Laboratorio de Biología Marina

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Influence of human activity on macrofaunal communities in the estuary of the Guadalquivir.

Influencia de la actividad humana sobre las comunidades de macrofauna en el estuario del Guadalquivir.

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PhD

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Los directores **Dr. José Carlos García Gómez**, catedrático del Departamento de Zoología de la Universidad de Sevilla y **Dr. Juan Emilio Sánchez Moyano** profesor titular en el Departamento de Zoología de la Universidad de Sevilla,

INFORMAN:

Que la presente memoria de tesis doctoral, titulada "**Influencia de la actividad humana sobre las comunidades de macrofauna en el estuario del Guadalquivir**" fue realizada por **Iñigo Javier Donázar Aramendía** 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.

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A mis padres

Nos ocupamos del mar
y tenemos dividida la tarea,
ella cuida de las olas
yo vigilo la marea

Javier Krahe

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Resumen General

Los estuarios son ambientes costeros altamente complejos y dinámicos con una enorme productividad. Sin embargo, debido al incremento del desarrollo urbano y agrícola, se encuentran entre los sistemas acuáticos más alterados y amenazados. La presente tesis doctoral centra su área de estudio en un buen ejemplo de un estuario altamente antropizado: el estuario del Guadalquivir. Este estuario además de ser una de las principales vías de entrada de metales pesados y nutrientes al Golfo de Cádiz, sufre dragados de mantenimiento periódicamente. Esta tesis recoge varios estudios sobre los efectos sobre las propiedades fisicoquímicas del sedimento y sobre la estructura de las comunidades de macrofauna, tanto de las operaciones de dragado dentro del estuario, como del posterior vertido de sedimentos en un vaciadero marino situado en mar abierto. Los resultados obtenidos mostraron grandes diferencias en los efectos de estas operaciones dentro del estuario comparado con la zona de vertido. Mientras que en la zona interior prácticamente no se encontraron efectos atribuibles a los dragados, en el vaciadero marino, la continua deposición de sedimento durante años ha creado un ambiente diferente con una comunidad más rica y diversa que la encontrada en zonas de referencia cercanas. La deposición de sedimentos llevada a cabo en 2015 incrementó la concentración de metales pesados en el vaciadero marino,

sin embargo, esta concentración nunca alcanzó los niveles mostrados por las áreas de referencia. Por otro lado, no se encontraron efectos sobre las cadenas tróficas ni en la zona de dragado ni en la de vertido.

La falta de efectos detectables dentro del Guadalquivir pudo ser debida al pobre estado de las comunidades bentónicas de esta zona sumado a la dificultad para detectar impactos en ambientes tan dinámicos como los estuarios. A pesar del estado de las comunidades betónicas, las redes tróficas del Guadalquivir mostraron una mayor complejidad y diversidad trófica que las encontradas en un estuario, *a priori*, mejor conservado como el Guadiana. La presente tesis doctoral pone de manifiesto la necesidad de llevar a cabo estrategias de gestión que impliquen a todas las administraciones para la mejora del estado de las comunidades del Guadalquivir, pero de una manera lenta, controlada y monitorizada, ya que cualquier cambio abrupto, aunque sea orientada a la mejora de las condiciones del estuario podría alterar el complejo equilibrio mostrado, al menos, en la ecología trófica del sistema.

General Abstract

Estuaries are highly complex dynamic and productive coastal environments. However, due to the increase in urban and agricultural development, they are among the most disturbed and threatened aquatic systems. This thesis focuses its study area on a good example of a highly anthropized estuary, the Guadalquivir estuary. This estuary, in addition to being one of the main routes of entry of heavy metals and nutrients to the Gulf of Cádiz, suffers periodically maintenance dredging works. This thesis includes several studies of the effects on the physicochemical properties of the sediment and on the structure of the macrofaunal communities, both of the dredging operations within the estuary, and of the subsequent disposal of sediments in a marine dump located in the open sea. Large differences were found in the effects of these operations within the estuary compared to the disposal area. While in the inner zone practically no effects attributable to dredging were found, the continuous deposition of sediment in the disposal area for years has created a different environment with a richer and more diverse community than that found in nearby reference areas. The deposition of sediments carried out in 2015 increased the concentration of heavy metals in the marine dump, however, this concentration never reached the levels shown by the reference areas.

On the other hand, no effects were found on the trophic food webs either in the dredged or in the disposal area.

The lack of detectable effects within the Guadalquivir could be related with the poor status of the benthic communities in this area, together with the difficulty of detecting impacts in dynamic environments such as the estuaries. Despite the status of the benthic communities, the trophic food web of the Guadalquivir showed a greater complexity and trophic diversity than those found in an estuary, a priori, better preserved as the Guadiana. This thesis highlights the need to carry out management strategies which involve all administrations for the improvement of the state of the Guadalquivir communities, but in a slow, controlled and monitored manner, since any abrupt change, although if it is oriented to the improvement of estuary conditions, it could alter the complex equilibrium shown, at least, in the trophic ecology of the system.

General introduction

Estuarine ecological scenario

Estuaries are a type of aquatic environment known as transitional waters, which also include deltas, coastal lagoons, fjords, etc. (Elliott et al., 2019). The concept of an estuary has been revisited in recent decades since it was defined by Pritchard in 1967, as this early definition did not adequately characterise some estuaries, such as those in Australia and Africa (Potter et al., 2010). Today, an estuary can be defined as ‘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’ (Elliott and Whitfield, 2011). These environments have properties of both fresh water and sea water environments but also have unique properties, making these areas full ecosystems rather than ecotones (Whitfield and Elliott, 2012).

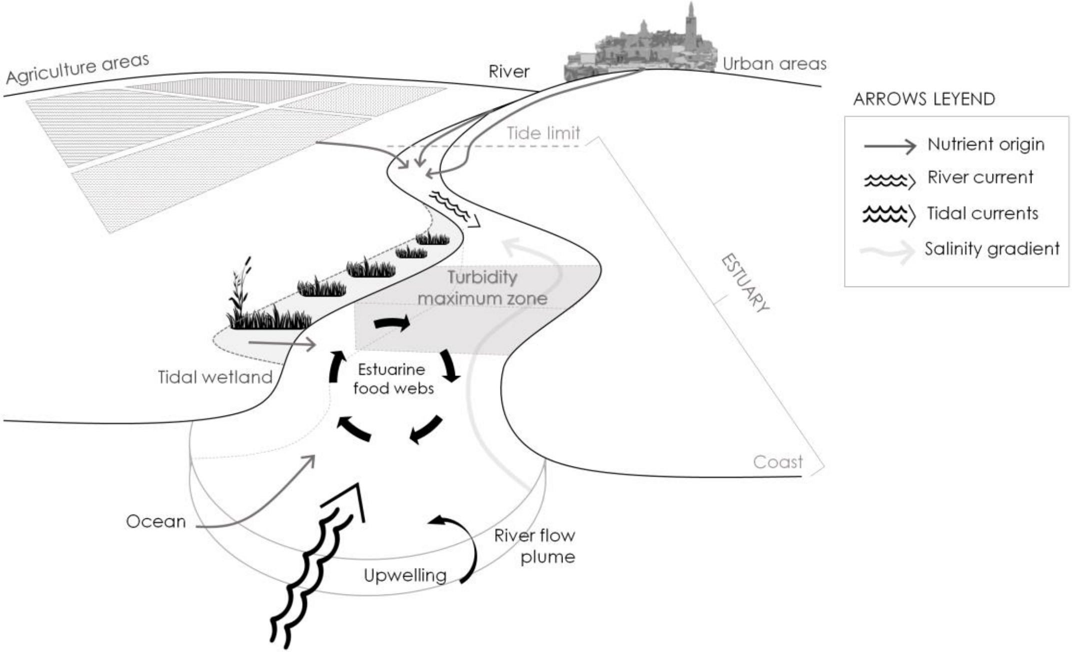


Figure 1. Sketch of the dominant forces that govern water, sediment and nutrients pathways in an estuary. Modified from Wolanski et al. (2006).

Estuary systems are driven by physical forces derived from water movements and generated by two opposite currents that depend on the tidal regime and river flow (Fig. 1) (Day et al., 2012). These factors affect water mixing, sedimentation rate and other physical factors, all of which influence the estuarine biota (Elliott and Whitfield, 2011). Estuaries are dynamic environments in which there are physicochemical gradients with a greater variability than in any other aquatic ecosystem (Elliott et al., 2019). The most remarkable of these gradients is probably that of salinity, but other gradients exist such as that of pH, dissolved oxygen, temperature, suspended solids, among others (Chapman and Wang, 2001). For example, turbidity limits primary production in many European estuaries and detritus is an important source for estuarine food webs (David et al., 2006). Daily tidal changes contribute to the variation in these gradients in the water column, as do fluctuations over seasons and other temporal scales, particularly in stratified estuaries (Whitfield and Elliott, 2012).

Despite the stressful nature of this environment, estuaries are among the most productive aquatic environments in the world (Dauvin, 2008). They are critical areas for feeding, development, migration and breeding for both resident and migrating species (Chapman and Wang, 2001). Although estuaries are considered to have a low number of species, this lack of richness is normally paired with high abundance values for some of these species (Chapman and Wang, 2001; Dauvin, 2007). The salinity gradient is the main factor conditioning the organisms living in this ecosystem, from the upstream fresh waters to the marine waters (Dauvin and Desroy, 2005). According to Attrill and Rundle (2002), animal communities in these environments are arranged in a *continuum* from the innermost areas to the mouth, with a more or less overlapping distribution of species. The dominant species are well adapted to this changeable scenario (Dauvin et al., 2009, 2012; Dauvin, 2007). Estuarine biota usually show strong resistance and resilience to the high variability in the physicochemical processes as well as to anthropogenic pressures (Dauvin et al., 2008). However, because of the lower number of species compared to other nearby ecosystems, a loss of species could lead to major consequences in estuarine food chains (Elliott and Whitfield, 2011).

In the estuarine environment, the trophic dynamics of organisms result in complex connections totally different from those of the sea environment (Wolanski, 2007). Estuarine food webs are usually complex, with a high number of primary producers and trophic interactions between organisms, with several generalist species and high levels of interchange between the water column and the sediment (Fig. 2) (Wolanski and Elliott, 2016). There are often a set of primary producers rather than a single one, as can be found in the ocean (e.g., phytoplankton, benthic algae, riverine plants, seagrasses, etc. (Maier et al., 2011). In highly turbid estuaries, light is limited and thus a wide set of primary producers cannot be supported; this scenario is usually accompanied by a strong presence of detritus (Elliott and Whitfield, 2011). Detritus is an important source of carbon, based on decomposed allochthonous and autochthonous material (David et al., 2006; Islam and Tanaka, 2006). Estuaries usually receive large amounts of organic matter from the sea, the river, the land and from human waste (Elliott and Whitfield, 2011). This detrital or photosynthetic primary production is consumed by lower heterotrophic organisms such as fungi, bacteria or zooplankton, which feed small planktivorous fishes and invertebrates with large predators at the top of the food web (Elliott and Whitfield, 2011; Mazumder et al., 2011; Vinagre et al., 2010). In highly heterotrophic estuaries, detrital material is the base of the microbial loop and also links the pelagic food web to the benthic food web (Wolanski and Elliott, 2016). There is a continuous interchange of organic and inorganic nutrients between the water column and the sediment (Hou et al., 2013; Lohrer and Wetz, 2003). In the benthic food web of estuaries, there are organisms that use the currents to feed off these primary producers (filter feeders), such as some bivalves; others that feed directly from the sediment (deposit feeders), such as worms, amphipods and clams; and finally others vertebrates and invertebrates that feed on all of these organisms (predators) (Herman et al., 1999).

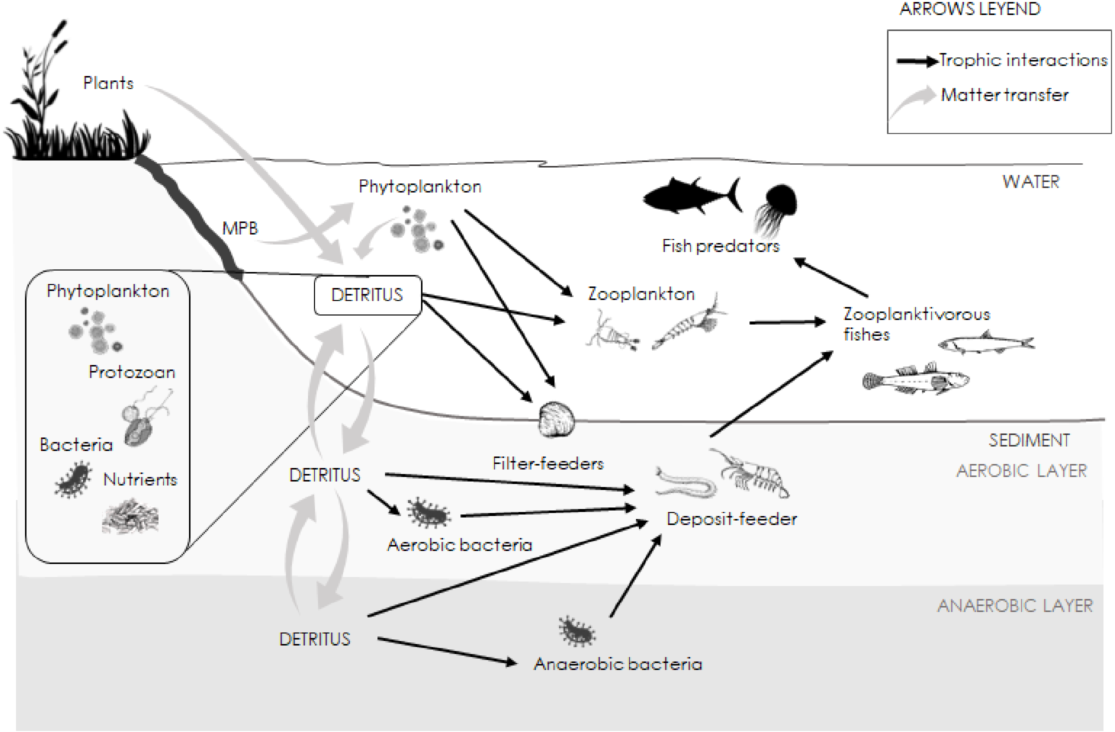


Figure 2. Sketch of an estuarine food web in a temperate estuary. MPB: Microphytobenthos.

Human impacts on estuarine ecosystems: the case of dredging and disposal

Estuaries are not only stressful environments for their inhabitants because of their variable physico-chemical properties, but also because of multiple anthropogenic pressures (Dauvin and Ruellet, 2009; Elliott and Whitfield, 2011). Coastal areas have been extensively occupied by humans for centuries and today, more than 60% of the Earth’s population lives in these areas leading to severe modifications in this environment (Ray, 2006). Estuaries have been favourite areas to urban and industrial development, as well as for vessel traffic, fisheries and tourism (Dauvin et al., 2006). Thus, these systems are threatened by habitat loss, eutrophication, resource extraction, chemical contamination, pollution, species invasions, sea level rise, and plastics, among other impacts (Bárcena et al., 2017; Ruiz et al., 2015; Ryder et al., 2007).

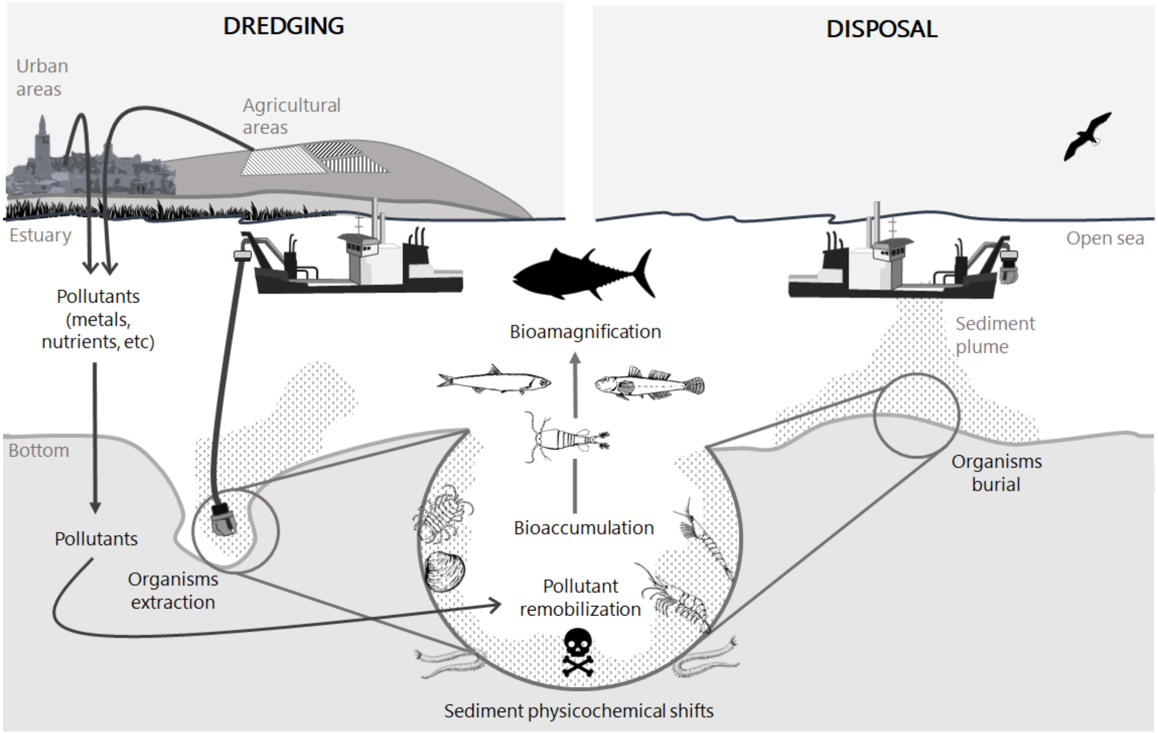


Figure 3. Dredging and disposal effects on sediment and organisms.

One of the main impacts in coastal areas is nutrient pollution, derived from multiple natural and human sources, e.g. aquaculture, agriculture, and urban industrial waste (Howarth et al., 2000; Seitzinger et al., 2005). Nutrient pollution in aquatic ecosystems has led to different impacts on biodiversity and ecological processes such as basal resource production, nutrient dynamics and energy transfer (Howarth et al., 2000; Warry et al., 2016). High biological activity as a consequence of nutrient inputs can lead to anoxia and hypoxia events, as well as overgrowth of seaweed, epiphytes and toxic algal blooms (Cardoso-Mohedano et al., 2015). Nutrient load inputs in estuaries are directly related to intensive agriculture and large populations (González-Ortegón and Drake, 2012) and also have the potential to alter the nutrient dynamics modifying the function and structure of the estuarine ecosystems (Mazumder et al., 2015). Over time, these nutrients could accumulate in the sediment and act as a reservoir that can be released back into the water column under different environmental conditions (Carpenter et al., 1998).

Estuarine sediments can not only act as a sink of nutrients, but also of other pollutants including heavy metals from diverse sources (Souza et al., 2015; Stephens et al., 2001). Heavy metals are very durable substances in the environment, and thus have received special attention (Nicolau et al., 2006). Estuaries are one of the aquatic environments most polluted by trace metals (Förstner and Wittmann, 1981; Mucha et al., 2005, 2003). Thus, heavy metal pollution has become a major issue in estuarine environments (Buruaem et al., 2012), due to their potential bioavailability and toxicity for biota, and their tendency to bioaccumulate (Bárcena et al., 2017; de Souza Machado et al., 2016). These pollutants influence the feeding habits of benthic and pelagic animals and may also influence the biodiversity and ecological health of these ecosystems (Birch, 2017). Since sediments accumulate nutrients and other pollutants, they play a crucial role in the transport of contaminants as suspended particles in the water column (Birch, 2017). Thus, slight changes in the water and sediment conditions may modify the mobility and bioavailability of metals (Cesar et al., 2014; Guerra et al., 2009; Katsiaras et al., 2015; Wilber et al., 2007). Moreover, the remobilisation of sediments may be favoured by physical advection events, e.g. wave action, storm surge, and dredging activities (de Souza Machado et al., 2016). As a consequence, these released contaminants can be bioaccumulated and biomagnified in marine organisms across the food chain (Roberts and Forrest, 1999).

Dredging and the subsequent disposal of dredged material are common practices in estuaries worldwide (OSPAR, 2008). These operations are considered a necessary activity in the management of aquatic systems (Ceia et al., 2013a). This approach is used for beach nourishment, construction, restoration of tidal marshes, for navigation safety and to support trade and economic sustainability (Bates et al., 2015; OSPAR, 2008; Vivan et al., 2009). However, dredging and disposal activities are a key environmental concern in coastal management (Marmin et al., 2016; Moog et al., 2015; Van Dolah et al., 1984). These practices are particularly developed in estuaries where sedimentation patterns are high and may be accelerated by human activities (Cesar et al., 2014). They are considered large-scale disturbances in these habitats, which can affect the water quality and biota (Lohrer and Wetz, 2003). Moreover, they can also increase the tidal range, the salinity gradients and change the concentration of suspended solids and sedimentary dynamics (van Maren et al., 2015).

Dredging operations on their own imply various impacts, but the relocation of the dredged material extracted is also considered a management problem (Fig. 3). There are several destinations for the relocation of dredged material, but disposal at licensed sites at sea is still a priority for economic reasons (Harvey et al., 1998; Katsiaras et al., 2015; Tornero and Hanke, 2016). Dredging and disposal of dredged material may cause environmental problems in estuarine and marine areas. Moreover, these impacts can also affect surrounding areas and may lead to important habitat changes through long-term effects (Powilleit et al., 2006). Ecosystems may be affected both physically through changes in the sedimentary composition of the seabed and water conditions with increasing turbidity and through the release of pollutants and nutrients that have been trapped in the sediment (Barrio

Froján et al., 2011; Ceia et al., 2013b; Karel, 1999; Lohrer and Wetz, 2003; Morgan et al., 2012; Rehitha et al., 2017; Sánchez-Moyano et al., 2004). In that sense, Cardoso-Mohedano et al. (2015) found that the impact of dredging combined with other anthropogenic impacts such as nutrient loading cause negative synergistic effects on water quality. Effects are highly variable, and depend on the dredging and disposal method used; the season, depth, duration and extent of the activity; the amount and physicochemical characteristics of the mobilised material and of the origin and receiving areas; the oceanographic and sedimentary conditions of the habitat; and finally the ecology of the inhabitant community (Katsiaras et al., 2015; Simonini et al., 2005 and references therein).

Dredging often has even more repercussions for benthic macrofauna due to the relative immobility of organisms (Simonini et al., 2005). These communities play a crucial role in the structure and function of the ecosystems, such as sediment stability, nutrient processing and contaminant sequestering (Ceia et al., 2013a; Kon et al., 2015; Thrush and Dayton, 2002). As stated above, macrofauna is one of the most important elements in estuarine food webs linking organic matter to the higher trophic levels, and acting as prey for epibenthic crustaceans, fishes and birds (Bolam et al., 2011; Herman et al., 1999). Humans also harvest many species of macrofauna such as shellfishes and crustaceans in estuaries (Herman et al., 1999). Benthic macrofauna are mostly fixed to the sediment or show low mobility. Often, they have relatively long live cycles, which integrate environmental influences over long periods of time. Thus, benthos and suprabenthos are recognised as good indicators of natural and anthropogenic changes in estuaries (Dauvin and Pezy, 2013). Macrofauna are often used in monitoring programs or in the evaluation of anthropogenic disturbances, not only as single species, but also analysing the whole structure of the community (Herman et al., 1999). Within the implementation of the European Water Framework Directive, improvement of our knowledge about human impacts is critical for marine management and conservation (Marmin et al., 2016). Therefore, monitoring is highly necessary in dredging and disposal activities, especially when the option chosen is the deposition of dredged material in one marine area (Bocchetti et al., 2008). However, sometimes it is difficult to interpret impacts in these environments, due to the changing variables and the complex interconnected interactions (Dauvin, 2008).

Assessing dredging impacts

An appropriate experimental design to evaluate environmental impacts is the BACI (before after control impact) approach. The principle is that the measured variable in the impacted area will cause a different pattern of change from before to after the impact compared to a similar unimpacted location, preferably more than one (Underwood, 1991, 1992). Furthermore, this approach can detect short-term (pulse) or long-term impacts (press). Conclusions drawn from this method are dependant on the parameters assessed (Dauvin et al., 2006). For example, suprabenthic fauna is a good source of short-term information, while sediment and benthic fauna are good indicators of both past and present disturbances (Dauvin et al., 2006).

These impacts can also be reflected in the food webs (Olsen et al., 2011; Warry et al., 2016). Physical disturbances to the sediment can generally cause releases of nutrients into the water column (Bancon-Montigny et al., 2019). Nutrient loading in estuaries may reduce primary production to a single basal source, converting a structured and compartmented ecosystem into a less stable food web (Layman et al., 2007b; Rooney et al., 2008, 2006). On the other hand, they can favour autotrophs and increase the nutritional quality of basal resources (Warry et al., 2016) and sometimes increase fish abundances (Howarth et al., 2000).

Assessing community trophic structure is one way to assess human impacts on marine environments (Hussey et al., 2014). Stable isotope analysis (SIA) is one of the primary tools to examine the structure and dynamics of food webs (Layman et al., 2012) and may represent an unifying methodology to compare anthropogenic pressure on different coastal ecosystems (Mancinelli and Vizzini, 2015) (Box 1). The combination of isotope analysis of carbon and nitrogen is a powerful tool to assess the diet composition of consumers, analysing isotopic signature of its potential prey, even quantitatively (Fry, 2006). Moreover, SIA has proven to be a useful and unifying tool to assess cumulative anthropogenic pressures on coastal ecosystems (Mancinelli and Vizzini, 2015) and can provide integrated temporal and spatial information of trophic ecology of species (Layman et al., 2007a). Moreover, stable isotopes can be used to track the source of nutrients in a food web, to assess chemical pollution, evaluate effects of invasive species, characterise the trophic niche of species or provide community wide measures (Layman et al., 2012; Mancinelli and Vizzini, 2015).

BOX 1. Isotope ecology as a tool to describe the trophic structure of macrofaunal communities

Stable isotopes are non-radioactive elements with the same number of protons but different numbers of neutrons in their nucleus, causing them to differ in their atomic weight. This analysis is based on the fact that atoms that make up living beings are derived from what they eat (Pasquaud et al., 2008) and reflect an integrated record of what organisms are eating over a certain period of time before sampling (Vander Zanden et al., 2015). Carbon and nitrogen ratios, notated as $\delta^{13}C$ and $\delta^{15}N$ respectively, are the most common isotopes used in aquatic trophic ecology and are defined as the relative proportion of the heavier isotope vs the lighter $^{13}C/^{12}C$ and/or $^{15}N/^{14}N$ compared to a standard (Pasquaud et al., 2008 and cites therein). These ratios change in different biogeochemical processes called isotopic fractioning and mixing; thus some substances are enriched in the heavier atom and others are impoverished (Fry, 2006).

Differences in ratios of the carbon isotopes are related to the carbon cycle (Fry, 2006). Primary producers differ in their carbon ratios because of different photosynthetic metabolism (C_3 vs C_4) and because of the isotopic signatures of the inorganic carbon available in their environment. Moreover, carbon ratios do not undergo significant changes during food web transfer, which allows their use in assessing the origin of organic matter sources (i.e. marine, riverine, terrigenous, benthonic, etc.). On the contrary, nitrogen undergoes a fractionation through the loss of lighter isotopes in excretion and metabolic processes resulting in an enrichment in the heavier isotope of the consumer with respect to their prey over the food chain. Thus, it used to indicate the trophic position of the organisms (Post, 2002). Moreover, nitrogen isotopes allow the assessment of nutrient pollution in aquatic ecosystems because anthropogenic waste is enriched in ^{15}N, which can be used as an indicator of human-derived pollution (Baeta et al., 2009).

Study area: The Guadalquivir Estuary

The study area of this thesis is located in the Guadalquivir Estuary (southwestern Spain), a good example of a highly stressed temperate estuary (Ruiz et al., 2015). It extends 110 Km from the mouth in the Gulf of Cádiz (Atlantic Ocean) to the upstream limit, the Alcalá del Río dam. This estuary has suffered deep natural and human modification throughout its history and is an area of human and environmental interest. An assessment of this area can be found in Llope (2017), Ruiz et al. (2015) and Contreras and Polo (2010). Briefly, the first modifications of this estuary were of a natural origin by sedimentation and infilling resulting in the Guadalquivir marshes and giving rise to the Doñana National Park (Ruiz et al., 2015). In the eighteenth century, since Seville harbour was the principal connection between the Spanish empire and the Americas, some meanders were cut short to facilitate the speed of the maritime traffic (Ruiz et al., 2015). These pressures increased in the twentieth century reducing the original marsh surface in favour of agriculture fields and new settlements, which led to increases in the spill of nutrients and other pollutants into the estuary (González-Ortegón and Drake, 2012; Mendiguchía et al., 2007). Finally, the reduction of fresh inputs into the river flow and the installation of dams along the river has resulted, among other severe disturbances, in a distortion of the dynamics that govern the estuary (Ruiz et al., 2015).

The Guadalquivir estuary provides access to Seville harbour, the only inland harbour in the Iberian Peninsula. All of these modifications have resulted in a well-mixed estuary composed of a 90-km long navigation channel with a mean depth of about 6.5 metres. The strongly modified course and sedimentary dynamics have made necessary some dredging works every few years to maintain navigability (Díez-Minguito, 2012). Part of the sediment dredged has been disposed of in an established location since 2010. The main channel has become isolated from the marshes, which have suffered a reduction of 85% with a decrease of freshwater inputs of 60% (Llope, 2017). Currently, intertidal marshes only account for 1.9% of the marsh surface located in a stretch of about 0.25 km wide and 15 km long (Gallego and García Novo, 2006). The mean tidal range amplitude of about 2.5 m creates a horizontal salinity gradient that governs the composition and spatial distribution of the aquatic communities (Llope, 2017). Moreover, Guadalquivir estuary is characterised by high turbidity levels and increased nutrient and heavy metal loadings, which make it a major provider of metals and organic matter to the Gulf of Cadiz (González-Ortegón et al., 2019; González-Ortegón et al., 2018). On top of this, in April 1998, an acid mining spill was accidentally discharged into the river through its tributaries (Tornero et al., 2014).

There have been several studies in the last fifteen years about the salinity and freshwater effects of the distribution of the biota in the

Guadalquivir, but these are mostly related to the suprabenthos (Drake et al., 2002). Studies about the endobenthos are scarce and more focussed on the intertidal or shallow subtidal areas (Baldó and Cuesta, 2005). These latter studies highlighted the poor richness and abundance of these organisms compared to other nearby estuaries, such as the Guadiana, which indicates the stress on the environment by both natural and anthropogenic pressures (Sánchez-Moyano and García-Asencio, 2011, 2010). Thus, human-derived pressures in this estuary seems to influence these communities. The high turbidity, currents and irregular flow as well as the maintenance-oriented dredging works may be the main factors related to these observations.

Objectives and Thesis structure

The main aim of this thesis is to evaluate the effects of human pressures on the macrofaunal communities in the Guadalquivir estuary (Box 2). We analysed the effects of dredging works carried out in the Guadalquivir estuary and the subsequent disposal of this dredge material in authorised marine areas. We combined a classical approach assessing the dredging and disposal impacts on the physicochemical and biological characteristics of the system, and incorporated a new approach based on the analysis of stable isotope values of carbon and nitrogen. We used SIA to assess the effects on the food web structure in the dredging disturbance and also analysed sediment toxicity, biomagnification and bioaccumulation within the food web as a result of the disposal event. Finally, we compared the food webs found in the Guadalquivir estuary with the Guadiana estuary as a reference for a less impacted ecosystem.

In addition to the introduction presented above, this thesis is composed of four 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 assess the effects of the maintenance dredging works carried out in summer 2015 in the Guadalquivir estuary. We evaluated both direct and indirect impacts through a BACI analysis in two salinity ranges. We analysed effects on and recovery of the biological and sediment physicochemical characteristics as well as the community food web through a stable isotope analysis. This chapter has been published in *Marine Environmental Research*.

In **chapter 2,** we assess the effect of the subsequent disposal of the dredged material extracted from the channel of the Guadalquivir in a recurrent marine dumping area. We assess the impacts on the physical and chemical characteristics of the sediment and water column, determining the variation of heavy-metal concentrations. Moreover, we analysed their toxicity and bioaccumulation within the food web.

In **chapter 3,** we analysed the effect of the material deposition from the previous chapter but this time focussing on the effects on the macrofaunal communities and their subsequent recovery. We also analysed the impacts on the trophic structure. We compared patterns observed before and after the disposal in the impacted area with two control areas close to the dumping site following a beyond BACI approach. This chapter has been published in *Marine Environmental Research*.

In **chapter 4,** we used stable isotope analysis to assess human pressures by contrasting the food web structure of the Guadalquivir estuary and the Guadiana estuary. These two estuaries are exposed to different degrees of urban and agricultural perturbations. This chapter has been published in *Scientific Reports*.

