dredging (Before, During and After) in the mesohaline zone. Significant terms p ≤ 0.05 are highlighted in bold.

MESOHALINE ZONE

As	estimate	SE	df	t.ratio	p.value
Before-During	0.0102	0.0105	5.42	0.972	0.6218
Before-After	0.0115	0.0135	4.4	0.851	0.6932
During-After	0.0013	0.0121	3.98	0.108	0.9936
Cd	estimate	SE	df	t.ratio	p.value
Before-During	4.67E-04	0.000423	7.06	1.103	0.5419
Before-After	5.36E-04	0.000519	4.36	1.033	0.5948
During-After	6.91E-05	0.000459	3.67	0.151	0.9876
Cr	estimate	SE	df	t.ratio	p.value
Before-During	-0.00492	0.00345	8.02	-1.428	0.3724
Before-After	-0.00811	0.00346	4.2	-2.344	0.1545
During-After	-0.00318	0.00378	6.81	-0.842	0.6914
Co	estimate	SE	df	t.ratio	p.value
Before-During	-0.000193	0.000868	5.13	-0.223	0.9732
Before-After	-0.000797	0.001121	4.32	-0.711	0.7697
During-After	-0.000604	0.001005	4.01	-0.601	0.8273
Cu	estimate	SE	df	t.ratio	p.value
Before-During	-0.00087	0.00158	4.67	-0.55	0.8513
Before-After	-0.00199	0.00281	6.91	-0.708	0.7666
During-After	-0.00112	0.00266	6.56	-0.422	0.9079
Fe	estimate	SE	df	t.ratio	p.value
Before-During	-0.0139	0.0191	5.6	-0.727	0.7581
Before-After	-0.0552	0.0272	6.18	-2.026	0.1846
During-After	-0.0412	0.0265	7	-1.558	0.3234
Ni	estimate	SE	df	t.ratio	p.value
Before-During	-0.00312	0.00375	5.78	-0.833	0.6987
Before-After	-0.00446	0.00457	4.19	-0.977	0.6261
During-After	-0.00134	0.00428	4.5	-0.312	0.9483
Pb	estimate	SE	df	t.ratio	p.value
Before-During	-0.000326	0.00662	7.46	-0.049	0.9987
Before-After	0.005565	0.00827	4.83	0.673	0.7887
During-After	0.005891	0.00703	3.75	0.838	0.7036
Zn	estimate	SE	df	t.ratio	p.value
Before-During	-0.00812	0.0065	8.41	-1.249	0.4582
Before-After	-0.03022	0.0103	5.46	-2.941	0.0643
During-After	-0.02211	0.0111	7.48	-1.986	0.1818

Table S.3. Pairwise comparisons to evaluate the most abundant early fish stages and macrofauna groups among the different periods of the dredging (Before, During and After) in every zone (polyhaline and mesohaline). Significant terms p ≤ 0.05 are highlighted in bold.

POLYHALINE ZONE						MESOHALINE ZONE					
Engraulis encrasicolus	estimate	SE	df	z.ratio	p.value	***Engraulis encrasicolus***	estimate	SE	df	z.ratio	p.value
Before-During	2.53	0.588	Inf	4.301	**0.0001**	Before-During	0.788	0.348	Inf	2.265	0.0608
Before-After	5.39	1.337	Inf	4.032	**0.0002**	Before-After	4.66	1.631	Inf	2.857	**0.0119**
During-After	2.86	1.431	Inf	1.999	0.1124	During-After	3.872	1.653	Inf	2.342	0.0502
***Pomatoschistus* spp.**	estimate	SE	df	z.ratio	p.value	***Pomatoschistus* spp.**	estimate	SE	df	z.ratio	p.value
Before-During	0.7359	0.534	Inf	1.377	0.353	Before-During	0.138	0.479	Inf	0.288	0.9553
Before-After	0.8053	0.609	Inf	1.323	0.3824	Before-After	0.763	0.502	Inf	1.522	0.2807
During-After	0.0694	0.659	Inf	0.105	0.9939	During-After	0.625	0.48	Inf	1.304	0.3932
Mysids	estimate	SE	df	z.ratio	p.value	**Mysids**	estimate	SE	df	z.ratio	p.value
Before-During	156	62.4	2.52	2.496	0.1915	Before-During	113.5	47	5.91	2.414	0.115
Before-After	273	63.7	2.66	4.28	0.0577	Before-After	159.2	35	3.37	4.55	**0.0321**
During-After	117	32.6	4.59	3.586	**0.0401**	During-After	45.7	41.5	5.55	1.1	0.5507
Decapods	estimate	SE	df	z.ratio	p.value	**Decapods**	estimate	SE	df	z.ratio	p.value
Before-During	40.5	33	3.15	1.229	0.5136	Before-During	13.4	7.21	3.09	1.857	0.2907
Before-After	68.8	30.2	2.27	2.28	0.2407	Before-After	9.48	9.42	3.98	1.006	0.6122
During-After	28.2	17.2	5.48	1.64	0.3071	During-After	-3.92	7.66	2.94	-0.512	0.8714
Isopods	estimate	SE	df	z.ratio	p.value	**Isopods**	estimate	SE	df	z.ratio	p.value
Before-During	2.148	0.808	3.17	2.659	0.1409	Before-During	16.89	6.11	3	2.765	0.1356
Before-After	0.363	1.675	2.83	0.216	0.9747	Before-After	7.05	7.66	3.99	0.92	0.6581
During-After	-1.786	1.566	2.26	-1.14	0.5748	During-After	-9.84	6	3.04	-1.641	0.356

4. EFFECTS OF FRESHET EVENTS ON THE BIOTIC AND ABIOTIC CONDITIONS IN A WELL-MIXED ESTUARY

Abstract

Freshwater input is one of the most important variables to generate elevated productivity in estuaries, influencing over many biogeochemical processes. However, when high discharges happen, it may also affect estuarine biota by altering its water physiochemical variables and displacing the organisms. The present study investigates the short-term effects of three different kind of freshets events on the early life stages of fish species, the rest of macrofauna and the environmental conditions in the Guadalquivir estuary. Freshet events generally produced a strong shift in physiochemical conditions of most the estuary section, compressing salinity gradient and increasing turbidity, and even decreasing oxygen concentration in some cases. This alteration influenced in the abundance and distribution of early life stages of fishes and the rest of macrofauna, reducing its nursery area. Still, the physicochemical conditions showed an almost complete or partial recovery (depending on the intensity, duration and period of the discharge) to the previous state in a short term (one month after the freshet approx.). Instead, different biological responses were found depending on the species and periods, such as displacement downstream, flushing out of the estuary or cue-guided attraction. In general, the estuarine species like gobies and other macrofauna groups coped better with the freshets than marine species like anchovies. Nonetheless, despite of the multiple environmental disturbances caused by the distinct natural freshet events, the estuary and their estuarine organisms showed a high resilience.

Resumen

El aporte de agua dulce es una de las variables más importantes para generar una elevada productividad en los estuarios, influyendo en muchos procesos biogeoquímicos. Sin embargo, cuando ocurren altas descargas, también puede afectar la biota estuarina alterando las variables fisicoquímicas del agua y desplazando a los organismos. El presente estudio investiga los efectos a corto plazo de tres tipos diferentes de eventos de alta descarga en las larvas y juveniles de peces, el resto de la macrofauna y las condiciones ambientales en el estuario del Guadalquivir. Las avenidas generalmente produjeron un fuerte cambio en las condiciones fisicoquímicas de la mayor parte de la sección del estuario, comprimiendo el gradiente de salinidad y aumentando la turbidez, e incluso disminuyendo la concentración de oxígeno en algunos casos. Esta alteración influyó en la abundancia y distribución de los peces y el resto de la macrofauna, reduciendo su área de cría. A pesar de ello, las condiciones fisicoquímicas mostraron una recuperación casi completa o parcial (dependiendo de la intensidad, duración y período de la avenida) al estado anterior en un corto periodo de tiempo (un mes después de la alta descarga aprox.). En cambio, se encontraron diferentes respuestas biológicas según la especie y los períodos, como fue el desplazamiento aguas abajo, la expulsión fuera del estuario o la atracción guiada por señales. En general, las especies estuarinas como los gobios y otros grupos de macrofauna se enfrentaron mejor a las avenidas que las especies marinas como el boquerón. No obstante, a pesar de las múltiples perturbaciones ambientales causadas por los distintos eventos de descargas naturales, el estuario y sus organismos estuarinos mostraron una alta resiliencia.

1. Introduction

Estuarine environments are regions at the interface of riverine and marine systems and are critical habitats for many resident and migratory species (Day et al., 2013). They may support high abundances of organisms due to their high productivity, playing an essential role in the nursery function of many species, especially for marine fishes (Strydom et al., 2003). These ecosystems can be highly dynamic and variable with vertical and horizontal gradients in salinity, temperature, dissolved oxygen and turbidity (Garel and Ferreira, 2015; Navarro et al., 2011). One of the main factors which explains much of the fluctuation in the estuarine dynamic is the freshwater inputs (Gillanders and Kingsford, 2002). Moreover, river flow brings nutrients and organic material into estuarine systems, which could increase primary and secondary production (Schlacher and Wooldridge, 1996; Statham, 2012), improving estuarine fish stocks. Thus, the intensity, duration and period of occurrence of the freshwater discharges influence notably in physiochemical structure of the habitat and biological composition of the community (Whitfield, 1994).

Notwithstanding, when high discharges happen due to heavy rainfalls, it may also influence estuarine biota by altering salinity gradients, pushing downstream suspended particulates and small organisms or even flushing them out of the estuary (Chícharo et al., 2006; González-Ortegón and Drake, 2011). The freshwater input during a freshet event generates currents much faster than usual which could be considered extreme environment for fish larvae (Teodósio and Garel, 2015). Advective forcing of small organisms from the inner zones of estuary, which offer refugee and suitable conditions for enhanced development, to nearshore areas could facilitate their predation and/or reduce their food availability (Axler et al., 2020). Also, external water currents may transport them to remote areas (Cowen et al., 2006), avoiding the possibility to return inside after the event. Even rapid salinity fluctuations represent a significant stress for marine organism, which depending on its osmoregulatory ability and its behavioural response, can be lethal (Serafy et al., 1997).

Interpreting the link between freshwater flows, environmental gradients and plankton drift within estuaries is crucial to its management and conservation as they undergo transformations due to climate change, particularly in regulated river systems (Bates et al., 2008; Hughes, 2003). This is the case of the Guadalquivir estuary, which is located in a Mediterranean climate region, where the predictions consider a decrease in mean annual precipitations, but the intensity of rainfall events will increase (Filippo and Lionello, 2008). Its freshwater inputs show an interannual variability in river discharges extremely high since the construction of a dam in 1930, although significant decreasing trend in the dam's discharges has been observed in the last 80 years (González-Ortegón et al., 2012). The river flow has an immediate effect on the estuarine salinity gradient, displacing it either seaward or upstream, being considered the most influential factor in the community structure and distribution (Fernández-Delgado et al., 2007). Previous studies have shown distinct approaches to analyse the effects of different hydrological regimes in the environmental characteristics of its inner estuarine zone [e.g. Drake et al. (2002); González-Ortegón et al. (2015, 2010)]. However, there are not studies on short-term effect in the inner and outer zones of the estuary during freshet events. In fact, although some studies have analysed the runoff effects in mesozooplankton assemblages in other estuaries [e.g. Hitchcock et al. (2016); Hoover et al. (2006); Ueda et al. (2004)], no studies have focused on early life stages of fish to our knowledge.

Estuarine communities are exposed to a variety of scales of spatial and temporal variability in these changeable systems, and the role of short-term processes, like freshets, are essential to understand the recruitment ecology of estuarine-dependent organisms. Thus, the present study aimed to investigate the short-term effects of three different kind of freshets events on the early life stages of fish species, the rest of macrofauna and the water physiochemical conditions, using the Guadalquivir estuary and its nearshore area as example.

2. Material and methods

2.1. Study area

The Guadalquivir estuary is located in South-West of Iberian Peninsula, in a particular temperate North Atlantic region at the entrance of the Mediterranean Sea (Figure 1). It extends 110 km inland from its mouth and it presents a convergent morphology with widths of 800 m near the mouth and 150 m at the head (Díez-Minguito et al., 2012). The main channel is mostly isolated of surrounding natural areas, with a navigable channel of 7.1 m average depth (Ruiz et al., 2015). It is a flood-dominated and well-mixed mesotidal system with 3.5 m amplitude range (spring tides) in the river mouth (Díez-Minguito et al., 2012), which presents a longitudinal salinity gradient with temporal displacement by tides, discharges and seasonal variations (González-Ortegón et al., 2014). Normally, it is a flood dominated system with discharges lower than 40 m^3/s, although it can change to fluvial-dominated if the freshwater inputs are higher than 400 m^3/s (Díez-Minguito et al., 2012). Its waters flow to the Gulf of Cádiz, and the freshwater input into the estuary from the Guadalquivir River (680 km long and 57,527 km^2 basin area) is controlled by Alcalá del Río Dam.

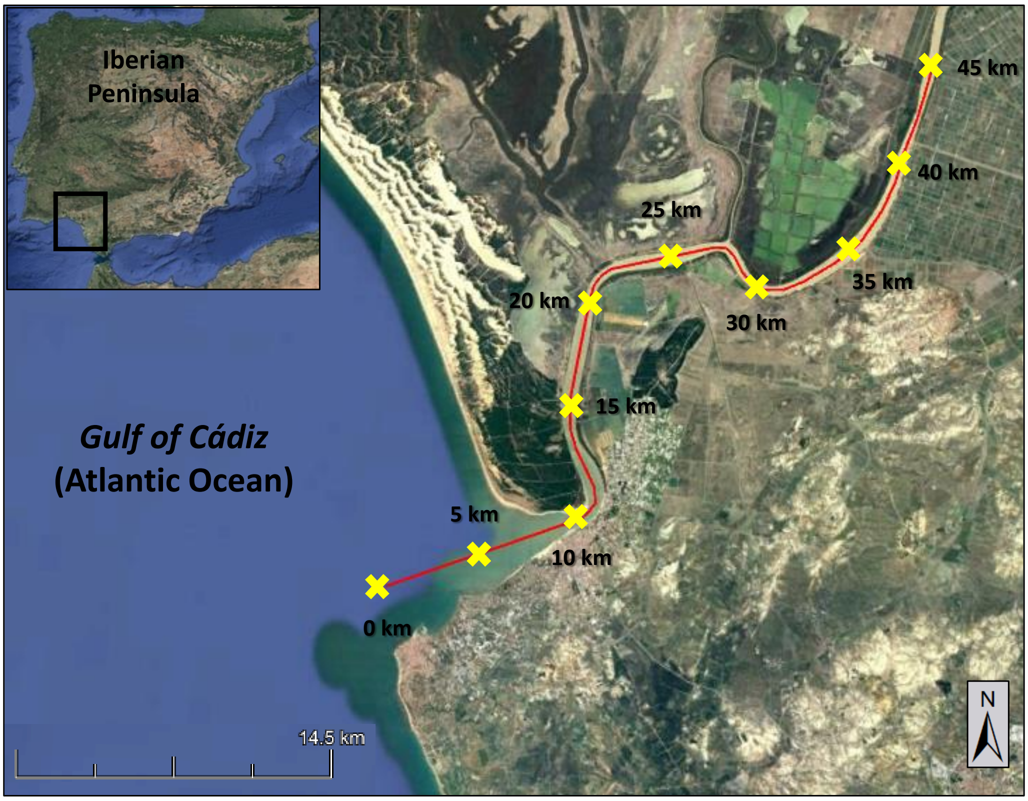

Figure 1. Study area of the Guadalquivir estuary with the distance section (kilometres) from outer station (0 km).

2.2. Field sampling

To analyze the short-term effect of high discharges in the whole water column, biological samples (early life stages of fish and macrozooplankton) and samples physiochemical variables were collected during high freshwater inputs.

The study was carried out inside of a monitoring program from October 2015 to September 2018, where samples were taken monthly. During this period, three events of high discharges were analysed. Freshwater input in the Guadalquivir estuary is registered and published in real time by Alcalá del Río station (data provided by Confederación Hidrográfica del Guadalquivir, http://www.chguadalquivir.es/saih/DatosHistoricos.aspx). When the freshwater input was at least 200 m^3/s for 24 hours, samples were taken as soon as the weather conditions allowed it. Thereafter, two more sampling times were carried out along the next month to analyse the short-term effect. Also, the monthly sample of the monitoring program before the high discharge were used to compare with the previous conditions. Additionally, in order to analyze the seasonal trends on abundances in the main organisms found in the estuary, comparisons with the monthly monitoring sampling were done using the same zones and periods of the year of the high discharge events, but in different years without any freshet event.

Samples were taken in 4 stations along the longitudinal section with 3-5 replicates of each one (Figure 1). One station was located in the outer zone of the estuary (≈36 PSU), close to the estuary mouth, and the other stations were spread along the salinity gradient, always sampling the water masses of 25, 15 and 5 PSU regardless of the geographical position it occupies by the tidal or water flow dynamic. We chose 5 PSU as the upper limit station in normal conditions (with low inflow) because previous studies, as well as our own preliminary study before the onset of the monitoring program, determined this isohaline as the end of the nursery for marine recruits in the Guadalquivir estuary, being negligible the density of early life stages of fishes upstream (Fernández-Delgado et al., 2007). During the high discharge, depending on the salinity alteration, samples were spread along the salinity gradient until reach freshwaters (≈0 PSU).

Samples were always collected in the flood tide with a plankton net of 1 m diameter and 1 mm mesh size equipped with a flow-meter General Oceanics 2030R. Oblique tows of 10-12 min (363 ± 82m^3; mean ± SD) were done with a boat at a speed of 2–2.5 knots. Samples were fixed in 70% ethanol and the early fish stages were sorted from the rest of macrofauna (mainly mysids, followed of decapods and isopods). Fishes were identified and quantified whenever possible, to species level, and macrofauna biomass was calculated in fresh weight.

Three replicated physicochemical profiles of the whole water column were recorded in every station with a multiprobe (depth, temperature, salinity, turbidity, dissolved oxygen concentration (DO), oxygen saturation (OS) and pH; Eureka™ Manta2). If the turbidity levels overcome the limit of the sensor (5400 NTU), water samples were taken at mid-depth with a Niskin bottle to measure total suspended solids (TSS). To measure total suspended solids, water was filtered through 0.7 µm pore pre-dried (24 h, 60 °C) filters (Whatman GF/F); thereafter filters were dried (24 h, 60 °C) and weighted.

2.3. Data analysis

A single profile for every physicochemical variable recorded with the multiprobe during the high discharge event was obtained with a generalised additive mixed model fitted in every station, using the replicated individual profiles as random and obtaining the common smoother (Zuur et al., 2015). This allows an appropriate single profile to represent the whole water column of every station. Results of physiochemical profiles of the water column along the estuary section were plotted in three dimensional figures with Ocean Data View software using “Weighted-average” as gridding method (Schlitzer, 2020).

Fish abundances were standardised by filtered volume and expressed as number of individuals/1000 m^3. Density data were organized in a species/sample abundance matrix, and a Bray–Curtis similarity matrix was calculated on fourth-root-transformed data as a distance measure among samples (Bray and Curtis, 1957). To identify the early fish species that most contributed to the similarity among the different periods of every freshet event, a Similarity Percentages [SIMPER (Clarke, 1993)] analysis was performed. General Additive Models [GAMs: (Hastie and Tibshirani, 1990)] were used to investigate the influence of freshets on the abundance and distribution of these most contributor species, in addition to the total early fish stages and the rest of macrofauna, in every event. The primary purpose of the GAM model was to implement the effects of the spatial distribution to allow temporal contrasts between abundances that were not confounded by the salinity gradient along the estuary section. We also used GAMs because the nature of relationships between distance and abundance were not necessarily linear. The experimental design included: one parametric factor "Time", with 4 levels in Freshet period ("Before, During, After 1 and After 2") and 3 levels in Control period (Month 1, Month 2 and Month 3 [equivalent to Before, After 1 and After 2 respectively]) and one spline smoothing function of the covariate "distance" interacting with every level of Time factor. The sampling units were the 3–5 replicate tows taken at each salinity station located at different distances along the longitudinal section of the estuary. Normal distribution was the most adequate for the biomass of the macrofauna, and the negative binomial distribution for count data of total early fish stages and more contributor species with the log of filtered volume as the offset variable. The cases with an excess of zero counts, the zero-inflated Poisson was selected. *Post hoc* pair wise of distinct levels of Time was analysed using the Tukey correction for multiple comparisons.

All spatio-temporal figures were performed using the package 'ggplot2', the statistical analysis of GAMs with the 'mgcv' and the multiple comparisons with 'emmeans' (Lenth, 2018) of the R 3.5.2 software (R Core Team, 2018). SIMPER analyses were carried out with PRIMERv6.1.11 and PERMANOVA+ v1.0.1 statistical package (Clarke and Gorley, 2006).

3. Results

3.1. Event 1 (discharge 2015)

Precipitations during the month of October 2015 were frequent, reaching values of 25 mm/day on several occasions (Figure 2). Freshwater input was lower than 50 m^3/s during the whole period except for the flooding at the beginning of November 2015, which reached discharges higher than 200 m^3/s. This event was a "pulse" with a short temporal freshet of 2 days.

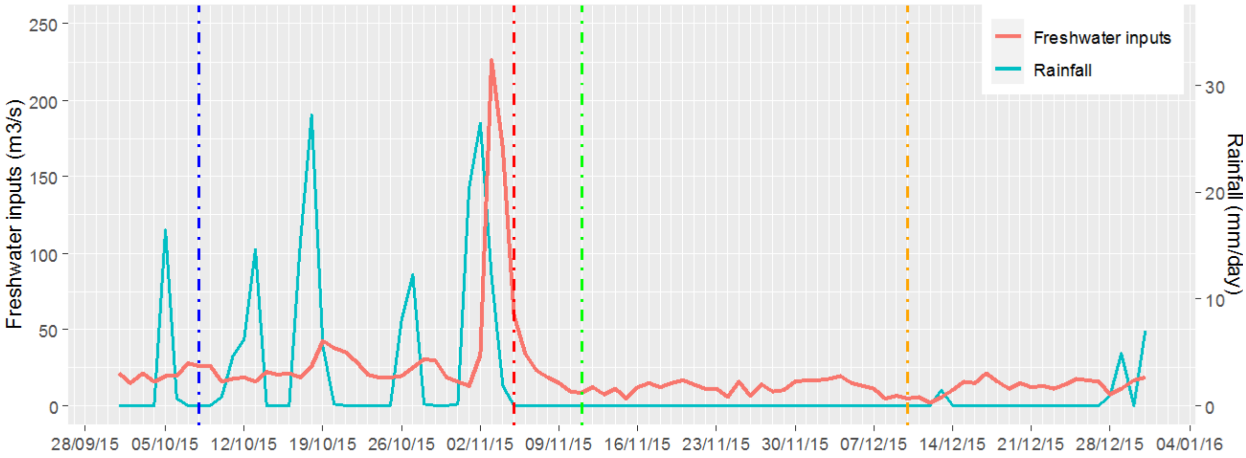

Figure 2. Temporal series of freshwater inputs in the Guadalquivir estuary and local rainfall from Alcalá del Río station during the event 1 in 2015. Vertical dashed lines indicate sampling dates [Blue: Before (08/10/15); Red: During (05/11/15); Green: After 1 (11/11/15); Orange: After 2 (11/12/15)].

Physiochemical variables showed spatio-temporal variations during the whole event (Figure 3). No results were obtained in the sampling day of After 2 due to technical problems with the multiprobe. Salinity gradient was compressed longitudinally close to the river mouth just after the high discharge. The isohaline of 1 PSU was registered 30 Km upstream. Salinity showed a strong vertical stratification with more than 15 PSU of differences between the surface and bottom layers in some zones. The isohaline of 5 PSU (and hence the practical space available for nursery function) almost recovered its position 6 days afterwards. However, the longitudinal and vertical structure of the salinity gradient maintained altered, mostly in downstream zones. Turbidity was gradually higher in upstream zones, where the discharge provoked a notable increase in the bottom, reaching values of 3500 NTU, although they restored partially in After 1. Before the high discharge pulse, pH was gradually lower downstream. During the elevated discharge, pH decreased and was homogenized along the longitudinal section. This lower pH was still held in the estuarine zone thereafter. The oxygen showed higher concentrations in upstream zones before the freshet. The concentration increased during the discharge in the whole sampling section, especially in the surface layers of the middle zone (5 and 30 Km). The oxygen saturation, which was homogeneous along the longitudinal section before the freshet, also changed afterwards, showing a gradual increment towards the estuary inlet. The temperature showed a temporal decline, with softer changes in the outer zone.

A total of 21 species of early life stages of fish were found, 15 in the freshet event and 17 in the control period. SIMPER analysis showed the characteristics species for both study

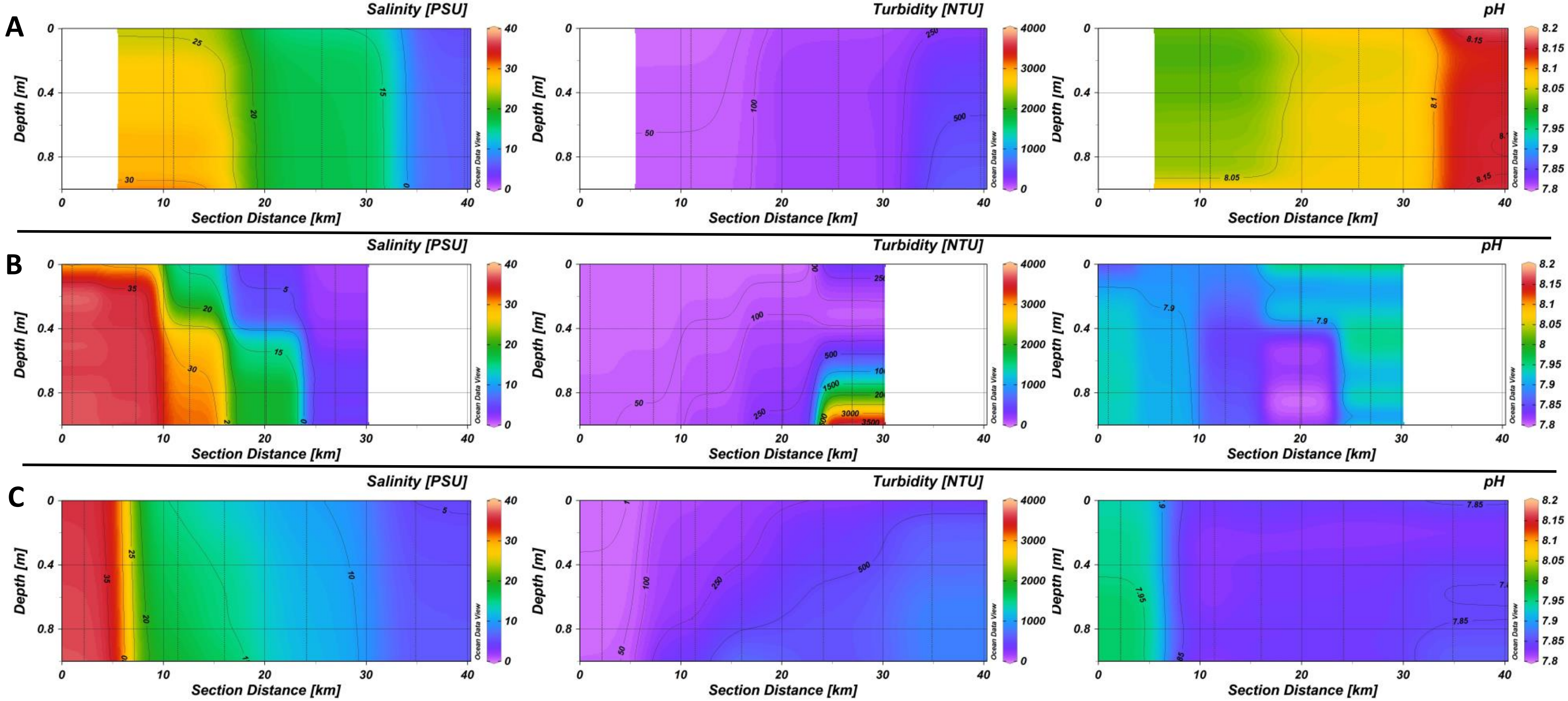

Figure 3.1. Physiochemical variables (salinity, turbidity and pH) recorded in the water column (normalize depth) along the longitudinal section of the estuary from the river mouth to the most upstream sampling station during the different sampling dates [A (Before: 08/10/15), B (During: 05/11/15), C (After 1: 11/11/15)] of the high discharge event 1 in 2015.