Box 2. Macrofaunal benthic community of the Guadalquivir estuary

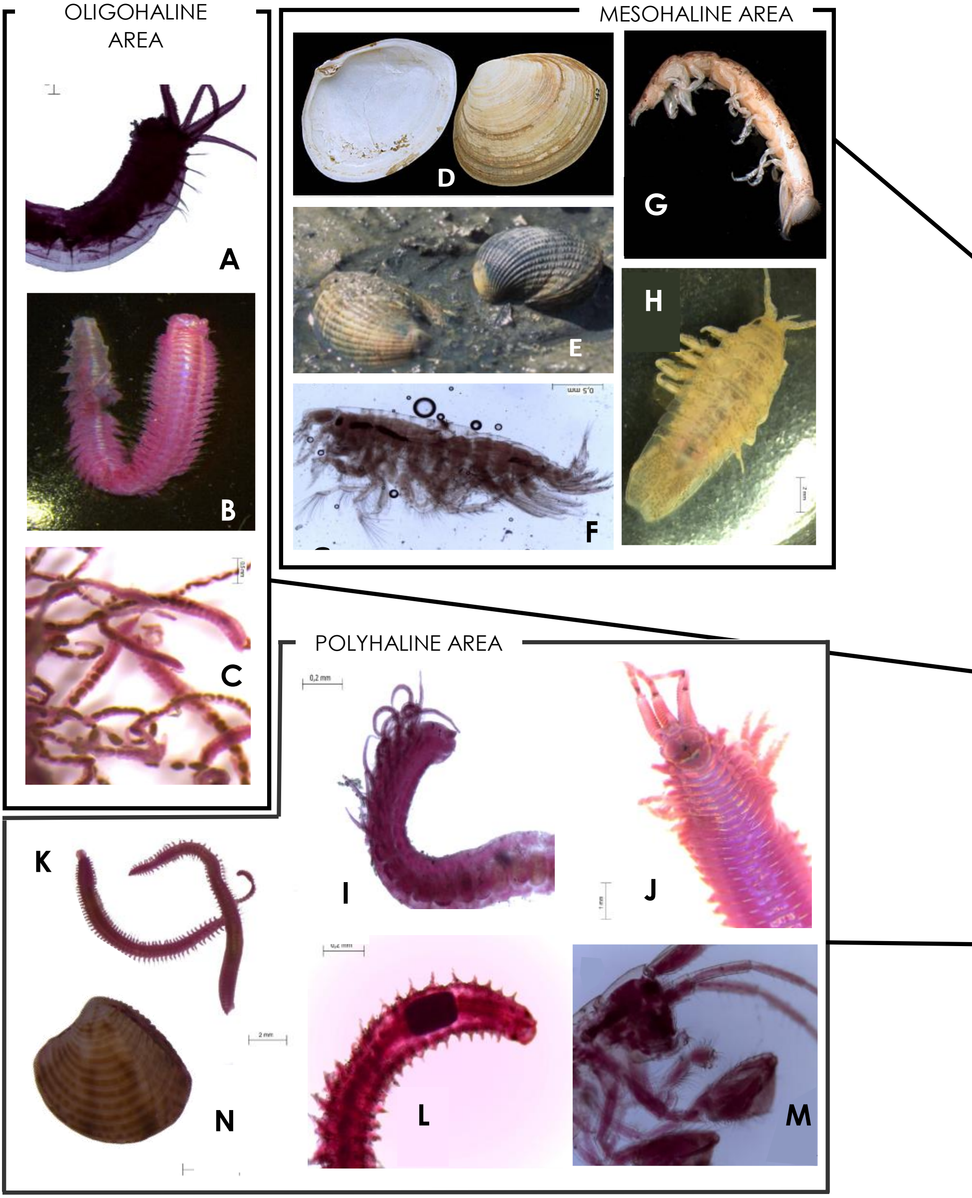
Here are presented the principal benthic communities found during surveys carried out in parallel to the development of this thesis (Fig 4). Samples were taken in different salinity zones with a van Veen grab.

The oligohaline area was predominantly composed by annelids such as *Alkmaria romijni* (A), *Hediste diversicolor* (B) and *Streblospio shrubsolii* (C).

Mollusc species were found for the first time when the mesohaline area was reached (*Scrobicularia plana* (D) and Cera*stoderma edulte* (E)). Crustaceans, such as the amphipod *Bathyporeia pilosa* (F) and the isopod *Cyathura carinata* (G) were observed, and the presence of the invasive isopod *Synidotea laticauda* (H) was also recorded.

The polyhaline area was characterised by a higher number of species, following the natural patterns observed in temperate estuaries. Among the organisms found, we particularly note the presence of annelids such as *Aonides oxycephala* (I), *Diopatra neapolitana* (J), *Nephtys hombergii* (K) and *Sphaerosyllis pirifera* (L); the amphipod *Melita palmata* (M); and the bivalve *Chamalea gallina* (N).

Finally, the most diverse community was found in the euhaline part of the estuary, where representatives of all the benthic groups were present: crustaceans such as *Harpinia pectinata* (O), *Ampelisca diadema* (P) or *Leucothoe incissa* (Q); the bivalve species *Nucula henleyi* (R), *Corbula gibba* (S) and *Angulus tenuis* (T); and echinoderms such as the brittle star *Amphiura chiajei* (U), the sea urchin *Echinocardium cordatum* (V) and the sea cucumbers *Oestergrenia digitata* (W).


OLIGOHALINE AREA
MESOHALINE AREA
POLYHALINE AREA
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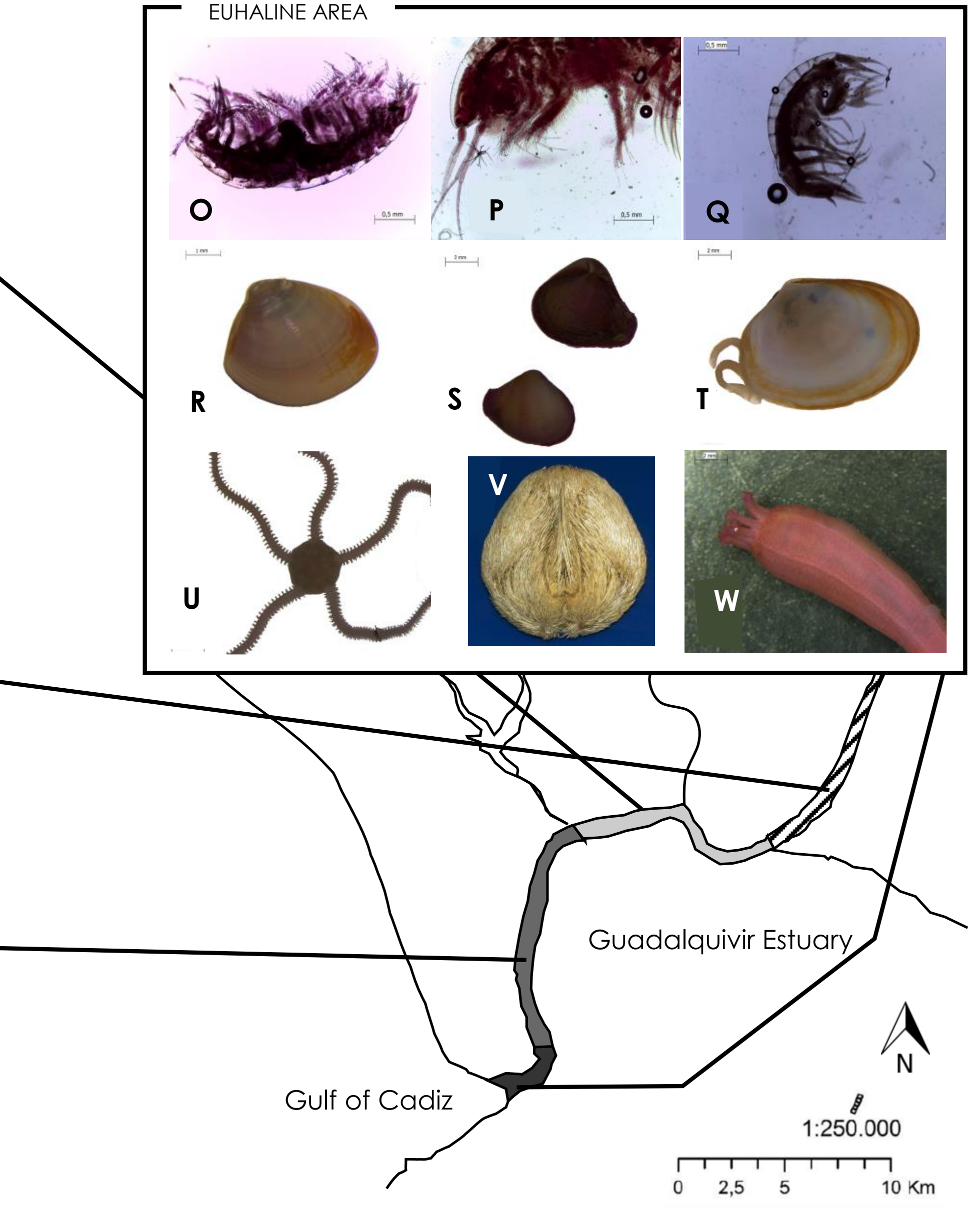
0,5 mm
0,2 mm
2 mm
1 mm


EUHALINE AREA
O
0,5 mm
P
0,5 mm
Q
0,5 mm
1 mm
R
2 mm
S
2 mm
T
U
V
2 mm
W
Guadalquivir Estuary
Gulf of Cadiz
N
1:250.000
0
2,5
5
10 Km

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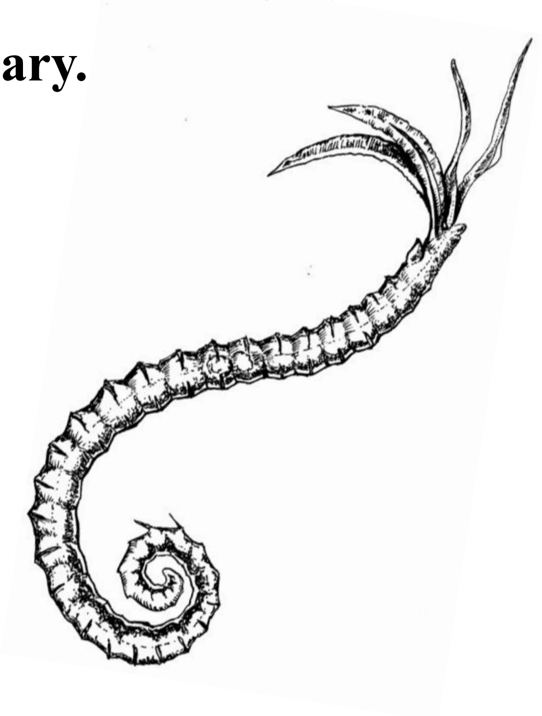
1 Maintenance dredging impacts on a highly stressed estuary.



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Resumen

Los estuarios son sistemas complejos donde implementar diagnósticos ambientales es una tarea difícil debido al gradiente salino y a las perturbaciones antropogénicas. En este sentido, para evaluar los efectos directos e indirectos de los dragados de mantenimiento realizados en el estuario del Guadalquivir, se usó un análisis “Before After Control Impact” en dos rangos de salinidad diferentes. El análisis no mostró efectos en las características fisicoquímicas del agua o el sedimento. Además, el estado tan pobre de la comunidad macrobentónica tampoco permitió detectar impactos significativos en la estructura de la misma. El análisis de la estructura trófica mediante el uso de isótopos estables mostró que los cambios observados a lo largo del tiempo parecían explicarse por la variación natural del sistema más que por las operaciones de dragado. Este articulo remarca la necesidad de definir una estrategia de conservación y gestión adecuada para mejorar el estado de las comunidades bentónicas del Guadalquivir.

Abstract

Understanding the effects of dredging in estuaries is a hard task due to the difficulty of implementing an adequate environmental diagnosis, as a consequence of the salinity gradient and anthropogenic disturbances. To assess the effects of maintenance dredging work on the Guadalquivir estuary (southwestern Spain), we used a Before-After-Control-Impact (BACI) approach to determine both direct and indirect effects in two salinity ranges. No effects were found on water and sediment physicochemical characteristics. The small impacts on dredged areas were followed by a rapid recovery of opportunistic species. The poor status of the benthos does not permit the detection of significant effects on macrofaunal community structure. The use of stable isotopes analysis to determine impacts on food web structure showed that changes over time seem to be explained by natural temporal variation rather than the dredging works. This paper emphasises the need to define proper management and conservation plans to improve the status of the benthic communities of the Guadalquivir estuary.

Introduction

Although estuaries are one of the most productive marine coastal environments in terms of biomass (Wolf, 1983; Wetzel et al., 2013), they often face perturbations (Dauvin et al., 2006; Sánchez-Moyano and García-Asencio, 2010). With more than 60% of Earth's population living in the coastal realm, estuarine ecosystems have been extensively altered by human activities (Ray, 2006). Furthermore, estuaries are dynamic and complex systems where high variability of the physical-chemical gradients makes them one of the most stressful aquatic environments (González-Ortegón et al., 2006; Dauvin, 2008). In this changeable scenario, characteristics of estuarine communities are strongly and directly related to parameters, such as turbidity, temperature and, particularly, salinity (Baldó and Cuesta, 2005; Dauvin, 2008). As a consequence, benthic community diversity is limited, but it is often associated with a high tolerance to variable environmental conditions (Dauvin, 2007). Interpreting disturbance effects in estuaries often is complex, because the dynamic geological, physical and chemical characteristics that rule those systems might be confused with anthropogenic impacts (Morrisey et al., 2003; Dauvin et al., 2006; Dauvin, 2008). An accurate evaluation of the anthropogenic impacts in estuaries is vital for the proper management of resources and maintaining good environmental health as well as reaching a "good environmental status" in the context of the requirements of the

European Water Framework Directive (Taupp and Wetzel, 2013; Rehitha et al., 2017).

The Guadalquivir estuary (southwestern Spain) is a good example of this kind of stressed scenario. In this system, mixed natural perturbations, such as a horizontal salinity gradient, govern the composition and spatial distribution of the aquatic communities, while human activities have deeply modified the ecosystem (González-Ortegón et al., 2006; Castañeda and Drake, 2008; Llope, 2017). They vary from desiccation of tidal marshes and isolation of the estuary course from the original tidal marshes, reduction of freshwater inputs and eutrophication from urban and agricultural waters to maintenance dredging work (Taglialatela et al., 2014; Llope, 2017). The Guadalquivir estuary is the only navigable river in Spain and gives access to Seville harbour. To maintain navigability, the Autoridad Portuaria de Sevilla (APS) has performed maintenance dredging work every one or two years since 1985 (Gallego and García Novo, 2006). Dredging operations represent a potential risk to the estuarine environment; effects basically depend on the method used, duration and extension, amount of dredge material and sediment characteristics. These activities may cause changes in the seabed and natural fluctuations in water conditions, population dynamics and sedimentary composition of the system and the surrounding areas (Sánchez-Moyano et al., 2004; Barrio Froján et al., 2011; Ceia et al., 2013; Rehitha et al., 2017). Dredging often has more repercussions on benthic communities due to the relative immobility of organisms (Simonini et al., 2005). Macrofaunal communities play a crucial role in the structure and functioning of ecosystems, such as sediment stability, nutrient processing and contaminant sequestering (Thrush and Dayton, 2002; Ceia et al., 2013). In estuaries, macrofauna are also an important link between organic matter and predators (Kon et al., 2015) acting as a food source for the next trophic level, generally secondary consumers such as fish and shellfish (Bolam et al., 2011).

Studies assessing dredging effects on macrofaunal assemblages are widely available (Klapan et al., 1975; Newell et al., 1998; Sánchez-Moyano et al., 2004; Bemvenuti et al., 2005; Ponti et al., 2009; Rehitha et al., 2017). However, more focused studies on dredging effects in different salinity ranges in estuaries are rare, despite the fact that salinity is the major environmental factor influencing the distribution of organisms in estuaries (Attrill, 2002). Most monitoring programs in estuaries have been developed in higher salinity ranges, while low salinity areas have been scarcely studied (Vinagre et al., 2015). Moreover, studies analysing dredging impacts on food web structure are few. Stable isotopes analysis is a useful tool to determine anthropogenic impacts on food web structure in aquatic ecosystems (Ke et al., 2016). Nitrogen and carbon isotopic ratios can be used for tracing the natural or anthropogenic sources of nutrients in estuaries (Castro et al., 2007; Kon et al., 2012; Van De Merwe et al., 2016). Also, the different rates of nutrient assimilation by different organisms can reflect estuarine status over temporal scales (Van De Merwe et al., 2016). For this reason, isotope analysis could be a useful tool to assess dredging impacts and the potential following recovery.

In this context, we analysed the effects of dredging work carried out in the Guadalquivir estuary in two different salinity gradient ranges with a Before-After Control-Impact (BACI) analysis (Underwood, 1991). We combined a classical approach assessing the dredging impact on the physicochemical and biological characteristics of the system, and we incorporated a new approach based on the analysis of stable isotope values of carbon and nitrogen. This study specifically aims to assess (i) effects of dredging on sediment and water characteristics and on macrofaunal communities and (ii) indirect effects on the surrounding shallower habitat and on the whole food web structure.

Methods

Study area

The Guadalquivir estuary is located in southwestern Spain. It extends from the mouth in Sanlúcar de Barrameda (Atlantic Ocean) to the Alcalá del Río dam, 110 km upstream. This estuary plays a critical role in the ecological and economic sustainability of very sensitive and protected areas of southwestern Spain (e.g., National Park of Doñana) (Tornero et al., 2014). The Guadalquivir estuary is a well-mixed and tidally dominated system (3.5 m tidal range at the mouth in spring tides) (Díez-Minguito, 2012), which presents a longitudinal salinity gradient with temporal displacement by tides, discharges and seasonal variations (González-Ortegón et al., 2014). In order to guarantee a minimum navigation depth of 6.5 m, the channel is dredged every one or two years (Ruiz et al., 2015). In summer 2015, a maintenance dredging operation was carried out in several estuarine sections. The dredging work was performed by trailer suction dredge. Our study was focused on two dredging sections, one in the polyhaline range (18–30 PSU) and the other in the oligohaline range (< 5 PSU), locally known as Salinas and La Gola, respectively (Fig. 1). Approximately 74,000 and 22,000 m^3 of dredged material were extracted in each range, respectively.

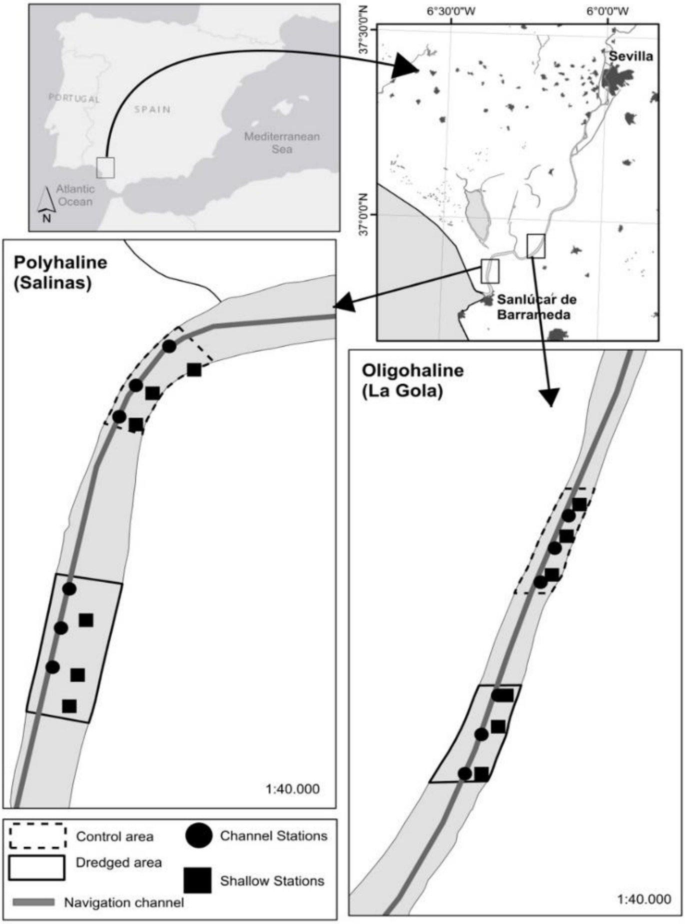

Figure 1. Location of the study area and sampling stations in both ranges of the salinity gradient.

Sampling design

Our sampling was designed according to a BACI approach (Underwood, 1994). In total, four sampling surveys were carried out: two pre-dredging (June and July 2015) and two post-dredging (October 2015 and August 2016) surveys. In both salinity ranges, two areas were established: one within the dredged section and the other (as a control) far away from the influence of these operations but always at the same salinity range intervals. Establishing more control areas in the same salinity ranges were not possible due to the areas not affected by the dredging being spatially limited (ca. 2 km). In each area, three stations were randomly located inside of the navigation channel and the other three in the shallower left margin in order to assess the direct and indirect effects of dredging in those habitats, respectively. Three samples were taken for macrofaunal analysis with a Van Veen grab (0.15 m^2 total sampling area per station and date). For posterior analysis, all stations were pooled together and were considered replicates of each area. Macrofaunal samples were sieved through a 0.5-mm mesh sieve, and infauna was preserved in ethanol (70%) and stained with rose bengal for subsequent identification and quantification at the lowest possible taxonomic level.

To relate the effects of dredging on sediment characteristics, one additional sample was taken for grain size distribution, particulate organic matter (POM) content and redox potential. Grain size distribution was measured as percentages of 100 g of dry sediment passed through a series of sieves (5 mm, 2 mm, 1 mm, 0.5 mm, 0.250 mm, 0.125 mm and 0.063 mm). Also, the median grain size (Q_{50}) and sorting coefficient (S_0) (Trask, 1950) were calculated. Granulometric typology was established according to the Wentworth geometric scale (Buchanan, 1984). The POM content was determined by calculating the weight difference between the dried sediment samples of three replicates (at 60 °C until dried weight stabilisation) and after combustion (500 °C for 4 h). Apparent redox potential was measured with a pH meter (WTW pH 1970i with SenTix ORP electrode).

For the heavy metals and trace element concentrations analyses, sediments were taken from the uppermost 2 cm. In the laboratory, sediment samples were air-dried, crushed and sieved though a 2-mm sieve and then ground to < 60 µm. These samples were digested with aqua regia (1:3 conc HNO_3: HCl) in a microwave digester. Quantification of elements in the extracts was achieved using a VARIAN ICP 720-ES (simultaneous ICP-OES with axially viewed plasma). The accuracy of the analytical methods was assessed via a reference soil sample from the Wageningen Evaluating Programs for Analytical Laboratories (WEPAL) for soils, International Soil-Analytical Exchange (ISE). The index of geoaccumulation (I_{geo}) has been used as a relative measure of metal pollution in sediments for Cr, Cu and Zn according to the regional background established by Ruiz

(2001) for unpolluted sandy and silty-clayey sediments and is given by: $I_{geo} = \log_2 (Cn/1.5\ Bn)$, where Cn is the value of the element n and Bn is the background data of that element. Following Ruiz (2001), the index values were divided into five groups: unpolluted ($I_{geo} < 1$); very lowly polluted ($1 < I_{geo} < 2$); lowly polluted ($2 < I_{geo} < 3$); moderately polluted ($3 < I_{geo} < 4$); highly polluted ($4 < I_{geo} < 5$) and very highly polluted ($I_{geo} > 5$). Comparisons between metal concentrations and sediment quality values (SQVs) proposed by Long et al. (1995) and DelValls and Chapman (1998) have also been performed. Heavy metals in water and sediment were only measured in the channel area in July and October 2015 and August 2016.

Water parameters were analysed from the bottom layer with a multiparametric probe Eureka Manta 2 with pH, dissolved oxygen, salinity and turbidity sensors. A 5-l water sample from 1 m above the bottom was collected with a Niskin bottle and then filtered through a GF/C Whatman glass fibre filter with an air vacuum pump; then, suspended organic matter (SUOM) and total suspended solids (TSS) were calculated. SUOM was determined with the same procedure as POM.

We investigated the possible impact of the dredging work on the nekton-benthonic food web of the two salinity ranges. Although sampling was carried out at the same time as the macrofaunal surveys, we did not differentiate control and dredging areas because the daily tide movements did not allow the establishment of control areas. We selected this community because it was more diverse and accessible to sample than strictly benthic fauna. Samples of the planktonic community were collected before dredging (July 2015) and twice after it (October 2015 and August 2016). Organisms were collected with a 1000-µm mesh zooplankton net with a 1 m mouth diameter. Oblique tows were performed from surface to bottom during flood tide in the main channel. All organisms were sorted by species, transferred to the laboratory in refrigerated containers and kept alive for 24 h to evacuate their gut contents. Sediment was taken from the upper 2 cm of a Van Veen grab sample for sediment organic matter (SOM) analysis. We rinsed samples with distilled water. Muscle tissue samples of fish larvae and shrimp abdomen were extracted. Pools of several organisms were used when individuals had low biomass values. Samples were dried at 60 °C and ground to a powder. Sediment samples were acidified with 0.1M HCl to remove carbonates and oven-dried. Subsamples of powdered materials were weighed to the nearest 0.3 µg and placed into tin capsules for $\delta^{13}C$ and $\delta^{15}N$ determinations. All samples were combusted at 1020 °C using a continuous flow isotope-ratio mass spectrometry system by means of Flash HT Plus elemental analyser coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific, Bremen, Germany).

Data analysis

Direct and indirect dredging effects were independently examined in both salinity ranges. Channel and shallower left margin habitats were also separately compared with their respective controls in both salinity ranges. Water and sediment variable differences were tested on Euclidean distances using a permutational univariate analysis of the variance (PERMANOVA) (Anderson, 2001a). PERMANOVA was chosen for univariate analyses because resulting sums of squares and F-ratios are exactly the same as Fisher's univariate F-statistic in traditional ANOVA and does not assume a normal distribution of errors (Anderson, 2005, 2001b; Scyphers et al., 2011). The experimental design included two crossed fixed factors: "Impact vs. Control" with two levels (Impact and Control areas), "Time" with two levels (Before and After the dredging work) and a random factor, the sampling dates "Dates" nested within "Time" with four levels (Jun 15, Jul 15, Oct 15 and Aug 16).

According to a BACI design, if the disposal had a permanent impact, the putatively impacted areas will change over time from the samplings before the dredging work to the samplings after with a different pattern when compared to control areas. This difference can be detected as a significant "Time" x "Impact vs. Control" interaction (Guerra et al., 2009).

Univariate measures, such as species richness (number of taxa, S), Shannon's diversity index (H', based on log *2*), total abundance (N, ind/m^2) and Pielou's evenness (J') were calculated. Significant interactions were tested using a permutational univariate analysis of the variance (PERMANOVA) with the same experimental design as above. The p-values were provided using unrestricted (9999) permutation of the abundance data based on the Euclidean distance matrix. When the number of total possible permutations to obtain the p-values were low (<100), we used the estimate obtained by Monte Carlo sampling (Anderson and Robinson, 2003). Significant interactions, if detected, were further explored in separate analyses, within the levels of the interacting factors; in other words, the significant interactions between "Impact vs. Control" and "Dates" were further analysed separately by impact area and the control area.

The effects on the multivariate structure of the communities were investigated using a PERMANOVA analysis based on the Bray-Curtis similarity index of square-root transformed abundance data with the same design as above. Macrobenthic communities were also investigated by a non-metric multidimensional scaling ordination (nMDS). SIMPER analysis was used to identify the species contributing most to any observed spatial or temporal pattern in the communities (Clarke, 1993).

Previously standardised sediment and water variables were examined using principal components analysis (PCA). Spearman correlations were done with the heavy metal concentrations and univariate community indices.

From the obtained results of stable isotopes analysis, we created graphical plots of the carbon and nitrogen signals (Fry, 2006). Only species found in all surveys in both ranges were used. Differences between stable isotopes signals of carbon and nitrogen were tested with non-parametric Kruskal-Wallis tests. All analyses were carried out in IBM SPSS for Windows and PRIMER v 6.0 software (Clarke and Gorley, 2006).

Results

Environmental variables

Water and sediment parameters are shown in Table S1. Granulometry of the sediments in the channel habitat of the oligohaline range, La Gola, oscillated between very fine sand and fine sand with a reduced bottom according to the redox potential. In the channel habitat of the polyhaline range, Salinas, sediments ranged from very fine sand to fine sand, while in the shallow habitat of both ranges, there was always very fine sand (Fig. 2). In the oligohaline range, the turbidity was higher than in the polyhaline range (Fig. 3).

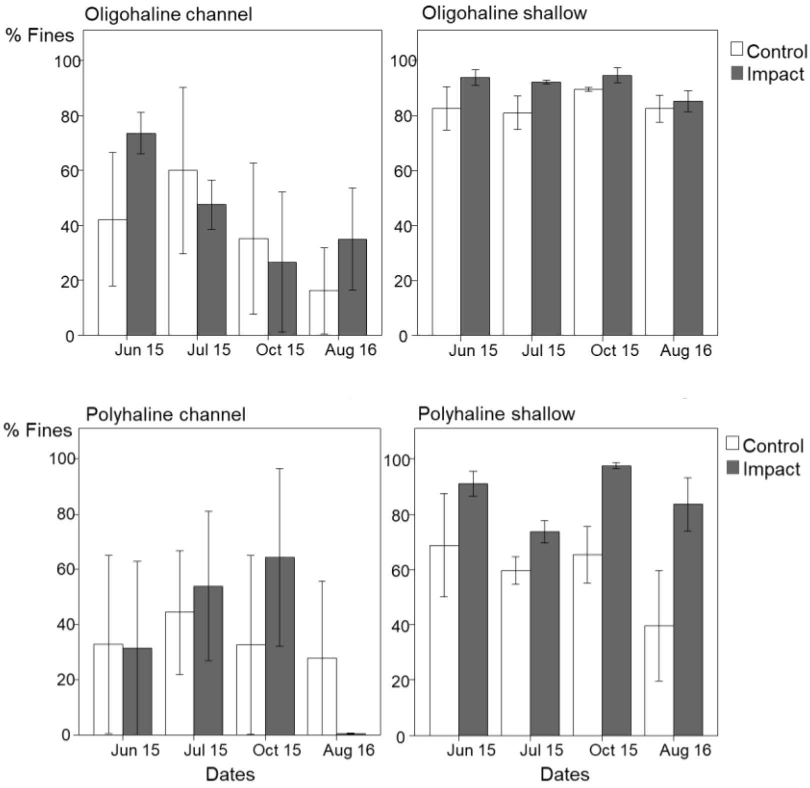

Figure 2. Mean (± standard error) of fine percentage (< 0.063 mm) of both channel and shallow habitats of the two salinity ranges.

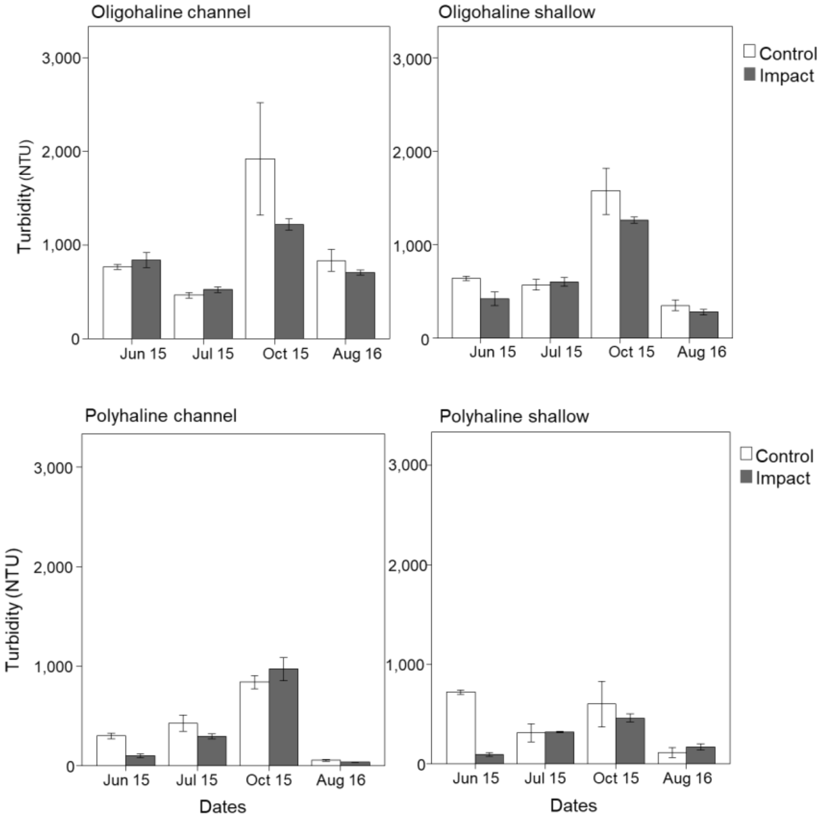

Figure 3. Mean turbidity values (± standard error) of both channel and shallower habitats of the two salinity ranges.

The PERMANOVA results for both ranges showed significant temporal differences ($p < 0.01$) in both areas and both habitats (control and dredged and shores and channel, respectively) for most of the water parameters, while sediment parameters remained constant. There were not significant interactions between the factors “Time” and “Impact vs. Control” for any variables. Heavy metal concentrations are shown in Table S2 and mean concentration variation of selected metals in Figure 4. Results showed generally higher concentrations after the operations in the dredging area of the polyhaline range. The PERMANOVA results of heavy metal concentrations for the two ranges did not show significant differences ($p > 0.01$) between the control and the dredging areas for all the heavy metals analysed. It also did not show interactions between the factors “Time” and “Impact vs. Control” in both salinity ranges. The I_{geo} index for Cr, Cu, Zn and Pb showed that the sediment was uncontaminated in the majority of the areas and sampling periods though some samples had higher levels of Pb. We found moderately contaminated values only in Salinas in August 2016. All heavy metal concentrations were below quality values of the sediment (SQV: Cd: 98 mg/kg, Cu: 270 mg/kg, Ni: 51.6 mg/kg, Pb: 84.6 mg/kg and Zn: 225 mg/kg (Delvalls and Chapman, 1998; Long et al., 1995; Tornero et al., 2014).