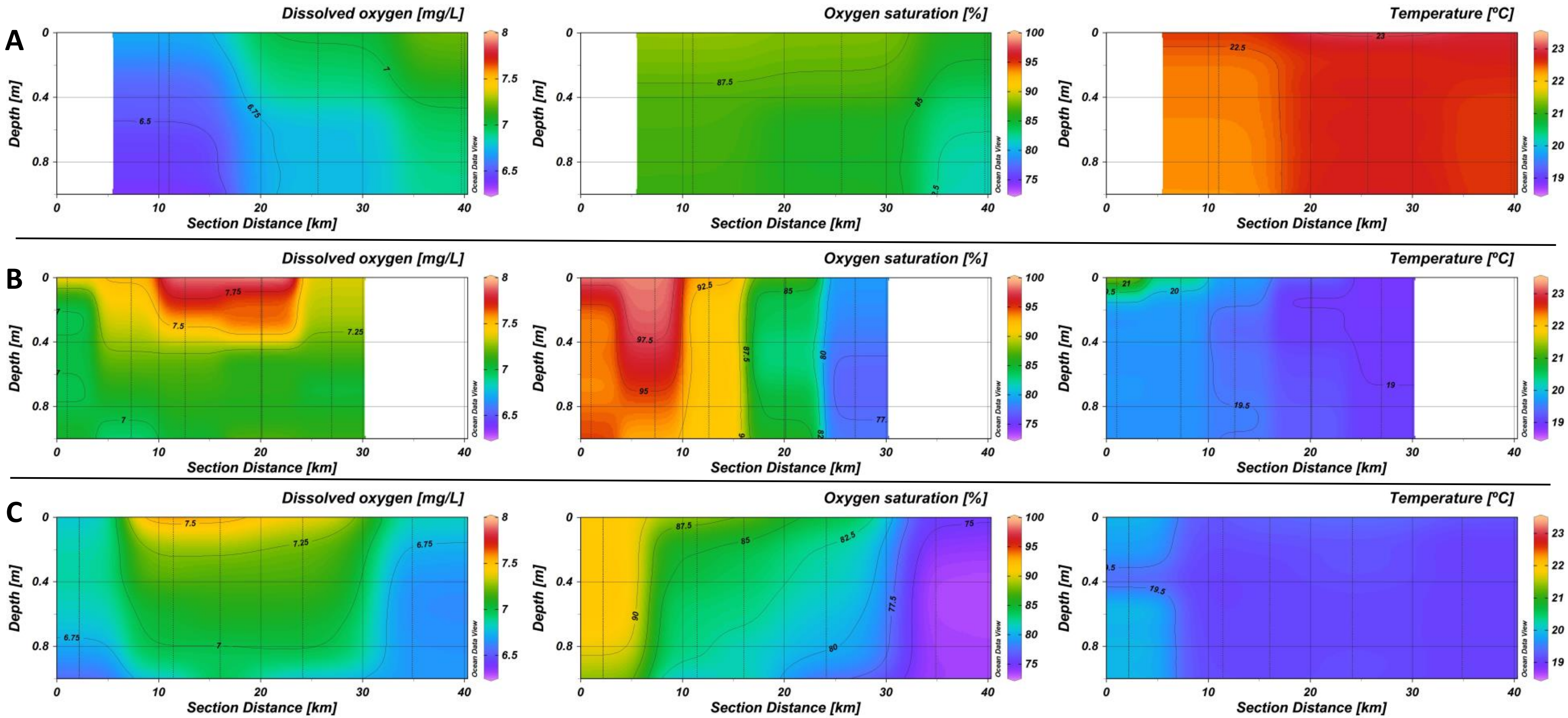

Figure 3.2. Physiochemical variables (dissolved oxygen, oxygen saturation and temperature) recorded in the water column (normalize depth) along the longitudinal section of the estuary from the river mouth to the most upstream sampling station during the different sampling dates [A (Before: 08/10/15), B (During: 05/11/15), C (After 1: 11/11/15)] of the high discharge event 1 in 2015.

periods, being *Pomatoschistus* spp., *Engraulis encrasicolus*, *Aphia minuta* and *Sardina pilchardus* the common species that more contributed to the similarity (Table 1). The spatial distribution of most organisms in the freshet event was displaced 10-15 downstream during the high discharge (Figure 4).

Table 1. Results of SIMPER analysis on the abundance of all larval and juvenile fish species from every period (freshet and control) of the high discharge event 1.

Average similarity: 49.16 **Freshet Event 1**

Species	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Engraulis encrasicolus	2.9	23.6	2.17	48.01	48.01
Pomatoschistus spp.	2.42	14.65	0.98	29.79	77.8
Aphia minuta	0.96	5.03	0.48	10.23	88.04
Sardina pilchardus	0.74	2.69	0.45	5.48	93.51

Average similarity: 34.65 **Control Event 1**

Species	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Engraulis encrasicolus	2.59	15.44	1.15	44.54	44.54
Pomatoschistus spp.	1.87	8.85	0.78	25.53	70.07
Aphia minuta	1.09	3.85	0.55	11.1	81.16
Anguilla anguilla	0.59	2.18	0.33	6.3	87.47
Sardina pilchardus	0.67	1.46	0.33	4.22	91.69

Statistical differences of the fixed effect "Time" for GAM on all variables were summarized in the plots by letter code and extended in Table S.1. The total abundance of early fish stages decreased significantly during the high discharge, although it almost recovered the density one week later (After 1), and also the distribution one month thereafter (After 2) (Figure 4.1.A). On the other hand, the analysis of the control period (Oct-Dec 2017) showed no changes in spatial distribution and a gradual temporal decrease, reaching significantly lower values in December. Macrofauna biomass did not show significant differences during the high discharge event, but it showed a temporal decrease in the control period (Figure 4.1.B). The spatio-temporal abundance of *Pomatoschistus* spp. fluctuated along the different periods showing a high increase in the lower zone (15-25 km) after the high discharge (Figure 4.1.C). The anchovy *Engraulis encrasicolus* was the species with highest density during the event. Its abundance declined significantly during the freshet and the sampling day After 1 (Figure 4.2.D). The density and distribution recovered completely, showing the same pattern as before the event in After 2. Instead, it showed a gradual decrease during those months in the control period. *Aphia minuta* was found mainly in the outer zone of the estuary during and after the freshet period (where no data were obtained before the high discharge due to technical problems) (Figure 4.2.E). Still, low abundance was found in the outer zone during October in the control period, whose density oscillated between sampling times. The spatio-temporal abundance of *Sardina pilchardus* did not show a significant variation in the freshet event, although their levels increased slightly in upstream zones thereafter. In the control groups, it was absent in October, and the abundance fluctuated significantly during the next months (Figure 4.2.F).

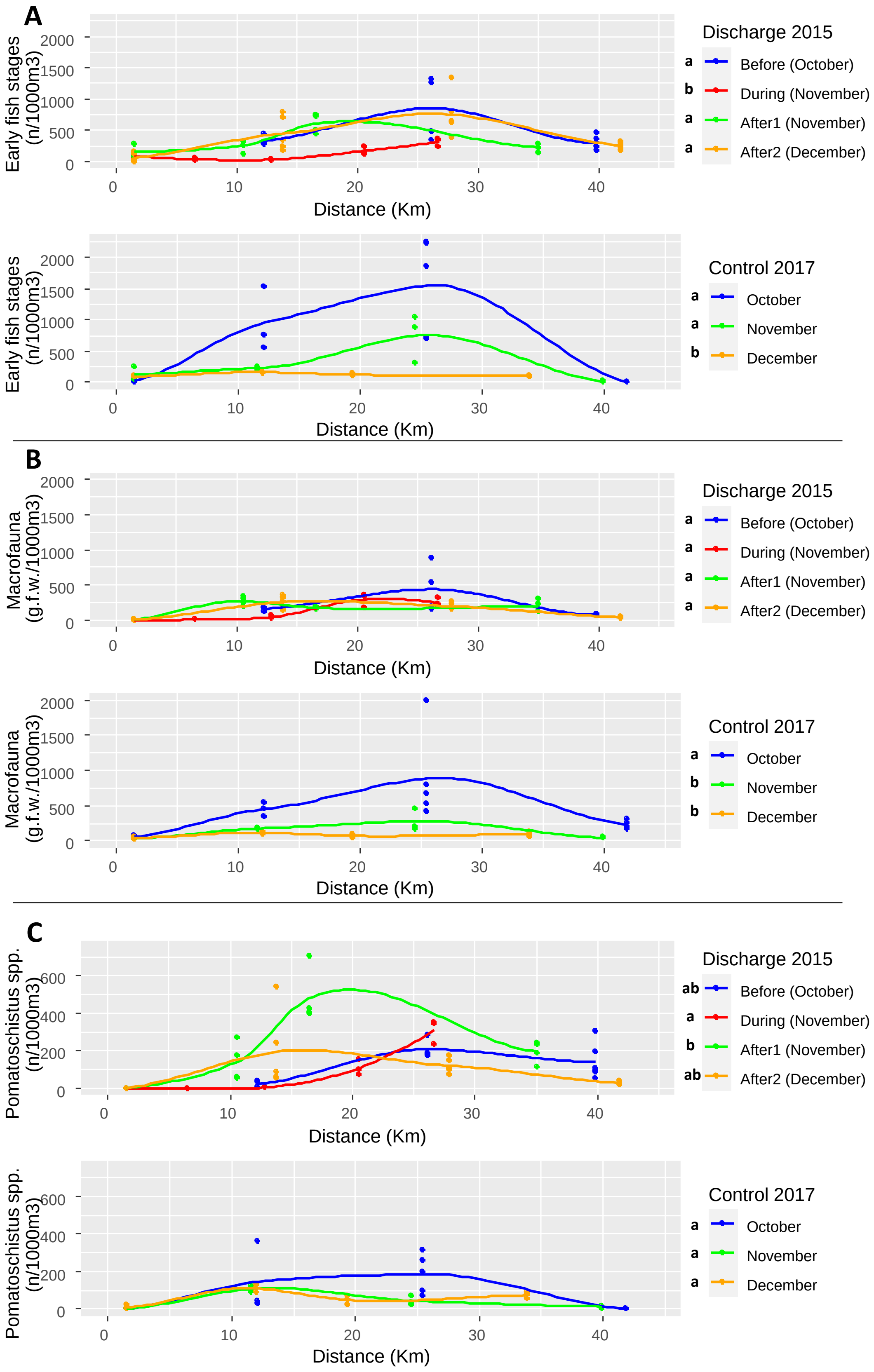

Figure 4.1. Spatial distribution of total early fish abundance (A), macrofauna biomass (B) and *Pomatoschistus* spp. (C) along the longitudinal section of the Guadalquivir estuary in the different sampling days during the high discharge (Before: 08/10/15; During: 05/11/15; After 1: 11/11/15; After 2: 11/12/15) and the control (October: 21/10/17; November: 30/11/2017; December: 13/12/17) periods of the freshet event 1. Different letters mean significant differences (p<0.05) between the levels of Time factor for GAM in every period.

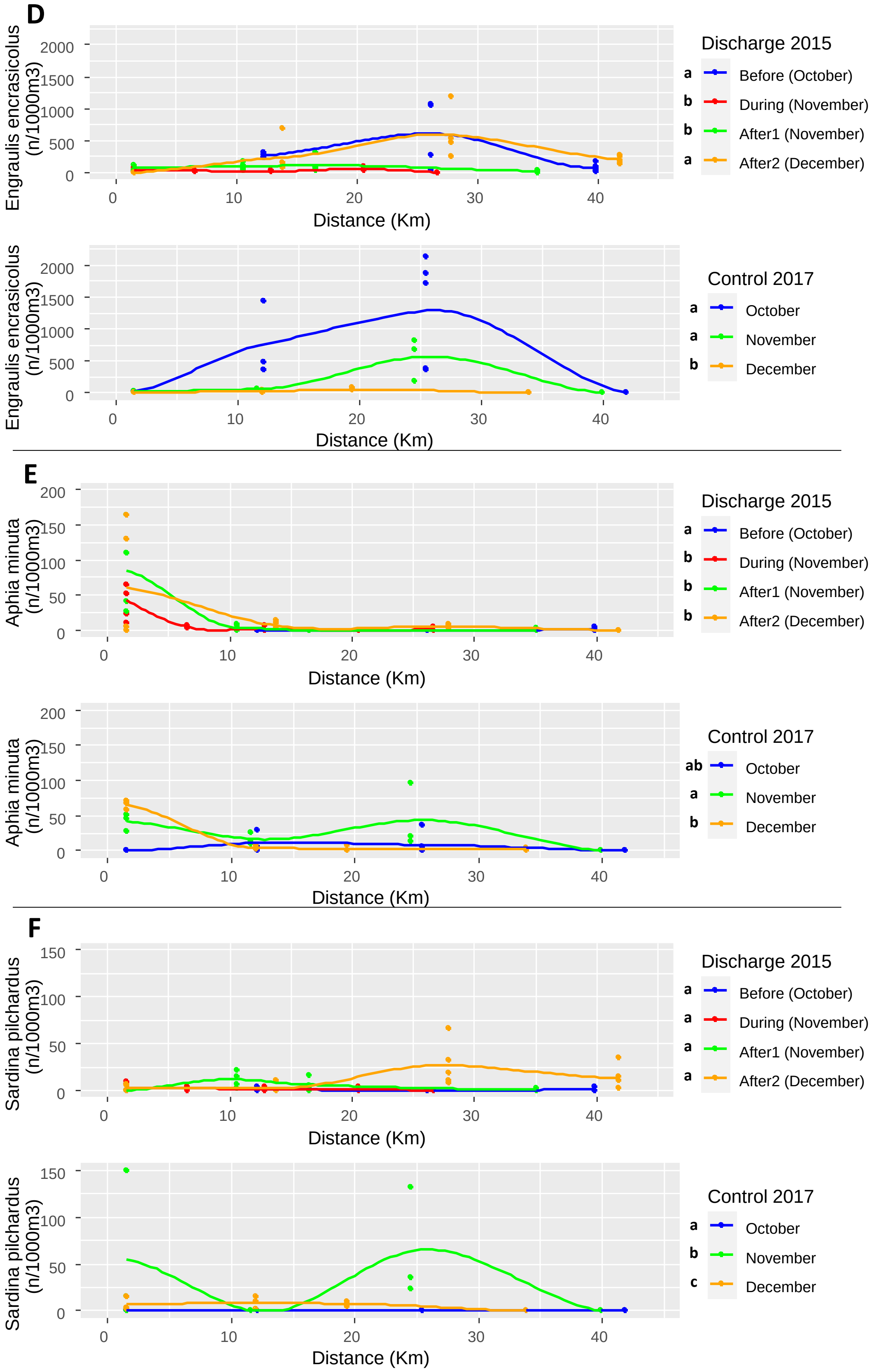

Figure 4.2. Spatial distribution of *Engraulis encrasicolus* (D), *Aphia minuta* (E) and *Sardina pilchardus* (F) along the longitudinal section of the Guadalquivir estuary in the different sampling days during the high discharge (Before: 08/10/15; During: 05/11/15; After 1: 11/11/15; After 2: 11/12/15) and the control (October: 21/10/17; November: 30/11/2017; December: 13/12/17) periods of the freshet event 1. Different letters mean significant differences ($p<0.05$) between the levels of Time factor for GAM in every period.

3.2. Event 2 (discharge 2016)

The event 2 happened in the same season as event 1, autumn, but with different characteristics. Precipitations were high and frequent during the month of November 2016, reaching values higher than 30 mm/day on several occasions (Figure 5). Increments in freshwater input were associated to rainfalls. The flow increased during one day to 150 m^3/s, before the first sampling day; afterwards, an event of high discharge happened at the end of November, with almost 250 m^3/s; finally, there were several low and temporally short discharges around 100 m^3/s during the first fortnight of December (Figure 5).

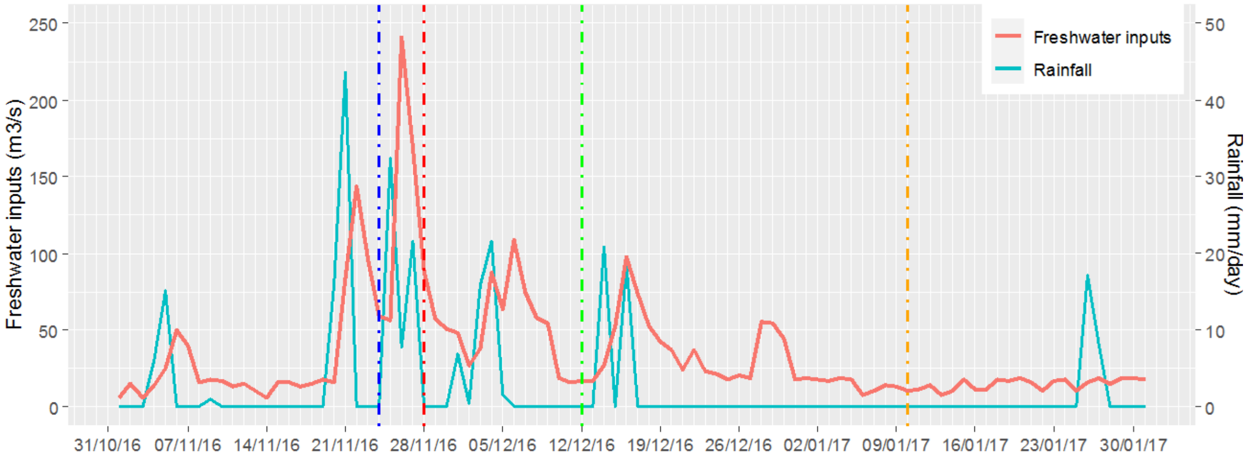

Figure 5. Temporal series of freshwater inputs in the Guadalquivir estuary and local rainfall from Alcalá del Río station during the event 2 in 2016. Vertical dashed lines indicate sampling dates [Blue: Before (24/11/16); Red: During (28/11/16); Green: After 1 (12/12/16); Orange: After 2 (10/01/17)].

Physiochemical variables showed spatio-temporal variations during the whole event (Figure 6), with a slight vertical stratification in some variables (mainly salinity and DO) in the Before sampling day, probably for the previous increment of freshwater input. Salinity gradient was reduced longitudinally, displacing the isohaline of 5 PSU from 30 Km upstream in Before to 10 Km during the high discharge. It was similar as event 1 but with a higher spatial reduction; the isohaline of 1 PSU was registered 20 Km upstream. Also, it provoked a strong vertical stratification with more than 15 PSU of difference between the surface and bottom layers in some zones. The longitudinal and vertical alteration was almost recovered 14 days after (After 1) the high discharge. Turbidity showed the same pattern as in the event 1, although the maximum values recorded were lower, with 2500 NTU, and they were observed more downstream. The pH was always stable in the outer zone with a value of 8. Instead, inner estuarine zone showed fluctuations. The higher values (8.1) registered in upstream zones in the first sampling, were then observed in the middle of the studied section during the high discharge with even higher values in the surface layers. Afterwards, the pH levels decreased to 7.8, and

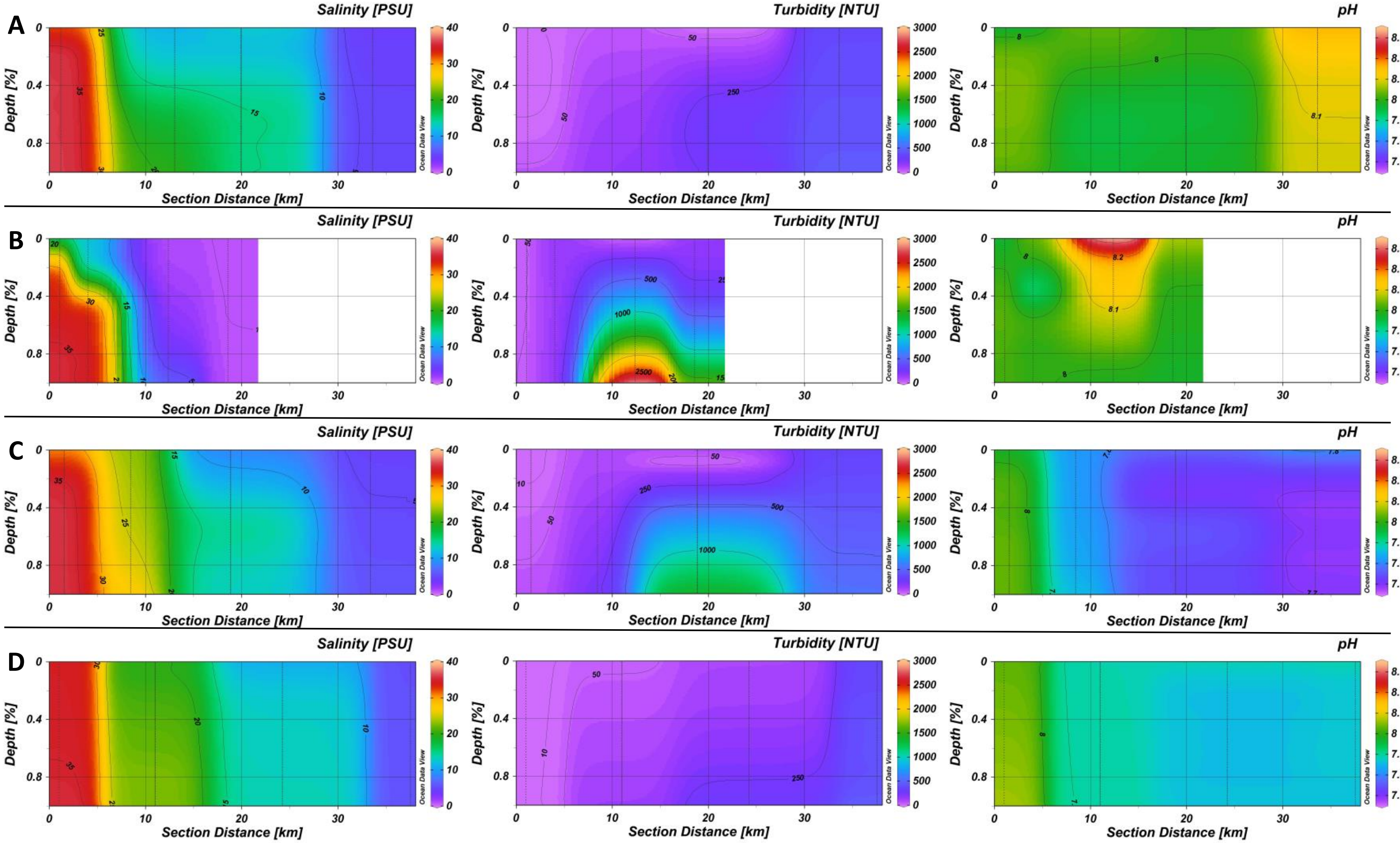

Figure 6.1. Physiochemical variables (salinity, turbidity and pH) recorded in the water column (normalize depth) along the longitudinal section of the estuary from the river mouth to the most upstream sampling station during the different sampling dates [A (Before: 24/11/16), B (During: 28/11/16), C (After 1: 12/12/16), D (After 2: 10/01/17)] of the high discharge event 2 in 2016.

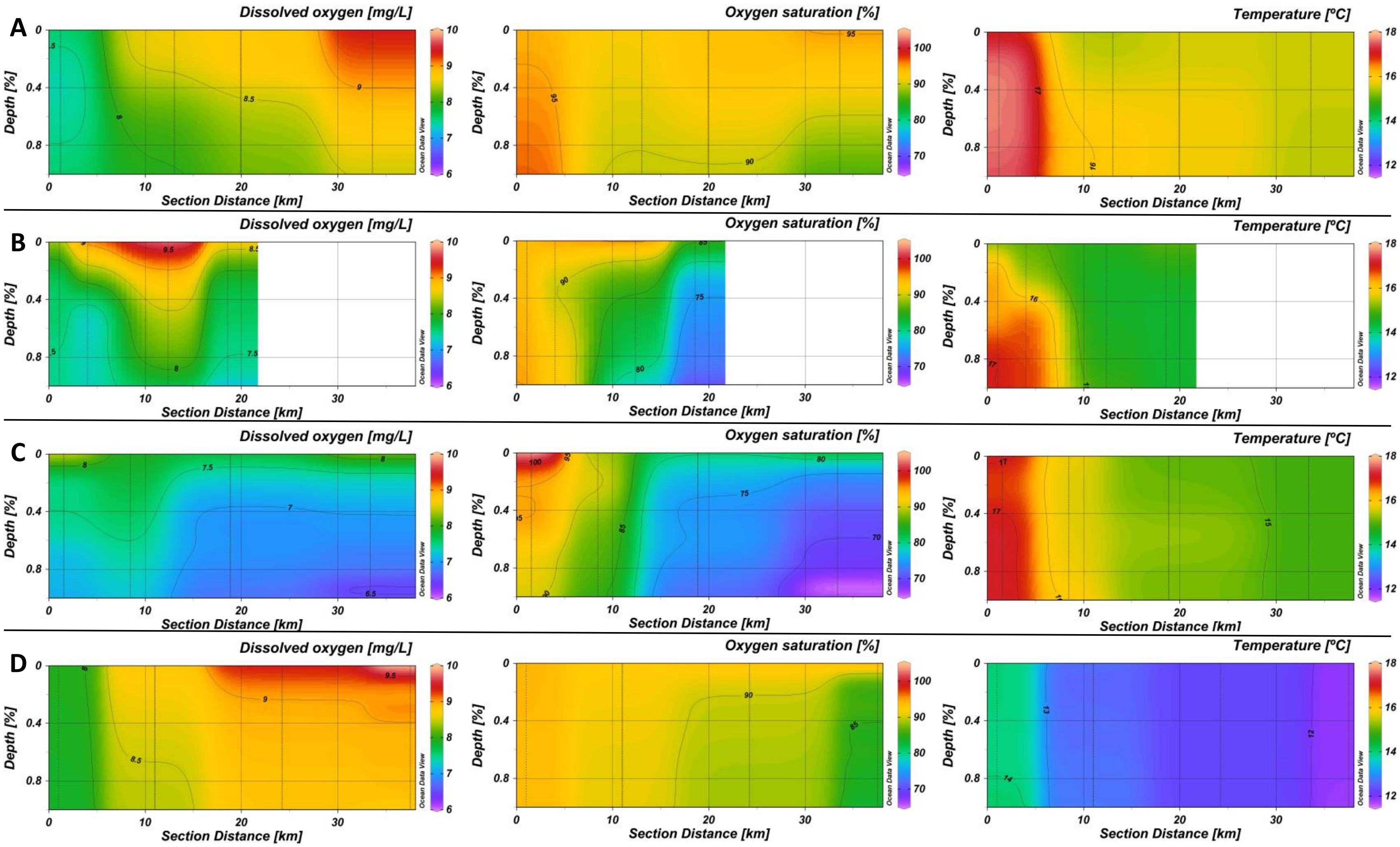

Figure 6.2. Physiochemical variables (dissolved oxygen, oxygen saturation and temperature) recorded in the water column (normalize depth) along the longitudinal section of the estuary from the river mouth to the most upstream sampling station during the different sampling dates [A (Before: 24/11/16), B (During: 28/11/16), C (After 1: 12/12/16), D (After 2: 10/01/17)] of the high discharge event 2 in 2016.

only showed a partial recovery one month later (7.9). The dissolved oxygen showed a similar pattern as the pH, with a displacement of the highest values (9 mg/L approx.) to the middle section during the high discharge. The levels decreased in the whole section in After 1 (never lower than 6 mg/L), but they almost recovered the same levels and distribution as before the event in the last sampling. A soft stratification with lower values in the bottom were observed in most samplings. Instead, oxygen saturation decreased during the high discharge, especially in the upstream zones, although it reached the previous percentages at the end. The temperature showed a slight and gradual increment downstream, while it declined temporally.