The PCA did not show any relationship between the dredging operation and the physicochemical variables (Fig. 5). In the oligohaline range, sample points were grouped, following the period, independently of the control or dredging area and channel or shallow habitat. The situation at the Salinas site was similar with major homogeneity between stations and/or periods

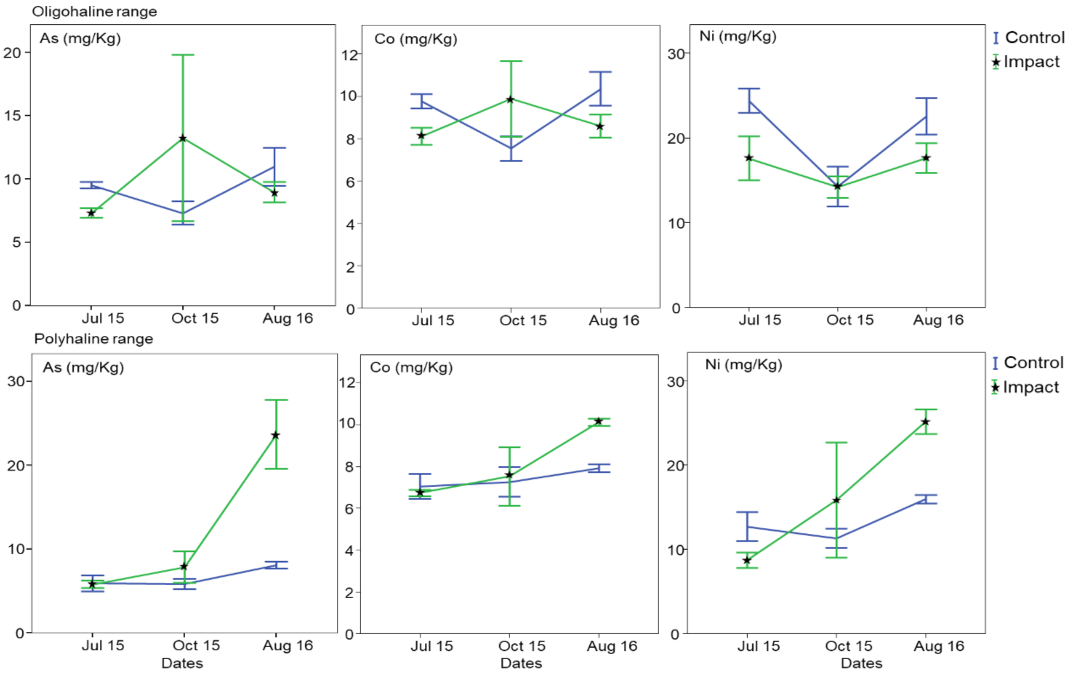

Figure 4. Mean concentration variation (± standard error) over the sampling dates of As, Co and Ni in both salinity ranges.

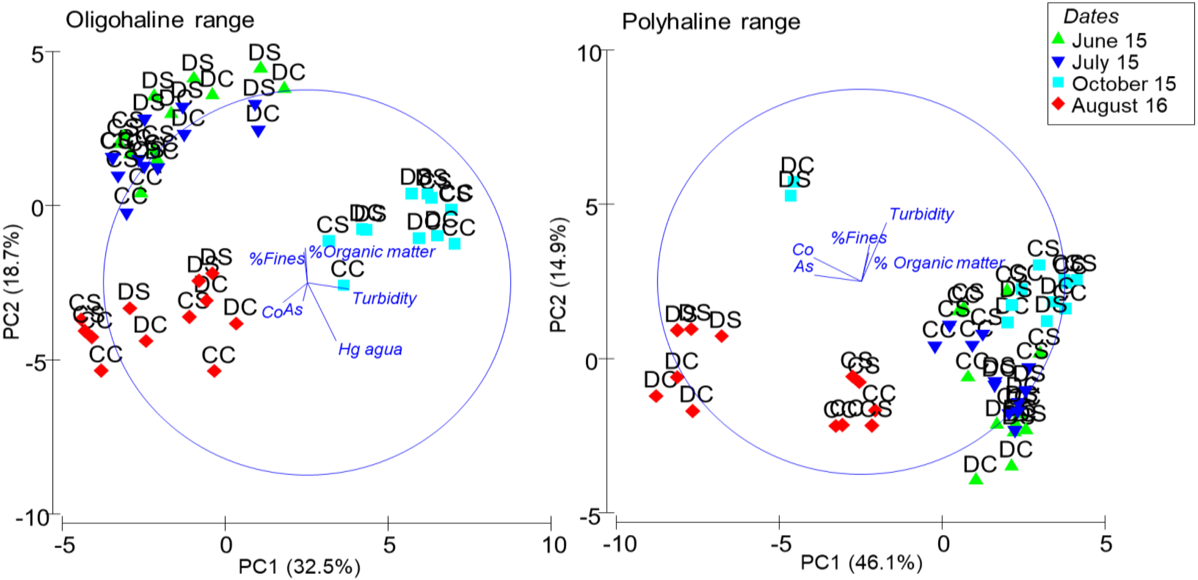

Figure 5. Principal components analysis (PCA) results for sediment and water parameters at all the stations in dredging and control areas and channel and shallow habitats over the sampling period. The percentage of variability explained by the two principal axes and vectors of a selection of parameters are given. (CC: Control channel, CS: Control shallow, DC: Dredging channel, DS: Dredging shallow).

Macrofaunal analysis

In total, 17 species were found in the oligohaline range and 38 in the polyhaline range. The most abundant group in all the samples was the annelids, especially the polychaetes *Alkmaria romijni* and *Streblospio shrubsolii*. Crustaceans also showed some importance in contributing to the diversity of the polyhaline range. There was practically no presence of molluscs in the oligohaline range, except some young specimens of the invasive species *Corbicula fluminea* (Table S3).

Univariate community indices are shown in Figure 6. Species richness showed differences in the oligohaline range in the channel area a month after the dredging operations in relation to previous sampling dates. In October 2015, there were no species present in the dredging area, while the control area did have species. In the shallow habitat, the number of species did not change over the sampling periods. In August 2016, a year after the dredging, the richness in the dredging area was similar to that of the pre-dredging period. A PERMANOVA analysis showed no significant interaction ($p > 0.05$) between “Time” and “Impact vs. Control” in both channel and shallow habitats (Table 1). On the other hand, in the polyhaline range, we always found some species in every survey, but, in October 2015, a reduction in the number of species was found in both habitats (Fig. 6).

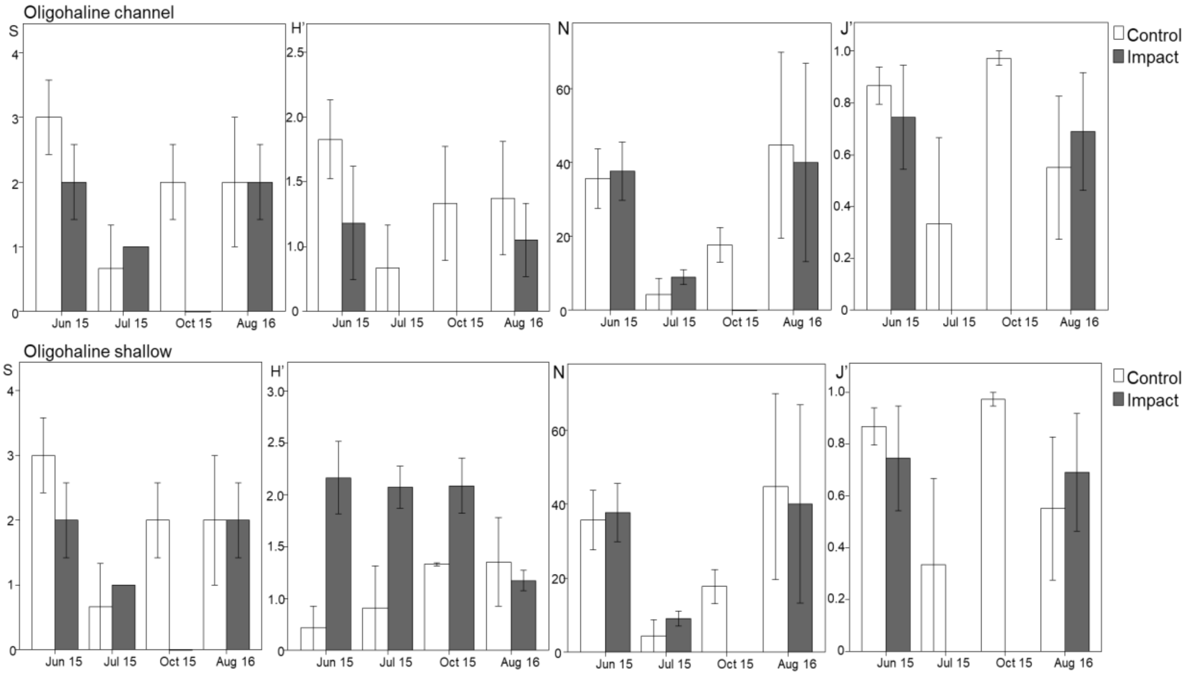

Figure 6. Mean (± standard error) values of the univariate community indices (S, N, H' and J') in the two salinity ranges over the sampling periods.

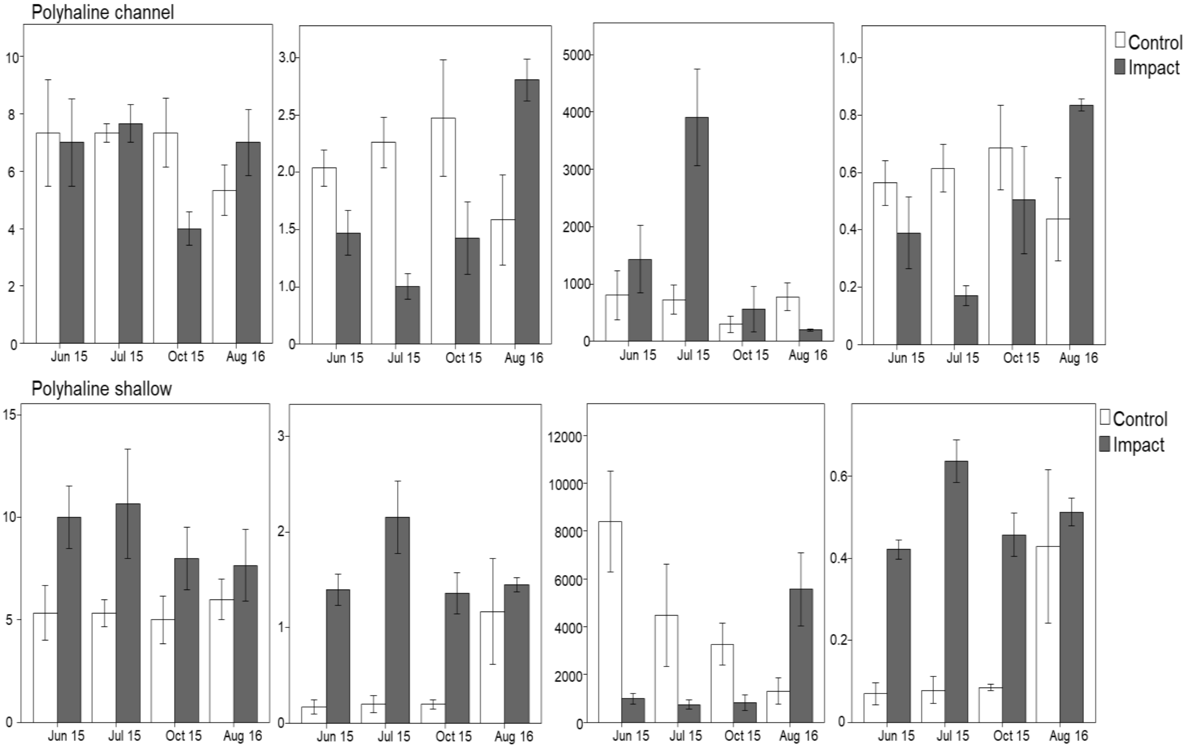

Figure 6 (continued)

The Shannon's diversity index showed low values in the oligohaline range in all the sampling periods (Fig. 6). The greatest values were found in the polyhaline range. The PERMANOVA results did not show a significant interaction between factors (Table 1). In the polyhaline range, the index values were higher (Fig. 6). In the dredging area of the channel, there was temporal variation over the sampling dates, while in the control, it was more stable. In the shallow habitat of the control area, we found low values due to the high abundance of the polychaete *S. shrubsolii*. The PERMANOVA results showed a significant interaction between "Impact vs. Control" and "Dates" in the channel habitat ($p = 0.0013$) (Table 1). Separate analysis of the "Impact" level showed significant differences over the sampling dates ($p = 0.0039$), whilst the "Control" level showed no differences.

Abundance (ind/m^2) values found in the oligohaline range were low in all areas and dates (<60 ind/m^2) (Fig. 6). The PERMANOVA analysis showed an interaction near significance between "Impact vs. Control" and "Dates" in the shallow habitat ($p = 0.051$) (Table 1). Separate analysis did not show significant differences over the sampling dates in both control or impact areas. The abundance values in the polyhaline range were higher than in the oligohaline range due to high numbers of the polychaete *S. shrubsolii* (Fig. 6). A PERMANOVA test showed a significant interaction between "Impact vs. Control" and "Dates" in both channel and shallow habitats ($p = 0.022$ and $p = 0.0296$, respectively) (Table 1). In the two separate analyses of control and impact stations of the two habitats, the impacted area showed significant differences over the sampling periods and controls did not.

Evenness showed greater values in the oligohaline range and followed similar trends as with Shannon's diversity and richness indices. A significant interaction was detected between the factors "Impact vs. Control" and "Dates" in the channel habitat of the polyhaline range ($p = 0.04$) (Table 1). Separate analysis also showed significant differences between sampling dates in the impacted area whilst controls did not.

The nMDS analysis in the oligohaline range showed the most of the stations with a similar macrofaunal community and no spatial or temporal patterns (Fig. 7). In the polyhaline range, there was more segregation between channel and shallow stations. The community of the shallow habitat, in both dredging and control areas, was similar in all sampling dates, while the channel habitat had more temporal variations. The community structure results did not show significant interactions in the oligohaline range. On the other hand, results showed a significant interaction between "Impact vs. Control" and "Dates" in both habitats of the polyhaline range ($p = 0.0197$ and $p = 0.0061$, respectively) (Table 2). Both separate analyses of the control and impact stations in the two habitats did not show significant differences in the control over the "Dates" while the "Impact" showed it.

Table 1. Univariate PERMANOVA results in both salinity ranges based on the Euclidean distance matrix of the richness data (S), Shannon's diversity (H'), total abundance (ind/m^2) (N) and Pielou's evenness (J'). *p estimation obtained by Monte Carlo sampling.

Oligohaline Channel

	df	MS	Pseudo-F	p	Unique perms
S					
Time	1	0.16667	2.94E-02	0.874*	3
Impact vs Control	1	2.6667	1.2308	0.36	204
Dates (Time)	2	5.6667	5.44	0.02	7150
Time x Impact vs Control	1	0.66667	0.30769	0.61	242
Dates(Time) x Impact vs Control	2	2.1667	2.08	0.15	9369
Res	16	1.0417			
Total	23				
N					
Time	1	9.20E+01	3.03E-02	0.8796*	3
Impact vs Control	1	92.042	1.3937	0.35	141
Dates (Time)	2	3033.4	5.3209	0.02	9957
Time x Impact vs Control	1	315.38	4.7754	0.15	801
Dates(Time) x Impact vs Control	2	66.042	0.11585	0.89	9951
Res	16	570.08			
Total	23				
H'					
Time	1	2.78E-03	2.34E-03	0.9664*	3
Impact vs Control	1	1.7103	12.66	0.08	800
Dates (Time)	2	1.1844	3.6732	0.05	9951
Time x Impact vs Control	1	1.14E-02	8.45E-02	0.72	799
Dates(Time) x Impact vs Control	2	0.1351	0.41899	0.66	9970
Res	16	0.32244			
Total	23				
J'					
Time	1	5.26E-04	1.17E-03	0.974*	3
Impact vs Control	1	0.78073	6.6382	0.12	799
Dates (Time)	2	0.44765	2.6904	0.1	9948
Time x Impact vs Control	1	4.52E-04	3.85E-03	0.82	793
Dates(Time) x Impact vs Control	2	0.11761	0.70686	0.51	9950
Res	16	0.16639			
Total	23				

Polyhaline Channel

	df	MS	Pseudo-F	p	Unique perms
S					
Time	1	1.20E+01	22.231	0.0437*	3
Impact vs Control	1	1.04E+00	0.10917	0.74	776
Dates (Time)	2	5.42E-01	0.1413	0.87	9950
Time x Impact vs Control	1	1.0417	0.10917	0.74	776
Dates(Time) x Impact vs Control	2	9.5417	2.4891	0.11	9953
Res	16	3.8333			
Total	23				
N					
Time	1	9.49E+06	4.3854	0.1682*	3
Impact vs Control	1	4.56E+06	1.6906	0.33	794
Dates (Time)	2	2.16E+06	3.7833	0.04	9947
Time x Impact vs Control	1	6.34E+06	2.3478	0.25	798
Dates(Time) x Impact vs Control	2	2.70E+06	4.72	0.02	9950
Res	16	5.72E+05			
Total	23				
H'					
Time	1	0.85436	7.4997	0.1113*	3
Impact vs Control	1	1.0158	0.48277	0.55	794
Dates (Time)	2	0.11392	0.45563	0.64	9951
Time x Impact vs Control	1	1.4878	0.70709	0.5	800
Dates(Time) x Impact vs Control	2	2.1042	8.4158	0	9956
Res	16	0.25003			
Total	23				
J'					
Time	1	0.19654	15.167	0.061*	3
Impact vs Control	1	6.09E-02	0.3959	0.59	800
Dates (Time)	2	1.30E-02	0.32005	0.73	9959
Time x Impact vs Control	1	0.25978	1.6892	0.32	794
Dates(Time) x Impact vs Control	2	0.15378	3.7981	0.04	9957
Res	16	4.05E-02			
Total	23				

Table 1 (continued)

Oligohaline Shallow

	df	MS	Pseudo-F	p	Unique perms
S					
Time	1	1.0417	5	0.1548*	3
Impact vs Control	1	35.042	168.2	0.07	748
Dates (Time)	2	0.20833	7.81E-02	0.93	9950
Time x Impact vs Control	1	0.375	1.8	0.31	108
Dates(Time) x Impact vs Control	2	0.20833	7.81E-02	0.93	9953
Res	16	2.6667			
Total	23				
N					
Time	1	5.01E+05	0.97118	0.4217*	3
Impact vs Control	1	5.85E+05	1.542	0.34	798
Dates (Time)	2	5.16E+05	4.2402	0.02	9954
Time x Impact vs Control	1	3.42E+05	0.90149	0.47	812
Dates(Time) x Impact vs Control	2	3.79E+05	3.1158	0.05	9954
Res	16	1.22E+05			
Total	23				
H'					
Time	1	2.46E-03	8.08E-03	0.9381*	3
Impact vs Control	1	3.8231	10.61	0.09	800
Dates (Time)	2	0.30438	1.2763	0.3	9956
Time x Impact vs Control	1	1.5611	4.3323	0.17	798
Dates(Time) x Impact vs Control	2	0.36033	1.5109	0.25	9965
Res	16	0.23848			
Total	23				
J'					
Time	1	4.02E-02	0.22745	0.6885*	3
Impact vs Control	1	0.4861	9.8016	0.1	798
Dates (Time)	2	0.17683	2.5882	0.1	9948
Time x Impact vs Control	1	0.7592	15.308	0.08	800
Dates(Time) x Impact vs Control	2	4.96E-02	0.72589	0.5	9958
Res	16	6.83E-02			
Total	23				

Polyhaline Shallow

	df	MS	Pseudo-F	p	Unique perms
S					
Time	1	8.1667	24.5	0.0387*	3
Impact vs Control	1	80.667	96.8	0.08	739
Dates (Time)	2	0.33333	4.57E-02	0.95	9675
Time x Impact vs Control	1	10.667	12.8	0.08	530
Dates(Time) x Impact vs Control	2	0.83333	0.11429	0.89	9928
Res	16	7.2917			
Total	23				
N					
Time	1	5.02E+06	0.53537	0.5388*	3
Impact vs Control	1	3.27E+07	1.4896	0.34	798
Dates (Time)	2	9.38E+06	1.9784	0.17	9936
Time x Impact vs Control	1	6.31E+07	2.8717	0.24	800
Dates(Time) x Impact vs Control	2	2.20E+07	4.6293	0.03	9941
Res	16	4.74E+06			
Total	23				
H'					
Time	1	2.40E-02	3.69E-02	0.867*	3
Impact vs Control	1	8.00E+00	16.279	0.08	794
Dates (Time)	2	0.65004	3.183	0.07	9940
Time x Impact vs Control	1	1.128	2.2956	0.24	796
Dates(Time) x Impact vs Control	2	0.49137	2.4061	0.13	9947
Res	16	2.04E-01			
Total	23				
J'					
Time	1	2.89E-02	0.36995	0.6083*	3
Impact vs Control	1	0.70139	14.877	0.08	801
Dates (Time)	2	7.82E-02	4.7287	0.01	9952
Time x Impact vs Control	1	7.70E-02	1.6334	0.33	795
Dates(Time) x Impact vs Control	2	4.71E-02	2.8504	0.08	9949
Res	16	1.65E-02			
Total	23				

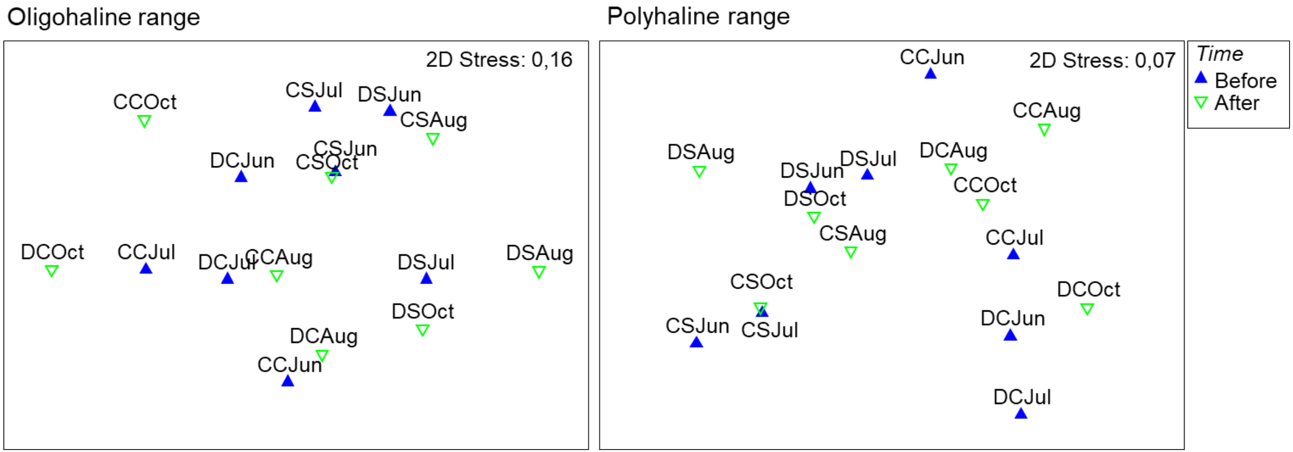

Figure 7. nMDS of the distance among centroids resemblance for the combined factor between the “Dates” and the two dredging and control areas before and after the dredging of both habitats based on abundance of the different species in both salinity ranges. (CC: Control channel, CS: Control shallow, DC: Dredging channel, DS: Dredging shallow).

SIMPER analysis of the polyhaline range (Table S4) showed no temporal trends in the channel habitat of the control area over the sampling dates. In the impacted area, results showed a general decrease in October 2015 of the abundances of species, such as the amphipod *Bathyporeia pilosa*, the isopod *Lekanesphaera levii* and *S. shrubsolii*, with respect to pre-dredging surveys, which were characterised by the dominance of these species. In August 2016, more species with no dominance patterns were observed. In the shallow habitat, differences found were mostly due to August 2016 where, in the impacted area, high abundances of the polychaetes *A. romijni* and *S. shrubsolii* and the isopod *Cyathura carinata* occurred.

The Spearman correlation between univariate community indices showed significant negative relationships between richness species and Co (-0.361), and abundance and As (-0.332), Co (-0.426) and Ni (-0.386). Concentrations of As, Co and Ni showed an increase in October in the dredging area of both salinity ranges, except Ni which showed a decrease in the oligohaline range. In the control areas, concentrations of these metals showed a decrease or remained at the same levels than the pre-operational measures. In August 2016, an increase in the concentrations was detected, except for Co and As in the dredging area of the oligohaline range (Fig.4).

Table 2. PERMANOVA results of the Bray-Curtis similarity matrix based on square-root transformed data in both channel and shallow habitats of both salinity ranges. *p estimation obtained by Monte Carlo sampling

Oligohaline Channel

	df	MS	Pseudo-F	p	Unique perms
Time	1	1095.5	0.14673	0.9809*	3
Impact vs Control	1	2307.7	0.79748	0.5166	801
Dates(Time)	2	7466	3.5928	0.0003	9933
TimexImpact vs Control	1	3075.3	1.0627	0.4183	800
Dates(Time)xImpact vs Control	2	2893.8	1.3926	0.1778	9922
Res	16	2078			
Total	23				

Polyhaline Channel

	df	MS	Pseudo-F	p	Unique perms
Time	1	3743.7	1.2603	0.3536*	3
Impact vs Control	1	7689.8	2.3883	0.1507	799
Dates(Time)	2	2970.6	2.2071	0.0367	9929
TimexImpact vs Control	1	3148	0.97769	0.4498	800
Dates(Time)xImpact vs Control	2	3219.8	2.3922	0.0197	9926
Res	16	1345.9			
Total	23				

Oligohaline Shallow

	df	MS	Pseudo-F	p	Unique perms
Time	1	4082.3	1.0272	0.444*	3
Impact vs Control	1	8354.1	4.8568	0.1106	801
Dates(Time)	2	3974.1	1.8304	0.1073	9945
TimexImpact vs Control	1	2368.2	1.3768	0.3253	798
Dates(Time)xImpact vs Control	2	1720.1	0.79227	0.5742	9941
Res	16	2171.1			
Total	23				

Polyhaline Shallow

	df	MS	Pseudo-F	p	Unique perms
Time	1	1684.4	1.4344	0.2797*	3
Impact vs Control	1	7760.4	3.9154	0.0814	801
Dates(Time)	2	1174.2	1.435	0.1399	9919
TimexImpact vs Control	1	3131.5	1.5799	0.2822	800
Dates(Time)xImpact vs Control	2	1982	2.4222	0.0061	9933
Res	16	818.27			
Total	23				

Stable isotope plots suggested a more widespread food web structure in the oligohaline range than in the polyhaline range (Fig. 8). This could suggest that organisms in La Gola occupied different trophic niches. The Salinas samples showed similar carbon and nitrogen isotope signals, suggesting similar trophic interactions. Plots also suggested a different organic matter origin in the polyhaline range than in the oligohaline due to the different carbon enrichment values observed in the food web of the polyhaline range.

The two salinity ranges did not show the same pattern over time. In the oligohaline range, some organisms suffered changes in the surveys after the dredging operations. There was a significant ($H = 7.64$; $p = 0.02$) decrease in nitrogen values of the mysid *Neomysis integer* in October, one month after the dredging. One year later, the mysid nitrogen signal was at the same level as before. Although plots also showed an increase after dredging on the vegetal matter nitrogen values, there were no significant differences. This value remained at the same level one year after dredging. Carbon values only showed enrichment in the anchovy (*Engraulis encrasicolus)* in October. One year later, the carbon signal was at pre-dredging levels. The other species did not show any changes. Conversely, in the polyhaline range, isotope signals of the organisms were similar across all sampling months. Only the mysid *Mesopodopsis slaberii* suffered a slight depletion in their carbon signal one year after the dredging work.

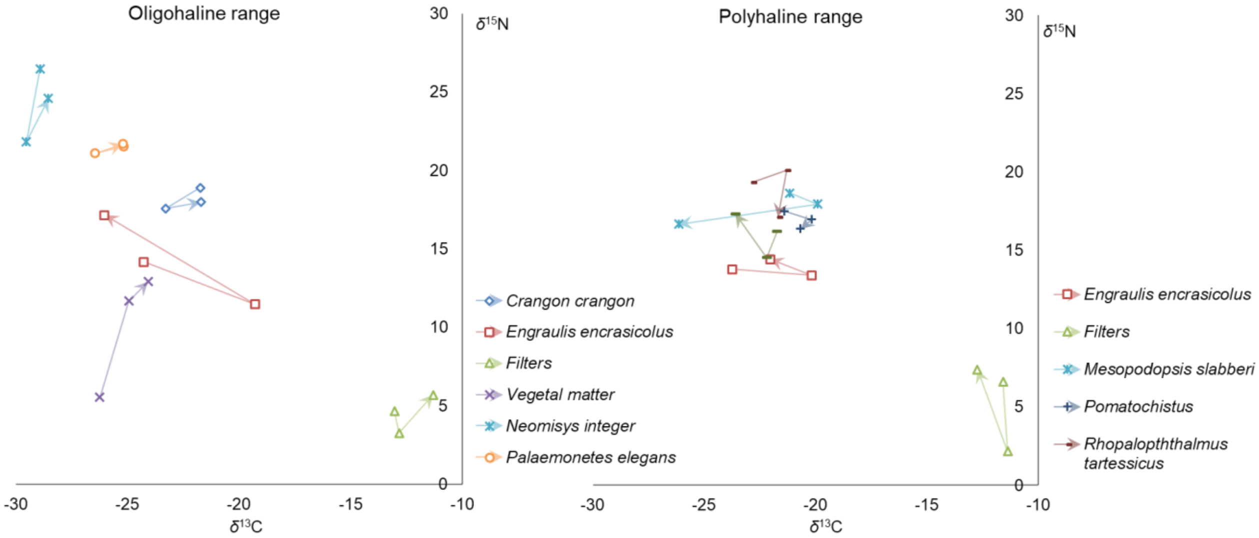

Figure 8. Means for δ13C (x-axis) and δ15N (y-axis) of the organisms collected in oligohaline (left) and polyhaline (right) sampling areas. Arrows represent isotopic variation over the three sampling periods (July 2015, October 2015 and August 2016).

Discussion

Increasing anthropogenic pressures on the benthic environment in estuaries has not always been an issue of concern (Rehitha et al., 2017). Our study assessed the grade of impact of dredging operations carried out in summer 2015 on benthic communities in two salinity ranges in the Guadalquivir estuary. The most noticeable feature observed in the dredging ranges, both in the channel and shallow habitats, is the absence of an evident effect in sediment and water parameters and the low impact in the biological communities independent of the salinity range. Also, the food web structures in both salinity ranges were not clearly affected by the dredging. Changes in the isotopic composition of the anchovies and the mysids could be explained by the natural variation of the system. The impossibility of the establishment of more replicated controls per dredging area makes it necessary to interpret these results with caution. When there are not replicated control areas under study, we do not have a measure of the natural random variability among any two different areas. In case the analysis leads to the identification of differences between the evolution of the control and the potentially impacted areas, these differences cannot be unconfoundedly assigned to an impact. When the differences are found to be not significant, this weakness is less critical given that the inclusion of more replicates of control areas will not change that the observed variation in dredging areas was overlapping with the natural variation.

Although we have no data about the very early effects immediately after dredging, the deepening of channels may significantly increase suspended matter concentrations in the long-term by the stirring up of bottom substratum or erosion from locations that were not sensitive to erosion before (de Jonge et al., 2014; Rehitha et al., 2017). In both ranges, water parameters (pH, oxygen and salinity) showed the expected values for a temperate estuary during the sampling period. Turbidity was notably higher in the oligohaline range than in the polyhaline range, because this range is in the maximum turbidity zone of the estuary (Vilas et al., 2008). The same pattern was also observed in the sediment characteristics. In both ranges, the granulometry remained stable in both the dredging and control areas. Our data suggest that sediment characteristics changed similarly in the control and dredging areas following natural variations. Therefore, dredging operations seem not to affect the water and granulometry, possibly due to the extraction method used, which minimized sediment overflow. However, the high flows originated by tides and the high chronic turbidity in the Guadalquivir estuary (Losada et al., 2017) could overshadow these effects.

Dredging operations may also release contaminants that were trapped in bottom sediments to the water column (Wasserman et al., 2016). However, all heavy metal concentrations were below SQV values. Furthermore, PCA analysis indicated that sampled points were grouped according to the period of survey instead of being grouped according to area (dredging vs. control). Our results agree with those reported by Guerra et al. (2009), who found that sediment deposited after dredging had the same contamination levels as before dredging operations. The higher levels of some heavy metal concentrations found after the operations in the dredging area of the polyhaline range were mostly due to the increase in August 2016, one year after dredging. Deepening of channels could lead to a greater dominance of fine fractions of sediment in dredging areas for a few hundred meters due to the dredge plume and lower current velocities, which favoured the deposition of fine sediment with higher levels of heavy metals (Klapan et al., 1975; Newell et al., 1998; Ponti et al., 2009; Crowe et al., 2016). However, our results did not show an increase of the percentage of fine sediments in this area. Moreover, the increase of concentration of pollutant one year after could indicate a possible input of contaminants from different sources. This fact has been pointed out by Tornero et al. (2014), who suggest that other sources, such as mining activities upstream, could explain As and Pb concentrations in clams in the Guadalquivir estuary. Areas affected by dredging work could experience drastic reductions in richness species, abundance and biomass or become completely defaunated (Klapan et al., 1975; Newell et al., 1998; Fraser et al., 2006; Gutperlet et al., 2017). In other studies, with similar volumes dredged, impacts were detected on community structure and other univariate community indices (Ceia et al., 2013; Ponti et al., 2009; Van Dolah et al., 1984). In October 2015, one month after the dredging work, there were no species in the dredging channel area of the oligohaline range. Direct removal of the species seems to be the explanation. Salinas had a more structured and rich community; as a consequence, the reduction suffered in October 2015 in the channel of the dredging area was more pronounced, but did not reach the azoic level, probably due to lateral and vertical migration of surrounding bottom communities (Hall, 1994). On the other hand, it seems that there was no effect in the shallow habitats of the dredging areas. This agrees with the results of Ponti et al. (2009), who found direct effects on dredging channels and no effects on nearby areas. Richness and abundance have been proven to be more effective to indicate the first impacts of a perturbation than the Shannon’s diversity index (Katsiaras et al., 2015), and our results showed that richness was the most useful index to describe shifts in the macrofaunal community in both salinity ranges.

The absence of any significant interaction between the factors “Time” and “Impact vs. Control” indicated there was not a permanent effect in the univariate indices or the community structure from the dredging operations (Underwood, 1994). Despite the absence of permanent effects, significant interactions were found between the factor “Dates” and “Impacts vs. Control” in some univariate community indices as well as in the community structure in the polyhaline range. This indicated different trends in the control and dredging areas over the random sampling dates. Separate analysis of the impact and controls always showed a significant variation in the impacted areas whilst the controls did not. SIMPER analysis showed a decrease of abundance of predominant species in the channel habitat of the impacted area one month after the dredging, which could indicate a possible impact. In spite of this, most of the differences were due to changes in August 2016 in the impacted areas of both channel and shallow habitats where a higher number species were found.

Dredging effects on macrofaunal communities and posterior recolonisation rates are site specific (Thrush and Dayton, 2002; Bemvenuti et al., 2005; Fraser et al., 2006; Gutperlet et al., 2015). Estuaries characterised by a muddy bottom and high dynamic areas often have more rapid recoveries than those with stable sand and gravel areas (Gutperlet et al., 2015; Rehitha et al., 2017). For example, rates reviewed by Newell et al. (1998) suggest a recovery time of 6–8 months for muddy estuaries, while communities with sand and gravel may take 2–3 years to re-establish. Our results were in concordance with these studies. One year after the dredging work, abundances in the oligohaline range as well as richness and Shannon’s diversity in the polyhaline range reached pre-dredging values.

Dredged habitats are often first colonised by opportunistic species (Sánchez-Moyano et al., 2004). According to Newell et al. (1998), a large population of small sedentary deposit feeders, like polychaetes, would be the first colonisers after cessation of the disturbance and then would progress towards the same levels as before the disturbance. In other studies, community recovery demonstrated that univariate community indices, such as abundances and richness, after a dredging impact could reach pre-operational levels after a certain period of time, but the ecological function could be not the same (Ceia et al., 2013). These shifts seem to be related to changes in sediment characteristics. Conversely, Sánchez-Moyano et al. (2004) observed a recovery in one month, reaching the same community structure and not only opportunistic species. In estuaries, Rehitha et al. (2017) detected changes in the granulometry toward more fine sediment in dredged areas as well as a reduction in species richness and diversity followed by a rapid colonisation of opportunistic species compared to non-dredging areas. They also reported that complex communities in the dredging areas could not be reached due to continuous dredging activities. In the Guadalquivir estuary, the benthic community, principally in the oligohaline range, was characterised by high abundances of the polychaetes *A. romijnii* and *S. shrubsolii*, even in the control areas. Therefore, these r-strategist species rapidly colonised the dredged areas, showing a rapid and complete recovery. This is consistent with Bemvenuti et al. (2005), who assessed that in areas that annually experience high changes in salinity (e.g., estuaries), fauna were reduced and community structure was altered by dredging activities, but there was also a rapid recovery due to the high resilience of the system. This is consistent with other studies (Fraser et al., 2006 and references therein).

Despite the low number of species present in all surveys is a major constraint for the isotopic analysis in this study, some conclusions can be made. Differences in the food web structures of the two salinity ranges could indicate the use of more carbon-depleted sources of organic matter with a possible terrestrial origin in the oligohaline range. Conversely, in Salinas, the marine inputs coming with the high tides can introduce more enriched carbon sources of organic matter into the food web (Selleslagh et al., 2015). The variation in isotope values in the oligohaline range suggests more complex feeding pathways than in the polyhaline range. In Salinas, the primary consumers could be feeding on the same organic matter sources, because similar isotope signals of secondary consumers could indicate similar diet composition. Conversely, in the oligohaline range, different nitrogen signals of the secondary consumers could suggest that they feed on a different suite of prey.

Changes in the isotopic signals of some organisms in the oligohaline range over the sampling periods could suggest an effect of dredging. A decrease in nitrogen levels of *N. integer* could suggest a change in trophic niche. Differences in trophic position of this species in October could be caused by the elimination of an intermediate consumer or a change in the degree of trophic omnivory (Post and Takimoto, 2007). The increase in the nitrogen signal of the vegetal matter could suggest the presence of more enriched nutrients with an anthropogenic origin. Dredging can resuspend fine sediments, nutrients and pollutants that had been trapped over the years (Ponti et al., 2009; Wasserman et al., 2016), making them available to the food web. In that sense, the variation in the carbon signal of *E. encrasicolus* would indicate that they use sources of organic matter with different origin over time (Dias et al., 2017). The multispecies approach of selecting organisms with different turnover rates would assess the dredging impact over a temporal scale (Modéran et al., 2012; Selleslagh et al., 2015). Changes in the diets of organisms are not immediately reflected by stable isotopes signals; higher trophic level organisms can show an integrated time response to nutrients better than primary producers (Van De Merwe et al., 2016). One year after the dredging work, mysid and vegetal matter isotope signals were still at the same levels as one month after; however, anchovies showed the same levels as pre-dredging measures. Despite changes in the oligohaline range in some isotope values one month post-dredging, variability in the patterns of isotope signals for carbon and nitrogen do not allow us to confirm an impact of dredging on food web structure. Thus, changes seem to be more related to natural variations rather than a dredging impact.

Conclusions

Maintenance dredging work is common activity that is necessary to maintain navigability and support trade. However, these human impacts may lead to several direct or indirect threats for estuarine ecosystems. The site-specific component of these impacts necessitates the study of these effects in every system (Fraser et al., 2006). In a highly variable scenario with anthropogenic and natural frequent perturbations, such as in the Guadalquivir estuary, macrofaunal communities often are characterised by low diversity and large populations of species well adapted to rapid recolonisation (Newell et al., 1998). The poor benthic community status in both salinity ranges in the Guadalquivir estuary explains the absence of a detectable effect on the community structure, diversity and richness and the quick recovery of the punctual affections by recolonisation of organisms of nearby areas. Moreover, the dredging work did not evidently affect the food web structure either. This poor status has been reported by other authors (Baldó and Drake, 2001; Sánchez-Moyano et al., 2017) and even in drastic impacts, such as acid mining spills released to the estuary, an impact on the benthic community was not detected (Baldó and Drake, 2001). In the management of estuaries, Ceia et al. (2013) reported that higher dredging frequency and extension means a longer recovery period for macrofaunal assemblages due to sediment structure destabilisation. However, the actual pressures on the

Guadalquivir estuary, beyond the maintenance dredging work (e.g., unnatural freshwater inputs in summer for rice agriculture, permanent turbidity and high regulation of the natural flow by upstream dams) does not permit the establishment of more complex communities. Therefore, in poor diversity systems, like the Guadalquivir estuary, and from economic and management efficiency perspectives, research efforts should focus on the most diverse areas, such as the polyhaline range. This study marks the need for a proper management plan that involves all the administrations for the improvement of the biological benthic communities of the Guadalquivir estuary.

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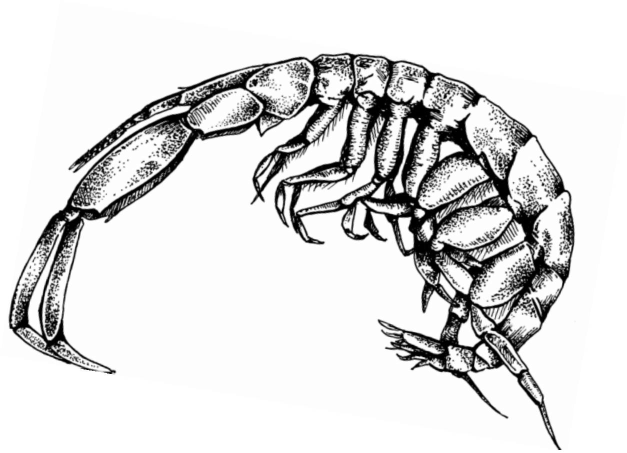
2 Environmental consequences of dredged-material disposal.



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Resumen

En este estudio se evalúan los posibles impactos de la deposición recurrente de material dragado del estuario del Guadalquivir en un vaciadero marino. A su vez, se analizan cambios en las características del sedimento, así como en procesos de bioacumulación y biomagnificación de metales pesados a través de la red trófica bentónica. Los resultados obtenidos mostraron un aumento significativo en la concentración de algunos metales pesados en el vaciadero marino, lo que podría atribuirse a la deposición de sedimentos procedentes del estuario. Este aumento también podría explicar una menor supervivencia de los anfípodos en el análisis ecotoxicológico. Las concentraciones de metales pesados en organismos detritívoros y depredadores indicaron cierta bioacumulación. Sin embargo, tanto estos patrones, como aquellos encontrados en los procesos de biomagnificación a través de la red trófica, no fueron concluyentes. La combinación de estudios que evalúen los cambios en las características de los sedimentos y sus posibles consecuencias para las redes tróficas parece ser un enfoque interesante que debería ser testado más a fondo en este tipo de estudios.

Abstract

In this study we assessed the effects of the recurrent disposal of dredged material from the Guadalquivir estuary (south-western Spain) in a marine disposal area. We analysed shifts in sediment characteristics as well as bioaccumulation and biomagnification of heavy metals through the benthic food web. Results showed that the significant increase in concentration of some heavy metals observed in the marine dump after the latest disposal event could be attributed to the deposition of river-dredged sediments. This increase could also explain the decreased amphipod survival in the ecotoxicology analysis. Heavy metal concentrations in organisms indicated some bioaccumulation in deposit feeders and predators but with no clear patterns nor biomagnification through the food web. Hence, combining studies that monitor shifts in sediment characteristics and their possible consequences for the food web seems to be an interesting approach that should be assessed further in this type of studies.

Introduction

Dredging activities are especially developed in navigable estuaries to maintain security in navigation (OSPAR, 2008; Bates et al., 2015). Subsequent direct disposal of the dredged material in open waters is still a priority management option (Harvey et al., 1998; Katsiaras et al., 2015). These activities can modify the physicochemical, biochemical and biological properties of the ecosystem (Bolam, 2012; Guerra et al., 2009). Therefore, dredging and disposal are serious environmental concerns in coastal management (Marmin et al., 2016; Moog et al., 2015; Van Dolah et al., 1984).

The rapid development of urban and industrial areas on rivers and coasts in the past century has resulted in contaminants being discharged into estuaries (Bárcena et al., 2017). Most contaminants accumulate in the sediments, which act as a repository and a resource of pollutants for the water column (Souza et al., 2015). Heavy metals represent substances of special interest due to their durability in the system (Nicolau et al., 2006). Thus, heavy metal pollution has become a major issue in estuarine environments (Buruaem et al., 2012) due to their potential toxicity and tendency to bioaccumulate (Bárcena et al., 2017). Slight changes in the water and sediment conditions, such as those that occur in dredging and disposal operations, may modify the mobility and bioavailability of metals (Cesar et al., 2014; Guerra et al., 2009; Katsiaras et al., 2015; Wilber et al., 2007). As a consequence, those released contaminants can concentrate and be biomagnified in marine organisms in the food chain (Roberts and Forrest, 1999). Dumping of dredged material may also cause increases in water column turbidity, enhance organic matter and change the sediment structure, which are some of the main impacts to the benthic environment (Cesar et al., 2014; OSPAR, 2008). Therefore, monitoring is highly necessary in dredging and dumping activities, especially when the option chosen is the deposition of dredged material in one marine site (Bocchetti et al., 2008).

In this study we assess the effect of the recurrent disposal of dredged material from the Guadalquivir estuary (south-western Spain) in a marine disposal area. This estuary, through a 90 km long navigation channel, is the entrance to Seville harbour, the only inland harbour on the Iberian Peninsula. Every few years it is necessary to perform maintenance dredging works to maintain navigability (Díez-Minguito, 2012). Part of the sediment dredged along the channel is disposed in an authorised marine dump established in 2010. Sediment in the estuary has been receiving historic metal contamination for centuries, and in April 1998 a toxic mining spill was accidentally discharged into the estuary (Tornero et al., 2014). Although there are systematic contamination controls carried out in these operations, there is a potential impact in the marine dumping area.

The divergent results obtained in disposal impact studies made it necessary to evaluate environmental effects case by case (Bolam et al., 2006; Bolam and Rees, 2003; Donázar-Aramendía et al., 2018; Harvey et al., 1998; Katsiaras et al., 2015; OSPAR, 2008; Simonini et al., 2005). Moreover, bioaccumulation and ecotoxicology assessment for different species is one of the procedures of increasing importance in monitoring programmes of sediment disposal (Marmin et al., 2014). The effects of bioaccumulation of heavy metals through the food web have been little studied. In this context, the aim of this study was (i) to assess the effect of the dredged material disposal on the physical and chemical characteristics of the sediment and water column, (ii) to determine the variation of heavy-metal concentrations and (iii) to analyse their toxicity and bioaccumulation within the food web. We hypothesise that the continuous discharge of riverine sediments in the same area would have changed the physical characteristics of the sediment. Also, the remobilisation of contaminants would have increased the metal contamination and toxicity, and these metals would have accumulated in the food web.

Methods

Study area

This historic marine dump is described by Donázar-Aramendía et al. (2018). Briefly, the marine dump is situated five miles offshore in an open water area off the river mouth of the Guadalquivir estuary, south-western Spain (Fig. 1). The dump has a rectangular shape with a surface area of 662.571 m^2. The bottom is 20 metres in depth with sediments composed principally of fine sand and mud (Usero et al., 2008). The seafloor is influence by terrigenous inputs of the Guadalquivir River and the hydrodynamic conditions of the Gulf of Cádiz, with predominantly west-to-east currents (Sainz and Ruiz, 2006). These features give the surrounding areas similar sedimentary characteristics and biological communities (Fa et al., 2003; Gonzalez and Dias, 2004). This dump has been receiving dredged material from harbours and the Guadalquivir estuary for seven years (2010: 338.652 m^3; 2011: 353.488 m^3; 2013: 354.795 $m^{3;}$ and 2015: present study), (data from Autoridad Portuaria de Sevilla). In 2015, dredging operations for maintenance of the navigation channel of the Guadalquivir estuary and Bonanza port were performed during summer of 2015. A volume of 314.275 m^3 of extracted sediment was discharged in the sea dump.

Sampling Design

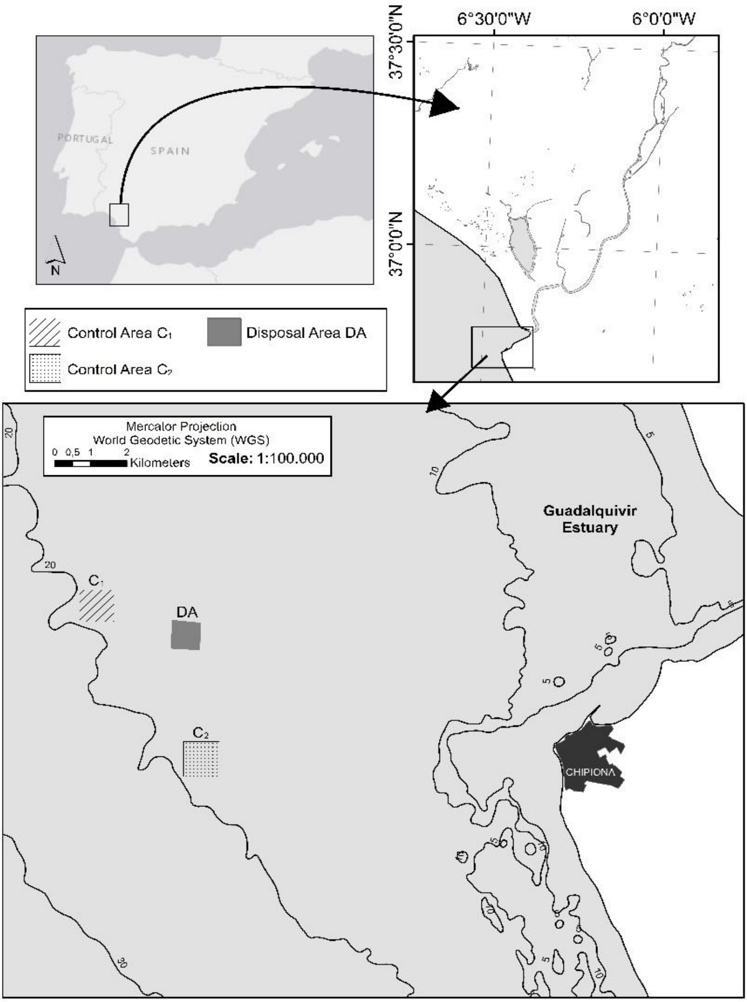

Figure 1. Location of the disposal and control areas.

In total, three sampling surveys were carried out: once before dumping (July 2015 to test the previous effects of the recurrent disposals), and twice more afterwards (October 2015, and August 2016, to analyse the effects of this latest perturbation on the system). Three sampling areas were established in the same environment: one within the marine disposal area (DA) and two controls located approximately three miles from the affected area (C_1 and C_2) (Fig 1). Controls were located at the same depth in a very similar area with equal hydrological and geomorphic characteristics (Sainz and Ruiz, 2006). Distances between controls and the disposal area were sufficient to avoid any possible impacts (Fig 1).

At each control and disposal area, three samples were taken randomly for sediment analysis with a van Veen grab (0.15 m^2 total sampling area per station and date). All stations were considered replicates of each area. Grain size distribution was measured as percentages of 100 g of dry sediment graded through a sieve net (5 mm, 2 mm, 1 mm, 0.5 mm, 0.250 mm, 0.125 mm, 0.063 mm). Particulate organic matter (POM) was determined by calculating the weight difference between the dry sediment sample of three replicates (at 60 ºC until dried weight stabilisation) and after combustion (500 ºC for 4 h). Apparent redox potential was measured by a pH meter (WTW pH 1970i) with SenTix ORP electrode. Water turbidity was analysed with a multiparametric probe (Eureka Manta 2).

For the heavy metal and trace element concentration analysis, another sample was taken from the uppermost 2 cm of the surface. In the laboratory, sediment samples were air-dried, crushed and sieved though a 2 mm sieve and then ground to <60 µm. These samples were digested with aqua regia (1:3 conc. HNO_3/HCl) in a microwave digester. Quantification of elements in the extracts was achieved using a VARIAN ICP 720-ES (simultaneous ICP-OES with axially viewed plasma). The accuracy of the analytical methods was assessed through a reference soil sample from the Wageningen Evaluating Programs for Analytical Laboratories for soils, International Soil-analytical Exchange (WEPAL; ISE).

The metallic content index (MCI) was calculated through the concentrations of the metal selected for multivariate analysis (As, Cd, Co, Cr, Cu, Ni, Pb and Zn). This index allows global comparison of the pollution levels of different sites and gives a concentration number for graphical representation. This index is calculated according to the formula MCIn = (M1·M2·…·Mn)1/n, where Mi is the concentration of metal i in an area (Consejería de Medio Ambiente, 2000).

The index of geoaccumulation (Igeo) has been used as a relative measure of metal pollution in sediments for Cr, Cu and Zn according to the regional background established by Ruiz (2001) for unpolluted sandy and silty-clayey sediments. Igeo = log2 (Cn/1.5 9 Bn), where Cn is the value of the element n, and Bn is the background data of that element. Following Ruiz (2001), the index values were divided into five groups: unpolluted (Igeo < 1); very lightly polluted (1 < Igeo < 2); lightly polluted (2 < Igeo < 3); moderately polluted (3 < Igeo < 4); highly polluted (4 < Igeo < 5) and very highly polluted (Igeo < 5).

For toxicity analysis, a ten-day static sediment toxicity test was conducted with the amphipod *Ampelisca brevicornis*, following the methods proposed by the 'Comisión Interministerial de Estrategias Marinas' (2014) for the three study areas. Test organisms were collected from a clean intertidal zone by sieving the sediments. They were then placed in the laboratory in a 20 L tank with filtered sea water and native sediment. Organisms were gradually acclimated with constant aeration (1 ºC/day) to the test temperature (22 ºC). Test were conducted in 2 L tanks containing 200 ml of sediment previously sieved through a 1 mm mesh, and 800 ml of overlying water. After the sediment was deposited, 20 individuals were added per replicate. Five replicates per station plus one more for control with clean sediment from the origin zone of the organism were established. After ten days the full tank was sieved and the survivors were counted. When the percentage survival was less than 70% and the survival in the control was over 90%, the sediment was considered toxic.

For the analysis of carbon and nitrogen isotopes and heavy metals from the biota, fauna was collected in three surveys with a van Veen grab (0.05 m^2) and an epibenthic sledge (46 × 25 cm, 2.5 mm net mesh size). The number of samples was enough to obtain a representative number of species of the benthic community. All samples were sieved through a 0.5 mm mesh sieve and organisms were sorted by species, transferred to the laboratory in refrigerated containers and kept alive for 24 h to evacuate their gut content. In order to determine the isotopic composition of sediment organic matter (SOM), sediment was taken from the upper 2 cm of the van Veen grab sample. In the laboratory, species identification was confirmed and fauna were rinsed in distilled water.

Organisms were dried at 60 °C for at least 48 h and ground to a powder. For the heavy-metal analysis, individuals of the same species were separated and 0.3 g of each dried sample was processed using the same procedure as used for the sediment.

Muscle tissue samples of fish and molluscs were dissected. A pool of several organisms was used when the individuals had low biomass values (<0.3 mg dry weight). Sediment samples were acidified with 0.1 M HCl to remove carbonates, and were then oven-dried at 60 °C. Subsamples of powdered materials were weighed to the nearest 0.3 mg and placed into tin capsules for $\delta\,^{13}C$ and $\delta\,^{15}N$ determinations.

All samples were combusted at 1020 °C using a continuous-flow isotope ratio mass spectrometry system by means of a Flash HT Plus elemental analyser coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific, Bremen, Germany).

Data analysis

To assess graphically the effect of the dumping on water turbidity, a generalised additive mixed model (GAMM) was fitted using three random replicates per area and date. Predictions of the model were represented graphically with the Ocean Data View (ODV) software (Schlitzer, 2019) to observe the temporal variations.

To examine the effects of dumping in the study area, a permutational univariate analysis of variance (PERMANOVA) (Anderson, 2001) was performed, based on Euclidean similarity matrixes of each sedimentary variable using 9999 permutations. When the total number of possible permutations to obtain the p-values were low (<150) we used the estimate obtained by Monte Carlo sampling (Anderson and Robinson, 2003). The experimental design included two crossed fixed factors: ‘Impact vs Control’ with two levels (the potentially impacted area and control areas); and ‘Time’ with two levels (before and after the latest dumping event). The design also included two random factors: ‘Area’ nested within ‘Impact vs Control’, with three levels (DA, C1, C2); and the sampling dates ‘Dates’ nested within ‘Time’, with three levels (Jul 15, Oct 15 and Aug 16). For trace metal concentration a PERMANOVA multivariate analysis of variance was also performed based on the Euclidean distance matrix of the concentrations of As, Cd, Co, Cr, Cu, Ni, Pb, Zn. This multivariate analysis was followed by a univariate analysis of each metal, with the same experimental design and methodology.

According to a Before After Control Impact (BACI) design, if the disposal has an impact, the disposal area will change over time from ‘before’ to ‘after’ with a different pattern when compared to control areas (C_1 and C_2). This difference can be detected as a significant ‘Impact vs Control’ × ’Time’ interaction (Guerra et al., 2009). Also, the control areas should not show differences between them. In this sense, it is important to notice that the factor ‘Area’ is nested within ‘Impact vs Control’ and, given the asymmetric design of this study, this terms only estimate the variance between the two control areas (Anderson et al., 2008). As reported by Donázar-Aramendía et al. (2018), when the saturated model (with all explained terms included in the model) generated negative estimates of some components of variation (this indicates that the model in turn is a poor one, according to Anderson et al., 2008), model selection was performed by excluding the affected terms, one by one, beginning with those of higher order (interactions), and provided that their p-values were higher than 0.25 (Anderson et al., 2008). Following this process with all the analyses performed here (both the multivariate and the univariate PERMANOVA), the factor ‘Area’ and its interactions with ‘Dates’ and with ‘Time’ were excluded from all models; hence, there were no differences between the control areas in any case. Significant interactions, if detected, were explored further in separate analyses, within the levels of the interacting factors; i.e., the significant interactions between ‘Impact vs Control’ and ‘Dates’ were further analysed separately by disposal area and control areas.

To test temporal and spatial variation of heavy-metal concentration in the three areas over all sampling dates, a principal component analysis (PCA) was also applied. All the analyses were carried out in IBM SPSS for Windows and PRIMER v 6.0 software (Clarke and Gorley, 2006).

To assess the bioaccumulation of trace metals in organisms, the bioaccumulation factor (BF) was calculated based on Negri et al. (2006). It was calculated by the ratio between the metal concentration in the organisms and that in the sediments. Organisms were grouped in trophic groups because it was not possible to find same species across all areas and dates. Bioaccumulation was considered when $BF > 1$ (Trevizani et al., 2016).

To test biomagnification of trace metals over the food web, linear regression models were used to examine the relationship between metal concentrations and trophic position (TP) of species. To estimate consumer TP in the disposal area and in the controls, we applied a novel Bayesian approach using the package ‘tRophicPosition’ in R (Quezada-Romegialli et al., 2018). This combines Markov Chain Monte Carlo Simulation with stable isotope data. This approach includes the variability of the baseline isotope values and the trophic discrimination factor, which is an advantage with respect to the classic approaches to estimate TP (Hetherington et al., 2018). We used isotope values of the sediment as baseline TP of consumers in the disposal area and in a combination of the two control areas since they did not show differences between them and numbers of species found were low. This package implements a Bayesian model:

$$\delta^{15}N_{consumer} = \delta^{15}N_{baseline} + \mathrm{TDF}(\mathrm{TP} - \lambda)$$

where λ is the baseline trophic level and TDF is the trophic discrimination factor from Post (2002). Posterior modes of TP were extracted and linear models calculated to test the relationship between TP and metal concentrations of the studied species. Metal concentrations were log-transformed.

Results

Water turbidity assessments did not show any temporal or spatial pattern (Fig 2.).

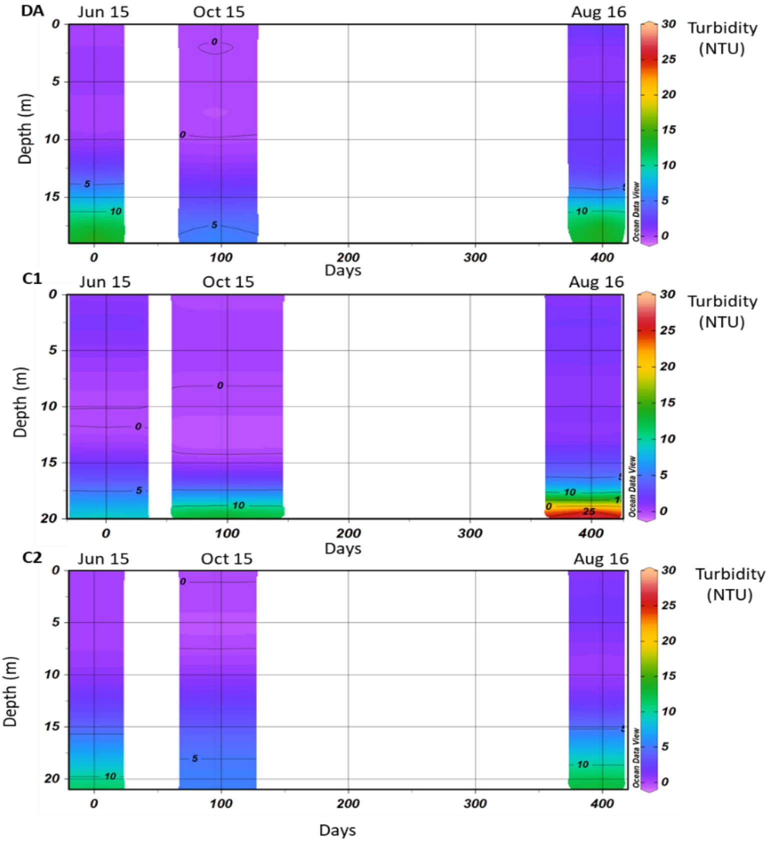

Figure 2. Patterns of variation of water turbidity NTU (Nephelometric Turbidity Units) in the three areas across the sampling dates.

Sediment analysis values showed that the disposal area had lower % fines (the fraction smaller than 0.063 mm) than the control areas at all the sampling dates (Fig. 3). Univariate PERMANOVA analysis showed significantly ($p < 0.01$) higher POM and % fines in controls than in the disposal area (Table 1). Also, there was no difference in these parameters between the two control areas despite the distance between them. In addition, there were no significant interactions between the factors ‘Impact vs Control’ and ‘Time’.

On the other hand, the control areas showed generally higher heavy-metal concentrations than did the disposal area (Figure 3). Control areas showed similar heavy-metal concentrations across all sampling dates (Table 2 and Fig. 3). In contrast, in October 2015, the disposal area showed an increase in heavy-metal concentration, which remained at the same level in August 2016 (Fig. 3). Only Hg showed no clear spatial or temporal patterns.

Table 1. Univariate PERMANOVA results in the studied areas based on the Euclidian distanced matrix of the sediment variables. *p estimation obtained by Monte Carlo sampling.

	df	MS	Pseudo-F	p	Unique perms
Redox					
Impact vs Control	1	2.7393	2.90E-03	0.9572	9845
Time	1	4.84E+04	0.46525	0.6249*	3
Dates(Time)	1	1.17E+05	123.78	0.0001	9841
Impact vs Control x Time	1	2.03E+00	2.15E-03	0.9622	9835
Res	22	944.93			
Total	26				
Turbidity					
Impact vs Control	1	44.754	0.18982	0.6731	9849
Time	1	185.36	0.11833	0.7894*	3
Dates(Time)	1	1732.8	7.3493	0.0061	9850
Impact vs Control x Time	1	8.1214	3.44E-02	0.8592	9847
Res	22	235.77			
Total	26				
OM					
Impact vs Control	1	62.707	14.195	0.0015	9847
Time	1	0.11267	3.41E-03	0.9616*	3
Dates(Time)	1	36.623	8.2904	0.0087	9845
Impact vs Control x Time	1	1.83E-02	4.14E-03	0.9506	9831
Res	22	4.4176			
Total	26				
Fines percentage					
Impact vs Control	1	3302.8	11.27	0.0053	9844
Time	1	31.504	0.92826	0.3501*	3
Dates(Time)	1	1.55E+00	5.29E-03	0.9464	9836
Impact vs Control x Time	1	41.652	0.14213	0.7183	9857
Res	22	293.05			
Total	26				

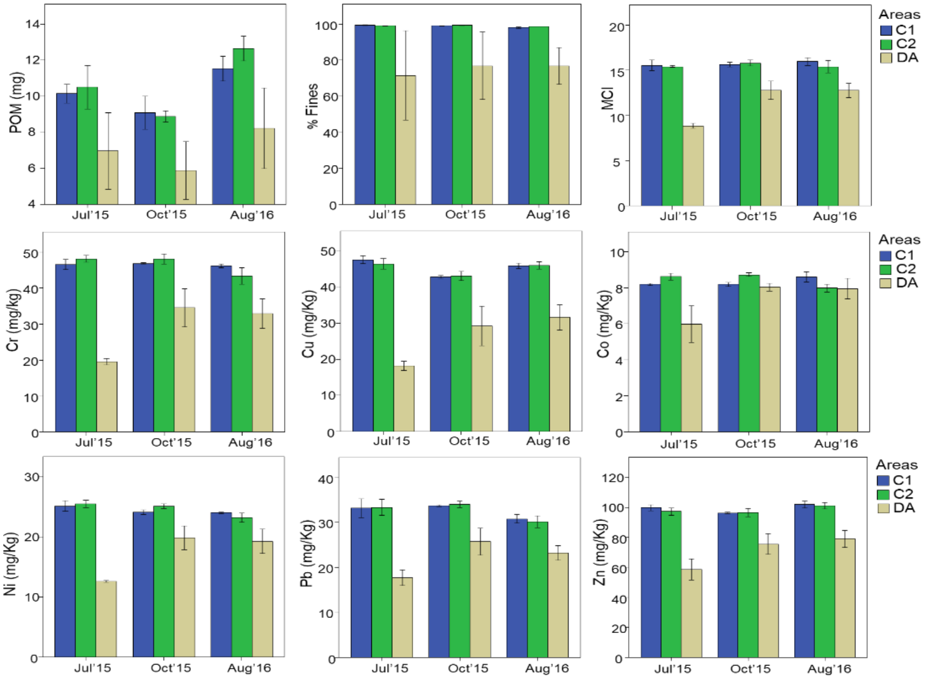

Figure 3. Patterns of variation of some variables of the sediment in the three areas across the sampling dates. Vertical bars represent ± standard error.