A total of 22 species of early life stages of fish were found, 21 in the freshet event and 17 in the control period. SIMPER analysis showed the characteristics species, being *Pomatoschistus* spp., *Engraulis encrasicolus*, *Aphia minuta*, *Sardina pilchardus* and *Solea senegalensis* the species that more contributed to the similarity in both periods (Table 2).

Table 4. Results of SIMPER analysis on the abundance of all larval and juvenile fish species from every period (freshet and control) of the high discharge event 2.

Average similarity: 34.38 **Freshet Event 2**

Species	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Pomatoschistus spp.	2.07	14	0.9	40.71	40.71
Engraulis encrasicolus	1.54	9.37	0.97	27.26	67.97
Aphia minuta	0.85	3.53	0.4	10.26	78.23
Sardina pilchardus	0.54	1.87	0.39	5.43	83.66
Solea senegalensis	0.73	1.64	0.33	4.78	88.45
Dicologoglossa cuneata	0.54	1.1	0.21	3.2	91.65

Average similarity: 39.85 **Control Event 2**

Species	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Pomatoschistus spp.	2	13.5	1.02	33.87	33.87
Aphia minuta	1.29	6.49	0.64	16.29	50.16
Engraulis encrasicolus	1.51	6.42	0.73	16.12	66.28
Sardina pilchardus	1.09	5.2	0.67	13.06	79.34
Solea senegalensis	0.72	2.5	0.46	6.27	85.6
Anguilla anguilla	0.62	2.29	0.47	5.74	91.35

The spatial distribution of most organisms in the freshet event was displaced to downstream zones during the high discharge, occupying only the first 15-20 Km (Figure 7). Statistical differences of the fixed effect “Time” for GAM on all variables were summarized in the plots by letter code and extended in Table S.2. The abundance of early fish stages showed a significant reduction during the high discharge, at the same time as compressed their distribution to the first 15 Km from estuary mouth (Figure 7.1.A). Afterwards, its abundance recovered partially, with distinct patterns in the distribution, which was elongated upstream a

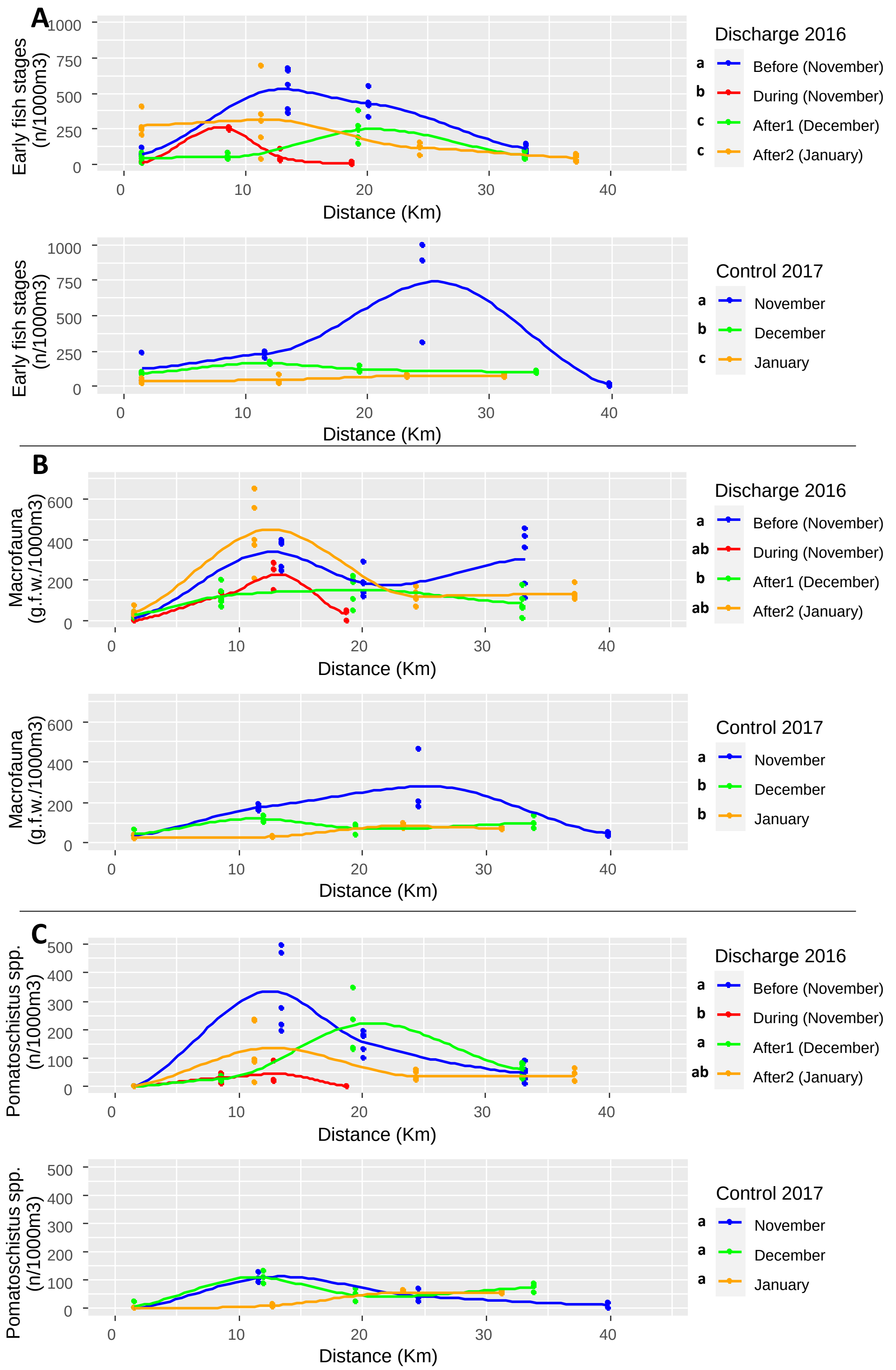

Figure 7.1. Spatial distribution of total early fish abundance (A), macrofauna biomass (B) and *Pomatoschistus* spp. (C) along the longitudinal section of the Guadalquivir estuary in the different sampling times during the high discharge (Before: 24/11/16; During: 28/11/16; After 1: 12/12/16; After 2: 10/01/17) and the control (November: 30/11/17; December: 13/12/17; January: 12/01/18) periods of the freshet event 2. Different

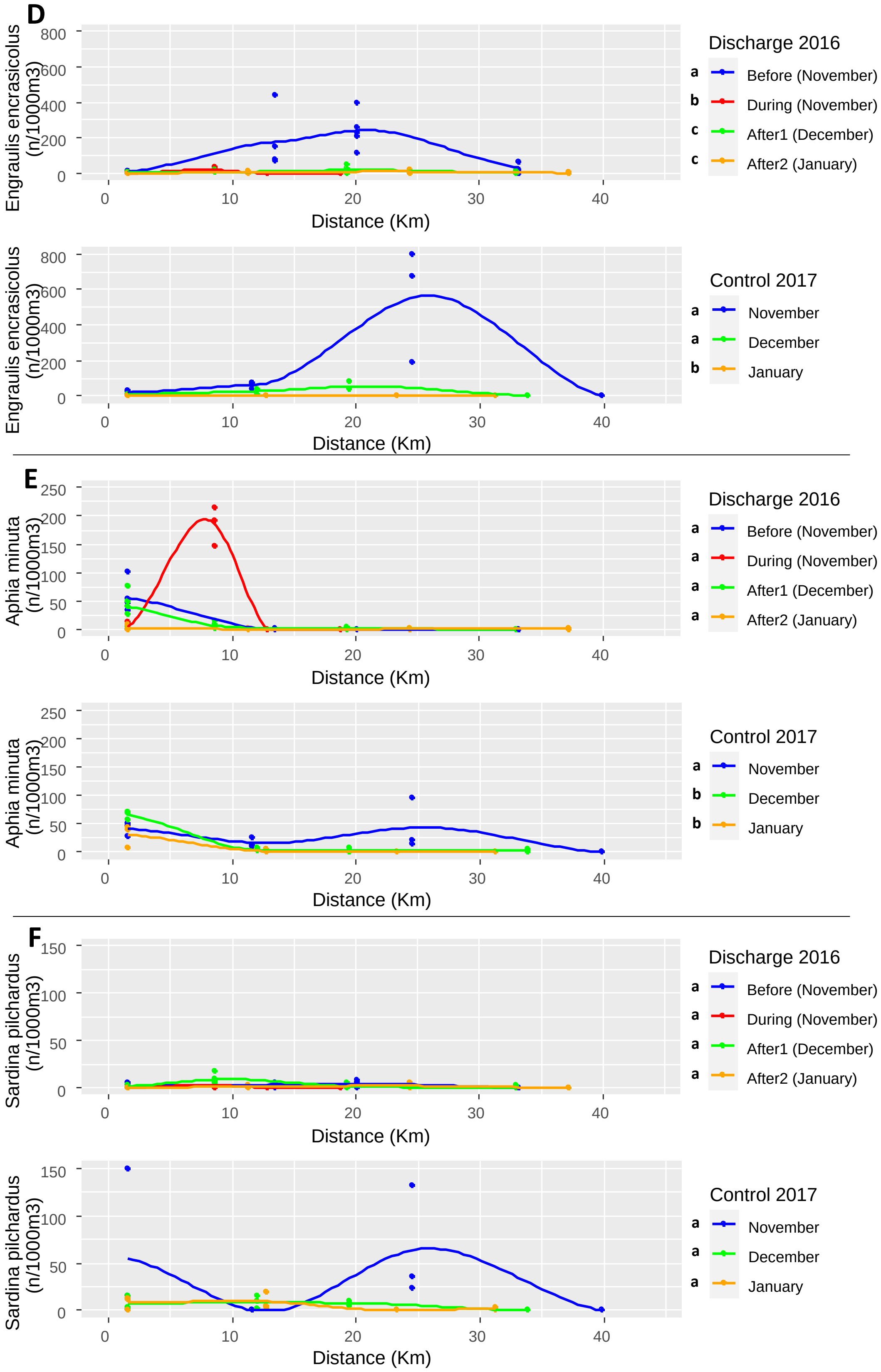

Figure 7.2. Spatial distribution of *Engraulis encrasicolus* (D) and *Aphia minuta* (E) along the longitudinal section of the Guadalquivir estuary in the different sampling times during the high discharge (Before: 24/11/16; During: 28/11/16; After 1: 12/12/16; After 2: 10/01/17) and the control (November: 30/11/17; December: 13/12/17; January: 12/01/18) periods of the freshet event 2. Different letters mean significant differences ($p<0.05$) between the levels of Time factor for GAM in every period.

fortnight after the freshet (After 1), while one month later (After 2), its higher densities were downstream. The control period showed a temporal decline in early fish abundance along the whole section. Macrofauna distribution was reduced to downstream zones during the high discharge, and its biomass decreased significantly in After 1 (Figure 7.1.B). The evolution to previous conditions was gradual, firstly with an elongation of the distribution (After 1), and secondly with a general increment (After 2), especially in the section around 10 and 20 Km from the estuary inlet. The goby *Pomatoschistus* spp. showed a high and significant reduction in the density during the freshet (Figure 7.1.C). The recovery to previous levels was fast (After 1), only two weeks thereafter, although it did not reach the same densities in downstream zones as Before. The control period did not show temporal differences. In contrast, the anchovy *Engraulis encrasicolus* disappeared during the high discharge, and some individuals were found along the section later, but with very low densities (Figure 7.2.D). The control period showed a temporal decreased, being absent during January. The species *Aphia minuta* was the only one which increased its density in the downstream zone of the estuary during the high discharge, although no significant differences were detected (Figura 7.2.E). *Sardina pilchardus* showed low densities along the event to find a significant effect (Figure 7.2.F). The control period showed some fluctuations with the month of November, but there was not significant. The abundance of *Solea senegalensis* during both periods was too low to find a pattern.

3.3. Event 3 (discharge 2018)

The event 3 happened during the end of winter and beginning of spring in 2018. In this case, the precipitations were elevated and continued during most of March, reaching values around 20 mm/day in numerous days (Figure 8). The freshwater input increased after the first rainfalls, maintaining high discharges during the whole month of March (mean: 420 m^3/s), with maximum values of 1080 m^3/s in a day (Figure 8).

Physiochemical variables showed spatio-temporal variations during the whole event (Figure 9). Salinity gradient was reduced longitudinally, displacing the isohaline of 5 PSU from 40 Km upstream in Before to 10 Km during the high discharge. In this case, no vertical stratification was registered during the freshet. In contrast, one week later (After 1), the salinity showed a vertical stratification in downstream zones, at the same time as the gradient recovered partially its previous elongation upstream. Still, the salinity did not recover the same structure as before one month thereafter (After 2). Turbidity showed a notable increment in the whole estuary section. In fact, the levels registered in the inner estuary during this event were higher than the maximum quantification limit of the multiprobe (5400 NTU), therefore, real levels would be

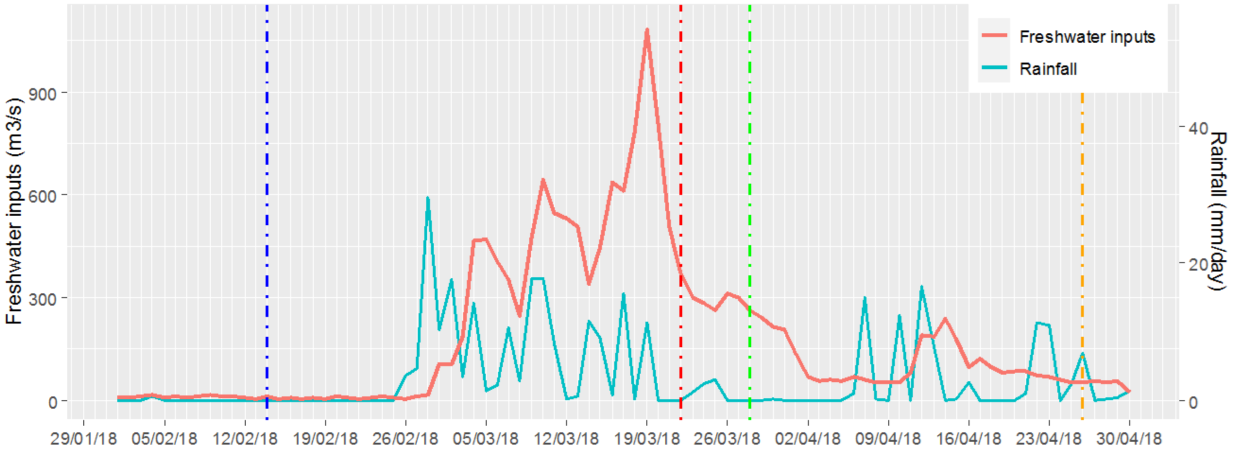

Figure 8. Temporal series of freshwater inputs in the Guadalquivir estuary and local rainfall from Alcalá del Río station during the event 3 in 2018. Vertical dashed lines indicate sampling dates [Blue: Before (14/02/18); Red: During (22/03/18); Green: After 1 (28/03/18); Orange: After 2 (26/04/18)].

even higher. Instead, total suspended solids (Figure S.1), which showed the same pattern as turbidity, reached a maximum concentration of 13774 mg/L at 13 Km upstream from the estuary mouth (samples collected in midwater). Lower levels were recorded in the surface layers and, mainly, in the outer zone of the estuary. Turbidity decreased one month later (After 2), although there were still higher levels than before the freshet, especially in the upstream zone. The pH changed in the whole section, associated to the salinity. It increased in the outer (8.2) and inner (8.1) zones and homogenized vertically. One week later, it decreased in most of the inner zone still showing values lower than before the freshet (7.7), with a gradual increment downstream. One month later, the levels showed a more similar structure along the estuary section as before the event, although the recovery was not completed maintaining vertical homogenization in some inner zones. The dissolved oxygen showed a notable decline during the high discharge, reaching hypoxic concentrations (< 2.5 mg/L) in the bottom areas of the middle section. Thereafter, the levels increased gradual and temporally, being in the upstream zone slower than the rest of the section, although it did not reach the concentrations (After 2: 6.5 mg/L) found previous to freshet (Before: 9.5 mg/L). The oxygen saturation showed the same pattern as oxygen concentration, but in this case, the recuperation in the outer zone was complete one month after de freshet. The temperature showed a fast temporary increase during the freshet due to the intrusion of inland waters, which in this period warm up faster than seawaters, and then followed the expected seasonal increase, from 11 ^{o}C in the first sampling on February to 19 ^{o}C in the last on April.

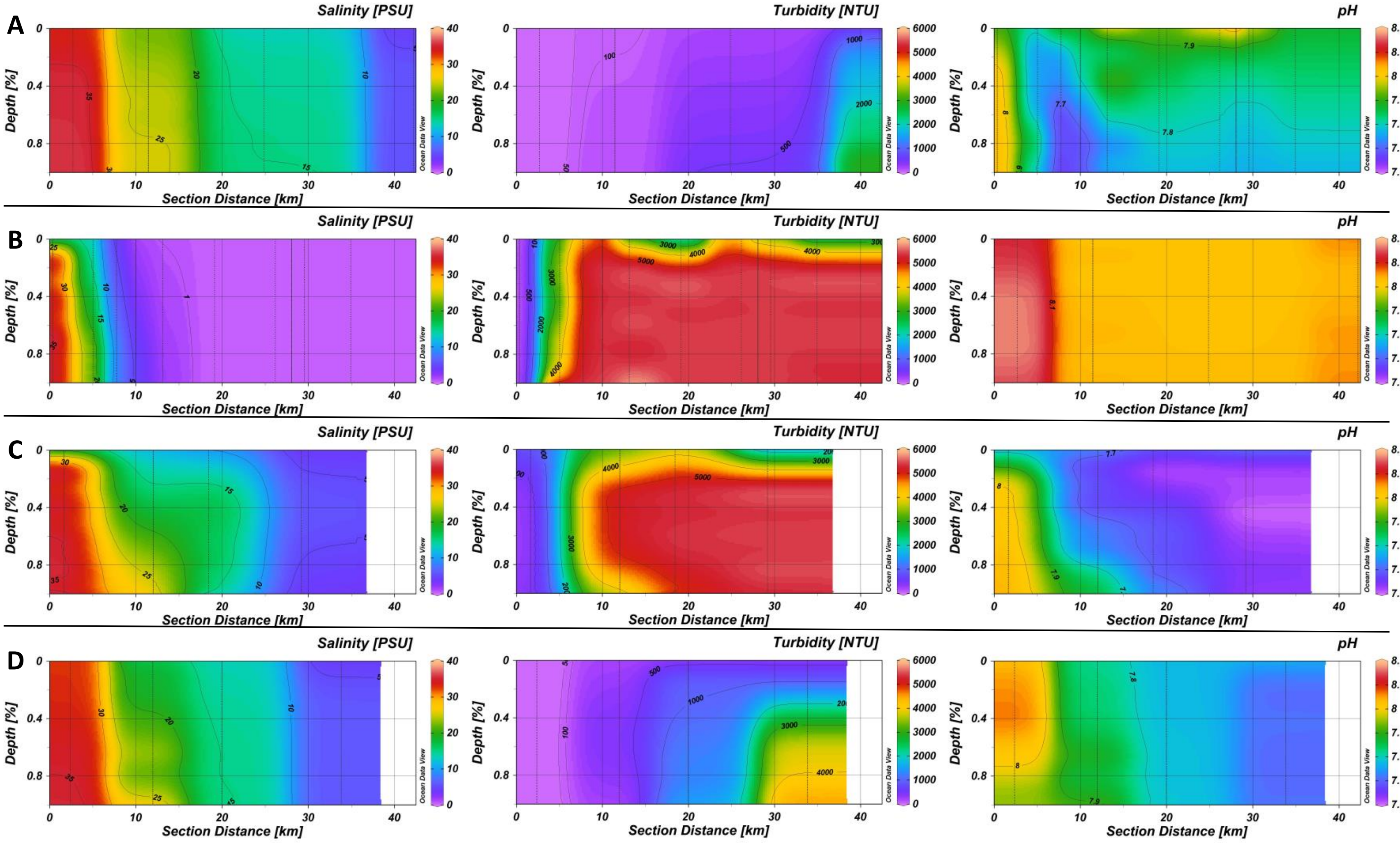

Figure 9.1. Physiochemical variables (salinity, turbidity and pH) recorded in the water column (normalize depth) along the longitudinal section of the estuary from the outer station to the most upstream sampling station during the different sampling dates [A (Before: 14/02/18), B (During: 22/03/18), C (After 1: 28/03/18), D (After 2: 26/04/18)] of the high discharge event 3 in 2018.

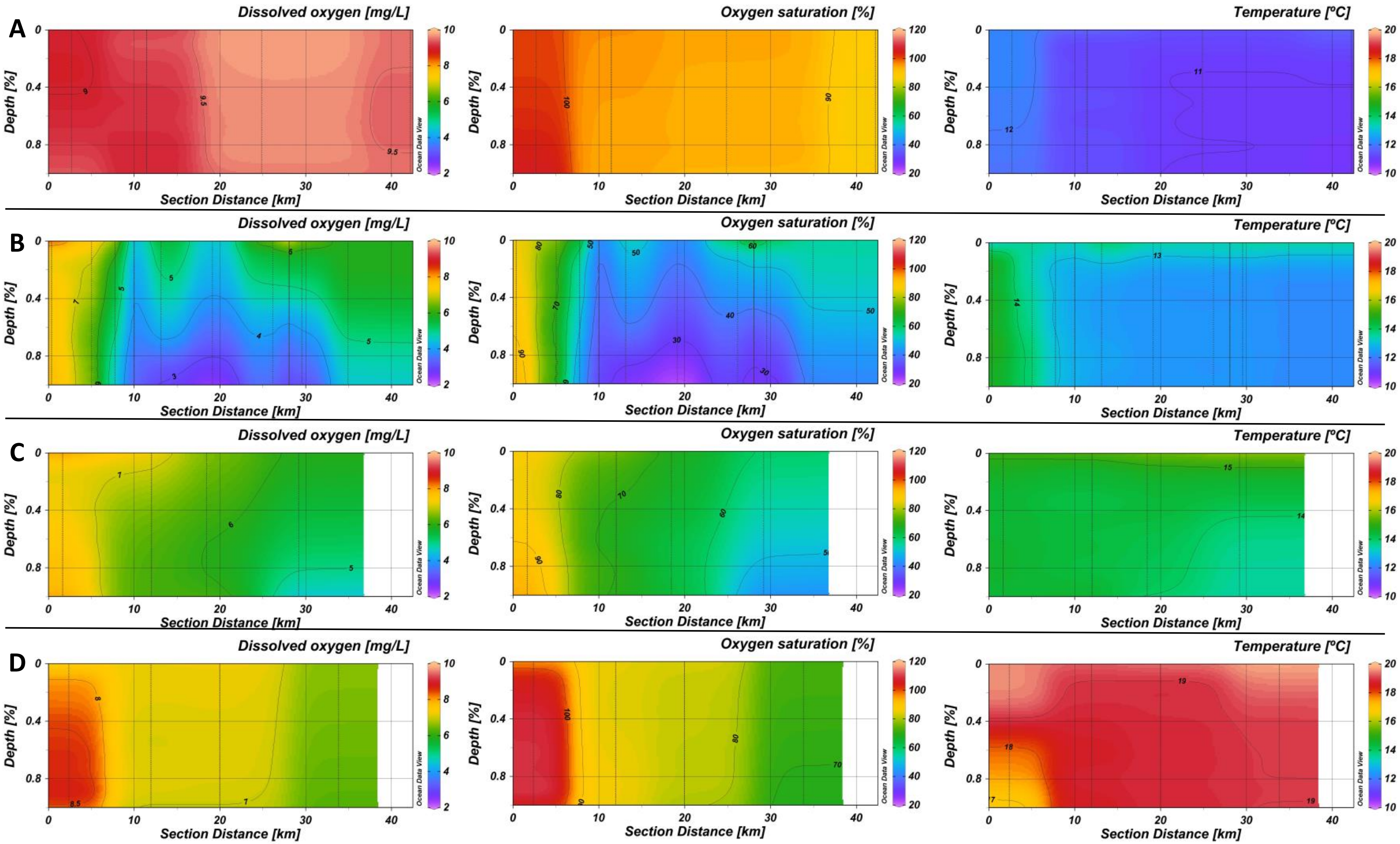

Figure 9.2. Physiochemical variables (dissolved oxygen, oxygen saturation and temperature) recorded in the water column (normalize depth) along the longitudinal section of the estuary from the outer station to the most upstream sampling station during the different dates [A (Before: 14/02/18), B (During: 22/03/18), C (After 1: 28/03/18), D (After 2: 26/04/18)] of the high discharge event 3 in 2018.

A total of 24 species of early life stages of fish were found, 21 in the freshet event and 20 in the control period. SIMPER analysis showed the characteristics species for both study periods, sharing *Pomatoschistus* spp., *Sardina pilchardus, Solea senegalensis* and *Diplodus vulgaris* (Table 3).

Table 3. Results of SIMPER analysis on the abundance of all larval and juvenile fish species from every period (freshet and control) of the high discharge event 3.

Average similarity: 44.30			**Freshet Event 3**		
Species	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Pomatoschistus spp.	4.44	22	1.82	49.65	49.65
Aphia minuta	1.42	3.99	0.64	9	58.65
Sardina pilchardus	1.23	3.63	0.67	8.19	66.84
Solea senegalensis	0.96	3.24	0.66	7.31	74.15
Gobius paganellus	1.2	2.88	0.58	6.49	80.65
Sparus aurata	0.89	2.86	0.52	6.46	87.11
Diplodus vulgaris	0.69	1.53	0.43	3.45	90.55

Average similarity: 51.35			**Control Event 3**		
Species	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Pomatoschistus spp.	3.05	16.35	1.77	31.85	31.85
Engraulis encrasicolus	2.22	13.68	2.16	26.63	58.48
Sardina pilchardus	1.8	7.21	1.02	14.04	72.52
Diplodus vulgaris	1.11	3.81	0.72	7.42	79.94
Solea senegalensis	0.95	3.04	0.7	5.92	85.86
Dicentrarchus labrax	1.1	2.39	0.51	4.65	90.5

The spatial distribution of most organisms in the freshet event was displaced to downstream zones during the high discharge, relegate them to the first 15 Km (Figure 10). The outer station could not be sampled in February and March of control period due to inclement weather in that zone. Statistical differences of the fixed effect "Time" for GAM on all variables were summarized in the plots by letter code and extended in Table S.3. Total abundance of early fish stages did not change significantly in the estuary section during the freshet, but its distribution was compressed downstream (Figure 10.1.A). In the two sampling times after the freshet, its density showed a notable and significant increment, and the distribution was elongated until 30 Km upstream. In the control period, the abundance increased temporally, but with much lower densities than the freshet period. Macrofauna biomass fluctuated between sampling times, showing a significant increment in the last cruise to compare with during the freshet (Figure 10.1.B). The distribution was compressed during the high discharge in the first 10 km from the estuary mouth. The distribution extended upstream two weeks later (After 1), and the densities incremented one month thereafter following the seasonal trend in this period. Also, control period showed a temporal increment of density, but with a more elongated

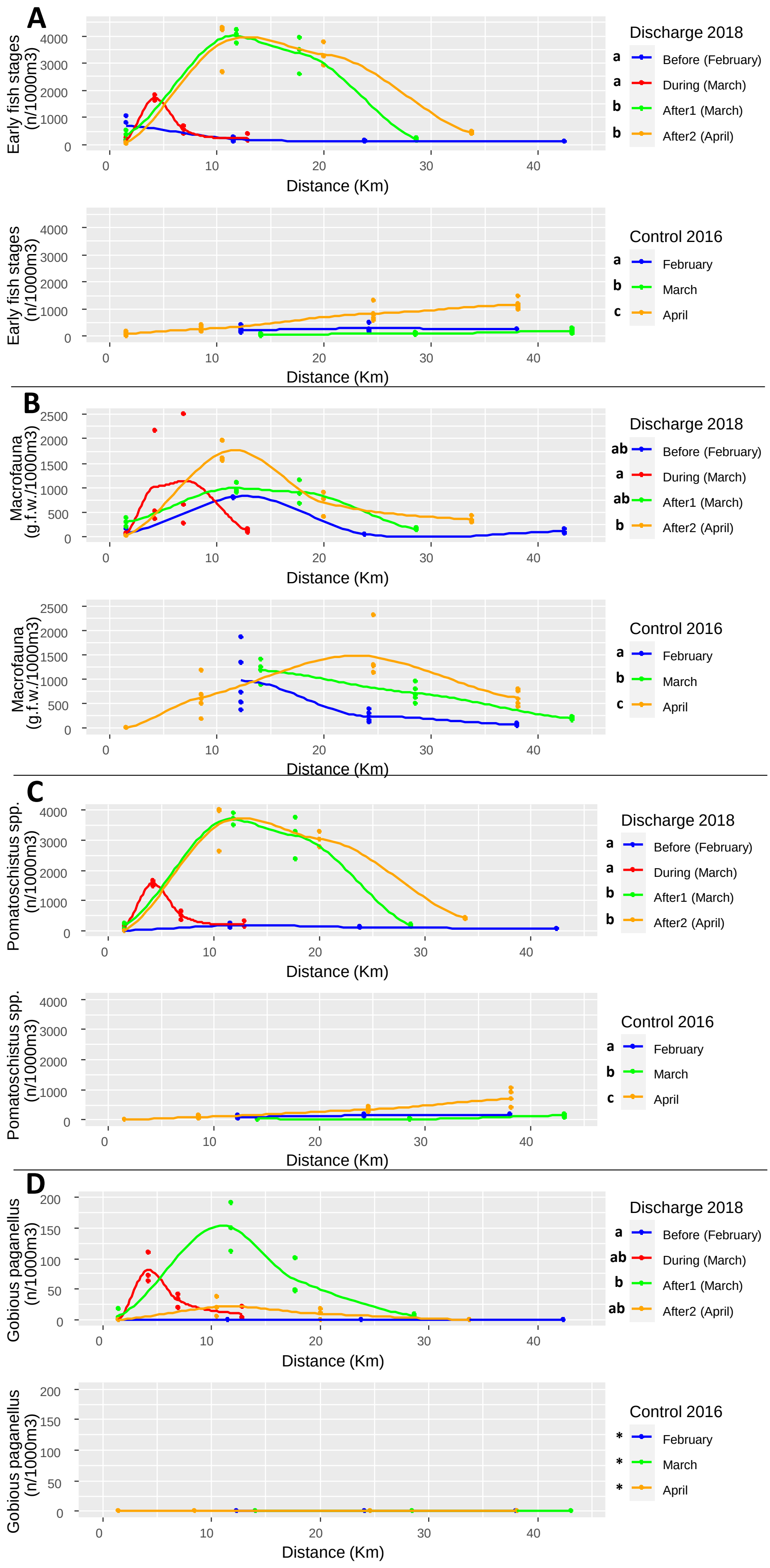

Figure 10.1. Spatial distribution of total early fish abundance (A), macrofauna biomass (B), *Pomatoschistus* spp. (C) and *Gobius paganellus* (D) along the longitudinal section of the Guadalquivir estuary in the different sampling days during the high discharge (Before: 14/02/18; During: 22/03/18; After 1: 28/03/18; After 2: 26/04/18) and the control (February: 22/02/16; March: 21/03/16; April: 21/04/16) periods of the freshet event 3. Different letters indicate significant differences (p<0.05) between the levels of Time factor for GAM in every period. "*" for species absent during the period.

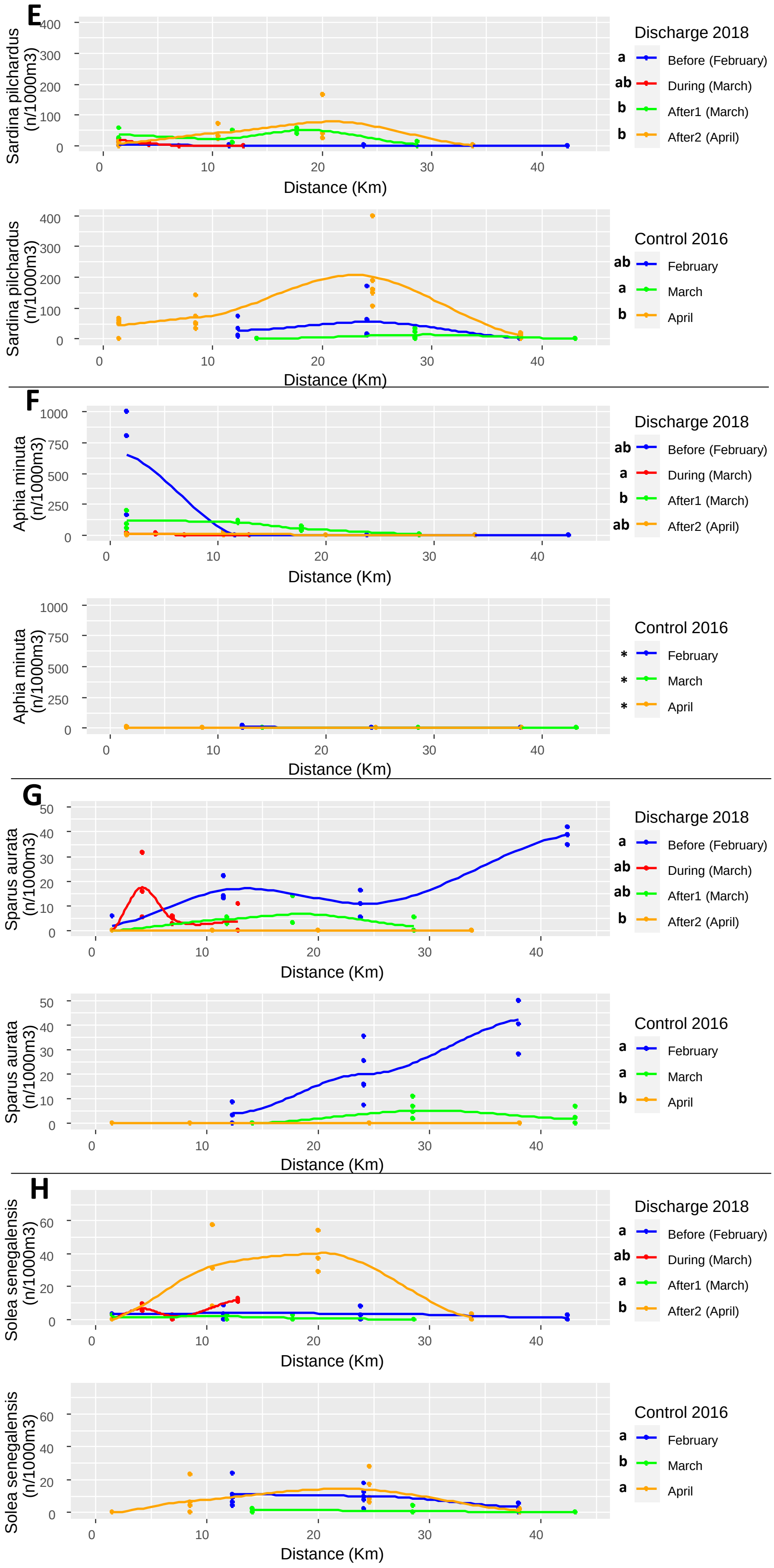

Figure 10.2. Spatial distribution of *Sardina pilchardus* (E), *Aphia minuta* (F), *Sparus aurata* (G) and *Solea senegalensis* (H) along the longitudinal section of the Guadalquivir estuary in the different sampling days during the high discharge (Before: 14/02/18; During: 22/03/18; After 1: 28/03/18; After 2: 26/04/18) and the control (February: 22/02/16; March: 21/03/16; April: 21/04/16) periods of the freshet event 3. Different letters indicate significant differences ($p<0.05$) between the levels of Time factor for GAM in every period. "*" for species absent during the period.

abundance upstream in April. The goby *Pomatoschistus* spp. was the fish species most dominant during this event, with the 80% of abundance. Therefore, it determined the observed pattern of total early fish abundance described previously (Figure 10.1.C). Another species of goby, *Gobius paganellus*, was absent before the freshet, showing an increment in the abundance during the freshet, mainly in downstream zone (Figure 10.1.D). Thereafter, its abundance and distribution continued increasing upstream, although its density decreased in all estuary section one month later (After 2). This species was not present during the control period. *Sardina pilchardus* was also not present in the estuary before the freshet (Figure 10.2.E). Individuals were found in the outer zone during the high discharge, but with low densities. Afterwards, it showed a slight but significant increase in the middle estuary section. The control period showed temporal fluctuations, being the month of April the most abundant. *Aphia minuta* was present in the outer zone with high densities before the freshet, but it disappeared during the high discharge in this case (Figure 10.2.F). Thereafter, it showed a low increment in downstream zone (After 1). This species was not found in the estuary during the control period, as happened with *Gobius paganellus*. The seabream *Sparus aurata* reduced its density gradually, being not present in the last sampling time (Figure 10.2.G). Instead, the distribution during the high discharge was reduced from upstream (40 Km) to downstream (5 Km) zones. Afterwards, it recovered partially the distribution but with lower densities as before, mainly in upstream zone. The control period showed a similar temporal decline for this species, but with a different distribution pattern. *Solea senegalensis* was almost absent before the high discharge (Figure 10.2.H). During the freshet, some individuals were found in downstream zones. Thereafter, it returned to previous conditions (After 1), but later, the density increased significantly in the middle estuary section (After 2). It showed a similar spatial distribution in the control period, with different density fluctuations between months, although its maximum abundances were lower than those found in April of the freshet period. The other contributor species caught presented too low densities to find patterns.

4. Discussion

This study was conducted to characterize the short response times and spatial distribution pattern of early life stage of fish and the rest of the macrofauna following high discharge events in a well-mixed estuary and its nearshore area. The differences in the intensity, duration and period of the year of every discharge were an opportunity to better understand the plankton dynamic in estuaries under distinct runoff scenarios. A shift on physiochemical conditions were observed in all cases, but the degree of biological response varied among events. Still, distribution of organisms showed a compression downstream during the high discharge similar as did the longitudinal salinity gradient in all studied freshets.

4.1. Physiochemical effects

High discharges sampled in the Guadalquivir estuary were associated to an increment of the precipitations in the region, which were more abundant and frequent in autumn and spring seasons during this study. Still, rainfalls do not always provoke an increment in the freshwater input due to the damming and water reservoir management of the watershed (González-Ortegón and Drake, 2011). This estuary is characterized by freshwater inputs lower than 40 m^3/s during 75% of the year (Díez-Minguito et al., 2012), hence all the freshet events sampled were in high river inflow conditions. In fact, among the different freshets, the event 3 changed the water current regime of the estuary from tidally-dominated to fluvially-dominated, as the monthly mean flow discharged was higher than 400 m^3/s (Díez-Minguito et al., 2012).

Under low freshwater input conditions, this estuary is considered, hydrologically, a well-mixed system (Vanney, 1970) with a longitudinal salinity gradient from seawater (≈36 PSU) in the estuary mouth to freshwater (≈0 PSU) 80 km upstream approximately (Díez-Minguito et al., 2013). The salinity was vertically stratified and longitudinally compressed in the downstream zone during all events, although its degree of change depended on the kind of freshet. The more intense and lasting was the discharge, the higher the compression of the salinity gradient. When freshwater inflow diminished, salt wedge intruded gradually further up the estuary, although with distinct recovery times. It depends on the spring-neap tidal cycle during which the discharge occurs, the magnitude of the freshwater discharge, and the atmospheric and oceanographic conditions at the mouth and the inner shelf (Díez-Minguito et al., 2013). While the event 1 and 2 took a few days, the event 3 spent more than one month, especially in the upstream zone.

The advance of the salt wedge during the flood tide was associated with a decrease in turbidity due to the advection of (less turbid) seawater, similar as other authors reported in a freshet event in the Guadiana estuary (Garel et al., 2009). Still, the recovery time of this variable was slower than the salinity. In the Guadalquivir estuary it has been reported two estuarine turbidity maximum (ETM) in low freshwater input conditions: at 35 km and 58 km (from estuary mouth; equivalent to 42 Km and 64 Km in our longitudinal section scale) approximately (Díez-Minguito et al., 2014). Although not all the estuarine section has been sampled in this study, the higher turbidity found near-bed during the high discharges could indicate a resuspension of sediment, including the displacement downstream, at least, of the lower ETM (Garel et al., 2009). Also, the high values registered in the whole water column in most of the estuarine section suggest elevated inputs of TSS load from the drainage basin. In fact, the supply was so high in the event 3 that it formed a turbid plume which spread several kilometres offshore from estuary mouth (Figure S.2). Previous studies in the Guadalquivir also have reported high concentrations of TSS during freshet events [e.g. 3500 mg/L in Navarro et al. (2012) or 5000 mg/L in Ruiz et al. (2017)], although in this case, the values were much higher (13774 mg/L), even than other estuaries [e.g. (Garel and Ferreira, 2015; Jalón-Rojas et al., 2015)]. For this reason, the Guadalquivir estuary has been characterized as a high turbidity system, especially in wet years (González-Ortegón et al., 2010; Losada et al., 2017).

On the other hand, high turbidity levels have been related with hypoxic conditions (Schmidt et al., 2019). The light penetration in the water column can be limited for the high turbidity, reducing the photosynthetic activity by phytoplankton (Ruiz et al., 2017), while the increment of organic and inorganic nutrients could increase the oxygen consumption by oxidation (Henrichs, 1992) or heterotrophic bacteria respiration (Atwood et al., 2012), which in its turn, it would increase CO_2 and reduce the pH. Therefore, the generalized decline of pH levels and oxygen concentration observed in the whole estuary section after the freshets could indicate a high bacteria proliferation. Still, only the event 3 showed hypoxic conditions. The extreme turbidity and the extended period of this discharge (1 month approx.) would have further increased photosynthesis limitation and oxygen consumption. Also, Navarro et al. (2012) observed similar effects during some freshet events during 2008-2010 in the Guadalquivir estuary, although in their case, the oxygen concentration reached even lower values (0.52 mg/L).

4.2. Biological effects

Freshets not only controlled the circulation of water masses and their physiochemical conditions, but also modify the structure and distribution of small organism populations. In general, the distribution of early life stages of fish and macrofauna species were associate to the salinity gradient, being almost absent in the freshwater masses during the high discharges despite of being located closer to the estuary mouth. Young recruits of marine species seemed to be pulled by freshwater flow, moving with the mass of estuarine water. They were almost absent in water masses lower than 5 PSU in the Guadalquivir estuary, even in low inflow conditions (Fernández-Delgado et al., 2007). On the other hand, no increments of densities were found in the outer station during the high discharge of any freshet despite the total abundance of organisms decreased generally in the inner estuarine zone. This suggest several possibilities for individuals not found, unless for a part of the community: i) they could have been flushed out further of the sampling area, as some studies found high abundance of early fish stages in the front of the plumes (Kingsford and Suthers, 1994; Sabatés, 1990), ii) they could have been predated in the nearshore areas and/or clearer zones downstream (Utne-Palm, 2002) or die by osmotic shocks (Serafy et al., 1997) and/or iii) they could have been used shelter zones as shoals, rocks or even submerged channels in the estuary mouth as reported Ueda et al. (2004) for some zooplankton species.

Different biological effects were found for the events 1 and 2 that for the event 3. In addition to the intensity of freshwater discharged, these events happened in different seasons. The events 1 and 2 took place during the autumn, when the temperature and the density of the macrofauna in the Guadalquivir estuary usually declines, while the event 3 was during the spring, when the temperature, the biological activity and recruitment start to increase (Drake et al., 2002). Still, the species *Pomatoschistus* spp., *Aphia minuta* and *Sardina pilchardus* were characteristics in all of them.

The fish species which better cope with the different freshet events was the goby *Pomatoschistus* spp. Although it showed different patterns during the high discharge in every event, its abundance and distribution recovered quickly or even increased notably in all of them. This goby is a benthic estuarine species whose bottom behaviour and the pectoral fin adduction may help them to deal with strong water currents settling in the bottom (Dolbeth et al., 2007), or even burrowing in the sediment (Magnhagen and Forsgren, 1991), being displaced downstream but no flushed out. These strategies would help to recover the distribution rapidly following the advance of salinity wedge formed by the flood tide. In addition, the pattern found during the event 3 suggests a strong call effect, because it coincided with its recruitment period, the early spring (González-Ortegón et al., 2012; Pampoulie et al., 1999), and its density increased remarkably in comparison with previous sampling day and also with the control period. Similar pattern was observed for the rock goby, *Gobius paganellus*, which is a species predominantly marine but can enter brackish waters (Azevedo and Simas, 2000), likely attracted by the concentration of prey downstream as small invertebrates observed in the macrofauna. The distribution of both gobies in high densities in the middle section of the estuary in the sampling day just after the high discharge showed their tolerance to high turbidity levels. In fact, we did not find many other organisms in this part of the section after the freshet; these turbid zones were also characterized by a low oxygen concentration (hypoxia in some instances), what could make them unsuitable (Schmidt et al., 2019). The transparent goby, *Aphia minuta*, showed variable responses depending on the period of the event. It increased in the estuary mouth during the freshets of autumn, while it disappeared during the high discharge of spring.

Differences could be doubt to the settlement periods of early life stages (La Mesa et al., 2005). Still, this species seems to be lured, showing increments of their abundances when environmental conditions were more variable, as González-Ortegón et al. (2010) found during high persistent turbidity events in the inner zone.

In contrast, most pelagic species which were abundant in inner zones did not recover the densities after the discharge. This was the case of the seabream *Sparus aurata*, which was displaced to lower zones and even flushed out. Its natural trend is to decrease during the spring, as showed the control period, but the freshet could have reduced its recruitment period, which can fluctuate in the region along the winter and early spring (Arias and Drake, 1990). Similar pattern was found for the anchovy *Engraulis encrasicolus*, although its negligible presence during the discharges suggest that it was completely flushed out of the estuary. Still, the event 1 showed a late recovery, while event 2 did not. The different recoveries could be because the discharge of event 1 was lower in intensity and duration, so individuals could be expelled closer to the estuary mouth, and therefore, return later. However, no density increments were found in the outer zone. Alternatively, high recruitment period of anchovy has been registered from May to November mainly, although this can oscillate interannually (Drake et al., 2007), enabling that a new recruitment stock from offshore could have been attracted and the previous estuarine population could have been predated in the nearshore area. The event 3 happened during the high larval recruitment period offshore of two important commercial fish in the Gulf of Cádiz, *Sardina pilchardus* and *Solea senegalensis* (Baldó et al., 2006). While *S. pilchardus* showed similar patterns during the event and control periods, *S. senegalensis* increased their densities in the freshet period one month after the discharge, which could suggest an attraction effect in this species due to the extent of estuarine cues by the large river plume (Kingsford and Suthers, 1994; Teodósio et al., 2016).

Also, the resto of macrofauna, as important food resource for many early fish stages (Baldó and Drake, 2002), can influence in their distribution (Drake et al., 2007). In fact, the spatial distribution along the section of the total abundance of early fishes and the rest of the macrofauna were very similar in most of the sampling days in all events. Macrofauna, when it was relative abundant before the freshet (event 2 and 3), tended to decrease slightly during the high discharge. But later, it recovered previous conditions quickly and its total abundance tended to increase (although the differences were not found to be significant), even when its normal trend was to decrease (event 2). This response, similar to that of gobies, shows the adaptation of estuarine species to these ecosystems, which better cope with this type of situation, probably avoiding being expelled from the estuary using shelter zones as a mechanism. Different studies have reported the benefits of freshet events in the lower trophic levels of estuaries by the nutrients and organic matter inputs, increasing the phytoplankton diversity and abundance, which in its turn, increase the secondary production as zooplankton (Chícharo et al., 2006; Hoover et al., 2006). However, the high turbidity levels found could difficult the photosynthetic activity and primary production in most of the water column of the estuary section (Ruiz et al., 2017), at least in the short-term studied. Instead, allochthonous carbon supplied by freshwater input can directly support zooplankton taxa with a more heterotrophic energy pathway (Abrantes et al., 2013; Hitchcock et al., 2016; Hoffman et al., 2008). Therefore, frequent high discharges in the Guadalquivir estuary can change in resource utilisation at the base of the food chain, making it one of the most productive systems of the region (Miró et al., 2020).

In summary, high freshet events generally generated a strong shift in physiochemical conditions of most estuary section, compressing salinity gradient, increasing turbidity and decreasing oxygen concentration. This alteration influenced in the abundance and distribution of early life stages of fishes and the rest of macrofauna, reducing its nursery area. Still, the physicochemical conditions showed an almost complete recovery in events 1 and 2, and partial in event 3 (depending on the intensity, duration and period of the discharge), to the previous state in a short term. Instead, different biological responses were found depending on the species and periods, being the benthic-estuarine ones which better coped with the freshets. Nonetheless, despite of the multiple environmental disturbances caused by the distinct natural freshet events, the estuary and their estuarine organisms showed a high resilience.

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Supplementary data

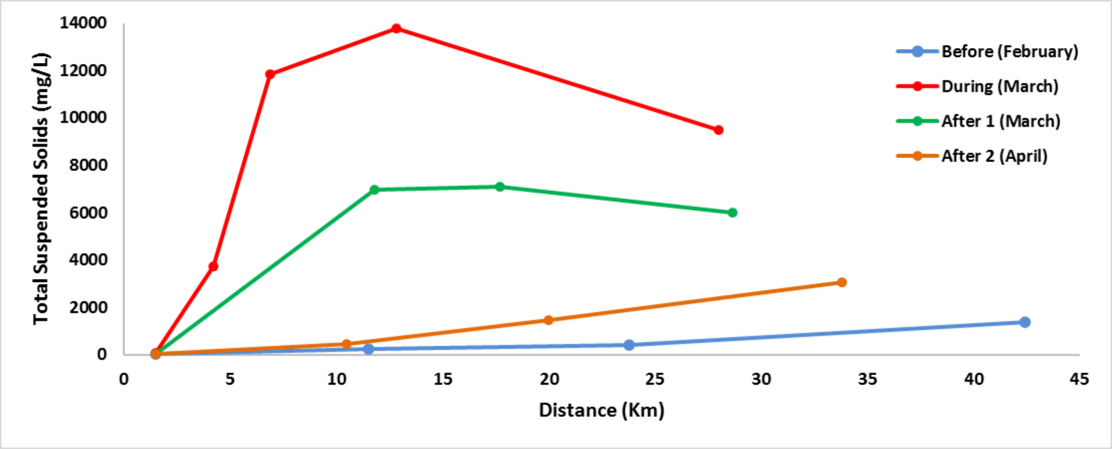

Figure S.1. Total suspended solids measured in different sampling moments along the estuary section of the Event 3.

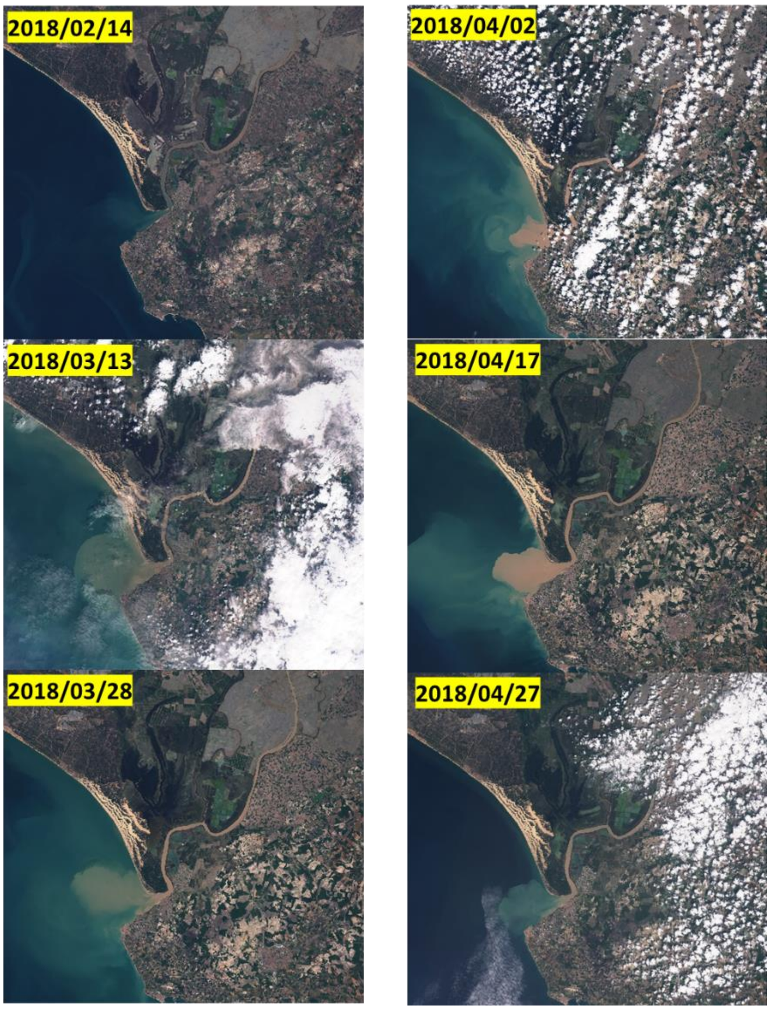

Figure S.2. Natural color images captured by the satellite Sentinel 2 in different dates during the progression of the river plume from the river mouth to the adjacent continental shelf in the Event 3.