Heavy-metal concentration did not show significant differences between the two control areas at any sampling date. Moreover, As, Cd, Hg, Sr concentrations did not show significant differences between the disposal and control areas. Multivariate PERMANOVA results of trace metal concentrations, as well as univariate analysis for MCI, Co, Cr, Cu, Ni, Pb, V and Zn showed a significant 'Impact vs Control' × 'Time' interaction (Table S5). Concentrations of these metals increased in October 2015, one month after the prompt disposal of 2015, while in the control areas they remained at the same preoperational levels (Fig. 3). In August 2016, most metal concentrations were at the same levels as in October 2015 in the disposal area (Fig. 3). PERMANOVA analysis of separated samples of the interacting factors showed significant differences before and after the prompt disposal in the disposal area and no differences in the controls for MCI, Co, Cr, Pb and Zn. Although concentrations of Cu, Ni and V showed significant differences before and after the prompt disposal both in disposal and control areas, the tendencies were different.

I_{geo} for Cr, Cu, Zn and Pb showed at all the sampled points and periods an unpolluted sediment ($I_{geo} < 1$). We only found for Cu a very lightly polluted I_{geo} ($1 < I_{geo} < 2$) in the two control areas.

PCA analysis, based on all sediment parameters, is plotted in Fig. 4. Although both control areas were several miles apart, they presented very similar environmental characteristics and were different from the disposal area. However, after the disposal of the dredged material in summer 2015 there was a homogenisation between the disposal area and the two control areas. PC1 axis accounted for 62.0% of the variation in the data; most variability from this axis could be attributed to heavy-metal concentrations according to eigenvector values. PC2 explained 11.9% of the variation, which was based mainly on granulometric characteristics and Hg concentration.

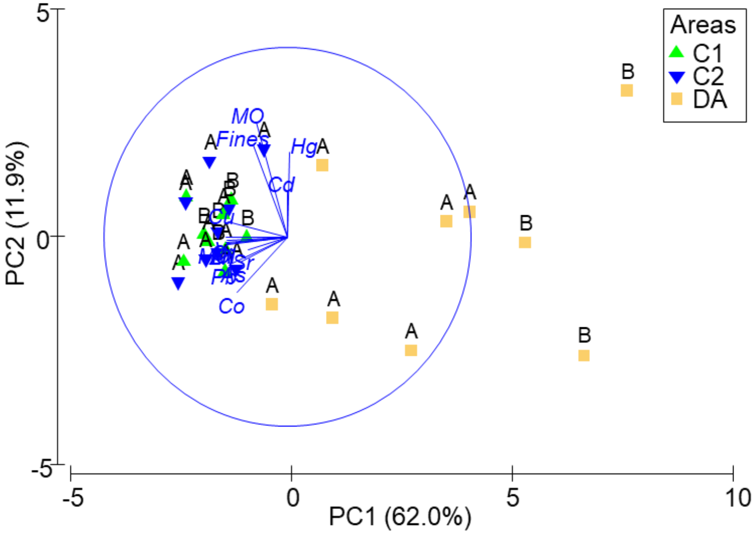

Figure 4. Outcome of PCA analysis for the three areas using the sediment variables before (B) and after (A) the prompt disposal in summer 2015.

Ecotoxicology analysis:

Results of the ecotoxicological analysis showed different % survival at the different stations across all periods (Fig. 5). Before the disposal, there was lower sediment toxicity in the disposal area than in the two control areas. After the dump, toxicity in the disposal area increased and reached the levels of the control stations, which presented lower toxicity than before the dump. In the last survey, a year after the dumping operations, survival was similar in the three areas and reached more than 80%.

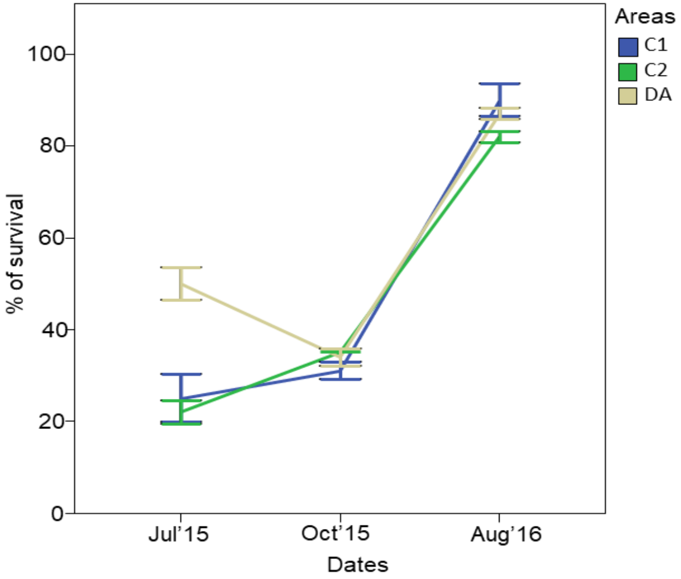

Figure 5. Results of the toxicity test of the sediment of the three areas at all sampling dates.

Metal concentrations in organisms

Since a certain biomass of organisms is necessary to analyse metal concentration, only the most abundant were taken for this purpose: the polychaetes *Sternaspis scutata*, *Nephtys hombergii* and *Glycera tesselata,* the echinoderms *Oestergrenia digitata* and *Amphiura chiajei*, the tanaid *Apseudopsis latreillii*, the decapods *Diogenes pugilator*, *Upogebia tipica* and *Goneplax rhomboides*, the mollusc *Turritella turbona* and a fish species of the family Gobidae. Organisms were classified according to these trophic groups: predator (P), deposit feeder (DF) and suspension feeder (SF). SF was not considered for bioaccumulation calculation as suspension feeders do not feed directly on the sediment.

Bioaccumulation of As, Cd, Cu, Hg, Sr and Zn was detected, and concentration levels and bioaccumulation are shown in Table 3. Concentration of As in organisms ranged from 3.86 to 63.19 mg/kg. The lowest metal concentration was found in predators in control areas in August 2016 (3.86 mg/kg) and the highest in DF in July 2015 in the three areas (42.75 mg/kg in DA to 63.19 mg/kg in C2). Generally, P showed lower values than DF. Bioaccumulation of As was observed in DF in all areas at all dates sampled except in October 2015 in the disposal area. In P, bioaccumulation was observed only in October 2015, both in control and in disposal areas.

The concentration of Cd ranged from 0.05 to 1.39 mg/kg. The lowest values were observed in P in C1 at the three sampling dates and the highest in DF in the control areas in July 2015. Bioaccumulation was detected in DF in the three areas in July 2015 but not in P. In October 2015 all trophic groups showed bioaccumulation in the three areas.

Values of Cu concentration ranged from 6.89 to 185.44 mg/kg. The highest concentration levels were detected in DF in the three areas in October 2015. Predators in the disposal area also showed higher values compared to those in the controls at all sampling dates. BFs were also higher in October 2015 in DF of the three areas. In the disposal area, P showed bioaccumulation of Cu at all sampling dates similar to DF except in July 2015. In contrast, in the controls P never showed bioaccumulation.

The highest concentrations of Hg were found in October 2015 in the three areas. In P this concentration was higher than in DF, reaching values of 5.04 mg/kg in the disposal area. In July 2015 these values were lower and became undetectable in August 2016. Both DF and P showed bioaccumulation in October 2015 in all three areas.

Sr concentrations ranged from 100.71 to 1625.78 mg/kg. Values were always higher in DF than in P, except P in the disposal area, which also showed high concentration compared to the other areas in August 2016. The highest BFs were found in DF in the disposal area at the three sampling dates.

A higher concentration of Zn was detected in October 2015 in all areas except in the DF of the disposal area. Bioaccumulation was always detected for Zn in all the trophic groups in the three areas except in one control area in July and August. Highest BFs were detected in P in the disposal area in October 2015. In the other sampling dates BFs were also higher in the disposal area than in the control areas.

Trophic position

TP was calculated at all sampling dates since there were insufficient species found to adjust the linear models. In the disposal area, the highest TP was detected in *U. tipica* and *G. tesselata*. In control areas the highest levels were shown by *O. digitata,* Gobidae and *N. hombergii*.

Linear models only detected a positive significant relationship between TP and metal concentration for Zn in the disposal area (estimate = 3.685, std = 1.457, t = 2.529, p = 0.0299).

Table 3. Mean values of trace metal concentration (mg/kg) in sediment and organisms grouped in trophic guilds. Bold letters indicate BF > 1 in organisms. Missing values indicate absence of species or insufficient biomass.

	Before			After		
	C1	C2	DA	C1	C2	DA
Sediment						
As	12.44	11.30	9.48	14.40	14.11	11.44
Cd	0.19	0.18	0.17	0.21	0.20	0.22
Co	8.19	8.62	5.98	8.40	8.35	7.99
Cr	46.57	48.14	19.51	46.51	45.75	33.73
Cu	47.63	46.52	18.13	44.40	44.61	30.44
Hg	0.15	0.11	0.10	0.06	0.19	0.12
Ni	25.14	25.51	12.59	24.09	24.14	19.57
Pb	33.17	33.36	17.74	32.26	32.07	24.49
Sr	262.80	273.67	186.36	289.75	293.02	293.15
V	61.90	65.92	29.02	69.21	67.71	51.19
Zn	99.84	97.28	58.67	99.15	98.76	77.24
Deposit feeders						
As	**43.11**	**63.19**	**42.75**	**19.31**	**19.81**	11.17
Cd	**1.26**	**1.39**	**0.64**	**0.42**	**0.45**	**0.25**
Co	6.18	**10.67**	3.61	4.08	4.63	1.22
Cr	20.20	22.15	6.70	10.16	9.00	3.07
Cu	32.36	35.07	14.74	**122.65**	**120.73**	**95.30**
Hg	0.11	**0.22**	**0.18**	**0.59**	**0.81**	**0.29**
Ni	15.27	19.36	7.75	9.49	8.94	3.07
Pb	18.25	22.43	7.66	13.45	10.88	3.31
Sr	252.29	**301.40**	**782.18**	**1090.92**	**1091.28**	**1386.86**
V	22.42	24.33	8.81	12.92	13.24	5.09
Zn	**110.53**	**106.43**	**84.73**	**121.85**	**167.24**	**91.98**
Predators						
As	4.94			**18.74**	12.09	**12.20**
Cd	0.13			0.13	**0.63**	**0.36**
Co	0.40			0.68	0.34	0.85
Cr	3.01			2.85	4.63	2.90
Cu	6.89			17.86	29.48	**89.55**
Hg	0.14			**1.01**	**1.34**	**2.52**
Ni	1.66			2.31	2.89	1.73
Pb	2.96			3.05	4.68	1.63
Sr	**326.53**			**377.98**	503.70	**880.83**
V	3.82			3.92	6.86	3.10
Zn	75.23			**133.46**	**134.99**	**148.16**

Discussion

Several studies assessing the impacts of dredged material disposal in marine environments have determined that their effects are mostly site-specific (Donázar-Aramendía et al., 2018; Marmin et al., 2014; OSPAR, 2008). Moreover, there have been few studies focusing on the impact of disposal in ongoing dumping areas (Bolam et al., 2011). Here we assessed both the effects of recurrent disposals in an active marine dump and the effects of a prompt deposition on sediment characteristics and their implication for the food web. There are various factors that may modulate the impact of disposals, such as the amount of sediment deposited and the season, water depth, hydrodynamics and the similarity of the dredged material to the native sediment (Marmin et al., 2014; Powilleit et al., 2006).

In fact, the main effects of dredge material disposal involve physicochemical disturbances in (i) a water column and (ii) the sea bed (Ceia et al., 2013)

In some studies, physicochemical effects on the water column due to dredged material disposal have been reported (Simboura et al., 2007). One of the main impacts resulting from disposal is the increase in turbidity, which can lead to changed metabolic rates of filter feeders and reduce larval recruitment and growth (Witt et al., 2004). However, our results indicated no effects on turbidity. We have no data about the very early effects in this sense immediately after disposal, but Fredette & French (2004) reported that water column impacts are minimal and short-term and could be comparable to natural process (OSPAR 2008). Hence, disturbances that affect macrofaunal communities are more related to the physicochemical characteristics of the sediment (OSPAR, 2008).

On the other hand, literature reviewed in OSPAR (2008), assessed that one of the main effects of disposal operations is changes in sediment structure, which may adversely affect organisms. This habitat alteration is due mainly to the deposition of fine-grained sediment. This agrees with more recent studies, which have found similar significant changes to finer grains (Katsiaras et al., 2015). Conversely, Simonini et al. (2005) and Smith and Rule (2001) found no influence on granulometry, Cruz-Motta and Collins (2004) found that although an increase in the coarse material in a disposal area was registered just after disposal, the main matrix of fine material was maintained. Harvey et al. (1998) found similar patterns after disposal; an increase in coarser sediment with more organic content than the natural bottom sediment was detected but differences decreased with time back to the natural conditions. In contrast, Vivan et al. (2009) found a shift in granulometry to more coarse sediment in the first month after disposal. Munari and Mistri, (2014) also found a change in the particle sizes of sediment from silty-clayey to sandy. In Spain, legislation encourages the selection of DAs with similar granulometric characteristics to that of the dredged material extracted (Buceta et al., 2015). A close match between grain size distribution of the dredged material and the native sediments has been reported to be an important factor in the ecological impacts of disposal operations (Wilber et al., 2007). Our results showed that the marine dump bottom is characterised by fine sand at all stations and there was no variation with time, although there was a high variability in the sediment characteristics of the disposal area, which indicates a higher degree of patchiness in this area. This granulometric composition is normal for open-water zones situated off the mouth of large estuaries and influenced by the terrigenous inputs of the river (Gray and Elliott, 2009). However, differences in grain size distribution and POM between the disposal area and the two control areas were found. While the two control areas had greater fines and POM content, the disposal area had more sand and lower POM. This shows that the sediments deposited had been dredged in the estuary from areas with some different sediment characteristics from those of the receptor area. The prior differences had probably been caused by the accumulation of disposals over the years. In spite of these differences, the prompt disposal in summer 2015 did not affect the physical characteristics of the dumping area.

Several studies have found shifts in the chemical characteristics of sediment due to variation in heavy-metal concentrations (Bolam et al., 2011; Katsiaras et al., 2015; Stronkhorst et al., 2003). The latter found changes depending on the trace metal analysed. Others, such as Roberts and Forrest (1999), did not find significant changes, or they found little indication of an impact on the disposal area (OSPAR, 2008). The significant increase in some heavy-metal concentrations observed in an area after disposal agree with these results and could be attributed to the deposition of river-dredged sediments with a higher concentration of those metals. In fact, González-Ortegón et al. (2019) suggested a high concentration of Cu and Ni due to the influence of the intense agriculture in the Guadalquivir river basin.

In spite of this, concentrations of heavy metals were never higher in the disposal area than in the two control areas. The higher levels of heavy-metal concentration found in the control areas could be due to the fact that heavy metals potentially show an affinity for mud particles and are accumulated where fine-grained sediments are present (George et al., 2007). Evidence of this process can be found in a wide variety of marine systems and localities (Boldrin et al., 1989; and references therein). Metal concentrations in the continental shelf of this area are explained by the contribution of the Tinto-Odiel, Guadiana and, mainly, the Guadalquivir estuary (González-ortegón et al., 2019).

Levels of toxic metal concentration at all sampling stations were similar to those found by Usero et al. (2008) in the Gulf of Cádiz and by Tornero et al. (2014) in the Guadalquivir. In this sense, according to Ruiz (2001) the base levels obtained in the Gulf of Cádiz, I_{geo} for Cr, Pb and Zn, showed 'unpolluted' sediment at all sampling periods and stations, and for Cu showed a 'very low pollution' in the two control areas.

Dauvin (2008) pointed out that the use of the amphipod toxicity test in European waters has to increase, since those tests have been more widely applied in estuarine habitats of North America or New Zealand rather than in Europe. Bioassays have showed different results when assessing the toxicity of dredging and disposal operations. For example, in Guerra et al. (2009), Stronkhorst et al. (2003), Bolam et al. (2011) and Roberts & Forrest (1999) there were no effects on amphipod survival in their toxicity tests; meanwhile in Cesar et al. (2014), bioassays suggested that the disposal of dredged material altered sediment quality and the toxicity was related to metal contamination. Organisms in disposal areas usually showed high resilience to perturbations (Bolam et al., 2011; Bolam and Rees, 2003). However, different chemical contents in disposed dredge sediments could impact the macrofaunal community, weakening their survival, reproduction and recruitment processes, due to the mobility and bioavailability of toxic heavy metals (Guerra et al., 2009; Katsiaras et al., 2015). Previous studies in this area had found lower increases in some species in the disposal area after this latest event (Donázar-Aramendía et al., 2018). The increase in the toxicity of the sediment after the prompt disposal in summer 2015 in the disposal area was attributed presumably to an increase in hazardous metal concentration. In preoperational samples, toxicity trials with sediment from the control areas showed a lower survival in amphipods than those with sediment from the disposal area, while after the prompt disposal the survival rates homogenised. This increase in the apparent toxicity in the disposal area was consistent with the observed increase in the concentration of some metals. In the final samples from August 2016, a universal increase in survival was observed; we do not have a clear explanation for this result, which could be related either to the condition of the experimental amphipods in their natural habitats or to some uncontrolled variable during experimentation. However, the environmental conditions and the treatments were equal for both control and disposal areas, and again there were no differences in survival between disposal and control areas, in contrast to preoperational trials.

Assessment of benthic condition is vital in monitoring programmes, since trace metals and other contaminants accumulate in marine sediment and may lead to an impact on the environment (Parnell et al., 2008). Macrofauna from benthic habitats are sensitive to sediment quality and make up a group in which different trophic strategies are present (Parnell et al., 2008; Gamito and Furtado, 2009). Several studies focusing on programmes monitoring bioaccumulation of pollutants in organisms have used few suspension feeding species such as clam cockles and mussels (Martín-Díaz et al., 2005). However, organisms which feed directly on the sediment may reflect more accurately the sediment toxicity. Macrofauna trophic structure includes different trophic levels of both prey and predator species. Moreover, since they are in an intermediate position in the food web, they may constitute a significant pathway for bioaccumulation and biomagnification of pollutants (Costa-Dias et al., 2010). Thus, contaminants can concentrate in their tissues and be transported up the food web (Parnell et al., 2008).

In this study we used a multispecies approach, with a measure of the TP of these species. However, a major issue when analysing metal content in organisms is that a relatively high biomass of each species is necessary, which was difficult to find in all the areas and dates. Despite of the low amount of data of trace metal concentration, mostly in predators, some conclusions may be extracted. We found bioaccumulation of some heavy metals in deposit feeders. Arsenic, cadmium, mercury, strontium and zinc were identifiable in the organisms in all areas, both before and after the disposal of summer 2015. Only copper was accumulated specifically after the disposal, but in all areas. On the other hand, predators showed bioaccumulation of the same metals but without a clear pattern, sometimes only in the disposal area and in one control. Only zinc and mercury were present in all areas after the disposal. Bioaccumulation of copper was seen only in the disposal area after the disposal of summer 2015, at high concentration compared to the organisms in the control areas. These patterns suggest that the bioaccumulation found both in the disposal area and in the controls is due mainly to metal transportation from the main rivers into the Gulf of Cádiz (González-Ortegón et al., 2019), rather than being an effect of the prompt disposal. In other studies, bioaccumulation found after a disposal event were low and/or returned to levels at the reference site after the operations; Stronkhorst et al. (2003) found bioaccumulation of mercury and zinc in starfish at the disposal site but at not more than twice the levels of those at the reference site. Bocchetti et al. (2008) and Fredette and French (2004) used suspension feeders as bioindicators of pollutant bioavailability after a disposal event. In both studies the low bioaccumulation of trace metals found returned to preoperational levels.

To our knowledge there have been few studies analysing biomagnification in dredging or disposal events. Our results showed that there were no patterns of biomagnification through the food web. We only found a significant relationship between the log-transformed concentration of zinc and the TP of the species in the disposal area. Thus, this could indicate an effect in the disposal area where these pollutants are amplified in the system. However, the low number of species found in this area with enough biomass to analyse both stable isotopes and trace metals makes more exhaustive research necessary. These results agree with Dauvin (2008), who found no biomagnification of Cd, Cu, Pb or Zn at any level of an estuarine food web. Conversely, in other studies, authors have found biomagnification of mercury in the estuarine food web (Coelho et al., 2013). These authors also pointed out that the age of the organisms or the time of exposure to the contaminants are important factors in these studies. This would explain the absence of patterns, being due to the relatively short lifetime of the macrofauna (Compton et al., 2017).

Conclusions

The historical depositions of dredged sediment from the Guadalquivir estuary in the disposal area have permanently modified the sediment characteristics (present study) and the biological communities (Donázar-Aramendía et al., 2018). These modifications include a transformation to a coarser granulometry in the disposal area compared to the reference areas. The coarser sediment has led to lower organic matter content and trace metal concentrations. The prompt disposal in summer 2015 increased significantly the concentration of some trace metals, which remained at the same level one year after the deposition. This increase in heavy metal content was reflected in the increase in toxicity of the sediment. In spite of these modifications, the same bioaccumulation patterns found in organisms in the disposal and control areas showed that the bioaccumulation seems to have an origin in the metals transported by the principal estuaries in the Gulf of Cádiz. The unique relationship between the zinc concentration and the TP of the organisms in the disposal area, which was absent in the controls, may indicate an impact that necessitates further research. Bioaccumulation analysis and ecotoxicology for different species are procedures with increasing importance in programmes monitoring sediment disposal (Marmin et al., 2014). As suggested by Donázar-Aramendía et al. (2018), the site-specific characteristics of these impacts make it necessary to continue investigating this topic, incorporating new approaches. For example, understanding food webs would allow better knowledge of bioaccumulation and biomagnification (Pasquaud et al., 2007).

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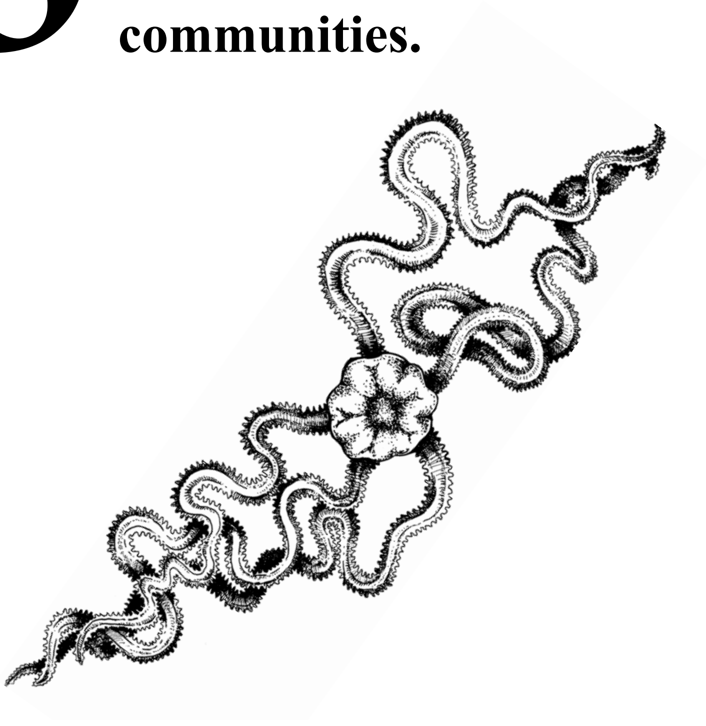
3 Impact of dredged-material disposal on soft-bottom communities.



Adapted from: Donázar-Aramendía, I., Sánchez-Moyano, J.E., García-Asencio, I., Miró, J.M., Megina, C., García-Gómez, J.C., 2018. Impact of dredged-material disposal on soft-bottom communities in a recurrent marine dumping area near to Guadalquivir estuary, Spain. Marine. Environmental. Research. 139, 64–78. https://doi.org/10.1016/j.marenvres.2018.05.010

Resumen

El estuario del Guadalquivir sufre dragados de mantenimiento periódicamente para garantizar la seguridad de la navegación. Desde 2010, parte del material dragado es depositado en un vaciadero marino cercano a la desembocadura del río. Este estudio evalúa el impacto de la deposición de material sobre la comunidad bentónica combinando una aproximación ecológica clásica con nuevas técnicas de análisis de isótopos estables. Para entender los efectos debidos a la deposición de sedimentos se compararon los cambios observados en el área de impacto con dos áreas naturales cercanas. Se detectaron cambios permanentes en la estructura de la comunidad de macrofauna así como en otros índices bióticos y de diversidad analizados. En contra de los esperado, estos índices mostraron valores mayores en el área de vertido. Por otro lado, las variaciones estaciones naturales observadas en las áreas de referencia no se detectaron en el área impactada. Finalmente, a pesar de los cambios estructurales observados en la comunidad betónica, no se detectaron impactos en la red trófica del sistema. Estos resultados confirman la alta variabilidad de los efectos de los vertidos de sedimentos en ambientes marinos. Por ello, se recomienda realizar una evaluación de los impactos de estas operaciones en cada evento de deposición combinando aproximaciones estructurales clásicas con nuevas técnicas que exploren los impactos a nivel funcional.

Abstract

This study assesses the effects of dredged material disposal in a recurrent marine dump near the Guadalquivir Estuary (south-western Spain). We compared the changes observed with two reference areas combining a classical ecological approach with new stable isotope techniques to analyse trophic structure. We detected permanent changes in the macrofaunal community structure as well as in the diversity and biotic indices applied, which showed higher values in the disposal area. The community in the marine dump had lost the natural temporal variations observed in the reference areas. These effects could be due to the last disposal event carried out in the summer of 2015 or to the recurrent disposals since 2010. Despite the structural changes shown by the benthic community, these impacts were not reflected in the food web structure of the marine dump. Our results confirm the high variability of disposal disturbances. Hence, we recommend performing studies in every disposal event, merging different functional and structural approaches.

Introduction

Both dredging and the dumping of dredged material are common practices around the world and are one of the most serious environmental concerns for coastal management (Marmin et al., 2016; Moog et al., 2015; Van Dolah et al., 1984). These practices are particularly developed in estuaries where sedimentation patterns are high and may be accelerated by human activities (Cesar et al., 2014). However, dredging is essential to maintain security in navigation and to support trade and economic sustainability (OSPAR, 2008; Bates et al., 2015). Although relocation of dredged material is one of the most important concerns in those activities, much of the material dredged is still disposed at sea for economic reasons (Harvey et al., 1998; Katsiaras et al., 2015; Tornero and Hanke, 2016). Dredging and disposal of dredge material may cause environmental problems in coastal and marine areas, both physically and through contaminants (e.g. Bolam et al., 2006; Bolam and Rees, 2003; Cesar et al., 2014; Fredette and French, 2004; Guerra et al., 2009, 2007; OSPAR, 2008). Within the implementation of the European Water Framework Directive, the improvement of our knowledge of human impacts is critical for marine management and conservation (Marmin et al., 2016).

Effects of dumping on coastal ecosystems depend on several factors, such as the disposal method, the amount of sediment disposed, the physical and chemical characteristics of the dredged and receiving sediment, depth, the oceanographic and sedimentary conditions of the receiving habitat, season and the adaptations and composition of the inhabitant community (Katsiaras et al., 2015; Simonini et al., 2005b, and references therein). Dredged material disposal may affect marine organisms through physical alterations such as the smothering of the sea bottom, in addition to increased turbidity and enrichment of pollutant, organic matter and nutrient concentrations in the sediment, among other impacts (Cesar et al., 2014, and references therein). Furthermore, these impacts may lead to important habitat changes through long term effects (Powilleit et al., 2009). Benthic macrofauna are the organisms that are most sensitive to these impacts because of their relative immobility (Simonini et al., 2005b; Taupp and Wetzel, 2013). However, effects on primary production and changes in species composition can have an impact on the whole food web structure (Pezy et al., 2017). Nutrient inputs often affect the basal resources, sometimes converting a complex food web to a more homogeneous system (Rooney et al., 2008). Analysing community trophic structure is one way to assess the nature and magnitude of human impacts (Hussey et al., 2014).

When a potential anthropogenic disturbance is repeated over time, the recurrence of this event is also an important factor too (Glasby and Underwood, 1996; Villnäs et al., 2013). On the first hand, sediment disposal after dredging operations can be considered a short-term or “pulse” disturbance, in the sense outlined by Glasby and Underwood (1996). On the other hand, the sediment disposed, if it differs from the native sediment, may constitute permanent or a long-term change in the affected habitat. Recurrent sediment disposal operations and the accumulation of dredged material could generate a series of short-term responses in the biological community, followed by a recovery (to some extent) and convergence to a previous state, a permanent change or a combination of these two processes (Underwood, 1994).

There are several studies addressing the effects of disposal in offshore environments (e.g. Smith and Rule, 2001; Stronkhorst et al., 2003; Zimmerman et al., 2003; Simonini et al., 2005b; Ware et al., 2010; Bolam et al., 2011; Bolam, 2012; Taupp and Wetzel, 2013; Cesar et al., 2014; Katsiaras et al., 2015; Marmin et al., 2016). However, several authors have highlighted the divergent results obtained in disposal impact studies and concluded that the potential environmental effects must be evaluated case by case (Bolam et al., 2006; Bolam and Rees, 2003; Harvey et al., 1998; Katsiaras et al., 2015; OSPAR, 2008; Simonini et al., 2005b). Furthermore, to our knowledge, the effects of disposal of a recurrent and active marine dump on the benthic community have not been widely studied (Bolam et al., 2011). In addition, the impact of sediment disposal on the food web has been studied even less (e.g. Pezy et al., 2017).

In this study we evaluated the impact of dredge sediment deposition on a recurrent marine disposal area in south-western Spain. The Guadalquivir estuary provides access to Seville harbour, the only inland harbour in the Iberian Peninsula, through a 90-km long navigation channel. The strongly modified course and sedimentary dynamics made some dredging work necessary every few years to maintain navigability (Díez-Minguito, 2012). Part of the sediment dredged along the channel is brought ashore for storage, while the other part goes to beach nourishment and some is disposed in an authorised marine area established in 2010. We compared the changes observed in the disposal area with two nearby control areas. Specifically, this study is focused on the following objectives: (i) to assess the response of the benthic community to recurrent sediment discharges (ii) to evaluate the effect of the most recent disposal event (iii) to determinate the effects on the food web structure and (iv) to describe the temporal variation of a biological community in a recurrently stressed area.

Methods

Study area

The marine disposal area is situated five miles offshore in an open water area in front of the river mouth of the Guadalquivir estuary, south-western Spain (Fig. 1). The bottom is 20 metres in depth.

Sediments are mainly composed of fine sand and mud and are affected by terrigenous inputs of the Guadalquivir River and the hydrodynamic conditions of the Gulf of Cádiz, with predominant east-ward currents (Sainz and Ruiz, 2006). These currents are derived from the North Atlantic surface waters and have the greatest effect on the sediment distribution (Sainz and Ruiz, 2006). The marine dump has a rectangular shape with a surface of 66.26 ha (Fig. 1). The sea bottom near this area has similar sedimentary characteristics (Gonzalez and Dias, 2004) and biological communities (Fa et al., 2003). This dump has been receiving dredge material from harbours and the Guadalquivir estuary in four events since 2010 (2010: 338.652 m^3, 2011: 353.488 m^3, 2013: 354.795 m^3 and 2015: present study), (data from Autoridad Portuaria de Sevilla (APS)).

From mid-august to the first week of September 2015, dredging operations for maintenance of the navigation channel of the Guadalquivir estuary and Bonanza port were performed and 314.275 m^3 of the extracted sediment was discharged in the disposal area.

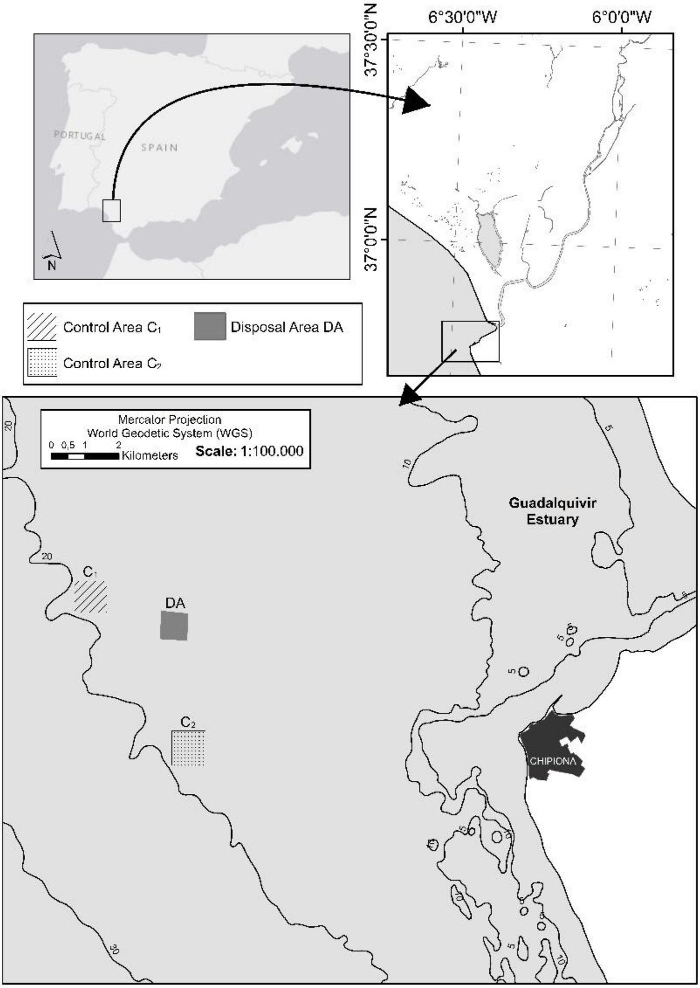


Figure 1. Location of the disposal and control areas.