Table S.1.1. Summary of contrasts in generalised additive model (GAM) results used to compare abundance and distribution of early life stages of fish species and rest of macrofauna in every sampling date (Before, During, After 1 and After 2) of the freshet event 1. Significant terms p ≤ 0.05 are highlighted in bold.

FRESHET EVENT 1

Early fish stages	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	**Early fish stages**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Before-During	1.7063	0.453	53.3	3.763	**0.0023**	s(distance):TimeBefore	1.897	1.989	5.604	0.053
Before-After1	0.1087	0.459	53.3	0.237	0.9953	s(distance):TimeDuring	2.818	3.329	28.888	**<.0001**
Before-After2	-0.0762	0.447	53.3	-0.17	0.9982	s(distance):TimeAfter1	2.259	2.557	12.952	**0.0121**
During-After1	-1.5976	0.407	53.3	-3.929	**0.0014**	s(distance):TimeAfter2	2.69	2.927	52.718	**<.0001**
During-After2	-1.7825	0.392	53.3	-4.542	**0.0002**					
After1-After2	-0.1849	0.4	53.3	-0.463	0.9668					
Macrofauna	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	**Macrofauna**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Before-During	0.4032	0.473	52	0.853	0.829	s(distance):TimeBefore	1.975	1.999	42.67	**<.0001**
Before-After1	0.8359	0.647	52	1.292	0.572	s(distance):TimeDuring	3.121	3.54	97.31	**<.0001**
Before-After2	-0.0117	0.553	52	-0.021	1	s(distance):TimeAfter1	2.94	2.997	58.8	**<.0001**
During-After1	0.4328	0.54	52	0.802	0.8534	s(distance):TimeAfter2	2.944	2.998	155.27	**<.0001**
During-After2	-0.4149	0.423	52	-0.982	0.7605					
After1-After2	-0.8476	0.611	52	-1.387	0.5131					
Pomatoschistus spp.	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Pomatoschistus spp.***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Before-During	0.248	0.562	53.3	0.442	0.9709	s(distance):TimeBefore	1.936	1.996	7.715	**0.022386**
Before-After1	-1.894	0.95	53.3	-1.993	0.2034	s(distance):TimeDuring	2.063	2.433	55.706	**<.0001**
Before-After2	-1.556	1.273	53.3	-1.223	0.6153	s(distance):TimeAfter1	2.799	2.963	18.643	**<.0001**
During-After1	-2.142	0.899	53.3	-2.382	0.093	s(distance):TimeAfter2	2.883	2.987	38.038	**<.0001**
During-After2	-1.804	1.235	53.3	-1.461	0.468					
After1-After2	0.337	1.453	53.3	0.232	0.9955					
Engraulis encrasiccolus	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Engraulis encrasiccolus***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Before-During	2.1053	0.722	52.7	2.915	**0.026**	s(distance):TimeBefore	1.922	1.994	21.334	**<.0001**
Before-After1	1.7601	0.597	52.7	2.95	**0.0237**	s(distance):TimeDuring	3.618	3.926	3.62	0.44665
Before-After2	-0.0288	0.703	52.7	-0.041	1	s(distance):TimeAfter1	1.894	2.186	9.825	**0.00899**
During-After1	-0.3452	0.599	52.7	-0.576	0.9387	s(distance):TimeAfter2	2.852	2.984	79.604	**<.0001**
During-After2	-2.1341	0.705	52.7	-3.026	**0.0194**					
After1-After2	-1.7889	0.576	52.7	-3.106	**0.0156**					
Sardina pilchardus	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Sardina pilchardus***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Before-During	-0.424	1.209	55.8	-0.351	0.985	s(distance):TimeBefore	1	1	0.204	0.6515
Before-After1	-2.085	1.37	55.8	-1.522	0.4315	s(distance):TimeDuring	1	1	1.658	0.1978
Before-After2	-2.537	1.031	55.8	-2.46	0.0777	s(distance):TimeAfter1	2.701	2.929	5.867	0.1101
During-After1	-1.661	1.321	55.8	-1.258	0.5934	s(distance):TimeAfter2	2.485	2.809	15.01	**0.0035**
During-After2	-2.113	0.965	55.8	-2.19	0.1387					
After1-After2	-0.451	1.16	55.8	-0.389	0.9798					
Aphia minuta	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Aphia minuta***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Before-During	-7886.127	2.316	58.24	-3404.539	**<.0001**	s(distance):TimeBefore	1	1	5435000	**<.0001**
Before-After1	-7888.214	2.922	58.24	-2700.053	**<.0001**	s(distance):TimeDuring	1.001	1.002	19.28	**<.0001**
Before-After2	-7891.938	3.269	58.24	-2413.919	**<.0001**	s(distance):TimeAfter1	1.01	1.02	50.83	**<.0001**
During-After1	-2.087	1.78	58.24	-1.172	0.6465	s(distance):TimeAfter2	2.751	2.94	134.70	**<.0001**
During-After2	-5.811	2.307	58.24	-2.519	0.0674					
After1-After2	-3.725	1.832	58.24	-2.033	0.1878					

Table S.1.2. Summary of contrast in generalised additive model (GAM) results used to compare abundance and distribution of early life stages of fish species and rest of macrofauna in every sampling date (Before, After 1 and After 2) of the control period of the event 1. Significant terms p ≤ 0.05 are highlighted in bold.

CONTROL 1

Early fish stages	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	**Early fish stages**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
October-November	1.35	0.711	31.7	1.895	0.1568	s(distance):TimeOctober	2.945	2.998	274.641	**<.0001**
October-December	2.9	0.577	31.7	5.015	**0.0001**	s(distance):TimeNovember	2.895	2.992	71.484	**<.0001**
November-December	1.55	0.476	31.7	3.253	**0.0074**	s(distance):TimeDecember	1.443	1.725	0.615	0.711
Macrofauna	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	**Macrofauna**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
October-November	1.06	0.388	31.1	2.733	**0.0269**	s(distance):TimeOctober	2.821	2.973	115.621	**<.0001**
October-December	2.25	0.336	31.1	6.698	**<.0001**	s(distance):TimeNovember	2.751	2.953	49.5	**<.0001**
November-December	1.19	0.337	31.1	3.525	**0.0037**	s(distance):TimeDecember	2.3	2.626	3.554	0.154
Pomatoschistus spp.	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Pomatoschistus spp.***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
October-November	1.304	1.78	30.7	0.734	0.7452	s(distance):TimeOctober	2.833	2.976	30.457	**<.0001**
October-December	2.033	1.32	30.7	1.541	0.2864	s(distance):TimeNovember	2.85	2.98	15.483	**0.00174**
November-December	0.729	1.3	30.7	0.559	0.8426	s(distance):TimeDecember	2.606	2.872	9.679	**0.01054**
Engraulis encrasiccolus	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Engraulis encrasiccolus***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
October-November	1.8	1.003	30.6	1.791	0.1894	s(distance):TimeOctober	2.922	2.996	136.52	**<.0001**
October-December	3.79	0.77	30.6	4.923	**0.0001**	s(distance):TimeNovember	2.91	2.994	60.94	**<.0001**
November-December	2	0.792	30.6	2.519	**0.0442**	s(distance):TimeDecember	2.561	2.851	13.52	**0.00289**
Sardina pilchardus	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Sardina pilchardus***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
October-November	-4.57E+10	0.616	38	-7.42E+10	**<.0001**	s(distance):TimeOctober	1.16E-24	2.31E-24	2.64E+17	**<.0001**
October-December	-4.57E+10	0.572	38	-7.99E+10	**<.0001**	s(distance):TimeNovember	1.03E+00	1.06E+00	5.17E+00	**0.0257**
November-December	2.00E+00	0.229	38	9	**<.0001**	s(distance):TimeDecember	1.00E+00	1.00E+00	2.43E-01	0.6219
Aphia minuta	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Aphia minuta***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
October-November	-0.497	3.061	31.5	-0.162	0.9856	s(distance):TimeOctober	2.627	2.924	2.391	0.524
October-December	2.194	2.995	31.5	0.733	0.7461	s(distance):TimeNovember	2.805	2.973	6.879	0.072
November-December	2.691	0.959	31.5	2.805	**0.0226**	s(distance):TimeDecember	2.106	2.425	32.744	**<.0001**

Table S.2.1. Summary of contrasts in generalised additive model (GAM) results used to compare abundance and distribution of early life stages of fish species and rest of macrofauna in every sampling date (Before, During, After 1 and After 2) of the freshet event 2. Significant terms p ≤ 0.05 are highlighted in bold.

FRESHET EVENT 2

Early fish stages	**estimate**	**SE**	**df**	**t.ratio**	**p.value**
Before-During	3.473	0.539	57.4	6.447	**<.0001**
Before-After1	1.16	0.328	57.4	3.538	**0.0044**
Before-After2	0.867	0.245	57.4	3.537	**0.0044**
During-After1	-2.313	0.581	57.4	-3.984	**0.0011**
During-After2	-2.606	0.538	57.4	-4.842	**0.0001**
After1-After2	-0.293	0.327	57.4	-0.896	0.8072
Macrofauna	**estimate**	**SE**	**df**	**t.ratio**	**p.value**
Before-During	1.07	0.692	56.7	1.547	0.4171
Before-After1	0.574	0.324	56.7	1.77	0.2981
Before-After2	-0.249	0.517	56.7	-0.481	0.963
During-After1	-0.496	0.689	56.7	-0.72	0.8886
During-After2	-1.319	0.798	56.7	-1.653	0.3579
After1-After2	-0.823	0.513	56.7	-1.605	0.3841
Pomatoschistus spp.	**estimate**	**SE**	**df**	**t.ratio**	**p.value**
Before-During	4.6755	1.056	61.7	4.428	**0.0002**
Before-After1	0.3633	0.981	61.7	0.37	0.9825
Before-After2	0.3126	1.919	61.7	0.163	0.9984
During-After1	-4.3122	1.085	61.7	-3.973	**0.0011**
During-After2	-4.363	1.974	61.7	-2.21	0.1319
After1-After2	-0.0508	1.935	61.7	-0.026	1
Engraulis encrasicolus	**estimate**	**SE**	**df**	**t.ratio**	**p.value**
Before-During	7.01	1.294	62.9	5.417	**<.0001**
Before-After1	2.69	0.427	62.9	6.309	**<.0001**
Before-After2	3.15	0.518	62.9	6.094	**<.0001**
During-After1	-4.31	1.306	62.9	-3.304	**0.0084**
During-After2	-3.85	1.338	62.9	-2.881	**0.0271**
After1-After2	0.46	0.547	62.9	0.841	0.8349
Aphia minuta	**estimate**	**SE**	**df**	**t.ratio**	**p.value**
Before-During	27.25	63.4	68.6	0.43	0.9732
Before-After1	-6.1	3.12	68.6	-1.955	0.2151
Before-After2	-4.84	3.23	68.6	-1.498	0.4443
During-After1	-33.35	63.33	68.6	-0.527	0.9524
During-After2	-32.09	63.33	68.6	-0.507	0.9572
After1-After2	1.26	1.1	68.6	1.15	0.66
Sardina pilchardus	**estimate**	**SE**	**df**	**t.ratio**	**p.value**
Before-During	2.06505	0.893	63.8	2.312	0.1061
Before-After1	0.31011	0.619	63.8	0.501	0.9585
Before-After2	0.30389	1.773	63.8	0.171	0.9982
During-After1	-1.75493	0.974	63.8	-1.802	0.2818
During-After2	-1.76116	1.926	63.8	-0.914	0.7973
After1-After2	-0.00622	1.815	63.8	-0.003	1

Early fish stages	**edf**	**Ref.df**	**Chi.sq**	**p-value**
s(distance):TimeBefore	2.725	2.935	73.35	**<.0001**
s(distance):TimeDuring	2.872	2.987	74.65	**<.0001**
s(distance):TimeAfter1	2.83	2.976	36.97	**<.0001**
s(distance):TimeAfter2	2.159	2.548	50.11	**<.0001**
Macrofauna	**edf**	**Ref.df**	**Chi.sq**	**p-value**
s(distance):TimeBefore	2.897	2.991	84.4	**<.0001**
s(distance):TimeDuring	2.785	2.963	29.6	**<.0001**
s(distance):TimeAfter1	2.645	2.902	31.84	**<.0001**
s(distance):TimeAfter2	2.943	2.997	55.94	**<.0001**
Pomatoschistus spp.	**edf**	**Ref.df**	**Chi.sq**	**p-value**
s(distance):TimeBefore	2.884	2.987	341.8	**<.0001**
s(distance):TimeDuring	3.775	4.131	39.47	**<.0001**
s(distance):TimeAfter1	2.752	2.939	253.45	**<.0001**
s(distance):TimeAfter2	2.872	2.984	154.98	**<.0001**
Engraulis encrasicolus	**edf**	**Ref.df**	**Chi.sq**	**p-value**
s(distance):TimeBefore	2.658	2.902	56.248	**<.0001**
s(distance):TimeDuring	3.829	4.221	19.621	**0.0154**
s(distance):TimeAfter1	2.242	2.602	6.745	**0.0434**
s(distance):TimeAfter2	2.418	2.773	9.46	**0.0248**
Aphia minuta	**edf**	**Ref.df**	**Chi.sq**	**p-value**
s(distance):TimeBefore	1	1	9.87	**0.00168**
s(distance):TimeDuring	1.882	1.986	68.43	**<.0001**
s(distance):TimeAfter1	1	1	37.968	**<.0001**
s(distance):TimeAfter2	1.513	1.831	5.009	0.05801
Sardina pilchardus	**edf**	**Ref.df**	**Chi.sq**	**p-value**
s(distance):TimeBefore	2.505	2.839	5.086	0.15153
s(distance):TimeDuring	2.433	3.005	2.372	0.48562
s(distance):TimeAfter1	2.739	2.955	15.382	**0.00119**
s(distance):TimeAfter2	2.505	2.867	2.01	0.56279

Table S.2.2. Summary of contrasts in generalised additive model (GAM) results used to compare abundance and distribution of early life stages of fish species and rest of macrofauna in every sampling date (Before, After 1 and After 2) of the control period of the event 2. Significant terms p ≤ 0.05 are highlighted in bold.

CONTROL 2

Early fish stages	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	**Early fish stages**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
November-December	1.345	0.464	27.4	2.9	**0.0193**	s(distance):TimeNovember	2.921	2.995	89.824	**<.0001**
November-January	2.18	0.455	27.4	4.796	**0.0001**	s(distance):TimeDecember	1.675	1.998	1.418	0.4894
December-January	0.836	0.194	27.4	4.297	**0.0006**	s(distance):TimeJanuary	1	1	4.332	**0.0374**
Macrofauna	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	**Macrofauna**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
November-December	1.167	0.298	26.5	3.913	**0.0016**	s(distance):TimeNovember	2.828	2.978	74.614	**<.0001**
November-January	1.699	0.273	26.5	6.228	**<.0001**	s(distance):TimeDecember	2.659	2.903	8.907	**0.0158**
December-January	0.532	0.19	26.5	2.796	**0.0249**	s(distance):TimeJanuary	1	1.001	16.136	**<.0001**
Pomatoschistus spp.	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Pomatoschistus spp.***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
November-December	0.965	1.263	24.8	0.764	0.7282	s(distance):TimeNovember	2.871	2.984	33.5	**<.0001**
November-January	1.66	1.294	24.8	1.283	0.4179	s(distance):TimeDecember	2.921	2.995	23.39	**<.0001**
December-January	0.695	0.414	24.8	1.677	0.2337	s(distance):TimeJanuary	2.45	2.772	22.91	**<.0001**
Engraulis encrasicolus	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Engraulis encrasicolus***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
November-December	1.65	0.762	26.4	2.17	0.0953	s(distance):TimeNovember	2.929	2.997	74.42	**<.0001**
November-January	5.62	0.882	26.4	6.375	**<.0001**	s(distance):TimeDecember	2.643	2.901	23.34	**<.0001**
December-January	3.97	0.583	26.4	6.803	**<.0001**	s(distance):TimeJanuary	1	1	0	0.984
Aphia minuta	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Aphia minuta***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
November-December	-2.79	9.331	27	-0.299	0.952	s(distance):TimeNovember	2.869	2.988	1.427	0.7
November-January	-2.048	9.324	27	-0.22	0.9738	s(distance):TimeDecember	2.129	2.528	3.584	0.234
December-January	0.742	0.698	27	1.063	0.5447	s(distance):TimeJanuary	1	1	3.714	0.054
Sardina pilchardus	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Sardina pilchardus***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
November-December	2.43	0.94	27.1	2.582	**0.04**	s(distance):TimeNovember	2.814	2.975	6.874	0.072172
November-January	4.28	1.32	27.1	3.234	**0.0087**	s(distance):TimeDecember	2.127	2.445	34.156	**<.0001**
December-January	1.86	1.14	27.1	1.621	0.2541	s(distance):TimeJanuary	1	1	14.154	**<.0001**

Table S.3.1. Summary of contrasts in generalised additive model (GAM) results used to compare abundance and distribution of early life stages of fish species and rest of macrofauna in every sampling date (Before, During, After 1 and After 2) of the freshet event 3. Significant terms p ≤ 0.05 are highlighted in bold.

FRESHET EVENT 3

Early fish stages	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	**Early fish stages**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Before-During	0.591	0.724	35	0.817	0.8462	s(distance):TimeBefore	2.364	2.7	41.81	**<.0001**
Before-After1	-3.138	0.257	35	-12.202	**<.0001**	s(distance):TimeDuring	6.895	7.577	178.14	**<.0001**
Before-After2	-3.384	0.422	35	-8.025	**<.0001**	s(distance):TimeAfter1	2.845	2.979	167.11	**<.0001**
During-After1	-3.729	0.723	35	-5.158	**0.0001**	s(distance):TimeAfter2	2.948	2.998	229.54	**<.0001**
During-After2	-3.975	0.797	35	-4.99	**0.0001**					
After1-After2	-0.246	0.421	35	-0.584	0.9362					
Macrofauna	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	**Macrofauna**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Before-During	2.296	1.292	32.8	1.777	0.3021	s(distance):TimeBefore	2.959	2.999	55.85	**<.0001**
Before-After1	-0.541	0.558	32.8	-0.971	0.7668	s(distance):TimeDuring	2.815	2.97	77.66	**<.0001**
Before-After2	-1.156	0.758	32.8	-1.524	0.4352	s(distance):TimeAfter1	2.478	2.778	32.53	**<.0001**
During-After1	-2.837	1.205	32.8	-2.355	0.1064	s(distance):TimeAfter2	2.946	2.998	79.28	**<.0001**
During-After2	-3.451	1.31	32.8	-2.635	0.0586					
After1-After2	-0.614	0.599	32.8	-1.026	0.7357					
Pomatoschistus spp.	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Pomatoschistus spp.***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Before-During	0.5149	0.74	35.5	0.696	0.898	s(distance):TimeBefore	2.968	2.999	69.61	**<.0001**
Before-After1	-2.8635	0.398	35.5	-7.187	**<.0001**	s(distance):TimeDuring	5.533	5.87	1393.31	**<.0001**
Before-After2	-2.8427	0.41	35.5	-6.941	**<.0001**	s(distance):TimeAfter1	2.997	3	388.81	**<.0001**
During-After1	-3.3785	0.638	35.5	-5.299	**<.0001**	s(distance):TimeAfter2	2.996	3	257.69	**<.0001**
During-After2	-3.3577	0.645	35.5	-5.209	**<.0001**					
After1-After2	0.0208	0.163	35.5	0.128	0.9992					
Sparus aurata	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Sparus aurata***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Before-During	1.002	0.394	44.8	2.542	0.067	s(distance):TimeBefore	1	1	8.636	**0.0033**
Before-After1	0.542	0.55	44.8	0.985	0.7585	s(distance):TimeDuring	1	1	2.793	0.0947
Before-After2	2.677	0.705	44.8	3.799	**0.0024**	s(distance):TimeAfter1	2.252	2.64	2.647	0.3742
During-After1	-0.46	0.555	44.8	-0.829	0.8405	s(distance):TimeAfter2	1	1	1.414	0.2344
During-After2	1.675	0.709	44.8	2.363	0.0992					
After1-After2	2.135	0.806	44.8	2.65	0.0522					
Sardina pilchardus	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Sardina pilchardus***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Before-During	19.27	36.203	36.3	0.532	0.9506	s(distance):TimeBefore	1	1	2.295	0.1299
Before-After1	-3.545	0.674	36.3	-5.258	**<.0001**	s(distance):TimeDuring	1.762	1.944	2.858	0.2045
Before-After2	-4.182	0.748	36.3	-5.59	**<.0001**	s(distance):TimeAfter1	2.235	2.566	9.336	**0.0304**
During-After1	-22.814	36.2	36.3	-0.63	0.9216	s(distance):TimeAfter2	2.67	2.922	22.062	**<.0001**
During-After2	-23.452	36.201	36.3	-0.648	0.9156					
After1-After2	-0.637	0.572	36.3	-1.115	0.683					
Aphia minuta	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Aphia minuta***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Before-During	-67.422	1466.947	42.9	-0.046	1	s(distance):TimeBefore	1	1	0.003	0.95966
Before-After1	-71.807	1466.947	42.9	-0.049	1	s(distance):TimeDuring	1.063	1.122	9.352	**0.00217**
Before-After2	-66.857	1466.949	42.9	-0.046	1	s(distance):TimeAfter1	2.636	2.9	66.592	**<.0001**
During-After1	-4.385	0.762	42.9	-5.754	**<.0001**	s(distance):TimeAfter2	2.395	2.678	6.113	0.17288
During-After2	0.565	2.856	42.9	0.198	0.9972					
After1-After2	4.95	2.757	42.9	1.795	0.2896					
Gobius paganellus	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Gobius paganellus***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Before-During	-1.99	4.47	33.4	-0.446	0.9699	s(distance):TimeBefore	2.291	2.727	0.956	0.76955
Before-After1	-4.67	1.17	33.4	-3.993	**0.0018**	s(distance):TimeDuring	2.867	2.983	15.501	**0.00115**
Before-After2	-3.49	2.64	33.4	-1.32	0.5569	s(distance):TimeAfter1	2.817	2.973	53.15	**<.0001**
During-After1	-2.68	4.33	33.4	-0.619	0.9253	s(distance):TimeAfter2	2.654	2.934	3.888	0.31105
During-After2	-1.49	4.93	33.4	-0.303	0.9902					
After1-After2	1.18	2.4	33.4	0.494	0.9598					
Solea senegalensis	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Solea senegalensis***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Before-During	-1.22	1.164	41.7	-1.044	0.7246	s(distance):TimeBefore	1.116	1.221	1.191	0.3783
Before-After1	1.08	0.811	41.7	1.327	0.5512	s(distance):TimeDuring	2.678	3.085	7.056	0.07257
Before-After2	-3.01	0.719	41.7	-4.19	**0.0008**	s(distance):TimeAfter1	1.758	2.126	1.032	0.62588
During-After1	2.29	1.334	41.7	1.718	0.3275	s(distance):TimeAfter2	2.769	2.957	15.315	**0.00197**
During-After2	-1.8	1.28	41.7	-1.403	0.5047					
After1-After2	-4.09	0.971	41.7	-4.212	**0.0007**					

Table S.3.2. Summary of contrasts in generalised additive model (GAM) results used to compare abundance and distribution of early life stages of fish species and rest of macrofauna in every sampling date (Before, After 1 and After 2) of the control period of the event 3. Significant terms p ≤ 0.05 are highlighted in bold.

CONTROL 3

Early life stages	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	**Early life stages**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
February-March	1.14	0.207	41.3	5.495	**<.0001**	s(distance):TimeFebruary	1	1.001	0.144	0.7045
February-April	-1.08	0.214	41.3	-5.026	**<.0001**	s(distance):TimeMarch	1.49	1.74	15.061	**0.00639**
March-April	-2.22	0.246	41.3	-9.012	**<.0001**	s(distance):TimeApril	2.188	2.566	94.608	**<.0001**
Macrofauna	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	**Macrofauna**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
February-March	-1.19	0.189	36.4	-6.28	**<.0001**	s(distance):TimeFebruary	1.001	1.002	94.62	**<.0001**
February-April	-1.761	0.199	36.4	-8.827	**<.0001**	s(distance):TimeMarch	1.696	1.908	60.99	**<.0001**
March-April	-0.571	0.229	36.4	-2.499	**0.0441**	s(distance):TimeApril	1.941	1.997	15.53	**<.0001**
Pomatoschistus spp.	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Pomatoschistus spp.***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
February-March	1.766	0.286	37	6.184	**<.0001**	s(distance):TimeFebruary	1	1	5.052	**0.0246**
February-April	-0.838	0.142	37	-5.903	**<.0001**	s(distance):TimeMarch	1.946	1.997	43.102	**<.0001**
March-April	-2.604	0.283	37	-9.198	**<.0001**	s(distance):TimeApril	1.001	1.001	68.43	**<.0001**
Sparus aurata	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Sparus aurata***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
February-March	4.85	5.74	44.1	0.846	0.6767	s(distance):TimeFebruary	1.02E+00	1.05E+00	46.53	**<.0001**
February-April	117.98	1.66	44.1	71.245	**<.0001**	s(distance):TimeMarch	1.85E+00	1.98E+00	2.168	0.37
March-April	113.12	5.97	44.1	18.942	**<.0001**	s(distance):TimeApril	1.42E-20	2.85E-20	0	0.99
Sardina pilchardus	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Sardina pilchardus***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
February-March	2.01	1.177	39.5	1.711	0.2139	s(distance):TimeFebruary	1.907	1.991	7.903	**0.0152**
February-April	-1.29	0.562	39.5	-2.3	0.0675	s(distance):TimeMarch	1.952	1.998	7.781	**0.0234**
March-April	-3.31	1.174	39.5	-2.816	**0.0202**	s(distance):TimeApril	2.608	2.887	25.463	**<.0001**
Solea senegalensis	**estimate**	**SE**	**df**	**t.ratio**	**p.value**	***Solea senegalensis***	**edf**	**Ref.df**	**Chi.sq**	**p-value**
February-March	2.147	0.592	41.2	3.626	**0.0022**	s(distance):TimeFebruary	1.001	1.001	3.128	0.077
February-April	-0.769	0.54	41.2	-1.426	0.3373	s(distance):TimeMarch	1.124	1.233	2.987	0.1146
March-April	-2.916	0.736	41.2	-3.963	**0.0008**	s(distance):TimeApril	2.718	2.929	9.937	**0.0162**

5. COMPARISON OF EARLY LIFE STAGES OF FISH AND ENVIRONMENTAL CONDITIONS BETWEEN ESTUARIES OF THE GULF OF CÁDIZ*

Abstract

Hydrological, geomorphological, physicochemical and biological factors influence the nursery function of estuaries. Our study compared the environmental conditions and the assemblages of early life stages of fish in the main four estuaries of the Gulf of Cadiz (Cadiz Bay, Guadalquivir, Odiel-Tinto and Guadiana). Samples were taken within each estuary and on their adjacent coast, during the dry-warm seasons of 2016, 2017 and 2018. Results showed that rivers with smaller basins had a very low freshwater input and their estuaries, Odiel-Tinto and Cadiz Bay, were essentially sea extensions into the land, containing similar physicochemical conditions to nearshore zones, as well as similar assemblages and densities of early life stages of fish. Open water masses of these estuaries do not have important nursery functions. In contrast, inner zones of estuaries with bigger basins and higher freshwater discharges, Guadalquivir and Guadiana, have different environmental characteristics and a long transition zone with a well-defined salinity gradient. Their assemblages and densities of early life stages of fish were different between them and with other estuaries. The Guadalquivir estuary held the highest abundance of larval and early juvenile fish, as well as macrozooplankton biomass. The most abundant fish species in all zones of every estuary was the anchovy *Engraulis encrasicolus*; the Guadalquivir inner zone had the highest density. High concentration of suspended organic matter, provided by freshwater input and correlated with total suspended solid, suspended inorganic matter and turbidity, was the physicochemical characteristic more typical of the Guadalquivir. This characteristic, in addition to the salinity gradient, could explain the highest densities of macrozooplankton found in this estuary, and consequently, of early fish stages. Recurrent jellyfish blooms were observed in Cadiz Bay and the inner zone of Guadiana, affecting their nursery functions. Odiel-Tinto showed altered physicochemical and biological characteristics, which may need further specific research.

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Resumen

Los factores hidrológicos, geomorfológicos, fisicoquímicos y biológicos influyen en la función de cría de los estuarios. Nuestro estudio comparó las condiciones ambientales y las comunidades de las larvas y juveniles de peces en los cuatro principales estuarios del Golfo de Cádiz (Bahía de Cádiz, Guadalquivir, Odiel-Tinto y Guadiana). Se tomaron muestras dentro de cada estuario y en su zona adyacente, durante los veranos de 2016, 2017 y 2018. Los resultados mostraron que los ríos con cuencas más pequeñas tenían una entrada de agua dulce muy baja y sus estuarios, Odiel-Tinto y Bahía de Cádiz, fueron esencialmente extensiones marinas tierra adentro. Estos contuvieron condiciones fisicoquímicas similares a las de las zonas cercanas a la costa, al igual que de comunidades y densidades de etapas tempranas de peces. Las masas de aguas abiertas de estos estuarios no mostraron funciones importantes de cría. Por el contrario, las zonas interiores de los estuarios con mayores cuencas y mayores aportes de agua dulce, Guadalquivir y Guadiana, tienen características ambientales diferentes y una zona de transición larga con un gradiente de salinidad bien definido. Sus comunidades y densidades de larvas y juveniles de peces eran diferentes entre ellos y también con los otros estuarios. El estuario del Guadalquivir contenía la mayor abundancia de larvas y juveniles, así como de biomasa de macrozooplancton. La especie más abundante en todas las zonas de cada estuario fue el boquerón *Engraulis encrasicolus*; la zona interior del Guadalquivir tuvo la mayor densidad. La alta concentración de materia orgánica en suspensión, proporcionada por el aporte de agua dulce y correlacionada con el total de sólidos en suspensión, materia inorgánica en suspensión y turbidez, fue la característica fisicoquímica más típica del Guadalquivir. Esta característica, además del gradiente de salinidad, podría explicar las mayores densidades de macrozooplancton encontradas en este estuario y, en consecuencia, de larvas y juveniles de peces. Se observaron explosiones demográficas de medusas recurrentemente en la Bahía de Cádiz y la zona interior del Guadiana, afectando sus funciones de cría. Odiel-Tinto mostró características fisicoquímicas y biológicas alteradas, que pueden necesitar estudios específicos.

1. Introduction

More than 60% of the Earth's population are living in coastal areas (Ray, 2006) and rapid urban and agriculture development (Lee et al., 2012; Wu et al., 2019) are affecting environmental conditions of rivers, wetlands, floodplains and estuaries by flow alteration and high sediment, nutrient or pollutant inputs (González-Ortegón et al., 2015; González-Ortegón and Drake, 2012; Rolls and Bond, 2017). Additionally, global climate change is imposing complementary modifications; recent studies, based on 30 years of historical data, have found a significant decrease in precipitations in south-central European and Mediterranean river basins (Xoplaki et al., 2004). This, together with anthropogenic water abstraction for diverse uses, has resulted in an increase in the number of days with low flow (Lobanova et al., 2018; Papadimitriou et al., 2016), especially during the summer (Wanders et al., 2015). These modifications have been reported to decrease the ecological status of affected ecosystems (Poff and Zimmerman, 2010), with fish being a taxonomic group of most concern (Schinegger et al., 2016). EU member states have legislated to manage and protect all running waters under the Water Framework Directive (WFD, European Commission, 2000), but the River Basin Management Plans from 2018 indicated that 60% of European water bodies failed to achieve good ecological status, as they are affected by a complex set of stressors (European Environment Agency, 2018).

Of the different aquatic ecosystems impacted, estuaries are particularly important because they play an essential role in the nursery function of many species, especially for marine fishes (Strydom et al., 2003). These ecosystems generally provide high food availability and good predator refuge for early life stages of these fishes (Boesch and Eugene, 1984; Elliot and Hemingway, 2002). However, human activity can modify this function, disturbing the hydrological regime and the characteristics of the transitional zone between coastal and riverine waters (Fernández-Delgado et al., 2007; Whitfield and Wooldridge, 1994; Whitfield et al., 2012). These modifications, in their turn, affect essential biological characteristics such as the base of food webs (*in situ* primary production and detrital organic matter) and how matter and energy transfer first to zooplankton and then to early life stages of fishes (Donázar-Aramendía et al., 2019; Warry et al., 2016). For instance, excess nutrient inputs can cause eutrophication with toxic algae blooms (Wolanski et al., 2006) or jellyfish proliferation (Purcell et al., 1999); sediment inputs can increase turbidity and, consequently, limit photosynthetic activity and primary productivity pushing the food webs to a more heterotrophic status (Ruiz et al., 2017; Soetaert et al., 2006).

No two estuaries have the same biotic and abiotic characteristics, since the quantity and quality of their habitats are often diverse (Vasconcelos et al., 2009). Geomorphological variables such as river and delta basin areas, barrier sands, estuary length, inlet width, mean depth or intertidal flat area also contribute to their diverse estuary functioning (Amezcua et al., 2019; Saintilan, 2004). As stated by Whitfield (1999), if fish consistently respond to the environment, the assemblages inhabiting similar types of estuaries in a particular region would be expected to reflect this similarity; hence, studies comparing assemblages of early stages of fish and environmental conditions between different estuaries of the same region are essential tools for understanding what factors influence their successful recruitment and for learning how to manage these factors sustainably, potentially improving ecosystem services (Harrison and Whitfield, 2006). Also, this kind of study shows which estuaries are more productive for the different species, which estuaries can supply more recruits to adult stocks and, consequently, which areas should be protected as a supporter ground for proper ecosystem function and fishery management in the region. However, most studies focus on adult fish, not in early life stages. Estuarine research on early fish stages that compare the assemblages and environmental conditions of two or more estuaries, using the same sampling methods, effort, periods and climatic regions, is still limited (but see Montoya-Maya and Strydom, 2009; Ramos et al., 2012; Strydom et al., 2003 for examples). Moreover, this kind of studies in temperate estuaries are scarce, with a few examples in South Africa (Grange et al., 2000; Harris and Cyrus, 2000; Whitfield, 1994) but none in Europe. Nearshore areas of estuaries can also be important rearing grounds (Able et al., 2013; Araújo et al., 2018), and some authors (Beck et al., 2001) proposed that a study of nursery function should also include a comparison with other surrounding habitats.

The Gulf of Cadiz (GoC) includes several permanently open estuaries with different freshwater inflow regimes and geomorphology types (González-Ortegón et al., 2018 and 2019) and, most likely, different environmental (salinity gradient, turbidity, organic matter, etc.) and biological (prey availability, chlorophyll concentration, etc.) characteristics (Drake et al., 2002; Drake and Arias, 1991; Faria et al., 2006). The proximity of these estuaries within this region and, hence, the similar influence of some external factors (relative geographical position, climate, larval supply coming from the same stock, connectivity, global water body circulation within the North Atlantic, etc.) enable an informative comparison of specific variables that govern the use of estuaries by early life stages of fishes.

The main goals of this study are i) to assess the potential function of these estuaries as nursery areas by comparing their early life stages of fish assemblages with their adjacent coastal zones, ii) to compare the structure and composition of early life stages of fish assemblages in estuaries with different hydrogeomorphological and environmental characteristics and iii) to identify which variables affect assemblage distributions and quantify their influence in the nursery success of each estuary by comparing their early life fish relative abundances while controlling for methodology and time period. The working hypotheses were: i) estuaries with similar characteristics would have similar early life stage assemblages of fish, but key environmental variables would generate differences in these assemblages; ii) inner estuarine zones would have higher relative abundance than their adjacent coastal zones.

2. Material and Methods

2.1. Study area

The study focused on a particular temperate North Atlantic region at the entrance of the Mediterranean Sea, the Gulf of Cadiz (South-west of the Iberian Peninsula), which exhibits some typical Atlantic and Mediterranean climate characteristics, and where the fishery has an important economic and employment role (Marti, 2018). Specifically, it was carried out in the four main, permanently open estuaries: Guadiana, Odiel-Tinto, Guadalquivir and Cadiz Bay (Figure 1) and their nearshore zones.

Guadiana estuary (37°13'33"N - 7°24'51"W) is a rock-bounded system approximately 80 km in length. It is a mesotidal well-mixed estuary with an amplitude range of 2 m, and its maximum depth varies between 5 and 17 m. It is oriented north–south and is the connection of Guadiana River (810 km long and 67,129 km^2 basin area) with the Atlantic Ocean (Garel and Ferreira, 2015). The input of freshwater to the estuary is controlled by Alqueva Dam, whose annual average discharge between 2016–2018 was 13.5 m^3/s (Pulo do Lobo station, 60 km from river mouth–Portuguese Environment Agency—http://snirh.pt), but it oscillates across seasons by the rainfalls (Chicharo et al., 2001; Faria et al., 2006).

Odiel-Tinto estuary (37°12'30"N - 6°56'31"W) is a salt-marshes system of 14,900 ha, whose main water channels are 25 km long in total. It is a mesotidal well-mixed estuary with an amplitude range of 2 m (Ruiz et al., 1998), and its channel depth oscillates between 3 and 15 m. It is formed by the Odiel (128 km) and Tinto (83 km) rivers (4,761 km^2 total basin area), whose mean water flows were approximately 4.1 m^3/s (Calañas station, 50 km from river mouth) and 2.8 m^3/s (Candón station + Villarrasa station, 40 km from river mouth), respectively during 2016–2018 (Agencia de Medio Ambiente y Agua de Andalucía, http://www.agenciamedioambienteyagua.es).

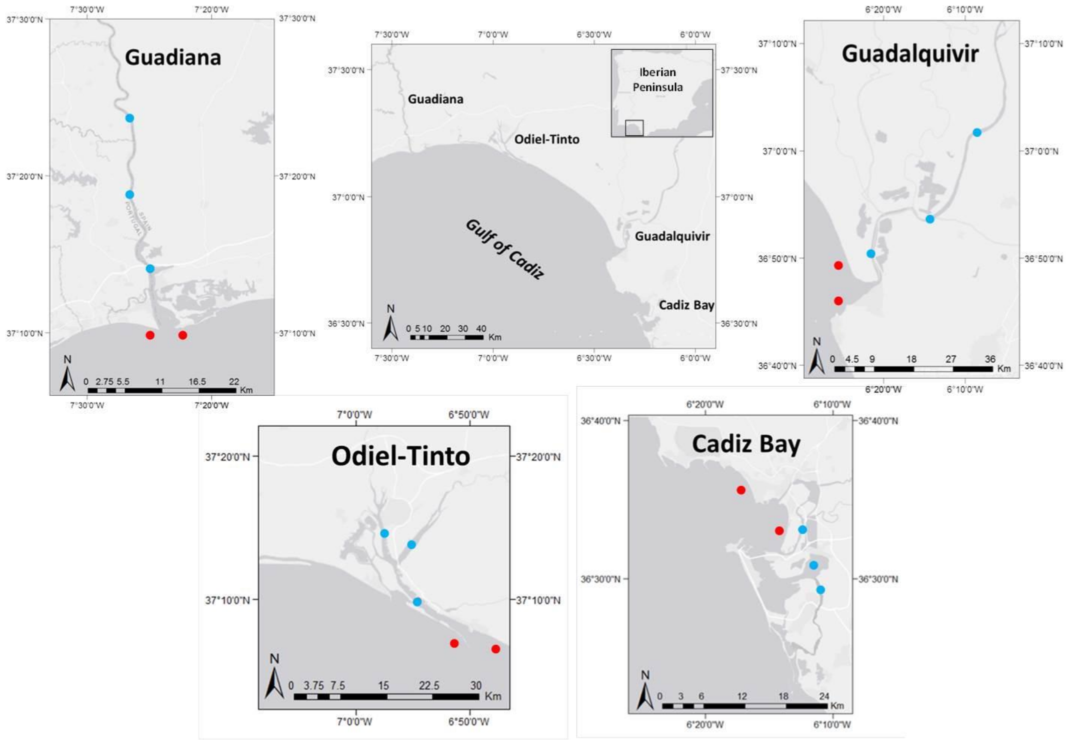

Figure 1. Study area in the Gulf of Cadiz with sampling stations (blue: inner estuarine stations; red: nearshore stations).

Guadalquivir estuary (36°51'27"N - 6°21'12"W) is a well-mixed mesotidal system with 110 km long channel and 3 m amplitude range (Díez-Minguito et al., 2012). The main channel is navigable and has an average depth of 7.1 m (Ruiz et al., 2015). Guadalquivir River (680 km long and 57,527 km^2 basin area) is connected to the estuary by Alcalá del Río Dam, which controls the water river flow with a mean discharge of 39.7 m^3/s (Alcalá del Río station, 110 km from the river mouth) during 2016–2018 (SAIH Confederación Hidrográfica del Guadalquivir, http://www.chguadalquivir.es/saih/DatosHistoricos.aspx).

Cadiz Bay (36°30'03"N—6°12'33"W) is a low-inflow, dynamically short and tidally driven estuary, with an amplitude range of 3 m, that can be divided into two basins: the inner and outer bays (Zarzuelo et al., 2015). The outer bay (7,000 ha) is affected by waves, tidal currents and low freshwater discharges of Guadalete River (166 km long and 5,960 km^2 basin area), with a mean water flow of approximately 4.1 m^3/s (Arcos Dam station + Guadalcaucín Dam station, 58 km from the river mouth) during 2016–2018 (SAIH Hidrosur, http://www.redhidrosurmedioambiente.es/saih/). Sandy bottoms (90%) and rocky shores and cliffs (10 %) characterise this area, which has a mean depth of 15 m (Sánchez-Lamadrid et al. 2002). The inner bay (5,000 ha) is a semi-enclosed coastal lagoon with shallow waters of 3 m average depth and an artificial channel that is 8 m deep. This zone is characterised by muddy bottoms, large intertidal flats, a dense tidal channel network and by the dominance of seagrasses and seaweeds meadows (mainly *Zostera noltii*, *Z. marina*, *Cymodocea nodosa* and *Caulerpa prolifera*; Brun et al., 2015).

Following the geological classification of estuary types by Roy et al. (2001), Cadiz Bay is considered as type I, while Odiel-Tinto, Guadalquivir and Guadiana as type II. In relation with the water bodies, Guadalquivir and Guadiana estuaries are characteristic for their longitudinal salinity gradient (Chícharo et al., 2001; Vanney, 1970), in contrast with Odiel-Tinto and Cadiz Bay, which are essentially marine (Cánovas et al., 2007; Zarzuelo et al., 2017) during the dry-warm season.

2.2. Field sampling

Sampling was carried out during the dry-warm season (June–July) of 2016, 2017 and 2018. Samples were taken from 20 stations with 3–5 replicates of each one: 5 stations per estuary, 3 in the inner zone of estuaries and 2 in the adjacent nearshore of the river mouth (Figure 1). In the estuaries with salinity gradient (Guadiana and Guadalquivir), stations were spread along the salinity range (between 25 and 5 PSU); in completely marine estuaries (Odiel-Tinto and Cadiz Bay), stations were spatially distributed, covering most of the area.

Samples were always collected in the flood tide with a plankton net of 1 m diameter and 1 mm mesh size equipped with a flow-meter General Oceanics 2030R. Oblique tows of 12 minutes (294 ± 77 m^3; mean ± SD) were done with a boat at a speed of 2–2.5 knots. Samples were fixed in 70% ethanol and the early fish stages were sorted from the rest of macrozooplankton organisms. Fishes were identified and quantified whenever possible, to species level, and macrozooplankton biomass was calculated in fresh weight.

Three replicated physicochemical profiles of the whole water column were recorded in every station with a multiprobe (depth, temperature, salinity, turbidity, dissolved oxygen concentration (DO), oxygen saturation (OS), pH and chlorophyll concentration (Chla); Eureka^{TM} Manta2). Water samples were taken at mid-depth with a Niskin bottle to measure chlorophyll (to calibrate the fluorimeter of the multiprobe) and total suspended solids (TSS) concentration. To measure total suspended solids (TSS), water was filtered through 0.7 µm pore pre-combusted (4 h, 500°C) filters (Whatman GF/F); thereafter filters were dried (24 h, 60 °C) and weighted. Suspended organic (SOM) and inorganic matter (SIM) were obtained as weight loss by ignition (500°C, 4h). Chlorophyll filters (Whatman GF/F) were kept frozen (-20°C) until fluorometric analysis by FP-8000 series Spectrofluorometer Software Manual (JASCO Corportion, 2011).

2.3. Data analysis

Fish abundances were standardised by filtered volume and expressed as number of individuals/1,000 m^3. Density data were organized in a species/sample abundance matrix, and a Bray–Curtis similarity matrix was calculated on fourth-root-transformed data with the addition of a dummy variable (value = 1) (Clarke et al., 2006), as a distance measure among samples (Bray and Curtis, 1957).

The differences in the multivariate structure of the early life stages of fish assemblages were analysed in a distance-based permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001; Mcardle and Anderson, 2001). The experimental design included 3 crossed fixed factors: location (with 4 levels, “Guadiana, Odiel-Tinto, Guadalquivir and Cadiz Bay” [GN/OT/GQ/CB]), zone (with 2 levels, “Inner estuary and Nearshore”) and year (with 3 levels, “2016, 2017 and 2018”), as well as 1 random factor, “Station”, nested within “Location”, “Zone” and “Year”. The sampling units were the 3–5 replicate tows randomly selected at each “Station”. Significant interactions, if detected, were further explored in separate analyses, within the levels of the interacting factors, i.e. the significant interactions between “Location” and “Zone” were further analysed separately by GN/OT/GQ/CB estuaries and inner estuary/nearshore zones. When appropriate, significant terms in the separated models were analysed individually using pair-wise comparison with the PERMANOVA test.

The distances between samples of every location were represented by principal coordinates analysis (PCO). SIMPER (Clarke, 1993) was used to identify the percentage contribution that each taxon made to the measures of similarity and dissimilarity among the different levels of the crossed fixed factors of location and zone. Multivariate analyses were performed using the software PRIMER v6.1.11 and PERMANOVA+ v1.0.1 statistical package (Clarke and Gorley, 2006).

We examined significant differences in total abundance (ind./1000 m^3) using PERMANOVA tests on Euclidean distance matrices, in an approach similar to parametric ANOVA (Anderson, 2001), using the same design as for assemblage structure. When appropriate, significant terms in the full model were analysed individually using pair-wise comparison with the PERMANOVA test.

To obtain a single value for physicochemical variables recorded with the multiprobe, to use them as covariates, a generalised additive mixed model was fitted in every station, using the replicated individual profiles as random and obtaining the common smoother (Zuur et al.

2015). This allows an appropriate single mean value to represent the complete water column. Results of all environmental variables, together with macrozooplankton and jellyfish abundance, were plotted with boxplot grouped by location and zone using the package "ggplot2" of R 3.5.2 software (R Development Core Team, 2018).

To identify which factors were the most important in characterising differences between the assemblages of each location and zone, a distance-based redundancy analysis (dbRDA) was carried out. A matrix was constructed using stations as columns and mean values of the environmental variables as rows. Hydrogeomorphological variables such as river length, estuary length, river basin area, mean bottom depth and daily annual mean of freshwater input (see data in study area section) were also included. Variables that were highly correlated ($r \geq 0.7$) were excluded (turbidity, TSS, SIM, OS, river and estuary length) from the analysis. Multicollinearity of the selected variables was further analysed with a variance inflation factor (VIF) test using the "vif" function from the "car" package (Fox and Weisberg, 2011) in R software. All variables from the selected best model had a VIF < 10 (Zuur et al., 2009). Selected variables were normalised and a similarity matrix based on the Euclidian distance was calculated. A similarity matrix based on Bray Curtis distance with the fourth-root-transformed mean value of every station in each location and zone during each year was constructed for early life stages of fish assemblage. Both matrices and the relationship analysis were performed using the software PRIMER v6.1.11.

3. Results

3.1. Assemblage analysis

In total, 20.839 fish individuals in early life stages belonging to 34 species, 20 genera, 19 families and 8 orders were caught both in estuarine and in nearshore zones at the 20 stations during the summer season of 2016, 2017 and 2018 (Table 1). Among the locations, a total of 23 species were collected in Guadalquivir, 21 in Guadiana, 19 in Odiel-Tinto and 16 in Cadiz Bay.

The assemblage structure varied among random stations within the same zone and also varied interactively between locations and zones and between locations and years; that is, the differences between inner estuary and nearshore zones were not the same in all locations, and the differences among years were also different in distinct locations (Table 2).

PERMANOVA analysis, separating nearshore and inner estuary levels of factor zone (Table 3), did not reveal differences between the nearshore zones of all locations but did show

Table 1. Spatial distribution and total mean densities (ind./1000 m^3) of fish early life stages in Cadiz Bay, Guadalquivir, Odiel-Tinto and Guadiana inner estuary and adjacent nearshore zones during the dry-warm season of 2016, 2017 and 2018.

Order	Family	Genera	Species	CADIZ BAY		GUADALQUIVIR		ODIEL-TINTO		GUADIANA	
				Nearshore	**Estuary**	**Nearshore**	**Estuary**	**Nearshore**	**Estuary**	**Nearshore**	**Estuary**
Perciformes	Ammodytidae		**Ammodytidae**	0	0	0.10	0	0	0	0	0
Anguilliformes	Anguillidae	Anguilla	***Anguilla anguilla***	0	0	0	0.47	0	0	0	0
Perciformes	Gobiidae	Aphia	***Aphia minuta***	0	0	2.47	0.06	0	0.06	0	0
Perciformes	Scianidae	Argyrosomus	***Argyrosomus regius***	0	0	0.85	13.16	0	0.07	0	0
Atheriniformes	Atherinidae	Atherina	***Atherina sp.***	0	0.10	0	0	0	0.06	0	0
Beloniformes	Belonidae	Belone	***Belone belone***	0	0.11	0	0	0	0	0	0
Perciformes	Blenniidae		**Blenniidae**	0.15	0	0.49	0	2.91	0.86	1.26	0
Perciformes	Callionymidae		***Callionymus sp.***	0	0	0	0	1.74	0.65	0.46	0
Clupeiformes	Clupeidae		**Clupeidae**	0	0	0	0.06	0	0	0.29	0.14
Perciformes	Moronidae	Dicentrarchus	***Dicentrarchus punctatus***	0	0.10	0	3.46	0	0	0	0
Pleuronectiformes	Soleidae	Dicologlossa	***Dicologlossa cuneata***	0	0	0.49	0	1.79	0	0.19	0
Perciformes	Sparidae	Diplodus	***Diplodus annularis***	3.32	5.57	1.69	0.29	6.94	1.04	5.38	0.08
Perciformes	Sparidae	Diplodus	***Diplodus sp.***	0	0.11	0	0	0	0	0.47	0
Clupeiformes	Engraulidae	Engraulis	***Engraulis encrasicolus***	24.52	25.25	48.57	761.31	39.67	10.63	21.41	162.09
Batrachoidiformes	Batrachoididae	Halobatrachus	***Halobatrachus didactylus***	0.41	0.73	0	0.09	0	0	0	0
Syngnathiformes	Syngnathidae	Hippocampus	***Hippocampus hippocampus***	0	0.95	0.12	0	0.12	0.91	0	1.66
Perciformes	Labridae		**Labridae**	0	0	0.24	0	0.10	0.27	0	0.23
Perciformes	Blenniidae	Lipophrys	***Lipophrys pholis***	0.71	0.31	0.23	0	0	0	0.10	0
Perciformes	Mugilidae	Chelon	***Chelon auratus***	0	0	0	0.12	0	0	0	0
Perciformes	Mugilidae	Chelon	***Chelon ramada***	0	0	0	0.07	0	0	0	0
Perciformes	Sparidae	Pagrus	***Pagrus pagrus***	0	0	0	0	0	0	0.49	0
Perciformes	Haemulidae	Pomadasys	***Pomadasys incisus***	0	0	1.01	0	1.46	0	0.93	0
Perciformes	Gobiidae	Pomatoschistus	***Pomatoschistus sp.***	0	0.95	0	409.42	0.23	0.73	0.19	89.64
Pleuronectiformes	Soleidae	Solea	***Solea senegalensis***	0	0.22	0.12	0.12	0.72	0.92	0.38	0.14
Pleuronectiformes	Soleidae		**Soleidae**	0	0	1.47	0	0.38	0.53	0.12	0.25
Syngnathiformes	Syngnathidae	Syngnathus	***Syngnathus acus***	0	0.45	0	0	0.25	0.20	0.10	4.07
Syngnathiformes	Syngnathidae	Syngnathus	***Syngnathus typhle***	0	0.55	0	0.35	0	0.06	0	13.74
Perciformes	Carangidae	Trachurus	***Trachurus trachurus***	0	0	0.38	0	2.02	0.15	0.12	0
Perciformes	Scianidae	Umbrina	***Umbrina cirrosa***	0.21	0	0.38	0.09	0	0	0	0
Unknown			**Unknown 1**	0	0	0	0	0	0	0.12	0
Unknown			**Unknown 2**	0	0	0	0	0	0	1.11	0
Unknown			**Unknown 3**	0	0	0	0	0.13	0	0	0
Unknown			**Unknown 4**	0	0.20	0	0	0.11	0.07	0	0
Unknown			**Unknown 5**	0	0	0	0	0	0	0.12	0

significant differences among inner estuaries. Pair-wise comparison showed that the two inner estuaries with a well-developed salinity gradient, GQ and GN, were different between them and the rest of estuaries, while the two estuaries with marine environment, OT and CB, were not different between them. Additionally, differences among years were significant both in inner estuaries and in their near coastal zones, without a clear common pattern.