Sampling design

Three sampling areas were established in the same environment: one within the marine disposal area (DA) and two controls located approximately three miles from the affected area (C_1 and C_2) (Fig 1). Controls were located at the same depth in a very similar area with equal hydrological and geomorphic characteristics (Sainz and Ruiz, 2006). Distances between controls and the disposal area were enough to avoid any possible impacts. In total, five sampling surveys were carried out: twice before the last dumping event (to test the previous affections of the recurrent disposals) and three times after the last disposal event of summer 2015 (to analyse both effects of this last perturbation and the temporal variation of the system). Before: June and July 2015. After: October 2015 (one month after the last disposal event), March and August 2016, (6 and 12 months after the event, respectively).

At each control and disposal area, three stations were randomly placed. In each station, three samples were taken for macrofaunal analysis with a van Veen grab (0.15 m^2 total sampling area per station and date). For posterior analysis, all stations were considered replicates of each area. Macrofaunal samples were sieved through a 0.5 mm size mesh. Infauna was preserved in ethanol (70%), and stained with Rose Bengal for subsequent identification and quantification to species level where possible. A sediment sample was taken for granulometric analysis. Grain size distribution was measured as percentages of 100 gr of dry sediment sieved through a sieve net (5 mm, 2 mm, 1 mm, 0.5mm, 0.250 mm, 0.125 mm, 0.063 mm).

For the isotope analysis of carbon and nitrogen, fauna were collected in three of the five surveys (June 2015, October 2015 and August 2016) with a van Veen grab (0.05 m^2) and an epibenthic sledge (46 x 25 cm, 2.5 mm net mesh size). The number of samples was sufficient to obtain a representative number of species of the benthic community. All samples were sieved by 0.5 mm mesh sieve and organisms were sorted by species, transferred to the laboratory in refrigerated containers and kept alive for 24 h to evacuate their gut content. In order to determine the isotopic composition of sediment organic matter (SOM), sediment was taken from the upper 2 cm of a van Veen grab sample. In the lab, species identification was confirmed and fauna were rinsed in distilled water. Muscle tissue samples of fish and molluscs were dissected. A pool of several organisms was used when the individuals had low biomass values (< 0.3 mg dry weight). Organisms were dried at 60 °C for at least 48h and ground to a powder. Sediment samples were acidified with 0.1 M HCl to remove carbonates, and were then oven dried at 60 °C. Subsamples of powdered materials were weighed to the nearest 0.3 mg and placed into tin capsules for $\delta\ ^{13}C$ and $\delta\ ^{15}N$ determinations. All samples were combusted at 1020 °C using a continuous-flow isotope-ratio mass spectrometry system by means of Flash HT Plus elemental analyser coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific, Bremen, Germany). From the obtained results we performed a descriptive approach to the food web structure in the three areas by means of isospace plots based on carbon and nitrogen isotopic signatures (Fry, 2006). Species selected were those that appeared both in the disposal and control areas and in the three surveys.

Data analysis

At disposal and control areas, univariate measures such as species richness (as number of taxa, S), Shannon's diversity index (H' based on log e) and total abundance (as ind/m^2, N), were calculated. Ecological quality status (EQS) was also assessed through the biotic indices AMBI (Borja et al., 2000), BENTIX (Simboura and Argyrou, 2010; Simboura and Zenetos, 2002) and BENFES (Sánchez-Moyano et al., 2017). The biotic indices are based on the assignation of ecological groups according to the sensitivity or tolerance toward to anthropogenic disturbances (Ponti et al., 2009). All indices were calculated for each station. AMBI and BENTIX are based on the relative abundance of species, and BENFES is based on presence/absence and identification at family level.

To examine the dumping effects in the study area, a Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson, 2001) based on Euclidian similarity matrices of S, N and H' indices using 9999 permutations was performed. 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 two crossed fixed factors: "Impact vs Control" with two levels (Impact and Control) and "Time" with two levels (Before and After the last dumping event). The design also included two random factors: "Area" nested within "Impact vs Control" with three levels (DA, C1, C2) and the sampling dates "Dates" nested within "Time" with five levels (Jun 15, Jul 15, Oct 15, Mar 16 and Aug 16).

The results of the multivariate communities' assemblages were also investigated using a PERMANOVA analysis, with the same experimental design as above, based on the Bray-Curtis similarity matrix from the square root transformed abundance sets. When the saturated model (with all explained terms included in the model) generated negative estimates of some components of variation (this indicates that the model in turn is a poor one, according to Anderson et al., 2008), a model selection was performed by excluding the affected terms, one by one, beginning with those of higher order (interactions), and provided that their p values were higher than 0.25 (Anderson et al., 2008). Following this process with all the analyses performed here (both the multivariate and the univariate PERMANOVA), the factor “Area” and its interactions with “Dates” and with “Time” were excluded from all four models. It is important to notice that “Area” is nested within “Impact vs Control” and, given the asymmetric design of this study (there are no replicated areas within “Impact” level), this term only estimates the variance between the two control areas (Anderson et al., 2008), which was negligible in this study. The analysis presented here does not include these terms. Significant interactions, if detected, were further explored in separate analyses, within the levels of the interacting factors; i.e. the significant interactions between “Impact vs Control” and “Dates” were further analysed separately by disposal area and the control area.

Macrobenthic communities were also examined by a non-metric multidimensional scaling ordination (nMDS) based on the Bray-Curtis similarity index. SIMPER analysis was used to identify the species contributing most to any observed spatial or temporal pattern in the communities (Clarke, 1993).

All analysis and data visualisations were performed using the statistical software package PRIMER version 6 and SPSS version 24.

Results

Sedimentary analysis showed that the disposal area had lower %fines (fraction lower than 0.063 mm) than the control areas (Fig 2). This difference remained across all sampling dates. On the other hand, biological analysis showed a total of 21,899 individuals in 128 species belonging to the phyla Annelida, Arthropoda, Mollusca, Echinodermata, Chordata, Platyhelminthes and Nemertea across the sampling dates. We did not find any predominant group except for some increments of particular species in all stations. For example, in October 2015 an increment of Arthropoda abundance was observed. The species with the maximum-recorded abundance per station were the polychaetes *Magelona papillicornis* (813 ind/m^2) and *Sternaspis scutata* (307 ind/m^2), the tanaidacean *Apseudopsis latreillii* (6313 ind/m^2) and the decapod *Upogebia tipica (*467 ind/m^2), and molluscs *Turritella turbona* (60 ind/m^2) and *Corbula gibba* (800 ind/m^2).

Percentage of fines, Shannon's diversity index, Richness and Total Abundance (ind/m^2) differences are shown in Figure 2 and Table 1. As mentioned earlier, PERMANOVA analysis of univariate indices showed that there were no differences in the three indices between the two control areas despite the distance between them. PERMANOVA results of the univariate indices also showed that the disposal area had significantly ("Impact vs Control", $p < 0.0001$) higher values of richness and Shannon's diversity than controls. Differences between areas remained over all the sampling surveys. Total abundance did not show significant differences between controls and disposal area, however we detected a different pattern of variation over the surveys in the disposal area than in controls (Impact vs Control x Dates, $p < 0.0001$). PERMANOVA analysis of separated samples of the disposal area did not show significant differences between dates ($p = 0.4485$). On the contrary, there was a significant difference ($p < 0.0001$) in the controls between sampling dates. In October 2016, a high number of individuals of the tanaid *Apseudopsis latreillii* occurred in the control areas (Fig. 3). More than 5000 ind/m^2 of this species were found in the control areas while this increase was lower in the disposal area (< 3000 ind/m^2).

The mean of biotic indices per area are shown in Table 2. The disposal area showed higher values (lower for AMBI) than control areas. However, limits of the ecological statuses for the AMBI and BENFES did not allow for the differentiation between areas or dates. AMBI showed a "Good" ecological status in all the areas across the sampling dates. BENFES showed a "High" status in all areas except in C1 in August 2016. Only BENTIX showed a "Good" ecological status in the disposal area in all the surveys and "Moderate "in October 2015. Control areas showed a "Moderate" status of this index in June, July and October 2015.

The MDS plot showed different tendencies between the areas (Fig. 4). There were similar communities in the two control areas, with clear temporal differences. However, the community in DA was clearly separated from the controls, even before the most recent disposal event, and showed a fuzzy temporal variability.

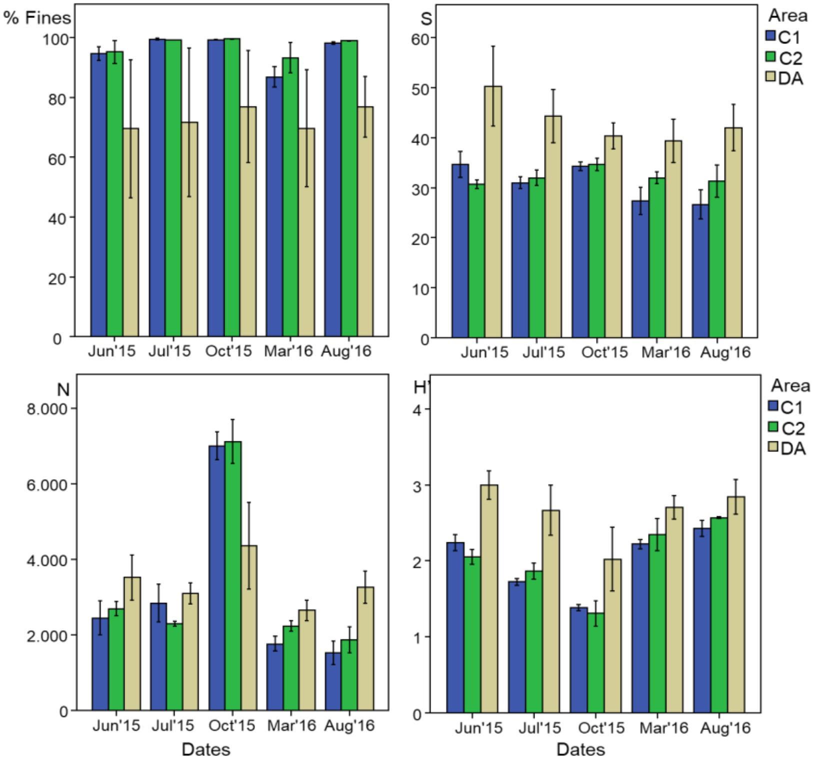


Figure 2. Patterns of variation of the %Fines and univariate indices (S, N, H') in the three areas across the sampling dates. Vertical bars represent ± Standard error.

Table 1. Univariate PERMANOVA results in the studied area based on the Euclidian similarity matrix of the richness data (S), Shannon's diversity (H') and total abundance (ind/m^2) (N). *p estimation obtained by Monte Carlo sampling.

	df	MS	Pseudo-F	p	Unique perms
S					
Impact vs Control	1	1470.20	58.96	0.0074	9555
Time	1	146.22	5.07	0.1081*	10
Dates(Time)	3	28.84	0.86	0.47	9951
Impact vs Control x Time	1	79.35	3.18	0.1721	9549
Impact vs Control x Dates(Time)	3	24.94	0.74	0.5343	9935
Res	35	33.44			
Total	44				
N					
Impact vs Control	1	8.00E+05	0.12	0.7558	9545
Time	1	3.07E+06	0.72	0.7241*	10
Dates(Time)	3	1.95E+07	33.44	0.0001	9960
Impact vs Control x Time	1	1.93E+06	0.28	0.6651	9568
Impact vs Control x Dates(Time)	3	6.78E+06	11.59	0.0001	9952
Res	35	5.85E+05			
Total	44				
H'					
Impact vs Control	1	4.309	104.9	0.0051	9812
Time	1	0.133	8.21E-02	0.79*	10
Dates(Time)	3	1.63	17.42	0.0001	9953
Impact vs Control x Time	1	0.343	8.34	0.0697	9854
Impact vs Control x Dates(Time)	3	4.11E-02	0.44	0.7343	
Res	35	9.33E-02			
Total	44				

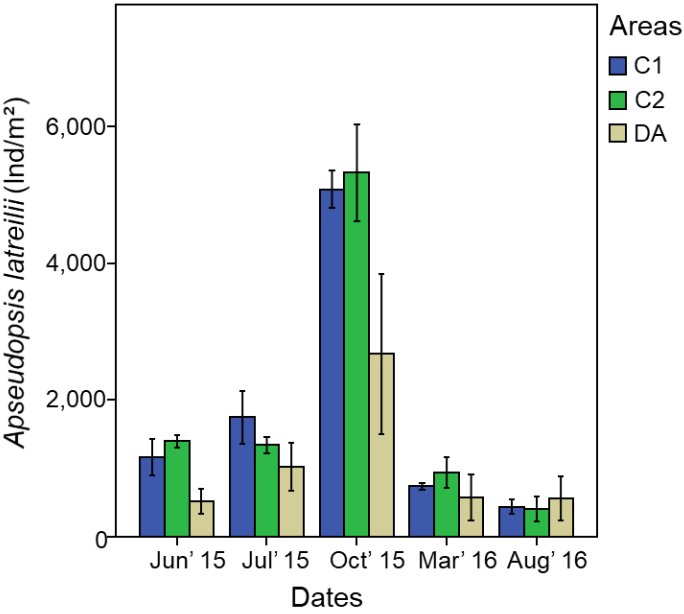


Figure 3. Abundance of the tanaid *Apseudopsis latreillii* across the sampling surveys.

Table 2. Results of the biotic indices AMBI, BENFES and BENTIX. Ecological Quality Status (EQS): Blue = "High", Green = "Good", Yellow = "Moderate"

	Jun 15			Jul 15			Oct 15			Mar 16			Aug 16		
	DA	C1	C2	DA	C1	C2	DA	C1	C2	DA	C1	C2	DA	C1	C2
AMBI	🟩 1.9	🟩 2.4	🟩 2.6	🟩 2.1	🟩 2.4	🟩 2.6	🟩 2.4	🟩 2.6	🟩 2.7	🟩 1.7	🟩 2.6	🟩 2.4	🟩 2.2	🟩 2.7	🟩 2.5
BENFES	🟦 174.0	🟦 124.7	🟦 108.3	🟦 158.0	🟦 113.3	🟦 117.3	🟦 148.3	🟦 117.0	🟦 124.7	🟦 146.3	🟦 100.7	🟦 113.6	🟦 152.7	🟩 98.3	🟦 117.7
BENTIX	🟩 4.0	🟨 3.3	🟨 3.0	🟩 3.8	🟨 3.1	🟨 3.0	🟨 3.2	🟨 2.7	🟨 2.6	🟩 4.4	🟨 3.4	🟩 3.5	🟩 3.9	🟩 3.5	🟩 4.0

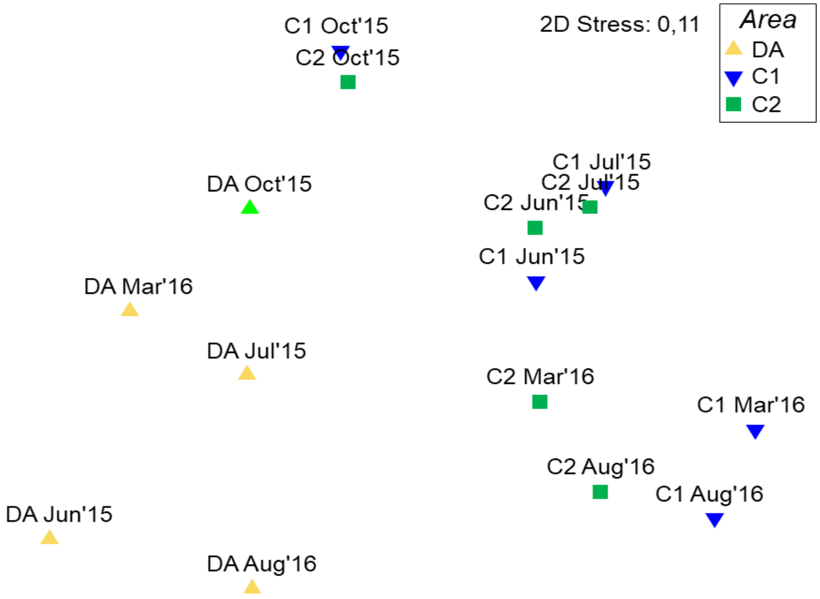


Figure 4. MDS of the distance among centroids resemblance for the factor 'Area x Dates', with representation based on the abundance of the different species.

PERMANOVA results for the community analysis again showed no differences between the two control areas. Results also showed significant differences between the community of the disposal area and controls (p = 0.0205) (Table 3). It must be noted that the pseudo-F for this term (Impact vs Control) is constructed using the mean squared of the interaction term "Impact vs Control x Dates" and provides a test for its significance over and above the potential variability in the effect among sampling dates; it is interpretable as significant even if the interaction between factors “Dates” and “Impact vs Control” were also significant ($p = 0.0024$). Instead, the results did not show a significant interaction between “Time” and “Impact vs Control” ($p = 0.4388$). These results indicate a different pattern of change in the control and the disposal area across the sampling dates, but not globally before and after the sediment disposal on October 2015. PERMANOVA results of the separated analysis of the disposal area showed no significant differences ($p = 0.1027$) across the sampling dates. Conversely, there were different significant community structures in the controls across the sampling dates ($p < 0.0001$).

SIMPER analysis (Table S6) showed dissimilarities between controls and the disposal area for each sampling date. Before the last dumping event, differences were mostly due to the higher abundance of the tanaid *Apseudopsis latreillii* and the polychaete *Sternaspis scutata* in the controls, and the polychaetes *Magelona papillicornis*, *Spiophanes kroyeri*, *Nepthys hombergii*, *Sigambra parva*, two species of the genus *Mediocorophium* and the cumacean *Eudorella truncatula* in the disposal area.

Table 3. PERMANOVA results of the Bray-Curtis similarity matrix based of the square root transformed data. *p estimation obtained by Monte Carlo sampling.

	df	MS	Pseudo-F	p	Unique perms
Impact vs Control	1	5,635	6.3079	0.0205	9626
Time	1	2,113	1.0245	0.4368*	10
Dates(Time)	3	2,062	4.4828	0.0001	9881
Impact vs ControlxTime	1	949.63	1.063	0.4388	9648
Impact vs ControlxDates(Time)	3	893.37	1.9422	0.0024	9873
Res	35	459.99			
Total	44				

SIMPER analysis comparing dates within controls (Table S7) showed that differences between June and July 2015 were, firstly, due to several species, such as *A. latreillii* and the mollusc *Hyala vitrea*, which began to increase their abundance in July 2015. Secondly, new species such as *U. tipica* and the bivalve *Hemilepton nitidum* occurred in this last month. In October, the abundance of several species experienced a high increase (*A. latreillii*, *Aonides oxycephala*, *Capitella capitata*, *U. tipica*, *H. nitidum*, *H. vitrea*). In March 2016 the abundances of these species and others decreased to June 2015 levels. The polychaete *Terebellides stroemi* occurred in this month. In August 2016, abundances of some species increased while others decreased:

e.g. *H. vitrea* and *C. gibba* began to increase while others, such as *A. latreillii*, *E. truncatula* and *T. stroemi* decreased.

On the other hand, in the disposal area (Table S8), SIMPER results showed that the most abundant species such as *A. latreillii* and *U. tipica* followed the same patterns as in the controls areas, but with a lower abundance increase. On the contrary, as with control areas, in October 2015, several species, such as *Mediocorophium sp.*, *Spiophanes kroyerii* and *Lumbrineris latreillei,* suffered a decrease in their abundance. In March 2016 *A. latreilii* decreased while *U. tipica* remained at the same value until August 2016, where it practically disappeared from this area in addition to *H. nitidum*. A general increase in the abundance of other species was observed.

Isotope graphs showed practically no differences in food webs between the three areas (Fig. 5). The carbon isotope signal of SOM in the disposal area showed different signals over time, whilst these signals were similar over time in the control areas. The disposal area showed a more enriched carbon value in June 2015, which became depleted over time. Graphs also suggest that trophic interactions were similar in the three areas (Fig. 5). However, the species *Apseudopsis latreillii* presented a clear difference in the disposal area; the nitrogen signal experienced an increase in the most recent survey, while it remained at the same levels in the control areas.

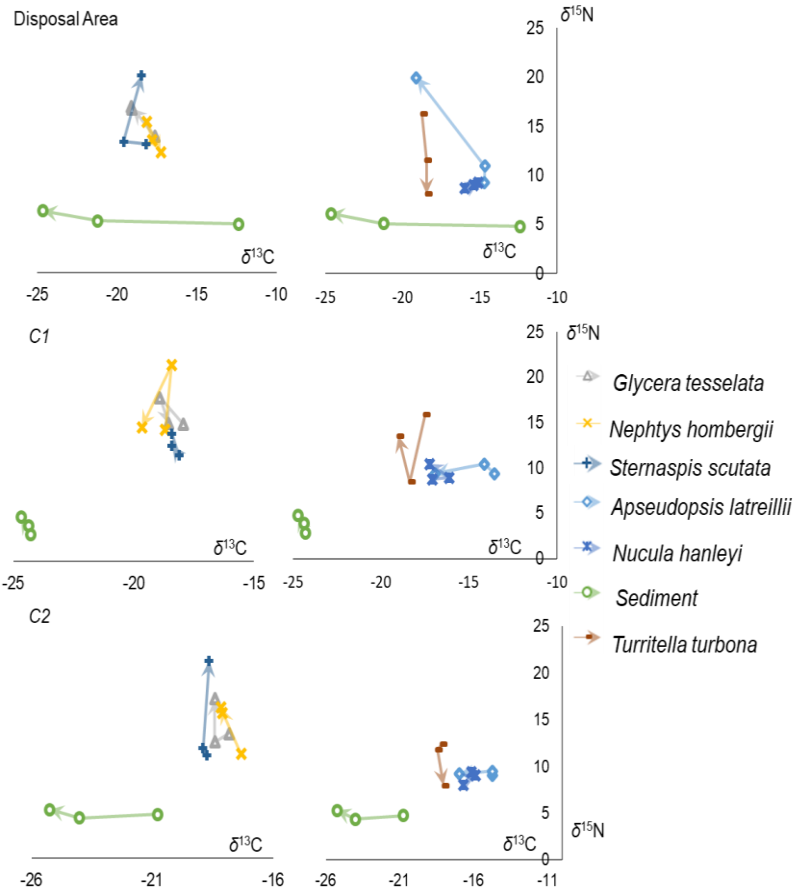


Figure 5. δ13C (x-axis) and δ15N (y-axis) means of the organisms collected in the Disposal area (upper), C1 (middle) and C2 (bottom). Arrows represents isotopic variation over the three sampling dates. Polychaetes have been separated for better visualisation (left column). The x-axis is not always on the same scale.

Discussion

The community structure in the disposal area displayed marked differences in relation to the nearby environment. Shannon's diversity, richness and the biotic indices showed that the disposal area had developed a more diverse and rich community with higher EQS values than control areas established a few kilometres away. On the other hand, the most recent disposal event, carried out in the summer of 2015, seemed not to permanently affect to any of the indices measured, nor the community structure. Furthermore, other findings in this disposal area include the fact that the natural shifts in abundance and the community structure of the control areas were absent in the disposal area. However, it is difficult to establish whether these effects could be due to the most recent disposal event, or to the recurrent disposals since 2010. On the other hand, these effects on the community have not been reflected clearly in the trophic food web. Although isotopic sediment signals in the disposal area could be influenced by the historical disposals, the isospace occupied by all the organisms was similar in the three areas. This suggests the same use of basal resources, as well as the same trophic interactions over time. Only a tanaid species showed an important increase in their nitrogen value a year after the disposal.

Our results showed that univariate indices such as diversity and richness reflected the changes driven by the historical disposal operations over time better than the biotic indices AMBI and BENFES. Only, BENTIX was able to differentiate the EQS of the disposal area from the controls, however, it did not show any pattern across the sampling dates. This agrees with Simboura et al. (2007), who found that BENTIX was useful for assessing the long term trends of community health in a metalliferous waste dumping. Total abundance and the Shannon's diversity indices also assessed the temporal changes over the sampling dates more effectively. This agrees with Katsiaras et al. (2015) and Ware et al. (2009), who pointed out that most sensitive ecological indicators of dumping effects were richness and abundance rather than AMBI or BENTIX. On the other hand, Taupp and Wetzel (2013) found that species richness, Shannon's diversity and AMBI among others were able to reflect the effects of dumping in estuaries.

Most of the species found in the control areas are characteristic of muddy bottoms, while a mixture of species characterised by both sandy and muddy bottoms were observed in the disposal area. The latter could be explained by the translocation of species from the original dredged material and by the immigration of species from the surrounding muddy community. In a soft bottom, habitat variability and structure are considered as key factors in biodiversity patterns (Hewitt et al., 2008; Reise, 2002; Zajac, 2008), and, generally, it is widely accepted that environments that show a great range of structural components can support a larger number of species and greater abundance (Carvalho et al., 2017). The sea bottom in the control areas was characterised by a very stable sedimentary and hydrological environment, typical of areas close to the mouth of great rivers, and host a biocoenosis typical of coastal terrigenous muds (Bellan-Santini et al., 1994) or *Amphiura* assemblages according to other authors, such as Thorson (1957). Consequently, these areas show a well-defined animal assemblage, so that the higher diversity and species richness in the disposal area could be due to the increase in structural complexity by the regular addition of more complex sediments characterised by lower % of fines. Johnson and Frid (1995), found similar results and suggested that it appears to be consistent with predictions of the Intermediate Disturbance Hypothesis (IDH) proposed by Connell (1978).

Differences in the temporal variation of the abundance in the disposal area compared to the controls could be explained by two factors: (1) Changes suffered by this area over the years has modified the natural seasonality of the species or (2) The most recent disposal event could lead to physical and chemical disturbances, which affect the macrofaunal community. Disposal could affect seasonal recruitment, affecting the species abundance (Manning et al., 2014). In fact, SIMPER analysis showed a decrease in the abundance of some species in the disposal area after the disposal of 2015. Furthermore, significant interaction between “Impact vs Control” and “Dates” in the abundance analysis, as well as the community structure, indicated a possible effect on the impacted area. Analyses revealed no changes in the disposal area over the dates, while control areas showed natural differences.

According to a Before After Control Impact (Underwood, 1991), if the putatively impacted area had changed over time from before to after the most recent disposal event, with a different pattern when compared to the controls, it would mean a permanent impact from the most recent disposal event. However, the absence of any interaction between the factors “Impact vs Control” and “Time” indicated that there was not a permanent effect from the most recent disposal. The relatively high diversity, richness and abundance found after the disposal could be attributed to a gradual and homogenous deposition of dredge sediment over a relatively large area. The thin layer created would permit the survival of a high proportion of different species, as in the study carried out by Simonini et al. (2005b). Organisms have the capacity to burrow into the sediment in order to avoid burial, making them capable of tolerating these perturbations (Powilleit et al., 2009). This would be in accordance with Marmin et al. (2016), who proposed that more dispersive disposals over a wider area were recommended, rather than depositions at a single point. Furthermore, organisms situated in a perturbed zone present more resilience to perturbations (Bolam and Rees, 2003). Furthermore, a rapid recovery after a disposal has been attributed to the great potential of juvenile settlement and adult colonisation from the edges (Bolam and Rees, 2003; Fredette and French, 2004; Munari and Mistri, 2014).

Several studies have found recovery in disposal areas in terms of abundance, richness and diversity, but not in terms of the community structure (OSPAR, 2008). Changes in macrofaunal community structure have been reported in many disposal effect studies: (e.g. Harvey et al., 1998; Zimmerman et al., 2003; Witt et al., 2004; Bolam et al., 2006; Powilleit et al., 2006; Ware et al., 2010; Taupp and Wetzel, 2013; Munari and Mistri, 2014; Katsiaras et al., 2015). However, other studies, such as Roberts and Forrest (1999), Smith and Rule (2001), Stronkhorst et al. (2003), Simonini et al. (2005b) and Bolam et al. (2011), found scarce indication of impact in their respective disposal areas. Our results pointed to an influence of the dredge material disposal in terms of community structure and a lack of temporal variation in the disposal area. Conversely, the control areas showed a very similar community trend with significant temporal variations. This could again be related to the changes established by the periodic sediment input from maintenance dredging for channel navigation in the Guadalquivir River, or at least to the most recent disposal event in the summer of 2015.

Recovery patterns after a disturbance, such as dredging and disposal operations, seem to take different pathways in every study, and depend on several factors (Newell et al., 1998). Bolam & Rees (2003) pointed out that communities of naturally stressed environments could recover better than more stable zones. Shallower zones with physical disturbances tend to take less than one year to recover in many cases, while deeper, more stable zones take more time to converge with the initial community structure. Despite the fact that an assessment of recovery in an on-going disposal area is difficult, our results are consistent with OSPAR (2008), where it was pointed out that community structure often fails to converge with natural reference areas. SIMPER analysis showed that one month after the last disposal event was the date with the highest similarity between the control and the disposal area. This could be explained by the decrease of some species in the disposal area that were contributing to these differences before. In this month, the abundance of some abundant species also increased in both control and disposal areas. Six months and a year after the summer 2015 disposal, the community structure of the disposal area showed more dissimilarity with the control areas. This was due to the different patterns of change in terms of species common to both the controls areas and the disposal area, and the appearance of species in the disposal area that were not present in the control areas.

The tanaid *Apseudopsis latreillii* was the main species contributing to the dissimilarity found between the disposal area and the control areas after the disposal of 2015. This organism did not experience such a pronounced increase in its abundance in the disposal area as it did in the two control areas. This species usually spawns during late summer and early autumn, reaching high densities in the soft bottoms of this geographical area (Sánchez-Moyano et al., 2007). de-la-Ossa-Carretero et al. (2010) indicated that *A. latreillii* responds to sewage disposal with a decrease in population density and, furthermore, smaller juvenile individuals appear to be particularly sensitive, since these individuals normally live near the surface and are more exposed to disturbance. Consequently, the coincidence of dumping with the reproductive peak may have adversely affected the populations of this species. However, another species that was very abundant among all studied areas was the decapod *Upogebia tipica* (and also, in smaller numbers, *U. deltaura*), which was not affected by the dumping. Upogebiidae usually need sandy and muddy substrates to construct long burrows (deeper than 20–30 cm) where they obtain shelter, protection from predators and appropriate conditions for feeding and reproduction (Coelho et al., 2000). *Upogebia* spp. occur in the coast of the Gulf of Cádiz between depths of 20 and 40 m and generally show a reproductive event during the spring-summer period with a planktonic larval phase of around three weeks (Pires et al., 2013). Their reproductive cycle prior to sediment disposal, and their deep burrows, could be the reasons why there were no differences between the control and the disposal areas. Similar results have been obtained with other abundant burrowing species, such as the polychaetes *Lumbrineris latreilli*, *Nephthys hombergii* and *Sternaspis scutata*.

Carbon isotopic signals of sediment in the disposal area could suggest a perturbation on the impacted area before the disposal of summer 2015 due to the periodical inputs of more carbon-enriched sediments from the estuary. However, this point was not reflected in the trophic food web, since analysed species showed similar carbon signal in the disposal area and in the two control areas. Since all species showed similar isotope signals over time, it suggests that the origin of organic matter was similar in the three areas. Furthermore, after the disposal in the summer of 2015, the isotopic signal of the sediment in the disposal area was similar to the control areas. Nitrogen isotopic values also suggest similar trophic niches for most organisms in the three areas across all sampling dates. However, *Apseudopsis latreillii* showed an important increase in their nitrogen values in the marine dump with respect to the control areas in the August 2016 survey. Variation in the tanaid isotopic signal could be attributed to the most recent disposal event, although more specific studies would be necessary to demonstrate this question. Change in diet composition is not immediately reflected by tissue isotopic values, which integrate trophic information over a certain period of time (Sampaio et al., 2010;

Sweeting et al., 2005). This could explain the lack of differences in the October 2015 survey. Since there were no changes in nitrogen signal of the basal resources, increase in the nitrogen signal of that species could be explained by a change in the degree of trophic omnivory (Post and Takimoto, 2007). These results contrast with other studies where changes in the trophic structure of disposal impacted areas have been found (Bolam, 2012; Munari and Mistri, 2014; Pezy et al., 2017; Simonini et al., 2005a). However, these studies were focused on functional traits and secondary production rather than a stable isotopic approach.

Conclusions

The recurrent disposals constitute pulse disturbances, but repeated periodically, which could produce: (1) recurrent pulse or short-term responses in the biological community followed by a convergence towards the reference communities, (2) a permanent change or (3) a combination of these two effects (Underwood, 1994). The results of this study suggest that a permanent change exists. However, we cannot discard some short-term effects of most recent disposal event such as the affection of the recruitment of some species.