Table 2. PERMANOVA results of the Bray-Curtis similarity matrix based of the fourth root transformed community data.

Source	df	MS	Pseudo-F	P(perm)	perms
Location	3	13199	4.64	>0.001	9915
Zone	1	27036	9.74	>0.001	9943
Year	2	13866	4.99	>0.004	9945
LocationxZone	3	14056	4.94	>0.001	9923
LocationxYear	6	5091	1.78	0.011	9898
ZonexYear	2	3886	1.4	0.166	9919
LocationxZonexYear	6	2429	0.85	0.697	9880
Station(ZonexLocationxYear)	36	2863	4.71	>0.001	9771
Res	205	607.4			
Total	264				

Table 3. PERMANOVA results of the Bray-Curtis similarity matrix based of the fourth root transformed community data for separate zone levels thereafter significant interaction Location x Zone.

	INNER ESTUARY					NEARSHORE				
Source	**df**	**MS**	**Pseudo-F**	**P(perm)**	**perms**	**df**	**MS**	**Pseudo-F**	**P(perm)**	**perms**
Location	3	28619	11.37	>0.001	9913	3	3534	1.02	0.435	9941
Year	2	5287	2.14	0.032	9934	2	11420	3.42	0.005	9938
LocationxYear	6	3859	1.53	0.05	9907	6	3658	1.06	0.409	9912
Station(LocationxYear)	24	2558	4.35	>0.001	9788	12	3474	5.46	>0.001	9846
Res	121	587.4				84	636.2			
Total	156					107				

PERMANOVA analysis, separating every location (Table 4), detected differences among nearshore and inner estuaries in GQ and GN but not in CB and OT. The PCO plots for each estuary clearly show these differences among the two types of estuaries (Figure 2). Year was significantly different in GN, OT and CB but not in GQ. *Post hoc* pairwise analysis of the factor year did not show any pattern in common for the three estuaries in their inner zones.

Table 4. PERMANOVA results of the Bray-Curtis similarity matrix based of the fourth root transformed community data for separate location levels thereafter significant interaction Location x Zone.

	BC			GQ			OT			GN		
Source	**df**	**Pseudo-F**	**P(perm)**	**df**	**Pseudo-F**	**P(perm)**	**df**	**Pseudo-F**	**P(perm)**	**df**	**Pseudo-F**	**P(perm)**
Zone	1	1.46	0.217	1	12.45	>0.001	1	2.49	0.058	1	5.17	0.011
Year	2	2.9	0.014	2	0.82	0.592	2	4.06	0.004	2	3.29	0.012
ZonexYear	2	0.88	0.561	2	0.9	0.54	2	1.12	0.372	2	1.23	0.295
Station(Zone	9	2.43	>0.001	9	10.15	>0.001	9	2.9	>0.001	9	5.9	>0.001
Res	31			60			60			54		
Total	45			74			74			68		

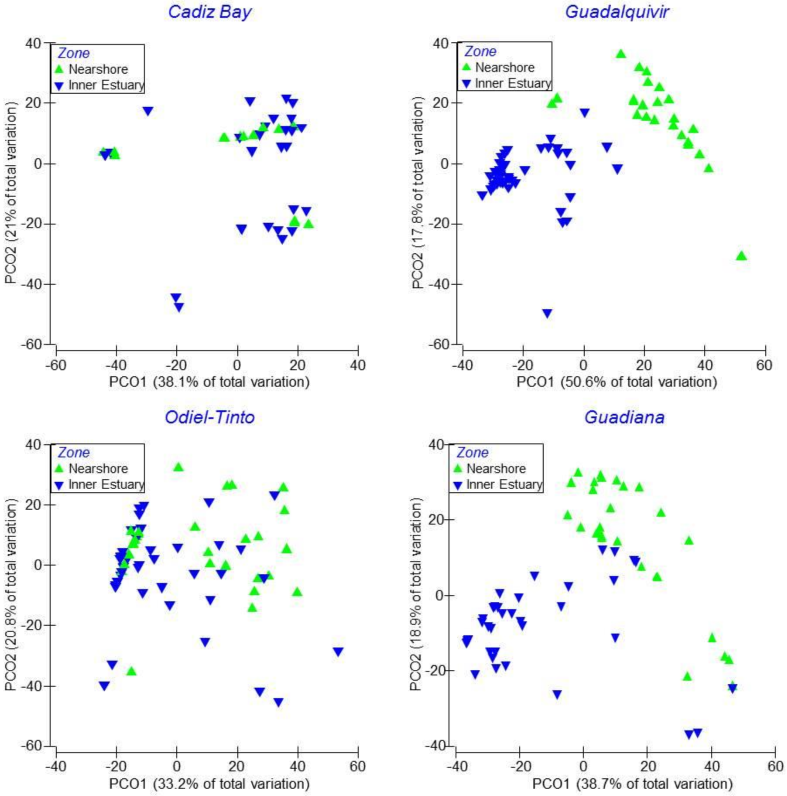

Figure 2. Ordination diagrams of Principal Coordinates Analysis (PCO) on early life fish stages of fish assemblage for each location (Cadiz Bay, Guadalquivir, Odiel-Tinto and Guadiana). Samples coded by zones.

SIMPER analysis of location and zones combined showed that the anchovy *Engraulis encrasicolus* was the species most consistent in all cases (Table 5). Additionally, in CB, the inner estuarine zone was also represented by *Diplodus annularis* and *Hippocampus hippocampus*, while the nearshore zone was dominated only by *E. encrasicolus* with more than a 95% of similarity contribution. In Odiel-Tinto estuary, other than *E. encrasicolus*, *D. annularis* and *Blennidae sp1* also contributed to the nearshore zone similarity, and *H. hippocampus*, *Blenniidae sp1* and *Solea senegalensis* to the similarity of the inner estuarine zone. Similar to OT, in the nearshore zones of both the GQ and GN estuaries, *E. encrasicolus* and *D. annularis* were the species that most contributed to their similarity. In the inner zones of estuaries with well-developed salinity gradient, the contribution of anchovies decreased (45.5% in GQ and 54.1% in GN), sharing the habitats with the goby *Pomatoschistus sp.*, which had a 40.9% in GQ and 24.2% in GN. Additionally, other species were characteristic of the GQ estuarine zone, such as *Argyrosomus regius*, and of GN, such as *Syngnathus typhle* and *S. acus*.

Total abundance of early fish life stages varied significantly between locations and zones, with higher values in the inner estuarine zones with a longitudinal salinity gradient, GQ and GN (Figure 3). In addition, the inner estuary of GQ was the zone with highest density—up to 5 times more than GN (t = 2.40; p = 0.03) and 25 times more than CB (t = 2.88; p = 0.01) and OT (t = 3.27; p < 0.01). No significant differences were found along years, and between nearshore zones.

Table 5. Results of SIMPER analysis on the abundance of all larval and juvenile fish species from every zone (Inner Estuary and Nearshore) of the four studied locations (Cadiz Bay, Guadalquivir, Odiel-Tinto and Guadiana).

NEARSHORE				**INNER ESTUARY**			
CADIZ BAY							
Average similarity: 33.18				Average similarity: 33.79			
Species	**Av.Abund**	**Contrib.%**	**Cum.%**	**Species**	**Av.Abund**	**Contrib.%**	**Cum.%**
Engraulis encrasicolus	1.49	95.56	95.56	*Engraulis encrasicolus*	1.66	78.68	78.68
				Diplodus annularis	0.6	13.37	92.05
				Hippocampus hippocampus	0.31	3.25	95.3
GUADALQUIVIR							
Average similarity: 28.69				Average similarity: 65.12			
Species	**Av.Abund**	**Contrib.%**	**Cum.%**	**Species**	**Av.Abund**	**Contrib.%**	**Cum.%**
Engraulis encrasicolus	1.81	91.73	91.73	*Engraulis encrasicolus*	4.31	45.45	45.45
Diplodus annularis	0.33	2.82	94.55	*Pomatoschistus sp.*	3.7	40.88	86.32
Aphia minuta	0.28	1.44	95.98	*Argyrosomus regius*	1.35	10.42	96.74
ODIEL TINTO							
Average similarity: 46.98				Average similarity: 40.44			
Species	**Av.Abund**	**Contrib.%**	**Cum.%**	**Species**	**Av.Abund**	**Contrib.%**	**Cum.%**
Engraulis encrasicolus	2.18	83.04	83.04	*Engraulis encrasicolus*	1.49	89.52	89.52
Diplodus annularis	0.8	8.49	91.54	*Hippocampus hippocampus*	0.28	3.07	92.58
Blenniidae sp1	0.54	3.86	95.4	*Blenniidae sp1*	0.26	1.78	94.36
				Solea senegalensis	0.23	1.52	95.88
GUADIANA							
Average similarity: 27.72				Average similarity: 45.49			
Species	**Av.Abund**	**Contrib.%**	**Cum.%**	**Species**	**Av.Abund**	**Contrib.%**	**Cum.%**
Engraulis encrasicolus	1.52	68.1	68.1	*Engraulis encrasicolus*	2.65	54.12	54.12
Diplodus annularis	0.83	20.54	88.64	*Pomatoschistus sp.*	1.89	24.21	78.33
Blenniidae sp1	0.34	6.28	94.91	*Syngnatus thyple*	1.18	14.5	92.83
Unknown 2	0.23	1.09	96.01	*Syngnatus acus*	0.51	3.62	96.45

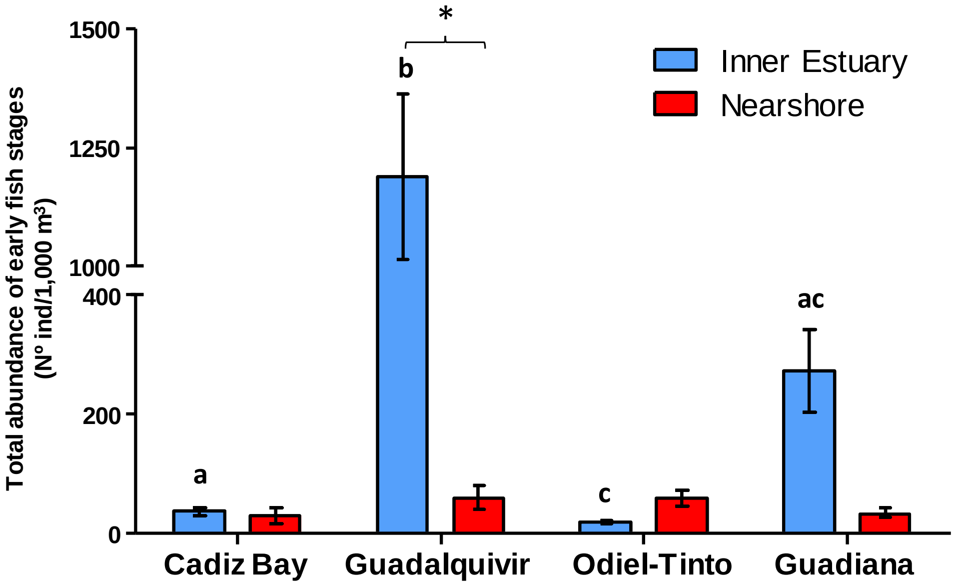

Figure 3. Total abundance (mean ± SE) of early life stages of fish per location and zone during all studied period (2016-2018). Asterisks indicate significant differences between inner estuary and nearshore zones of every location. Different letters indicate significant differences among locations within each zone.

3.2. Environmental analysis

Boxplot of all variables were presented by location and zone for the complete study period (Figure 4). Salinity clearly showed the different structure of the studied estuaries, with lower values and a wide range of variation in the inner zones of those estuaries with well-developed salinity gradient, like GQ and GN. The nearshores of all estuaries and inner zones of OT and CB showed a salinity of the typical marine environment. Related to this, the two estuaries with a well-developed salinity gradient showed higher turbidity, which was also directly correlated with TSS, SOM and SIM. The highest values were found in GQ inner estuary, followed by the inner zone of GN. In both estuaries, turbidity increased gradually in upstream stations. In the rest of the zones, the water was clearer. As expected, inner zones showed higher temperatures in every estuary, while nearshore zones presented a gradient with lower temperature in GN and gradually increasing towards the more inner part of the GoC. Generally, the estuaries presented higher values of DO and OS in their nearshore than in their inner estuarine zones, with a more conspicuous difference in GQ. With regard to chlorophyll concentration, CB showed similar values in the internal and external bay, and OT and GN showed clearly higher values in their inner estuarine zones, while this pattern was inverted in GQ. With respect to macrozooplankton biomass, the estuarine zones of GQ and GN showed high densities, being higher in GQ than in GN. Similar taxonomic groups were found in both estuaries, mainly isopods, decapods and mysids, with this last order being the most abundant. Jellyfish were found in the GN and CB estuaries. GN had the highest densities, with *Blackfordia*

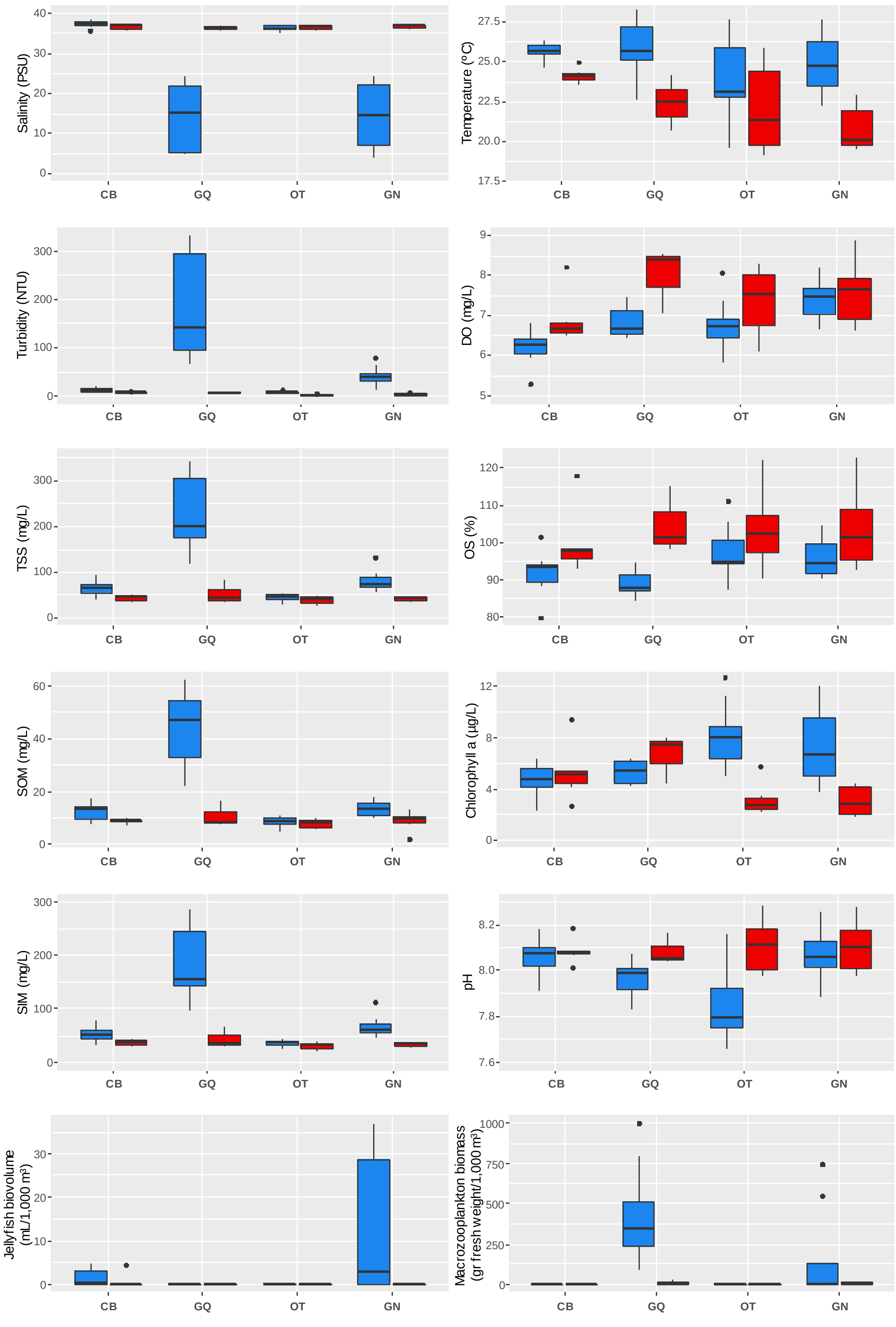

Figure 4. Boxplot of all variables (TSS: total suspended solid; SOM: suspended organic matter; SIM: suspended inorganic matter; DO: dissolved oxygen; OS: oxygen saturation) at each location (CB: Cadiz Bay; GQ: Guadalquivir; OT: Odiel-Tinto; GN: Guadiana) and zone (inner estuary: blue; nearshore: red) during the study period (dry-warm seasons of 2016, 2017 and 2018).

virginica as the principal species along with ctenophores of the genus *Bolinopsis*. Also, large individuals of the jellyfish *Catostylus tagi* were found in high densities (we are not providing an estimation of these densities because it cannot be properly estimated with a plankton net). Although there were not important amounts of jellyfish in 2016 (just *C. tagi* in polyhaline station), in 2017 and 2018, there were blooms of the exotic *B. virginica* invading the polyhaline and mesohaline waters (24–7 PSU) of the inner estuary, with mean densities of 1,918 ± 621 and 2,346 ± 717 ind./100 m^3 (mean ± SE), respectively, and a maximum density of 5,290 ind./100 m^3. Otherwise, the CB estuary contained high densities of the ctenophores *Bolinopsis sp*., although this was just in the inner bay of 2017 (71 ± 13 ind./100 m^3) and both zones in 2018 (inner bay: 3 ± 1 ind./100 m^3; outer bay: 32 ± 20 ind./100 m^3). The same species of ctenophores was also caught in GQ nearshore samples but with low total biovolume.

The structure of fish larval assemblages was related to the selected environmental variables (Figure 5). The ordination analysis obtained a correlation of 42.8%, and the first two

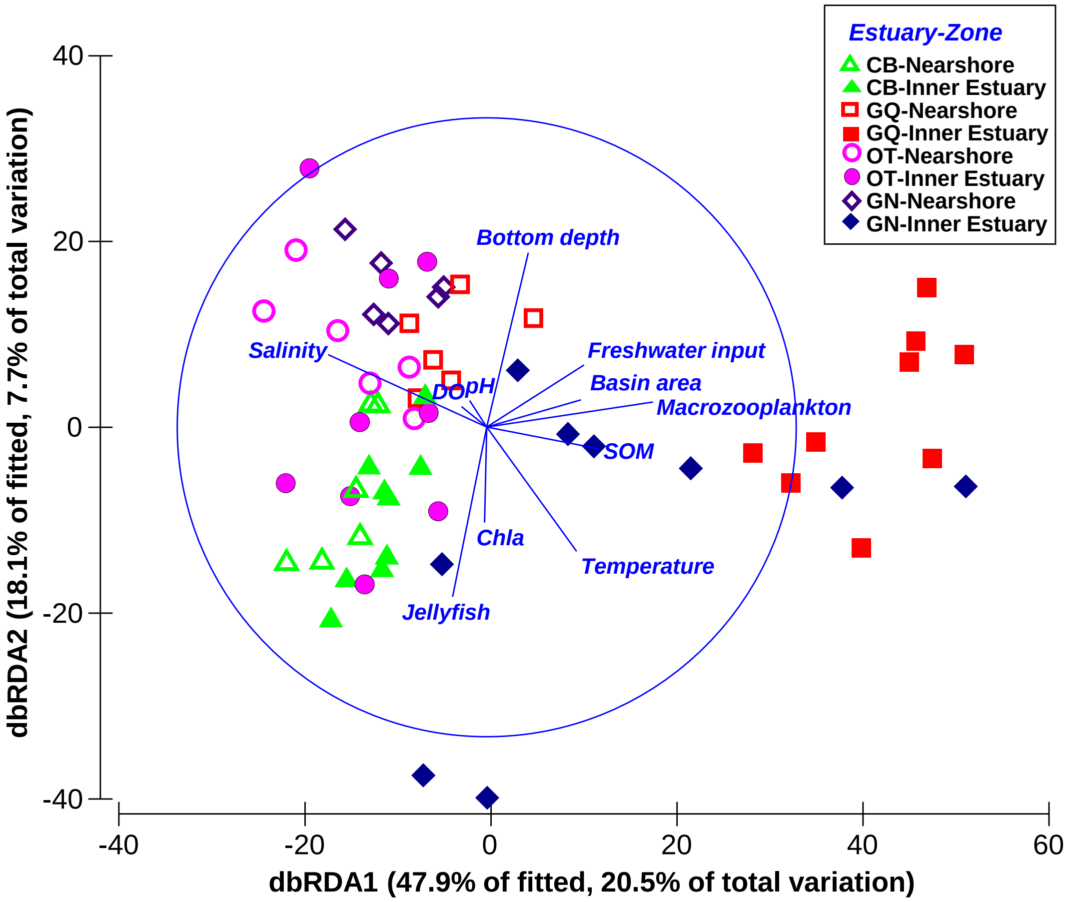

Figure 5. Ordination of sampling stations along the first two axes of the distance-based redundancy analysis (dbRDA) relating all species with environmental variables (blue arrows) during 2016, 2017 and 2018. Chla: chlorophyll-a; SOM: suspended organic matter; DO: dissolved oxygen; CB: Cadiz Bay; GQ: Guadalquivir; OT: Odiel-Tinto; GN: Guadiana.

dbRDA axes accounted for 28.2% of the total variation. Salinity, which was inversely related with freshwater input, basin area (directly correlated with estuary length and river length), macrozooplankton and SOM (directly correlated with turbidity, TSS and SOM) separate the inner GN and GQ assemblages from the rest of zones in the first axis. Along axis two, variables such as jellyfish, temperature and Chla grouped assemblages of both CB zones and several stations of GN inner estuary; inversely related to these variables, pH, DO (directly correlated with OS) and bottom depth associated the assemblages of the rest of nearshore zones.

4. Discussion

Assemblages of early fish stages showed clear differences among locations and zones across the Gulf of Cadiz. This variation is significantly related to some hydrogeomorphological, physicochemical and biological variables, especially salinity gradient generate by freshwater input, temperature, bottom depth, suspended organic matter, chlorophyll-a, macrozooplankton and jellyfish. Nevertheless, other variables may also influence the assemblage variations such as some intrinsic characteristics of each system or some effects derived from anthropic pressures. However, the differences observed were not as initially hypothesised. During the marked dry season characterising this region, rivers with smaller basins, showed a very low freshwater input and their estuaries, OT and CB, were essentially sea extensions into the land, with similar physicochemical conditions to nearshore zones, as well as similar assemblages of early life stages of fish. In contrast, GQ and GN estuaries were distinct between them and the rest of the zones. As expected, the regional climatic and environmental conditions impose similar characteristics for all external zones and we highly influenced by the general conditions in the GoC. The main environmental variability among coastal areas was the temperature gradient, increasing as it enters the GoC (Vargas et al., 2003). Some differences were also observed in DO and OS, with lower values in CB, probably associated to its particular embayment morphology, which potentially reduced water renovation. The lower mean bottom depth of its inner bay allowed an increased temperature as well. Still, these differences did not appear to limit the biological community and did not generate significant differences in the distribution of early life stages of fish, the structure of their assemblages and their total densities. This homogenous condition in nearshore zones helps to discern more accurately which environmental variables (biotic and abiotic) of the inner zones cause differences in the assemblages. The main captured species in all nearshore zones and inner estuaries without a salinity gradient (OT and CB) was *Engraulis encrasicolus*, followed by individuals of families Sparidae (*Diplodus annularis*) and Blennidae. A similar species composition was found by Baldó et al. (2006) in a study of ichthyoplankton in offshore and coastal waters of the Gulf of Cadiz; *E. encrasicolus* was the most abundant species during the same season, although they found higher abundances in GQ and OT adjacent coastal zones than in GN (a trend also observed in this study but without statistical significance). All these zones showed similar marine environmental conditions, characterised by high and homogeneous salinity, clear waters and low macrozooplankton biomass. Only the OT inner estuary seems to behave in a different manner, showing the lowest average density and pH. However, this is an essentially marine estuary with comparatively high chlorophyll concentration, which, in its non-turbid waters, would tend to increase pH during the day by means of a more intense photosynthetic activity. This estuary is strongly affected by long-term metal mining activity which discharges an acid drainage into the estuary and imposes a heavy contamination of metals (Nieto et al., 2007). Also, bottom depth seems to influence its assemblages, as other authors have found for demersal fish composition (Araújo et al., 2002); stations close to the estuary inlet were the deepest of the study, due to the navigation channel for vessels to the inner port.

Conversely, larval and early juvenile fish assemblages in GN and GQ inner estuaries were significantly different from each other and all other estuarine and nearshore zones. These estuaries, which drain the two largest basins in the region, maintain continuous freshwater input even in the dry season, which maintains a well-developed salinity gradient. Salinity has been shown to strongly influence biological communities, especially the fish assemblages (Amezcua et al., 2019; Araújo et al., 2018; Barletta et al., 2004) and the nursery function of estuaries (França et al., 2009; Whitfield and Harrison, 2003). One of the more abundant species, and the only characteristic species of these estuaries with reduced salinity, was the goby *Pomatoschistus* sp. It is a benthic resident estuarine species (Dolbeth et al., 2007; Pampoulie et al., 1999). Previous studies in GQ and GN have reported the high contribution of the goby to their assemblage structures (Drake et al., 2002; Faria et al., 2006), but it also inhabits coastal areas and lagoons, and it has been recorded in intertidal channels of the CB saltmarshes (Drake and Arias, 1991). With a comparative purpose, this research focused on a habitat well represented in all studied estuaries—the open water masses (from the bottom to the surface), which includes the larger water bodies, main channels and seagrass meadows (Brun et al., 2015).

Although both estuaries presented a similar transitional salinity zone, some distinctive features arose between them, showing differences to their densities and assemblages of early life stages of fish. For example, species such as *Argyrosomus regius* and *Dicentrarchus punctatus*, which are important for the fishery sector in the region (Catalán et al., 2006;

González-Quirós et al., 2011), are characteristics of GQ inner zone. In GN, species of Syngnathidae were more representative, where the tidal saltmarshes and seagrass meadows (Cunha et al., 2013) could provide favourable habitats for these species (Foster and Vincent, 2004). Even more illustrative is the case of the anchovy *E. encrasicolus*. This species was the most abundant in the region during the studied period and was dominant in all zones. It is a marine migrant species that is very important for the fisheries of the region (Ruiz et al., 2017), and its recruitment period is during the warm-dry season (Baldó et al., 2006; Drake et al., 2007). Its importance as a dominant and abundant species has been previously reported in the Guadiana estuary (Faria et al., 2006), Cadiz Bay (Drake and Arias, 1991), Guadalquivir estuary (Drake et al., 2007) and in coastal water in the Gulf of Cadiz (Baldó et al., 2006). Our comparative approach, using the same methodology, sampling gears and study period during three consecutive warm-dry seasons, supports the importance of this species in the whole region and all estuaries, showing the Guadalquivir estuary as its main nursery area with a notable highest density.

De Carvalho-Souza et al. (2018) showed that environmental conditions such as moderate turbidity levels (<100 NTU), stable freshwater discharges (30–40 m^3/s), mesohaline waters, high food availability of mysids (e.g. *Mesopodopsis slabberi* and *Rhopalophthalmus tartessicus*) and warm temperature, were the most significant to explain the abundance of *E. encrasicolus* in the Guadalquivir estuary. However, the Guadiana inner estuary also presented similar environmental characteristics but exhibited a notably lower macrozooplankton biomass than Guadalquivir with an average abundance four times lower. A similar pattern has been found for mesozooplankton, with different studies estimating a much higher copepods density in GQ (~30,000 ind/m^3; Taglialatela et al., 2014) than in GN (~7,000 ind/m^3; Chícharo et al., 2006) during the dry-warm season. The main physicochemical differences between these two estuaries were higher concentration of TSS and the associated values of SIM, SOM and turbidity in GQ, as well as a higher input of freshwater. Freshwater input not only affects salinity, but is also a main source of nutrients, sediment and organic matter, which influence the biological community and the nursery function of estuaries (González-Ortegón and Drake, 2012). The contribution of sediment by the drainage is higher in Guadalquivir due to the geomorphology of the basin and its high soil erosion rate because of traditional agriculture land use (Carmona, 2015). For the heterotrophic portion of the estuarine food web, a higher load of sediment has been described to dilute higher quality food resources (e.g. cellular phytoplankton) and may constitute a difficulty for mesozooplankton and hyperbenthos (mainly copepods and mysids) (Fockedey and Mees, 1999; Gasparini et al., 1999). Still, the associated higher concentration of suspended organic matter (detritus) can also provide food to zooplankton [directly (Islam and Tanaka, 2006; Vilas et al., 2008) or through microbial food web (Goosen et al., 1999)], which, in its turn, support early life stages of fishes. Baldó and Drake (2002) showed that copepods and mysids were the two greatest principal preys, respectively, of the post-larval stage of *E. encrasicolus* in the Guadalquivir estuary; the same prey but in the opposite order was found for the juvenile stage. Donázar-Aramendía et al. (2019), in their comparison between the food webs structure of GQ and GN using stable isotopes, found that allochthonous organic matter in the form of detritus can play an essential role in the food webs of the GQ, compensating for the limited phytoplankton production due to higher turbidity, thus maintaining a high biomass of copepods and mysids. These authors also found evidence of higher diversity of basal resources in GQ food webs, whose positive relationship with fish production had been previously suggested (Hoffman et al., 2015).

On the other hand, high turbidity levels in the Guadalquivir estuary has been reported to limit light availability for phytoplankton, reducing the photic depth of the water column and constraining its primary production (Ruiz et al., 2017). However, the chlorophyll concentration measured in this study was not particularly low, having similar or even higher values than other clearer zones. OS and pH levels, in contrast, were the lowest in inner GQ (except for anomalous pH in internal OT), indicating low photosynthetic activity in the water column. A previous study proposed that Chla in GQ estuary could be supported by upstream water reservoirs or irrigation channels from adjacent agriculture fields, in addition to autochthonous production when environmental conditions of turbidity and temperature were favourable for photosynthesis (González-Ortegón and Drake, 2012). However, recent studies of phytoplankton assemblages in the Guadalquivir estuary reveal that diatoms of the genera *Navicula* and *Nitzschia* are some of the most abundant cells in its waters (Cañavate et al., 2019); they have been documented as the most common epipelic microphytobenthos (MPB) of the biraphid diatoms (Underwood, 2001). The resuspension of MPB due to flood tide and wind action is typical in highly turbid estuaries (Brito et al., 2012; Irigoien and Castel, 1997; Statham, 2012), where its chlorophyll contribution to water column has been reported to represent an important proportion when compared to the contribution of real phytoplankton (Cadée and Hegeman, 1974; De Jonge and Van Beusekom, 1992). Due to high turbidity levels in GQ, photosynthesis is limited by phytoplankton in the water column, but not in the riversides where a dense mat of MPB developed (personal observation), the water depth is lower and the ebb tide exposes intertidal mudflats to sunlight. Up to now, there has not been a precise estimation of the contribution of the MPB to the primary production of the system and the chlorophyll concentration in the water column, but the evidence presented here supports that this would be particularly important in the GQ estuary.

Gelatinous zooplankton is a final important factor for assemblages of early stages of fish in the Gulf of Cadiz estuaries and the potential nursery function that they can play. The Guadiana estuary contained high densities of jellyfish, which decreased the larval and juvenile fish densities and also increased the density variations among years and stations (Personal observation; Chícharo et al., 2009; Muha et al., 2017). A similar effect was found for the macrozooplankton biomass. In the years when blooms of *Blackfordia virginica* were observed in polyhaline and mesohaline waters (2017 and 2018), oligohaline stations (< 6 PSU), where the medusas were absent, contained higher concentrations of macrozooplankton and early stages of fish in comparison with the whole inner estuarine zone during 2016. These results show that the jellyfish bloom in the lower part of the estuary works like a barrier (~30 km) for the zooplankton assemblages, which moved and concentrated upstream. The lower densities of this species recorded previously (>100 ind./100m^3 and a maximum density of 3,700 ind./100m^3; Chícharo et al., 2009) may indicate an intensification of this invasion. According to Muha et al. (2017), before construction of Alqueva Dam, this species was not present in the GN estuary, but after the dam's construction, low river discharges provided one of the main factors for its expansion in this ecosystem. Indeed, Guadiana estuary received a mean annual freshwater input three times lower than Guadalquivir estuary despite having a greater river basin area. Ecohydrological studies in the GN estuary demand higher discharges from Alqueva Dam during the dry-warm season to yield a higher plankton productivity and biomass and control jellyfish proliferation (Muha et al., 2012; Wolanski et al., 2006). Currently, this jellyfish has not been reported in the GQ estuary despite being a species that inhabits brackish waters (Marques et al., 2017; Mills and Sommer, 1995), the proximity to GN and the long history of this invasion (it was reported for the first time in 2001; Muha et al., 2012), which could have facilitated its colonisation. The higher freshwater discharges and muddy bottoms could hinder the settlement of the polypoid phase, which needs a hard substrate. Nevertheless, *B. virginica* is still a potential risk for the GQ estuary and its nursery function. In contrast, the ctenophore *Bolinopsis* sp. was found in more locations of the GoC, in addition to inner zone of GN. CB estuary showed high densities in both zones, and it was also present in GQ nearshore zone with low biovolume. However, it has been observed with important abundances in both zones of GQ during other periods (personal observation; Diz et al., 2015). Jellyfish are key elements in plankton assemblages when demographic blooms occur with a significant impact by predation and competition with zooplanktivorous consumers such as fish larvae (Boero, 2013;

Purcell and Arai, 2001; Robinson et al., 2014). The case of GN is of particular concern, as this is one of the most productive estuaries in the region, with a large transitional zone and well-developed salinity gradient, which is recurrently blocked by jellyfish blooms during the main recruitment period in the region. This must necessarily affect the nursery function and the fishery industry and can be partially responsible for the quantitative differences found between the GN and GQ nursery grounds.

In conclusion, estuaries have the capability to work as a nursery ground, but not all of them succeed because it depends on the characteristics that their habitats provide, which are strongly affected by anthropogenic alterations and the preferences of fish. The four main estuaries of the Gulf of Cadiz are impacted by human activity, as are most estuaries around the world; significantly, damming and water abstraction may have deep effects on environmental conditions, the assemblages of zooplankton and early life stages of fish, and the use that the latter do of estuaries as nursery ground. Estuaries whose rivers discharge enough freshwater to preserve a well-developed horizontal salinity gradient (i.e. the Guadalquivir and Guadiana estuaries) maintain different and denser assemblages in their inner zones. Open water masses of estuaries with scarce river flow maintain essentially marine environmental conditions (i.e. the Odiel-Tinto and Cadiz Bay estuaries) similar to the nearshore zones, and cannot be considered to have an important nursery function. Additionally, the occurrence of jellyfish blooms is also enhanced by water scarcity and could affect the biological community by interfering with the nursery function of estuaries and the fishery industry, as is the case in the Guadiana estuary. Otherwise, high levels of suspended sediment and turbidity limit pelagic primary production, which may be partially compensated by other sources such as microphytobenthos. Still, suspended organic matter associated with this sediment feeds the base of the food web through the heterotrophic pathway, which could maintain a high productivity of ecosystems, as in Guadalquivir estuary. This estuary presented the densest amount of early fish stages and macrozooplankton in the GoC, with the anchovy *Engraulis encrasicolus* being the most abundant species during the period studied, which provides the most important recruitment stock for the fishery in the region. As a practical conclusion to manage nursery grounds in the GoC, the present fishery production in the region may be supported by a productive equilibrium depending on riverine inputs of sediment into Guadalquivir estuary. The high and quasi-permanent turbidity in this estuary is a present concern, and its reduction is a commonly claimed objective. Any modification in this sense, even with the objective to improve its ecological status, should be done with caution and be closely monitored. On the other hand, the other potentially highly productive estuary in the region, Guadiana, is currently impoverished due to recurrent blooms of jellyfish, associated with scarce freshwater discharges into the estuary. An improvement of discharge management, as previously claimed by several authors, is possible and would notably improve the nursery function, ecological status and fishery production in the region.

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GENERAL DISCUSSION

The main aim of this thesis was to increase the knowledge of recruitment ecology processes for early life stages of fish in estuaries, using the Guadalquivir as study area. For that, spatio-temporal dynamic was analysed with different approaches (monthly monitoring program, comparisons with surrounding areas and estuaries, recruitment strategies) and under distinct natural (freshets) and anthropic (maintenance dredging) perturbations. Main findings and their interpretations are discussed below to integrate them in the present literature.

Estuaries as nursery areas

Estuaries have the capability to work as a nursery grounds, but not all of them succeed because it depends on the characteristics that their habitats provide and the preferences of fishes. In some cases, low river inflow in estuaries with small basins provoke a scarce supply of freshwater input which did not generate a longitudinal salinity gradient, typical of these ecosystems. This was the case of the Odiel-Tinto and Cádiz Bay estuaries during the dry-warm season, which presented similar water physiochemical conditions as nearshore areas, being essentially sea extensions into the land. These estuaries showed similar early life stages of fish assemblages and abundances than surrounding zones, therefore, according to (Beck et al., 2001), they cannot be considered to have an important nursery function (at least in their open water masses). Instead, Guadiana and Guadalquivir estuaries, which have higher watersheds and consequently higher freshwater inputs, contained well-developed salinity gradients. These brackish waters contained different early fish assemblages and higher densities than nearshore areas, which showed an important nursery function of both estuaries for multiple marine fish species. Hence, the freshwater inputs play an essential role in the saline characteristics of the estuaries and their capacities to rear early life stages of fish. Nonetheless, both estuaries have registered a temporal decline during the last decades in their freshwater inputs due to the extensive urban and agricultural development around their river basins (Dias et al., 2004; González-Ortegón et al., 2012), which in its turns, has influenced to their biological communities (Faria et al., 2006; Fernández-Delgado et al., 2007). In fact, during the monitoring program in the Guadalquivir estuary, temporal displacement of the salinity gradient was observed, although it was not always related with the tide or discharges of freshwater, which suggested other influential factors such as water abstraction directly from the estuary in its upstream zones for irrigation supply. Still, although both estuaries presented a similar transitional salinity zone, some distinctive features arose between them, which made the Guadalquivir estuary much more productive. Among them, it was the recurrent jellyfish blooms observed in poly and mesohaline waters of Guadiana estuary during the high recruitment period which reduced its nursery function. In contrast, the Guadalquivir estuary contained high densities of gelatinous plankton in nearshore areas and in oligohaline waters, where the early fish abundance and the rest of macrofauna was generally lower, so influenced to a lesser extent in its secondary production.

High productivity in the Guadalquivir estuary

The freshwater inputs, in addition to generate the longitudinal salinity gradient, supplied high amounts of nutrients and organic matter, which feed the trophic webs. In the Guadalquivir estuary, due to the high turbidity levels found, pelagic primary production is limited (Ruiz et al., 2017). However, chlorophyll concentrations were higher than expected in the water column of its inner zone. González-Ortegón and Drake (2012) suggested that it could be supported by upstream water reservoirs or irrigation channels from adjacent agriculture fields, in addition to autochthonous production when environmental conditions of turbidity and temperature were favourable for photosynthesis. Notwithstanding, recent studies of phytoplankton assemblages in the Guadalquivir estuary reveal that some of the most abundant cells in its waters (Cañavate et al., 2019) are considered common microphytobenthos taxa (Underwood, 2001). This evidence, in addition to the association of chlorophyll and total suspended sediments found and the personal observations of dense matts of benthic algae in the intertidal mud flats, suggest that most of the chlorophyll found in the water column may come from microphytobenthos resuspended by wind and tidal forces (Brito et al., 2012; Irigoien and Castel, 1997), as also proposed Díez-Minguito and de Swart (2020).

Although pelagic primary production may be partially compensated by other sources such as microphytobenthos, allochthonous carbon supplied by the freshwater input can directly support zooplankton taxa with a more heterotrophic energy pathway (Abrantes et al., 2013; Hitchcock et al., 2016; Hoffman et al., 2008). Suspended organic matter in form of detritus has been reported to contribute in the diet of meso- and macrozooplankton species (Donázar-Aramendía et al., 2019; Vilas et al., 2008), which in its turn, are prey to most of the early life stages of fish (Baldó and Drake, 2002). Peak productivity in temperate estuaries is usually linked to spring and early summer (Strydom, 2014). Similar trends were found in this research, although the increment of the different trophic levels was not synchronic, suggesting different prey-predator interactions. Macrozooplankton usually increased before the early life stages of fish, and declined when this reached its maximum density peak, showing a bottom-up control, while mesozooplankton generally achieved its peak when both higher trophic levels were decreasing, showing a top-down control. Instead, suspended organic matter concentration was usually high and constant in this estuary along the whole year, which seems to indicate it does not limit the base of the food web. This characteristic, in addition to the salinity gradient, provoked that the Guadalquivir was the estuary most productive in the Gulf of Cádiz.

Early life stages of fish assemblages

The Guadalquivir estuary have been shown to contain an environment favourable for the development of multiple fish species, many of them economically important for the fishery sector. The differences found in the early life stages of fish assemblage and abundance between its inner and nearshore zones, confirmed its high functionality as a nursery ground. The nearshore zone showed similar environmental condition between the distinct stations spread out the estuary inlet, therefore, no differences were found in their assemblages. Instead, inner zone was more diverse and productive, especially in poly and mesohaline water masses. The marine species more dominant in both zones, as well as in all the other estuaries sampled, was the anchovy *Engraulis encrasicolus*. This marine estuarine-opportunist species showed a high recruitment period from late spring to autumn, as also reported by Drake et al. (2007). In contrast, the densities found in the estuarine zone of the marine species *Sardina pilchardus* were much lower in our study than those registered by Drake et al. (2002) during 1997-1999, suggesting a dramatic temporal decline. Similar trend has been reported around the Iberian Peninsula waters for adult stocks during the last decade (ICES, 2019), whose populations could shift to northwards Atlantic waters for the increment of the sea surface temperature in the region (Alheit et al., 2012; Beare et al., 2004; Montero-Serra et al., 2015). Other dominant species that maintained a high contribution to catches along the whole year were the gobies *Pomatoschistus* spp., although their presence in the nearshore zone was negligible. These gobies are considered estuarine-resident species, however, several studies have documented seaward migration during the early spring for reproduction in other areas (Guelinckx et al., 2008; Pampoulie et al., 1999). No clear evidence was found in this research, but the scarce presence of adults in the estuary during the winter and the elevated abundance of juvenile during their recruitment period after freshet events suggest that they could migrate further of nearshore zone to reproduce.

Mechanisms for horizontal movements

Selective Tidal-Stream Transport (STST), in combination with vertical migration is one of the mechanism more used by invertebrates and small fishes in estuaries for horizontal movements (Forward and Tankersley, 2001). However, no evidence of vertical migrations was found for the species most abundant in the Guadalquivir estuary, *Pomatoschistus* spp. and *Engraulis encrasicolus*. The benthic gobies were mostly found in the bottom, while the pelagic anchovies were in the surface layer, regardless to the tidal condition (ebb or flood). Hence, they had to use alternative strategies to common STST.

In the case of the gobies, higher abundances were found in both sides, where the transition from ebb to flood usually occurs more quickly (Scully and Friedrichs, 2007), enabling more time for their upstream transport. Their locomotion by short hops and darts (Asriaens et al., 1993), in combination with burrowing behaviour (Magnhagen and Forsgren, 1991), could be used to avoid water currents unwished remaining close to the bottom. In addition, the influence observed of temperature and dissolved oxygen variables, separated or combined, could work as cues for these species to know when they have to go with the flow up or downstream.

On the other hand, the anchovy showed an advection transport downstream. However, considering this study and the information already available for this estuary (Drake et al., 2007), a clear strategy for the anchovies to ingress upper into the estuary has not been observed. Still, lateral shallowest areas, where higher densities and larger sizes have been found, present low water velocities (Garel and Ferreira, 2013). This would allow the anchovies for a more efficient swim against the current and a better control of their position, suggesting an ontogenetic transition in behavioural capability (Teodósio and Garel, 2015). This would be particularly applicable in neap tides, when anchovies could compensate the downstream transport during the spring using shallower areas as proposed by (Teodósio and Garel, 2015). In addition, the physiochemical changes between zones during the flood, as the shift in water current velocity and its consequently salinity variations, could work as cues in this species.

In general, the results showed that these species may have opposite mechanism to move across the estuary section. This antagonistic behaviour avoids the overlap of their physical habitats, enhancing the use of resources offered by the ecosystem and making these species particularly compatible.

Anthropic and natural disturbances

Estuaries have long been regarded as naturally stressed environments because of the high degree of variability in their physiochemical characteristics, for example oxygen, temperature, salinity and turbidity in the water column and bed sediment dynamics. These wide environmental variations make difficult to detect anthropic stress from natural changes, unless the human impact is severe, leading to the ‘Estuarine Quality Paradox’ (Elliott and Quintino, 2007). This was the case of the study of the maintenance dredging in the poly and mesohaline waters, which we could not detect a severe impact in the macrofauna community and even in the physiochemical variables. In fact, the increment of the turbidity levels, which is the most common effect in this kind of disturbances (Magris and Ban, 2019; Wenger et al., 2017), was not detected in short and medium terms despite of the dredger resuspended sediments from the bottom layers, suggesting a short persistence and local spatial extension of those sediments in the water column. Also, the high flows originated by tides and the high chronic turbidity in the Guadalquivir estuary (Losada et al., 2017) could have overshadow the sediment resuspension effect caused by the dredging in our sampling zones. Nonetheless, in addition to the environmental perturbation in the water column, organisms can suffer direct mechanical impacts by the trailer suction. In our case, epibenthic species, like *Pomatoschistus spp.* or decapods, tended to decrease, probably because they are prone to a high entrainment (Armstrong et al., 1982; Drabble, 2012). Still, it seemed to cause a low incidence in the epibenthic populations and negligible in pelagic ones, as also reported (Burton et al., 1992) in Delaware River.

In contrast, this estuary showed natural fluctuations which caused disturbances of higher order than the maintenance dredging such as the multiple freshets registered during this research, indicating that the characteristics of natural stress in estuaries can be similar or stronger to those for anthropogenic stress (Elliott and Quintino, 2007). High discharge events generally generated a strong shift in physiochemical conditions of most estuary section, compressing salinity gradient, increasing turbidity and even decreasing oxygen concentration in extreme cases. This alteration influenced in the abundance and distribution of early life stages of fishes and the rest of macrofauna, reducing its nursery area. In this kind of disturbance, marine pelagic fish species showed more dramatic effects than estuarine epibenthic species. The permanent surface behaviour of anchovies for horizontal displacements observed and the plumes caused by the discharges, suggested that individuals not found in the outer zones during the freshets could be expelled out. Contrary, the permanent benthic behaviour observed in the gobies could help them to maintain inside of the estuary but with downstream displacements, and also, to recover their previous distribution more quickly than other species using the advance of the salinity wedge during flood tides.

Additionally, long-term effects of the highest freshet event were observed in the two major contributor species of this estuary. While *Pomatoschistus* spp. showed the highest recruitment abundance and duration along the spring and summer in all inner stations, compared with previous years, *Engraulis encrasicolus* registered the lowest recruitment abundance and duration in the inner zone. The high turbidity levels stayed long after the freshet and overlapped with their high recruitment periods, what could have had a negative influence in this species, contrary to *Pomatoschistus* spp. The gobies, as estuarine resident species, seemed to cope better with this kind of disturbances. However, the marine opportunistic species as anchovies, seemed to be less adapted to these environmental variability and conditions. Generally, the estuarine biota is well-adapted to multiple stress because of the inherent variability of estuaries so these ecosystems may be regarded as resilient (Elliott and Quintino, 2007). Still, early life stages of marine fish species have showed an important contribution to the assemblage and productivity of this estuary, what make it necessary to consider this kind of disturbances for its better management, and consequently, to enhance the fishery stocks of the region.

Perspectives: research and management

The results obtained in this thesis have revealed the importance of the Guadalquivir estuary as a nursery area in the region, however, numerous questions about fish recruitment are still unknown in this ecosystem. For example, an interesting aim would be the quantification of the contribution of primary producers and the organic matter to higher trophic levels like early life stages of fish, which would explain the energy pathways (auto- or heterotrophic) of this high productive estuary. Also, the origin of the chlorophyll (phytoplankton or microphytobenthos) and organic matter found in the water column would help to manage these important food resources. In addition, although it is the most productive estuary in the Gulf of Cádiz, little is known about the real contribution of the early life stages of fish reared in it to the adult stocks offshore. On the other hand, the mechanisms that the anchovy use for horizontal movements in the Guadalquivir is still not completely understood despite of being the most abundant marine fish species in the region, as well as their location during freshet events. In general, there is still a lack of knowledge in the recruitment processes of many fish species and their responses to different scenarios. This kind of information would be essential for the success of estuarine and fishery management plans.

Notwithstanding, this thesis has found new answers for multiple questions and supplied more information about the complex environmental interactions of the Guadalquivir estuary. Hence, the knowledge generated in this research should be used for future management plans in the region, for a better use of the important resources that this ecosystem offers to the distinct users.

Due to the important nursery function of the Guadalquivir estuary and its high environmental variability, we suggest performing different monitoring programs with different approaches to better understand the ecological responses to distinct situations or perturbances. Furthermore, the results of across-river distribution of main fish species helps to design appropriate sampling protocols for future macrofauna studies in this estuary.

Multiple threats have been observed in this research for the sustainable development of this ecosystem. One of the most important is the supply of freshwater, whose inputs should be managed properly to ensure a stable salinity gradient that creates diverse and productive environmental conditions. High turbidity levels have been widely discussed as a widespread problem for primary production in this estuary. However, organic matter, which is associated to this turbidity, is an important food resource in the base of its trophic web. Therefore, the management of the turbidity variable should take into account the organic matter concentration as well, to avoid altering its productivity. On the other hand, maintenance dredging has caused scarce perturbations in poly and mesohaline waters. However, in order to minimize its effects, it is suggested to avoid dredging during the sensitive breeding, and recruitment periods of marine organisms. Finally, biological invasions as the recurrent demographic blooms of gelatinous plankton observed in the different estuaries sampled should be monitored to assess the potential ecological interactions with their nursery functions.

The Guadalquivir estuary is a critical resource area under intense and increasing pressure from a variety of uses and users and generally exists without an operative comprehensive management plan. Tidal shoreline systems are managed by a complex framework of regulatory agencies that are each responsible for a few resources rather than the coastal zone as a whole ecosystem. Still, estuaries are highly influenced by the quantity and quality of freshwater inputs. Thus, an integrative management plan which include also the users and agencies of the river basin would be necessary for the sustainability of this important ecosystem.

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GENERAL CONCLUSIONS

1. The Guadalquivir estuary showed different early life stages of fishes between its nearshore and estuarine zones, as well as a much higher density in this latter, showing an important nursery function for many marine fish species.
2. Biotic and abiotic characteristics in the nearshore station were similar, while the longitudinal salinity gradient in the inner zone, and associated environmental variability, provoked differences between their water masses. Poly and mesohaline waters contained different assemblages that oligohaline waters, as well as higher diversity and density of early fish stages.
3. Different trophic interactions were found along the temporal dynamic of mesozooplankton, macrozooplankton and early life stages of fish abundances. Early life stages of fish showed top-down control over the macro- and mesozooplankton, at the same time that macrozooplankton showed bottom-up control over the early fish stages, and top-down control over the mesozooplankton.
4. Although pelagic primary production can be limited in the water column, high chlorophyll concentration found in the systems indicate that other sources of primary production as microphytobenthos in the intertidal mud flats can contribute significantly in the primary production of the ecosystem.
5. Allochthonous carbon, in form of organic matter, supplied by freshwater input seems to directly support macrofauna taxa with a more heterotrophic energy pathway in the Guadalquivir estuary.
6. Estuaries with no transitional salinity gradients as Odiel-Tinto and Cádiz Bay showed similar biotic and abiotic characteristics than surrounding areas. Therefore, they cannot be considered to have an important nursery function during the dry-warm season. Instead, estuaries with well-developed salinity gradients, as Guadalquivir and Guadiana, presented different early fish assemblages and higher abundances than their nearshore zones, being important nursery grounds in the region.
7. Among the main estuaries in the Gulf of Cádiz (Guadalquivir, Guadiana, Odiel-Tinto and Cádiz Bay), the Guadalquivir estuary contained the highest amount of early life stages and macrofauna during the dry-warm season.
8. The marine species *Engraulis encrasicolus* was the most abundant species in all estuaries and their surrounding areas. The estuarine *Pomatoschistus* spp. were the second most abundant species in the brackish waters of the estuaries with longitudinal salinity gradient (Guadiana and Guadalquivir).

9. No vertical migrations, in combination with the selective tidal-stream transport, was found for horizontal movements inside the estuary in *Engraulis encrasicolus* and *Pomatoschistus* spp. The benthic gobies were mostly found in the bottom, while the pelagic anchovies were in the surface layer, regardless to the tidal condition (ebb or flood). They must use alternative strategies.
10. Maintenance dredging operations in the poly and mesohaline zones of the Guadalquivir estuary did not cause severe impacts in the water physiochemical conditions and macrofauna. Still, some effects were observed in epibenthic taxa (gobies and decapods), which tended to decrease, while most pelagic organisms did not show alterations.
11. Freshet events with freshwater inputs higher than 200 m^3/s for 24 hours compressed the salinity gradient downstream, increased the turbidity levels and caused larger river turbidity plumes, at the same time that they reduced temporally the nursery area of the Guadalquivir estuary.
12. Depending on the intensity of the freshet and the species present during the event, early life stages of fish could be displaced downstream or flushed out the estuary. In some cases, a net loss of individuals was detected (by predation, osmotic shock or any other process). In general, estuarine species (e.g. gobies and other macrofauna groups) coped better with this perturbances than the marine organisms (e.g. anchovies).

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