The impact of depositing dredge material in marine environments depends on various factors, such as the amount of sediment, dumping season, water depth, currents and similarity of dredge material and the native sediment (Powilleit et al., 2006; Marmin et al., 2014). For this reason, the impacts of dredge-material disposal are mostly site-specific (OSPAR, 2008). There are few studies assessing the effects of dumping in a historic marine dump with well-established macrofaunal communities such as Ware et al. (2009, 2010) for example. Our data suggest that in the disposal area, where a disposal operation occurs periodically, there have been changes that have affected biological characteristics permanently. Contrary to other studies, the continuous disposal of dredge material from the Guadalquivir estuary has increased richness and diversity indices, although these historical impacts, as well as the most recent disposal event, could have led to the natural loss of seasonality in the marine dump. Conversely, the food web has not been affected. Furthermore, disposal timing is an important factor to consider. To minimise the impact on soft-bottom communities, dredging and disposal operations should avoid the main reproduction and recruitment periods and be developed, where technical aspects make it possible, in winter months (Sánchez-Moyano et al., 2004; Ceia et al., 2013). Our results confirm the high variability of impacts due to disposal operations and confirm again the ‘site-specific’ character of these perturbations. We suggest that impact studies are needed in every disposal operation work across temporal and spatial scales. Classic approaches and new techniques are also required to assess the effects of these anthropogenic impacts at different levels in macrofaunal soft-bottom communities such as functional and trophic traits. Using stable isotope techniques to assess effects of these perturbations appears to be an interesting tool that has never been used in dredging and dumping studies.

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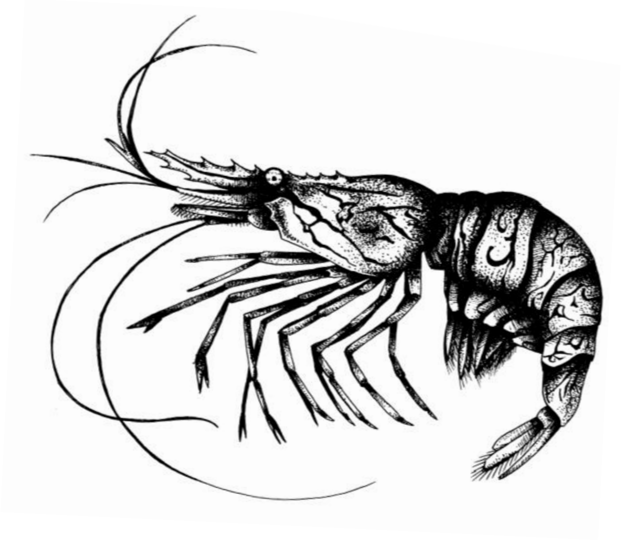
4 Human pressures are reflected in food webs structure.



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Resumen

Los estuarios se encuentran entre los sistemas acuáticos más alterados y amenazados debido al incremento del desarrollo urbano y agrícola en ambientes costeros. En este estudio se utilizaron isótopos estables para analizar la influencia de las presiones humanas sobre estos sistemas, comparando dos estuarios sometidos a diferentes grados de presión antrópica. Como resultado se encontraron interacciones tróficas más complejas en el estuario más modificado (Guadalquivir). Además, una mayor dispersión a lo largo del eje del carbono sugirió que los consumidores primarios de este estuario explotan fuentes de materia orgánica con diversos orígenes, mientras que la mayor diferencia en las señales de nitrógeno sugieren que los consumidores se alimentan de tipos de presas diferentes. Por el contrario, en el estuario relativamente poco alterado (Guadiana) se encontraron señales isotópicas similares en los consumidores, lo que sugiere que poseen una dieta similar soportada por fuentes de materia orgánica con el mismo origen. Los datos preliminares recogidos en este estudio suponen un paso para comprender las interacciones tróficas en estos ambientes, algo fundamental para poder definir estrategias de gestión y conservación adecuadas en estuarios altamente antropizados.

Abstract

As a result of the increased urban and agricultural development in coastal environments, estuaries are among the most modified and threatened aquatic ecosystems. This study used stable isotopes to examine the effects of human impacts by contrasting the food web structures of two Iberian estuaries exposed to different degrees of human pressure. More complex feeding pathways were found in the more altered estuary (Guadalquivir). Greater spread among species along the carbon axis suggests that the primary consumers exploit organic matter with various origins, whereas different nitrogen signals of the secondary consumers suggest that they feed on different suites of prey. In contrast, the similar isotopic signals of secondary consumers in the relatively little influenced estuary (Guadiana) suggests similarity in diet composition and feeding on the same organic matter sources. Understanding trophic interactions in estuaries is vital for defining proper management and conservation, and the preliminary data provided here are one step in this direction.

Introduction

Estuaries are some of the most biologically productive ecosystems in the world (França et al., 2011; Robb, 2014; Wetzel et al., 2013). They play an important role in the continental shelf environment, acting as nursery habitats and providing other habitats with invaluable ecosystem services (Costanza et al., 1997; Dauvin et al., 2014; Kostecki et al., 2010). However, with more than 60% of Earth's population living in coastal areas, estuarine ecosystems have been extensively altered by human activities (Ray, 2006). Rapid urban and agricultural development is the major factor contributing to wetland loss and the deterioration of water quality in these coastal areas (Lee et al., 2012; Morris et al., 2015). Nutrient load inputs to estuaries are directly related to intensive agriculture and large populations (González-Ortegón and Drake, 2012) and it have the potential to alter the nutrient dynamics modifying the function and structure of the estuarine ecosystems (Mazumder et al., 2015).

Elevated loads of nutrient input of anthropogenic origin into aquatic ecosystems may affect different ecological processes such as basal resource production, nutrient dynamics and energy transfer (Warry et al., 2016). These impacts can alter a system's trophic structure (Olsen et al., 2011; Warry et al., 2016) defined as the distribution of organisms in terms of biomass among producers and consumers (Warry et al., 2016). For example, nutrient loading of ecosystems may shift primary production to a single basal source, which is exploited by fewer intermediate consumers, thereby converting a structured and compartmented ecosystem into one with a less stable food web (Layman et al., 2007b; Rooney et al., 2008, 2006). In contrast, these impacts can favour autotrophs and increase the nutritional quality of basal resources (Warry et al., 2016). Analysing community trophic structure is one way to assess the nature and magnitude of human impacts (Hussey et al., 2014). Additionally, trophic niches, which describe the overall trophic role of species within an ecosystem, including all the trophic interactions, and it is often realised as the dietary resource base of consumers (Layman et al., 2007a; Leibold, 1995; Warry et al., 2016), respond quickly to modification of basal resources and biotic interactions (Bearhop et al., 2004; Evangelista et al., 2014; Warry et al., 2016). These niches therefore provide insights into the functional effects of nutrient loading in aquatic ecosystems.

Stable isotope analysis (SIA) is one of the primary tools used to examine the structure and dynamics of food webs (Layman et al., 2012) and may represent a unifying methodology with which to compare anthropogenic pressures among different coastal ecosystems (Mancinelli and Vizzini, 2015). SIA provide time- and space-integrated information on the trophic interactions of species (Layman et al., 2007a) in disturbed, undisturbed or restored ecosystems (Nigro et al., 2017). An example of SIA applicability is the assessment of the effects of invasive species on the trophic structure of native communities. It can be useful to analyse both direct predatory behaviour and indirect impacts on local food webs and to predict potential spread by comparing trophic niche metrics with those of the native species (Alomar et al., 2016; Mancinelli and Vizzini, 2015). Moreover, stable isotopes is used to track the source of nutrients in a food web, to characterize the trophic niche of species (isotopic niche (Bearhop et al., 2004; Layman et al., 2007a)). In this context, analysis of $\delta^{15}N$ and $\delta^{13}C$ stable isotope ratios is frequently used in estuarine systems to assess nutrient pollution, organic matter origin and trophic interactions (Baeta et al., 2017; Baker et al., 2013; França et al., 2011; Morris et al., 2015). $\delta^{13}C$ is typically used to determine the origin of carbon sources (Post, 2002). $\delta^{15}N$ values allow the study of trophic levels of consumers (Post, 2002), and the enriched nitrogen isotopic composition of biota can be an indicator of anthropogenic wastewater (Baeta et al., 2017; Watson et al., 2018). However, nutrient contamination studies that include multiple taxa and different trophic levels in the food webs are not common (Connolly et al., 2013).

Another approach based on SIA used to quantitatively characterize the community trophic niche aspects of food webs includes the “Layman metrics” (Layman et al., 2007a). As Layman *et al.* (2007)

proposed, these metrics provide an integrated estimate of multiple anthropogenic-related impacts on food webs, including parameters such as trophic diversity or food web stability and trophic resilience (Alomar et al., 2016; Layman et al., 2012, 2007a; Mancinelli and Vizzini, 2015). While these metrics have been applied in marine ecosystems, their use in estuarine systems remains limited. As Mitchell et al. (2015) suggested, more studies are needed to assess the implications of this approach as a monitoring and management tool.

In this study, we used SIA to contrast the food web structure of two Iberian estuaries exposed to different degrees of urban and agricultural perturbations. First, we hypothesized that the higher nitrogen isotope values of focal species would reflect greater anthropogenic pressures within the estuary. Second, we hypothesized that human impacts in the more impacted estuary would result in a more homogeneous basal resource pool. Third, we predicted a more complex food web in the less impacted estuary.

Study systems

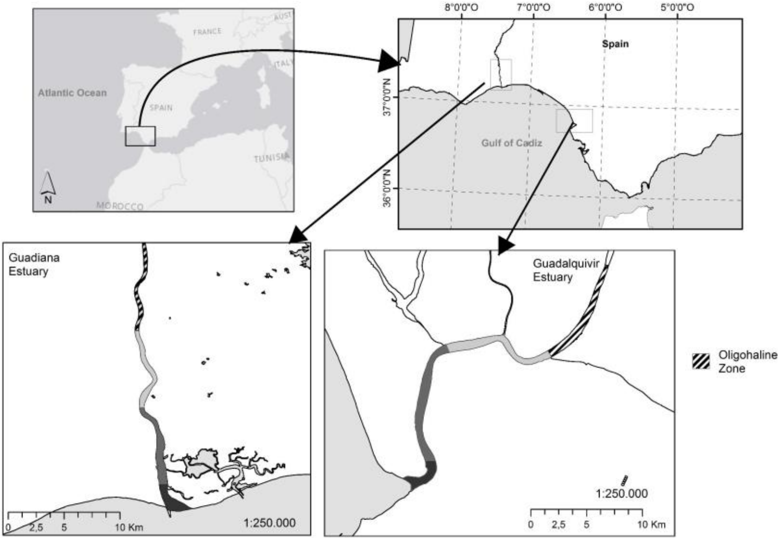

Figure 1. Sampling locations in the oligohaline zone of the Guadiana estuary (left) and Guadalquivir estuary (right). The salinity gradient of the estuaries is provided in grey scale from the euhaline zone (black) to the oligohaline zone (hatched area). The maximum turbidity zone is within the oligohaline zone.

The Guadiana and Guadalquivir Rivers are the largest rivers in the southern Iberian Peninsula. The estuaries of these two rivers are located in the Mediterranean climate region, and both flow to the Gulf of Cadiz on the Atlantic coast (Fig. 1). Both estuaries have hydrological regimes regulated by dams. The flow is low in summer, with episodic freshwater runoff in winter (González-Ortegón et al., 2014; Wolanski et al., 2006). The estuaries are mesotidal and vertically well mixed with a longitudinal salinity gradient. Despite the similarities of these estuaries, they have not been subjected to the same level of disturbances over recent years.

The Guadalquivir estuary is an example of a highly impacted estuarine environment. It crosses extensive rural areas and has been exposed to increasing human activity (Ruiz et al., 2013). Such activity includes desiccation of tidal marshes, isolation of the estuary course from the original tidal marshes, a reduction in freshwater inputs, and eutrophication from urban and agricultural wastes due to continual dredging work (Llope, 2017; Taglialatela et al., 2014). All these impacts have caused the Guadalquivir estuary to be characterized by high turbidity levels and increased nutrient loadings (Díez-Minguito, 2012; Prieto et al., 2009).

In contrast, the Guadiana estuary is characterized by relatively lower anthropogenic pressures (Morais, 2008; Sánchez-Moyano et al., 2017). Although it is also influenced by agriculture, agroindustrial activities, and dams (Barbosa et al., 2009; Sánchez-Moyano and García-Asencio, 2011), this estuary has been catalogued as one of the least polluted European estuaries (Sánchez-Moyano and García-Asencio, 2011; Vasconcelos et al., 2007). Furthermore, it is considered one of the best preserved and most vulnerable estuaries of the Iberian Peninsula (Barbosa et al., 2009). Comparative studies of both rivers have found concentrations of N one order of magnitude higher in the Guadalquivir estuary than in the Guadiana estuary associated with the influence of agricultural runoff in the waters (González-ortegón et al., 2019), as well as, twice as high pollution in modern sediments of the Guadalquivir estuary (Hanebuth et al., 2018). Although there are differences in human pressures between the estuaries, their biological communities contain a very similar set of species (Miró et al., 2018). For this reason, the Guadiana estuary has been used as a reference area in other biological studies (Sánchez-Moyano et al., 2017). To understand human impacts on each estuary, analysis carried out in summer 2017 showed that in the Guadalquivir estuary, ammonia ranged from 0.11 to 0.13 mg/L (0.12 ± 0.012 mg/L mean ± SD), and nitrate ranged from 1.33 to 3.73 mg/L (2.68 ± 1.23 mg/L mean ± SD), while in the Guadiana estuary, the levels were under the detection limits (0.05 mg/L for ammonia and 0.15 mg/L for nitrate). Turbidity in the Guadalquivir estuary is significantly higher than that in the Guadiana estuary (316± 94 NTU (Nephelometric Turbidity Unit) in the Guadalquivir estuary, while turbidity in the Guadiana estuary was 80 ± 31.21 NTU).

Methods

Sampling

To eliminate any seasonal bias, sampling was carried out in summer 2017. Sampling was performed in the oligohaline zone of the estuaries to avoid marine influences on species isotopic niche breadth and assemblage architecture (Warry et al., 2016). Several samples of the planktonic community were collected along the oligohaline zone to characterize the possible variation within this area. Samples were collected with a zooplankton net with a 1-m mouth diameter and 1000-µm mesh size. Twelve oblique tows were performed from the surface to the bottom during flood tide in the main channel at a constant speed of 2 knots. Parallel, copepods were collected with a bongo net with a 200-µm mesh size following the same process. Samples of soft bottom community were collected with a van Veen grab (0.05 m^2) although only the clam *C. fluminea*, found in the Guadiana estuary, had enough biomass for isotopic characterization. All organisms were sorted by species, and juvenile anchovies (*Engraulis encrasicolus*) were sorted into three size classes: large, juveniles of 34.2 to 43 mm; medium, postlarvae of 27.8 to 31.5 mm; and small, postlarvae of 18.5 to 25.1 mm. The organisms were transferred to the laboratory in refrigerated containers and kept alive for 24 h to allow stomach evacuation to avoid any possible interference with the isotope signatures of their prey.

Permission to gather the samples was obtained from the local authority "Consejeria de Medio Ambiente de Andalucía". There are no ethical concerns associated with our study based on Directive 2010/63/UE and order ECC/566/2015. Primary producers were sampled by first sieving the sample collected with the bongo net through a sieving column and then selecting the vegetal matter under the stereoscopic microscope. Three sediment organic matter samples were collected with the van Veen grab from the uppermost 2 cm of the sediment. Other possible primary producers were extracted from the literature (see supplementary information).

Isotope analyses

We rinsed animal and plant samples with distilled water. Muscle tissue samples of fish, clams and shrimp abdomens were used for isotopic analysis. Multiple organisms (>50) were pooled when the individuals had low biomass values (Supplementary Table S10). Samples were dried at 60 °C and ground to a powder. Sediment samples were acidified with 0.1 M HCl to remove carbonates and then oven dried. Organismal tissues were not acidified to avoid alterations in isotopic values (Schlacher and Connolly, 2014). Subsamples of powdered materials were weighed to the nearest 0.3 µg and placed into tin capsules for $\delta^{13}C$ and $\delta^{15}N$ analysis. Isotope analyses were carried out at the Laboratorio de Isótopos Estables of the Estación Biológica de

Doñana (LIE-EBD, Spain; www.ebd.csic.es/lie/index.html). All samples were combusted at 1020 °C using a continuous flow isotope-ratio mass spectrometry system with a Flash HT Plus elemental analyser coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific, Bremen, Germany).

To investigate changes in trophic diversity within both estuaries, analysis of community niche space was performed using a novel Bayesian approach with the metrics proposed by Layman et al. (2007) for quantitative comparison of food webs (Jackson et al., 2011). This method returns a posterior distribution of estimates of the original metrics, which include the $\delta^{13}C$ range (CR), $\delta^{15}N$ range (NR), mean distance to the centroid (CD), mean nearest neighbour distance (M-NND) and SD of the M-NND (SD-NND). The Bayesian inference technique provides measures of uncertainty for these metrics reported as sampling error for the estimates of the means. Thus, the technique permits robust statistical comparisons to be made between communities independently of the number of groups within the communities (Abrantes et al., 2014; Jackson et al., 2011). Briefly, the CR is indicative of niche diversification at the base of food webs. The NR is a representation of the vertical structure of a food web, and larger ranges suggest more trophic levels and a greater degree of trophic diversity. The CD provides a measure of the average degree of trophic diversity within a food web. M-NND represents trophic redundancy, and food webs with species with similar trophic ecologies will show smaller values. Finally, SD-NND is a measure of the evenness of the food web, and large values suggest more diversification of trophic niches (see Layman et al., (2007a) for more details).

The total convex hull area (TA) and the standard ellipse area (SEAc) were also calculated (c indicates that the SEA was corrected for a small sample size). The two metrics were estimated as quantitative proxies of the isotopic niche width, although the SEA is less sensitive to outliers and sample size than the TA (Jackson et al., 2011). Differences in the SEAc between the communities were evaluated via Bayesian interference (SEA_B) according to (Jackson et al., 2011). All measures were calculated using the SIBER package in R.

Differences in the isotopic values between estuaries were investigated using two-way permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001). Each $\delta^{13}C$ and $\delta^{15}N$ isotope variable was analysed based on two fixed factors: estuaries (Guadalquivir (GDQ)/Guadiana (GDN)) and species. Posterior pairwise tests were used to test for differences between species in the estuaries. The Monte Carlo P-value was used instead when small unique values in the permutation distribution were available (<100) (Anderson et al., 2008). The tests were based on Euclidean distance matrices of the untransformed data using 9999 permutations. Statistical analyses were conducted using Primer v.6 and PERMANOVA (Primer-E Ltd., Plymouth, UK).

Results and discussion

Our results suggest that the higher human pressures found in the Guadalquivir estuary lead to more complex feeding pathways, as shown by a greater trophic niche width (-23.05 to -29.32 and 6.75 to 21.34 max and min values of $\delta^{13}C$ and $\delta^{15}N$ respectively in the Guadalquivir estuary while in the Guadiana they were -25.81 to -27.20 for $\delta^{13}C$ and 5.83 to 17.28 for $\delta^{15}N$) and by the greater variability in organism position within isotope niche space (Figs. 2 and 3). A greater distribution among species along the carbon axis suggests that primary consumers exploit organic matter of various origins, whereas the different nitrogen signals of secondary consumers suggest that they feed on different prey items (Fig. 2). There was greater intraspecific variability in isotope signals in this estuary, suggesting more variation in diet composition. In contrast, the similar isotope signals of secondary consumers in the Guadiana estuary suggest similarity in their diet composition, implying that they feed on the same organic matter sources.

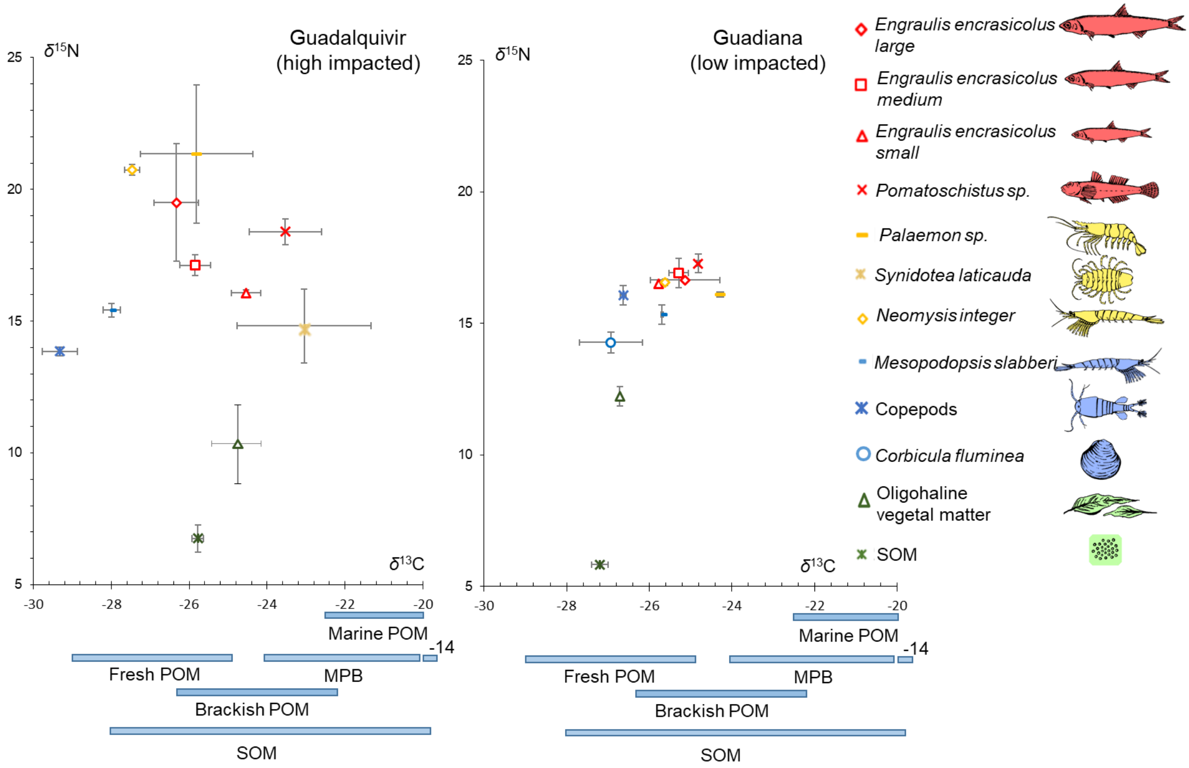
Guadalquivir
(high impacted)
Guadiana
(low impacted)
δ15N
δ13C
25
20
15
10
5
-30
-28
-26
-24
-22
-20
Marine POM
-14
Fresh POM
MPB
Brackish POM
SOM
Engraulis encrasicolus large
Engraulis encrasicolus medium
Engraulis encrasicolus small
Pomatoschistus sp.
Palaemon sp.
Synidotea laticauda
Neomysis integer
Mesopodopsis slabberi
Copepods
Corbicula fluminea
Oligohaline vegetal matter
SOM

Figure 2. $\delta^{13}C$ and $\delta^{15}N$ (mean± standard deviation) of the primary producers, invertebrates and fishes collected in the Guadalquivir (left) and Guadiana (right) estuaries in summer 2017. Horizontal bars below the x-axis represent the $\delta^{13}C$ ranges of primary producers extracted from the literature (Supplementary Table S9). POM: particulate organic matter, SOM: sedimentary organic matter, MPB: microphytobenthos (upper limit of MPB range, -14‰, is out of the axis limit). Figures of the different organisms are provided for a better understanding of the species. Colours indicated trophic position: Secondary consumers: red Primary and/or secondary consumers: yellow. Primary consumers: blue. Producers: green.

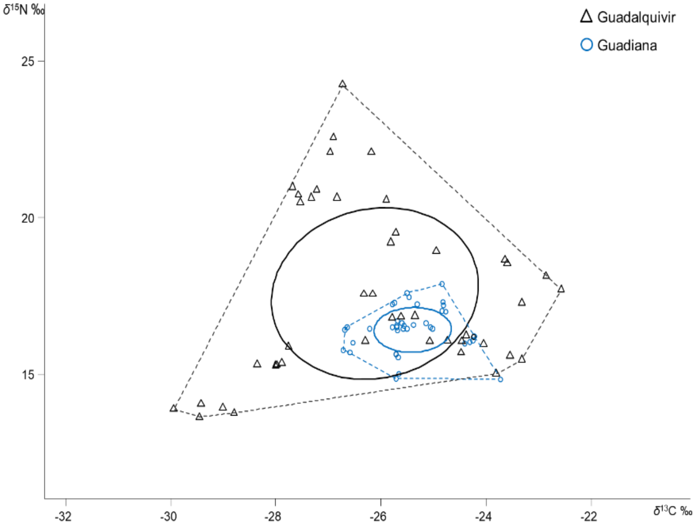

Figure 3. Trophic niche width according to the convex hull area (dotted lines) and standard ellipse areas corrected for a small sample size (SEAc) for the Guadalquivir (black lines) and Guadiana estuaries (blue lines). Triangles represent individuals of all the species measured in the Guadalquivir estuary, and circles represent species found in the Guadiana estuary.

The trophic structure of both estuarine communities, measured with the standard ellipse area (SEAc, where c indicates that the SEA was corrected for a small sample size) and the total area (TA), were distinct (Guadalquivir TA=41.03 vs Guadiana TA=5.74) (Fig. 3). The total overlap between the ellipses was the size of the Guadiana ellipse; it was much smaller and located inside the Guadalquivir SEAc (Guadiana SEAc=1.66 vs Guadalquivir SEAc=16.89, Fig. 3).

Furthermore, the probability that the Bayesian standard ellipse (SEA_B) value of Guadalquivir was larger than the SEA_B value of Guadiana was 100% (Supplementary Fig. S1). The community metrics (Layman et al., 2007a) also showed large differences between estuaries (Fig. 4). All indices were smaller in the Guadiana estuary than in the Guadalquivir estuary. Therefore, the smaller SEA and mean distance to the centroid (CD) in the Guadiana estuary suggest a more compact food web and lower trophic diversity than in the Guadalquivir (Abrantes et al., 2014). Trophic redundancy (low mean nearest neighbour distance (M-NND)) and its standard deviation (SD-NND) were also higher in the Guadiana estuary.

Individuals within populations can exhibit variation in their trophic niche (Evangelista et al., 2014). Variance in stable isotope values among individuals within populations can be used as a proxy of diet variation. This individual specialization is determined by biotic interactions such as predation and competition and by resource diversity (Araújo et al., 2011; Evangelista et al., 2014). Experimental and comparative studies suggested that while intraspecific competition increases individual specialization, interspecific competition reduces it (Araújo et al., 2011). In contrast, an increase in ecological opportunity, defined as the diversity of available resources, favours individual specialization (Araújo et al., 2011).. Although other factors can influence the intraspecific variability of SIA, such as the size of individuals (Chouvelon et al., 2014; Pasquaud et al., 2008), the higher diversity of resources in the Guadalquivir estuary seems to explain the higher intraspecific variability. Moreover, species that feed on more than one trophic level can also increase the intraspecific variability (Pasquaud et al., 2008), which is consistent with the higher trophic diversity found in the Guadalquivir estuary.

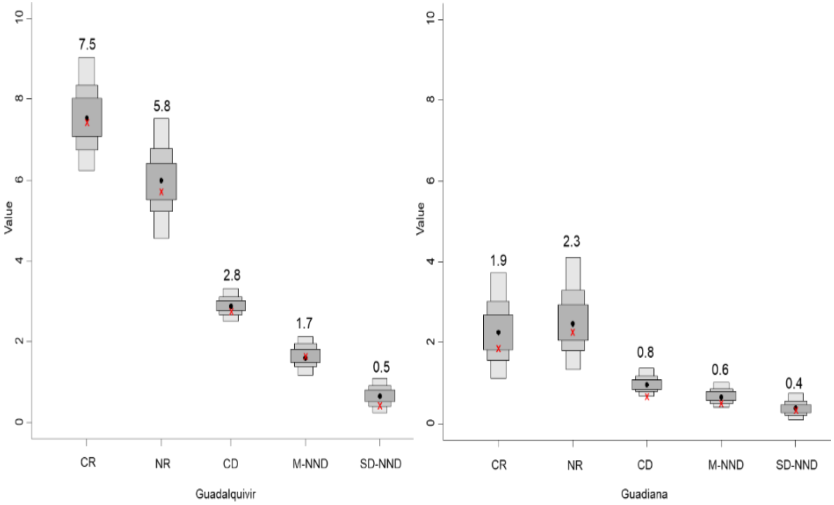

Figure 4. Bayesian results for the estuarine community-wide metrics that provide information on trophic diversity: carbon range (CR), nitrogen range (NR), mean distance to the centroid (CD) and trophic redundancy measured as the mean nearest neighbour distance (M-NND) and its standard deviation (SD-NND). Black dots are the modes, and boxes indicate the 50%, 75% and 95% credible intervals. The numbers above the red crosses represent the values of the crosses, which are the true population values.

Organisms in the Guadalquivir food web tended to have higher δ^{15}N values than those in the Guadiana food web, as reflected by the higher SEA position on the nitrogen axis of the Guadalquivir estuary. The high nitrogen range (NR) value in the Guadalquivir estuary suggests more trophic levels and more energy transfer to higher trophic levels (Cooper and Wissel, 2012), and could be explained by the higher nutrient load. The similarity of the δ^{15}N values of sedimentary organic matter (SOM) and vegetal matter in the two estuaries, in contrast to our hypothesis, suggests that this baseline variation does not drive the differences. However, the quick turnover rate of nitrogen in the primary producers could hide differences between estuaries in those resources since these values could be a snapshot of the temporal variation in the two systems (Van De Merwe et al., 2016). Organisms at higher trophic levels integrate the stable isotope values of primary producers over time in their tissues, which helps to capture potential variation in basal carbon isotope signatures (Van De Merwe et al., 2016). For this reason, the use of fish larvae and juveniles in planktonic communities is often a better long term indicator of water nitrogen content (Baeta et al., 2017; Hoffman et al., 2012).

Individual species, however, exhibited small differences between estuaries (Table 1). The variation was greatest in the large size class of the anchovy (*Engraulis encrasicolus)*, the mysid (*Neomysis integer*) and the shrimp (*Palaemon sp.)*. Differences in trophic position between estuaries of the mysid species *Neomysis integer* could be explained by the addition of an intermediate consumer to its diet or a change in its degree of trophic omnivory in the Guadalquivir estuary (Post and Takimoto, 2007). *N. integer* has been described as an opportunistic omnivore species that utilizes mesozooplankton and detritus as food sources and is able to feed on juveniles of the other mysid species such as *Mesopodopsis slabberi* (Vilas et al., 2008). In contrast, *M. slabberi*, which showed no difference between estuaries, feeds mostly on primary producers[56]. In the case of the anchovy *E. encrasicolus*, only the largest size class showed enriched $\delta^{15}N$ in the Guadalquivir estuary. This result could be explained by the addition of an intermediate consumer such as the juveniles of the invasive isopod *Synidotea laticauda* which were only found in the Guadalquivir estuary (Fig. 2) or a change in trophic omnivory. In the Guadalquivir estuary, *E. encrasicolus* larvae change their diet from copepods to mysids as they grow (Baldó and Drake, 2002), which could explain the $\delta^{15}N$ differences. Additionally, these differences could be explained in part by *E. encrasicolus* entering these two estuaries in early life history stages from the same spawning area (Baldó et al., 2006) and leaving in a later developmental period (Baldó and Drake, 2002; Drake et al., 2007). Consequently, the larger individuals of this species would feed longer on enriched sources in the Guadalquivir and would show larger differences with respect to the Guadiana than would the smaller individuals.

Table 1. $\delta^{13}C$ and $\delta^{15}N$ means (standard deviations) per species in the two estuaries and PERMANOVA pairwise results (t) of the comparison of each species between the two estuaries (* and ** indicate significant differences of species in their isotopic signal, where *$p<0.05$ and **$p<0.01$). Trophic position: producer (P), primary consumer (C1), and secondary consumer (C2).

	Organisms Type	**Guadalquivir**	**Guadiana**		**Guadalquivir**	**Guadiana**	
Species		**$\delta^{13}C$**	**$\delta^{13}C$**	**t**	**$\delta^{15}N$**	**$\delta^{15}N$**	**t**
***Engraulis encrasicolus* large**	C2	-26.33 (0.57)	-25.14 (0.84)	2.61*	19.5 (2.23)	16.64 (1.05)	2.6*
Engraulis encrasicolus medium	C2	-25.85 (0.4)	-25.78 (0.27)	2.75*	17.12 (0.4)	16.49 (0.04)	0.68
Engraulis encrasicolus small	C2	-24.54 (0.39)	-25.29 (0.23)	5.92**	16.07 (0.1)	16.92 (0.56)	8.52**
Pomatoschistus sp.	C2	-23.53 (0.92)	-24.82 (0.03)	3.13*	18.39 (0.48)	17.28 (0.36)	4.15**
Palaemon sp.	C2/C1	-25.81 (1.45)	-24.29 (0.07)	2.34*	21.34 (2.62)	16.1 (0.1)	4.46**
Synidotea laticauda	C2/C1	-23.05 (1.72)			14.82 (1.4)		
Neomysis integer	C2/C1	-27.46 (0.19)	-25.62 (0.06)	20.62**	20.74 (0.2)	16.55 (0.12)	40.34**
Mesopodopsis slabberi	C1	-27.99 (0.22)	-25.69 (0.03)	22.84**	15.42 (0.26)	15.34 (0.37)	0.41
Copepods	C1	-29.32 (0.44)	-26.63 (0.07)	13.39**	13.85 (0.16)	16.07 (0.37)	12.26**
Corbicula fluminea	C1		-26.93 (0.76)			14.28 (0.39)	
Oligohaline vegetal matter	P	-24.89 (0.60)	-26.72 (0.02)	8.98**	10.25 (2.53)	12.22 (0.37)	1.92
SOM	P	-25.78 (0.05)	-27.2 (0.06)	28.96**	6.75 (0.52)	5.83 (0.06)	3.32

In the Guadiana estuary, the $\delta^{13}C$ values of all organisms were similar, as indicated by the low carbon range (CR) and high redundancy values. These similar values could indicate the use of freshwater or brackish water particulate organic matter (POM), rather than marine inputs, as a carbon source; the latter would have more enriched carbon values. The similar isotope signatures between consumers suggest a more confined suite of prey resources than in the Guadalquivir estuary; these prey resources potentially include copepods and mysids (*Mesopodopsis slabberi*). Potential high trophic redundancy in this estuary showed by the Layman's metrics could indicate a higher capability of species to play similar trophic roles and could support resistance to disturbances without the loss of connectivity in the food web (Vinagre et al., 2010). However, pairing this information with stomach content analysis is necessary to truly assess trophic redundancy (Matich et al., 2017). Moreover, the small number of links between primary consumers and secondary consumers may lead to an increase in fragility (Vinagre et al., 2010). Niche width collapse and homogenization in the energy flow pathway have been described in fragmented systems, resulting in a less stable food web structure (Layman et al., 2007b). Low SEA values and low trophic diversity in estuarine fish food webs have been related to the low availability of aquatic producers as a consequence of the high level of suspended solids, which would limit primary production, which is not the case for

Guadiana (Abrantes et al., 2014). The simplification of the complex food web is also related to an increase in the vulnerability to environmental changes that can affect productivity and secondary extinctions (Careddu et al., 2017).

Conversely, in the Guadalquivir estuary, consumers showed greater differences in their carbon stable isotope signals, a greater niche width, more trophic diversity and lower redundancy values. These differences could indicate that the organic matter sources are different (Dias et al., 2017). Thus, the Guadalquivir estuary would have a relatively more reticulated food web with multiple trophic pathways towards upper-level consumers.

Since the high turbidity present in the Guadalquivir estuary limits primary production, the allochthonous organic matter inputs in the oligohaline zone may be an important basal source. This detritus contribution can compensate limited phytoplankton production in highly turbid estuaries (David et al., 2006; Kudryavtsev and Subbotovskaya, 2015). In the maximum turbidity zone of estuaries (MTZ), primary production was primarily bacterial, fed by detrital terrestrial and estuarine organic matter(David et al., 2006; Islam and Tanaka, 2006). In the MTZ sediment, where organic matter aggregates by flocculation (Kudryavtsev and Subbotovskaya, 2015), particles act as substrates for microorganisms that serve as prey for protozoa and other microorganisms (Artolozaga et al., 2002); the detrital energy is thus transferred to copepods and can also act as a food source for mysids (Kudryavtsev and Subbotovskaya, 2015). The high abundance of copepods and mysids (David et al., 2006; Islam and Tanaka, 2006) is supported by energy from detrital sources, which differs from nutritionally poor systems, in which food webs are based on algae in lower-turbidity areas (Islam and Tanaka, 2006). This is also the case for the Guadalquivir estuary, in which the MTZ is located within the oligohaline zone (Vilas et al., 2008). In this estuary, the high biomass of copepods and mysids who feed on these detrital sources would support fish larvae and other crustaceans (De Carvalho-Souza et al., 2018; Vilas et al., 2009). This finding agrees with those of other studies that have found that the turbidity maximum zone is a significant nursery area that positively influences fish growth and condition (Escalas et al., 2015; Islam and Tanaka, 2006). In addition, a positive relationship between the number of organic matter basal sources and fish production (Hoffman et al., 2015) has been suggested.

These results could also explain the differences between estuaries in the mysid species; when detritus is present, *M. slabberi* shows a detritivorous/herbivorous tendency, and *N. integer* exhibits omnivorous behaviour with a carnivorous feeding tendency (David et al., 2006). These authors described a 2- or 3-stage route from bacteria and vegetal matter to copepods. This route agrees with the high isotopic signatures of nitrogen of some species in the Guadalquivir estuary. In contrast, *M. slabberi* probably directly feed on detritus or phytoplankton, which would explain the lack of differences between estuaries in terms of the trophic enrichment factors.

The greater trophic niche width of the planktonic community, as well as the higher trophic diversity, could thus be explained by the different organic matter sources that are present in the Guadalquivir estuary (Post, 2002). Furthermore, another possible organic matter source would be linked to microphytobenthos in Guadalquivir mudflats. This particular organic matter source has been reported in other estuaries as one of the principal basal sources for the pelagic food web (David et al., 2016). In contrast, the lower turbidity in the Guadiana estuary would permit higher phytoplankton primary production in the water column, which would be the base for copepods and *M. slabberi*, in turn sustaining all the secondary consumers. The smaller mudflats would also limit the contribution of microphytobenthos to the food web, but the overlapping values of carbon isotopes in the basal resources make it difficult to identify the main resources. Therefore, these conclusions are a first overview of organic matter origins for this two estuarine food webs.

The species found in each estuary were a good representation of the native planktonic macrofaunal communities. The same species were found in both estuaries except of the invasive isopod *S. laticauda* mentioned before (found in the Guadalquivir estuary) and the invasive clam *Corbicula fluminea* (found in the Guadiana estuary) (Cuesta et al., 1996; Pérez-Quintero, 2008; Ruiz-delgado et al., 2016). Stable isotope studies assessing the effects of invasive species have been more frequently used in terrestrial and freshwater systems (Mancinelli and Vizzini, 2014). However, recent studies have applied stable isotopes to assess the effects of invasive species on marine ecosystems (Mancinelli and Vizzini, 2015). For example, the trophic niche of the benthic food web was wider in sites invaded by the macroalgae *Caulerpa cylindracea* than in non-invaded sites due to an increase in the diversity of basal resource pools (Alomar et al., 2016). In contrast, another study found a compacted food web structure in *Caulerpa prolifera* meadows (Deudero et al., 2014). There is a large degree of overlap in the utilization of basal sources, which is related to intra- and interspecific competition (Deudero et al., 2014) and is characteristic of degraded systems (Layman et al., 2007b). In this sense, our results showed that the invasive species *S. laticauda* may have an effect on the food web in the Guadalquivir estuary. First, this organism has isotopic signatures similar to those found in small anchovies (*E. encrasicolus*), suggesting that they are potential competitors. This potential risk caused by an invasive species has been also suggested (Carrozzo et al., 2014). Second, small individuals of this isopod could act as prey and could explain higher nitrogen signatures found in secondary consumers in the

Guadalquivir. Additionally, this species showed the most enriched carbon signatures (Fig. 2), suggesting that it feeds on microphytobenthos and/or more marine basal sources than other species, which would explain the higher trophic diversity of the Guadalquivir estuary. In contrast, the impacts of *C. fluminea*, the invasive species found in the Guadiana estuary, do not appear to be readily visible in this study, and further investigation into the impacts of this species on planktonic food webs is certainly warranted.

Although our results show that the isotopic signal of nitrogen in the planktonic community generally seems to reflect the higher anthropogenic pressure present in the Guadalquivir estuary (Table 1), other factors could contribute to these differences. In contrast to our hypothesis and previous related research (Rooney et al., 2008), even though the Guadalquivir estuary has higher human pressure, the food web is more complex, with more feeding pathways, a greater niche width, more trophic diversity and lower trophic redundancy than the Guadiana estuary. The different organic matter sources present in the Guadalquivir estuary and the detrital processes in the MTZ could explain these distinctions. These results could be related to the higher nitrogen loads in the Guadalquivir estuary, which would have a positive effect on food web consumers by improving the nutritional quality and palatability of basal resources (Cebrian et al., 2009). This finding also agrees with Warry *et al.* (2016) that found higher trophic diversity and less redundancy in fish food webs in estuaries with high nitrogen loads and suggested that the same pattern may be found in systems where the nitrogen loads are high and there is not a single dominant organic source.

Conclusions

This study concludes that the Bayesian approach to the "Layman metrics" (Layman et al., 2007a) is a useful tool with which to detect ecological differences in food webs between estuaries under different human pressures, as has been demonstrated in other studies (Abrantes et al., 2014). Furthermore, stable isotope analysis revealed differences in the trophic interactions of species that were present in both estuaries, which is important information which complements traditional species surveys (Nigro et al., 2017) by providing key, additional ecological information about differences between these estuaries. Because this study is a comparison made during the summer season, the results obtained must be considered carefully, keeping in mind that the conclusions obtained are applicable to the oligohaline zone. Nevertheless, the important trophic differences observed between these two systems allow us to extract some conclusions and also to point out some characteristics that would be worth being further investigated. Thus, more extensive research on the spatial and temporal variability of the origin of basal resources, as well as the bottom-up interactions of estuarine food webs and their relationships with other environmental factors, is needed to better understand the food web dynamics in both systems. Understanding the trophic interactions present in estuaries with a strong human presence is crucially important for defining proper management and conservation strategies (Vermeiren et al., 2015). Furthermore, the management of factors that influence an estuary, such as freshwater discharges and urban and agricultural wastes, can regulate inputs of basal resources and modulate a phytoplankton or detrital dominated food web.

One important environmental implication of this research is that, even under altered conditions, the community of the Guadalquivir estuary seems to have reached a comparatively complex structure, which ensures a high productivity and some important ecosystem services such as the nursery function (Drake et al., 2007; Fernández-Delgado et al., 2007). A word of caution should be included here for the future environmental management of this estuary since, any change, even with the objective to improve the environmental quality, should be done slowly and closely monitored. For instance, the high and permanent turbidity in this estuary is a present concern and its reduction is a commonly claimed objective (González-Ortegón et al., 2010; Ruiz et al., 2015). However, the possibility that introduced sediment could be partly associated with the main sources of carbon for the community (allochthonous organic matter), makes it advisable to proceed with any potential restoration measure with caution, since any abrupt change in the present equilibrium would probably affect the nursery function and the fisheries production in the nearby marine areas. Assessing trophic structure and its relationships with other factors is crucial for understanding the consequences of increasing human pressure on estuaries.

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General discussion

In this thesis, we assessed the effects of human pressures in a highly stressed estuary, namely the Guadalquivir. Although dredging impacts on estuaries have been widely studied, assessing impacts on different salinity ranges is less common. Similarly, there are few studies assessing impacts of dredged material disposal in recurrent and ongoing dumping areas. Moreover, the site-specific characteristics of these impacts made it necessary to carry out studies to improve the knowledge of these impacts in estuaries and coastal habitats. Additionally, to our knowledge, stable isotopes have never been used to characterise impacts on the trophic structure of dredging and disposal disturbance.

Specifically, we assess the effects of maintenance dredging works performed in the Guadalquivir estuary in summer 2015 (chapter 1) and the effects of the subsequent disposal of the dredged material in a recurrent marine dump used since 2010 (chapter 2 and 3). Finally, we analysed weather human impacts in the Guadalquivir are reflected in the food web structure of the Guadalquivir estuary by contrasting it with a reference estuary, namely the Guadiana (chapter 4).

To obtain a clearer picture of the true contributions of the present thesis, a global analysis relating the different results obtained in each chapter is discussed here.

Effects of dredging and disposal on sediments

In the Guadalquivir estuary, the dredging operations did not show evident effects on the physicochemical characteristics of the sediment and water column in the estuary and barely affected the biological communities. This lack of evident effects was observed in both salinity ranges studied (oligohaline and polyhaline) as well as in both directly affected subtidal areas and indirectly affected shallower areas. This could be explained because estuaries with high sediment loads would be filled rapidly with fluvial sediments or marine sediments (Chapman and Wang, 2001). Moreover, the poor status of the benthic communities did not allow a clear detection of an impact.

In contrast, the recurrent disposals in the authorised marine dumping area have permanently changed the sea bottom into a coarser sediment with lower particulate organic matter and lower heavy metal concentrations than the surrounding control areas. In spite of the significant increase in the concentration of some trace metals in the disposal area after the last disposal event (which seemed to be paired with an increase in the toxicity of the sediment), levels of those metals were never higher than in the control areas. This pattern can probably be explained by the fact that the Guadalquivir estuary is one of the major providers of trace metals in the Gulf of Cadiz (González-Ortegón et al., 2019). Moreover, trace metals show affinity for mud particles and are accumulated where fine-grained sediments are present (George et al., 2007). The permanent changes observed in the sediment granulometry of the disposal area before the last disposal of 2015 indicated depositions of coarser material coming from the more external parts of the estuary, which are more exposed to receiving sandy sediment from the sea bottom with the tidal currents (Allen et al., 1980; Chapman and Wang, 2001). Therefore, dredging operations do not seem to affect the granulometry while disposal does. In Spain, legislation suggests that disposal areas should have similar granulometric characteristics to that of the dredged material extracted (Buceta et al., 2015). This requirement has been obviated in this area in the past, thus it is important that the administration controls the management of dredged sediments to minimise impacts in open sea disposals.

Effects of dredging and disposal on benthic communities: structure and trophic pathways

Biological communities showed the same patterns as the sediment in the estuary and the disposal area. Before the dredging, the macrofaunal community of the Guadalquivir showed a poor status in both salinity ranges and we only detected a small impact in the polyhaline zone in the disposal area after dredging. In contrast, in the marine dump we detected a significant, more diverse and rich macrofaunal community compared to the controls. This was composed of a mixture of local sandy species and muddy species probably translocated from inside the estuary. Hence, the increase in the structural complexity of the sediment due to the addition of coarser sediment than the surrounding habitats probably favoured this high diversity and abundance (Carvalho et al., 2017).

The last disposal event in summer 2015 did not lead to a permanent change from those differences found previously. Statistical analyses reveal that the community in the disposal area did not show temporal variations over the months sampled. Thus, the natural increase observed in the abundance of organisms in the control areas, especially the tanaid *Apseudopsis latreillii*, was not observed in the disposal area. This could be related to this last event with disturbances in the physicochemical environment in this area through the increase in metal concentration, toxicity and other factors. Different chemical contents in disposed dredge sediments could impact the macrofaunal community, weakening their survival, reproduction and recruitment processes, due to the mobility and bioavailability of toxic heavy metals (Guerra et al., 2009; Katsiaras et al., 2015). However, we cannot discard the possibility that the recurrent disposals also influenced the temporal variation in this area.

Analysis of stable isotope signatures of organisms on the different sampling dates in both disturbance operations also did not reveal any clear patterns. Although some changes were observed in the isotopic signatures of some organisms inside the estuary, this seemed to be more related to natural variation rather than dredging impacts. However, stable isotopes revealed differences in carbon sources in the two salinity ranges. Moreover, more variation between isotope signatures of consumers in the oligohaline range suggested more complex trophic interactions than in the polyhaline range. In the disposal area, the isotope signal of the sediment seemed to be modified in comparison to control areas and the increase in the nitrogen signal of only one species could be related to the remobilisation of nutrients because of the disposal (Sampaio et al., 2010). However, since these increases were not detected in the basal sources of the food web, we are not able to tie this pattern to the disposal.

Despite a significant increase in metal concentrations in the disposal area after the disposal of summer 2015, we were not able to tie this human disturbance to the bioaccumulation found in this area. Since we also found bioaccumulation patterns in control areas, it seems that the principal provider of these metals is the natural transport from the

Guadalquivir (González-Ortegón et al., 2019). Metal concentrations in organisms sampled in relation to their trophic position also did not show patterns that could be related to the disposal operations. However, we have to consider these results with caution because of the low numbers of species sampled and the lack of samples of all trophic positions on all the sampling dates. Sampling sufficient biomass to perform metal content analysis in macrofaunal organisms is sometimes difficult due to their small size.

Our results indicated that stable isotope measures in organisms did not clearly reveal any impacts on the structure of food web in either dredging or disposal areas. Nevertheless, we think this is a promising tool to assess human impacts across food webs (Mancinelli and Vizzini, 2015), since the site-specific character of these impacts make it necessary to apply stable isotopes in other dredging and disposal events worldwide. Moreover, the use of stable isotopes in species with different turnover rates could be useful to assess impacts at both short- and long-term temporal scales (Van De Merwe et al., 2016).

Recovery patterns after dredging and disposal

After the disturbances, both dredging and disposal areas showed different recovery patterns. Few effects found in the polyhaline range of the Guadalquivir were recovered one year after dredging. In the dynamic bottom of estuaries as well as in other muddy bottoms, recovery patterns are usually faster than in sandy bottoms (Gutperlet et al., 2015; Pezy et al., 2017; Rehitha et al., 2017). Estuarine species also showed more resilience to perturbations since they are more physiologically adapted compared to those in more stable environments (Elliott and Whitfield, 2011). In the oligohaline zone, there was low richness, principally characterised by high abundances of opportunistic polychaetes such as *Streblospio shrubsolii* and *Alkmaria romijni*. Therefore, these r-strategist species could rapidly colonise the dredged areas from undisturbed areas (Bemvenuti et al., 2005 and cites therein). In the disposal area, the recovery pattern seemed to be related to the settlement of juveniles, the migration of organisms from the edges (Bolam and Rees, 2003; Munari and Mistri, 2014) and the survival of some organisms that were able to burrow through the sediment (Powilleit et al., 2009). However, the continuous disposals seemed prevent the community from converging with nearby natural areas, in agreement with other disposal studies (OSPAR, 2008).

As has been assessed in many dredging and disposal studies, these perturbations and the subsequent recovery are “site-specific” (Bemvenuti et al., 2005; Bolam et al., 2006; Collier et al., 2014; Fraser et al., 2006; Gutperlet et al., 2015; Munari and Mistri, 2014; Newell et al., 1998; OSPAR, 2008; Roberts and Forrest, 1999; Ware et al., 2010). The poor benthic community status in both salinity ranges in the Guadalquivir estuary explains the absence of a detectable effect on the community structure, diversity and richness and the quick recovery of the punctual effects by recolonisation of organisms from nearby areas. As other authors have pointed out, it is difficult to assess human impacts in systems where anthropogenic and natural stresses act together (Pezy et al., 2017). This concept has been called the 'Estuarine Quality Paradox'(Elliott and Quintino, 2007) and has been reported in other studies (Dauvin and Ruellet, 2009; Dauvin, 2007; Pezy et al., 2017). On the contrary, in stable environments such as where the disposal area is located, changes in the physicochemical characteristics of the sediment through several disposal events have led to a permanent modification of the benthic macrofaunal community.

Trophic structure differences between estuaries with different human pressures

Since stable isotopes have become an important tool to analyse human pressures in food webs (Mancinelli and Vizzini, 2015), we applied this technique to compare the food web of the oligohaline in the Guadalquivir estuary with the Guadiana estuary, which is considered one of the less polluted estuaries in the Iberian Peninsula (Vasconcelos et al., 2007) and has been used as a reference estuary in other studies (Sánchez-Moyano et al., 2017). In contrast to our hypothesis, the Guadalquivir estuary food web showed more complex feeding pathways, greater spread among nitrogen and carbon axes and more intraspecific trophic variability. This suggested that organisms in the oligohaline zone of the Guadalquivir estuary exploited organic matter of different origins and fed on a different suite of prey compared to the Guadiana oligohaline zone. Thus, this study revealed that the use of trophic structure and other trophic metrics (Layman et al., 2007) is a powerful tool to assess differences between food webs under different human pressures.

The trophic ecology results from the Guadalquivir estuary contrast with the poor benthic community found in the oligohaline area in the dredging studies. Thus, this study highlighted the need to fully understand the functioning of estuarine ecosystems to define proper management strategies. The altered condition of the Guadalquivir estuary, which is reflected in the benthic communities, was not reflected in the food web. On the contrary, this system showed comparatively complex food web structure, which would support the high productivity and nursery function of this estuary (Baldó and Drake, 2002; González-Ortegón et al., 2006).

Perspectives: research and management

We suggest that using measures of stable isotopes of carbon and metal concentrations in the possible basal sources of the food web, as well as in several key species with high biomass and/or abundance in the possibly impacted area, is a good integrated approach if it is combined with classical ecological analysis of the biological communities. Although the use of stable isotopes and metal concentration in organisms did not clearly indicate the effect of dredging and disposal disturbances, we think it is necessary to implement this technique in other impact studies worldwide because the effects of dredging and disposal are highly site-specific. We suggest monitoring possible changes in metal concentrations in both sediment and organisms and their relationship to the food web in bioaccumulation and biomagnification processes on both short- and long-term scales.

The results of this thesis have revealed that the benthic communities of the Guadalquivir estuary have low diversity and richness. On the other hand, the complex food web found in this estuary compared to the Guadiana estuary make it necessary to maintain a monitoring program controlling all the components of this estuarine environment to assess the dynamics and their relationships to this stressful natural and anthropogenic scenario.

Dredging has caused no or little perturbations inside the Guadalquivir estuary in both salinity ranges. However, we have no information from the very initial stages right after the dredging activity. We would like to study the first impacts of this disturbance and how the macrofauna respond in the early stages after dredging. Moreover, it would also be interesting and useful to assess how organisms are able to recover in the first days after the impact.

There is a lack of information about the mechanisms of response found in disposal studies and their ecological consequences (Bolam et al., 2015). Thus, it would be interesting to examine whether the structural shifts found in the communities of the disposal area are reflected in the functionality of the ecosystem.

In this thesis, we found that the Guadalquivir estuary seems to harbour more organic matter sources than a priori a less impacted estuary. Since the detritus food web could be an important carbon source, as well as the microphytobenthos (at least in the oligohaline zone), it would be interesting to assess temporal and spatial variations of the basal sources of the food web in the whole estuary. Moreover, a quantitative assessment of the proportion of the diet of the key species found in the estuary would elucidate the key sources accounting for the high productivity of this system.

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General conclusions

1. The poor benthic community status in the oligohaline range in the Guadalquivir estuary explains the absence of a detectable effect of dredging activities either on the community structure or in the diversity and richness indices. This status would also explain the quick recovery of the punctual effects on the polyhaline range by recolonisation of organisms from nearby areas. Thus, research efforts should focus on the most diverse areas, such as the polyhaline range.

2. In the marine dump, where a disposal operation has occurred periodically since 2010, there have been changes that have affected biological and sediment characteristics permanently. This area showed higher richness and diversity indices compared to nearby reference areas. Moreover, the disposal area showed a loss of the natural seasonality which may have been caused by these historical impacts, as well as by the most recent disposal event. Shifts in the sediment of the marine dump showed coarser granulometry and lower organic matter content and trace metal concentrations compared to the reference areas.

3. The punctual disposal in the summer 2015 significantly increased the concentration of some trace metals, which remained at the same level one year after the deposition. This increase in heavy metal content was reflected in an increase in the toxicity of the sediment.

4. The same bioaccumulation patterns found in organisms in the disposal and control areas indicated an origin in the metals transported by the principal estuaries in the Gulf of Cádiz.

5. The unique relationship between the zinc concentration and the trophic position of the organisms in the disposal area was absent in the controls, which may indicate an impact requiring further research.

6. Dredging and disposal operations did not evidently affect the food web structure in either salinity ranges sampled. Moreover, only hints of an effect were found in the isotopic signature of the sediment and in a tanaid *Apseudopsis latreillii* in the disposal area.

7. Our results confirm the high variability of impacts due to disposal operations and reconfirm the ‘site-specific’ character of these perturbations. Thus, impact studies are needed in every

disposal operation work across temporal and spatial scales. Moreover, to minimise the impact on soft-bottom communities, dredging and disposal operations should avoid the main reproduction and recruitment periods and be developed, where technically possible, in the winter months.

8. Classic approaches and new techniques are required to fully assess the effects of these anthropogenic impacts at different levels in macrofaunal soft-bottom communities.

9. Guadalquivir estuary food web showed more complex feeding pathways, greater spread among nitrogen and carbon axes and more intraspecific trophic variability than the Guadiana estuary. This suggested that organisms in the oligohaline zone of the Guadalquivir estuary exploited organic matter with different origins and fed on a different suite of prey compared to the Guadiana oligohaline zone.

10. Similar isotopic signals of secondary consumers in the relatively little influenced estuary (Guadiana) suggested similarity in diet composition and feeding on the same organic matter sources.

11. A Bayesian approach to the "Layman metrics" is useful tool to detect ecological differences in food webs between estuaries under different human pressures.

12. The actual pressures on the Guadalquivir estuary, beyond the maintenance dredging work (e.g., unnatural freshwater inputs in summer for rice agriculture, permanent turbidity and high regulation of the natural flow by upstream dams), does not permit the establishment of more complex communities. Thus, a proper management plan involving all administrations is necessary for the improvement of the benthic communities of the Guadalquivir estuary

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Supplementary data

Table S1. Means of water and sediment parameter over the sampling dates in both salinity ranges. (CC: Control channel, CS: Control shallow, DC: Dredging channel, DS: Dredging shallow, SUOM: Suspended organic matter, TSS: Total suspended solids).

		T (ºC)	pH	Salinity (PSU)	Oxigen (mg/l)	Redox	Turbidity (NTU)	% Organic matter	% Fines	Q50	Sel	SUOM	TSS
Oligohaline range													
CC	mean	24.87	8.08	5.27	6.73	-59.89	998.31	2.51	38.40	0.18	1.44	0.06	0.84
	SD	3.19	0.08	1.95	0.44	114.88	732.58	1.88	40.47	0.09	0.63	0.02	0.58
DC	mean	24.84	8.06	7.10	6.82	-113.14	822.60	2.42	45.67	0.15	1.31	0.05	0.69
	SD	3.11	0.08	2.41	0.46	94.17	277.02	1.12	30.95	0.05	0.26	0.01	0.65
CS	mean	24.98	8.08	4.94	6.75	-137.49	782.99	4.08	83.93	0.10	1.18	0.06	0.84
	SD	3.26	0.08	1.78	0.46	68.25	525.80	1.72	8.87	0.00	0.09	0.02	0.58
DS	mean	25.28	8.06	6.88	7.05	-147.28	642.80	3.98	91.44	0.10	1.20	0.05	0.69
	SD	3.32	0.06	1.97	0.53	66.42	400.62	1.07	5.71	0.00	0.01	0.01	0.65
Polyhaline range													
CC	mean	24.62	8.00	17.34	6.57	-26.97	405.04	3.20	34.50	0.16	1.29	0.04	0.35
	SD	2.93	0.12	5.15	0.45	78.68	307.60	2.21	43.32	0.05	0.19	0.01	0.25
DC	mean	24.62	8.00	21.88	6.46	-35.61	351.97	3.37	37.65	0.17	1.36	0.02	0.14
	SD	2.96	0.12	7.57	0.40	78.56	396.67	2.31	46.39	0.07	0.30	0.00	0.09
CS	mean	24.91	8.01	16.16	6.87	-124.08	438.75	3.29	58.37	0.11	1.15	0.04	0.35
	SD	3.00	0.12	5.33	0.49	83.63	310.85	1.53	24.81	0.02	0.14	0.01	0.25
DS	mean	24.60	8.00	22.53	6.52	-98.13	263.62	5.74	86.48	0.10	1.18	0.02	0.14
	SD	2.97	0.13	7.61	0.44	57.92	151.90	2.75	12.52	0.01	0.04	0.00	0.09

Table S2. Means of heavy metal concentrations (mg/kg) before and after the dredging operations in both salinity ranges at control and dredging areas. (CC: Control channel, DC: Dredging channel)

		As		Cd		Co		Cr		Cu		Hg		Ni		Pb		Sr		V		Zn	
Oligohaline range		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
CC	mean	9.53	9.16	0.18	0.18	9.77	8.94	37.03	25.81	29.28	18.69	0.00	0.24	24.37	18.40	24.23	21.48	249.75	216.98	50.54	39.10	74.64	61.24
	SD	0.44	2.79	0.02	0.07	0.60	1.87	2.06	12.20	2.01	11.85	0.00	0.29	2.45	5.72	4.53	5.71	16.73	79.77	2.85	18.80	7.89	20.42
DC	mean	7.32	11.11	0.18	0.20	8.11	9.23	24.76	19.24	16.17	14.49	0.01	0.21	17.56	15.89	20.38	18.29	202.57	199.95	35.58	30.05	53.63	52.59
	SD	0.67	7.62	0.07	0.08	0.71	2.17	9.77	8.51	7.12	7.94	0.01	0.21	4.47	3.04	4.78	4.35	36.84	71.54	11.24	13.54	12.65	12.70
Polyhaline range																							
CC	mean	5.93	6.95	0.22	0.15	7.04	7.58	15.06	17.80	9.91	13.37	0.00	0.23	12.67	13.63	16.95	16.36	185.30	211.68	23.25	28.40	43.47	50.93
	SD	1.67	1.45	0.06	0.05	1.05	0.88	6.50	7.84	5.02	9.20	0.00	0.16	3.00	2.88	3.20	2.05	31.54	57.02	7.69	12.11	9.09	12.73
DC	mean	5.80	15.77	0.13	0.24	6.73	8.82	7.81	32.36	4.06	27.42	0.22	0.21	8.70	20.50	16.13	24.80	168.01	261.42	12.43	47.66	35.33	76.97
	SD	0.79	9.97	0.12	0.15	0.25	2.10	0.70	18.30	0.54	17.71	0.19	0.21	1.59	9.19	1.36	7.53	78.69	62.91	1.82	24.95	4.63	32.59

Table S3. Means of the species abundances before and after the dredging operations in control and dredging areas of both channel and shallow habitats. (CC: Control channel, CS: Control shallow, DC: Dredging channel, DS: Dredging shallow).

	Oligohaline								Polyhaline							
Species	CC		DC		CS		DS		CC		DC		CS		DS	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Oligochaeta	0.00	0.00	0.00	0.00	34.44	17.78	43.33	10.00	0.00	0.00	0.00	0.00	2.22	2.22	0.00	0.00
Capitella capitata	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	7.78	10.00	5.56	31.11
Glycera tesselata	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	0.00	0.00	0.00	2.22	0.00	0.00	0.00
Syllidia armata	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11
Nephtys hombergii	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	4.44	0.00	2.22
Hediste diversicolor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	0.00	1.11	1.11	42.22	1.11	7.78	1.11
Aonides oxyephala	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.44
Polydora hoplura	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	205.56	50.00	1.11	8.89	0.00	2.22	5.56	0.00
Streblospio shrubsolii	1.11	3.33	0.00	0.00	1.11	1.11	5.56	31.11	71.11	51.11	234.44	58.89	6338.89	2128.89	526.67	1274.44
Alkmaria romijni	6.67	10.00	2.22	16.67	1.11	6.67	26.67	550.00	5.56	21.11	1.11	7.78	11.11	30.00	92.22	1477.78
Lagis koreni	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	0.00	2.22	1.11
Ampelisca diadema	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	0.00
Bathyporeia pilosa	0.00	1.11	0.00	0.00	0.00	0.00	3.33	0.00	127.78	188.89	2367.78	258.89	1.11	21.11	10.00	1.11
Corophium orientale	2.22	4.44	1.11	0.00	1.11	34.44	21.11	2.22	1.11	0.00	2.22	3.33	0.00	1.11	11.11	2.22
Monocorophium acherusicum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.33	0.00
Haustorius arenarius	0.00	4.44	1.11	0.00	0.00	0.00	0.00	0.00	0.00	4.44	0.00	3.33	0.00	16.67	0.00	0.00
Melita palmata	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	0.00	0.00	0.00	32.22	0.00
Parapleustes assimilis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.44	0.00	0.00	0.00	0.00	0.00	1.11	0.00
Alpheus macrocheles	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	1.11
Crangon crangon	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.22	1.11	0.00	0.00	1.11	0.00	0.00	0.00
Carcinus maenas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	0.00

Table S3. (continued)

	Oligohaline								Polyhaline							
Species	CC		DC		CS		DS		CC		DC		CS		DS	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Palaemon adspersus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	1.11	0.00	1.11	0.00	1.11	0.00	5.56	1.11
Nepinnotheres pinnotheres	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11
Liocarcinus cf. marmoreus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	0.00	0.00	0.00	0.00	0.00
Cyathura carinata	0.00	2.22	3.33	2.22	5.56	30.00	10.00	44.44	251.11	194.44	6.67	20.00	24.44	38.89	120.00	355.56
Eurydice pulchra	1.11	1.11	1.11	0.00	0.00	0.00	0.00	0.00	0.00	3.33	10.00	6.67	0.00	0.00	0.00	0.00
Paragnathia formica	0.00	0.00	0.00	0.00	0.00	0.00	1.11	0.00	0.00	1.11	0.00	0.00	0.00	0.00	0.00	0.00
Synidotea laticauda	0.00	0.00	7.78	1.11	0.00	0.00	0.00	0.00	6.67	14.44	1.11	1.11	0.00	1.11	6.67	3.33
Lekanesphaera hoestlandti	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.11	1.11	0.00	0.00	0.00	0.00	6.67	0.00
Lekanesphaera hookeri	1.11	0.00	1.11	0.00	0.00	0.00	2.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lekanesphaera levii	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	62.22	0.00	21.11	0.00	1.11	0.00	0.00	0.00
Lekanesphaera rugicauda	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.22	0.00	0.00	0.00	0.00	0.00
Mesopodopsis slabberi	5.56	1.11	5.56	0.00	0.00	0.00	3.33	1.11	4.44	2.22	4.44	2.22	2.22	1.11	2.22	0.00
Neomysis integer	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rhopalophthalmus tartessicus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.33	0.00	0.00	0.00	0.00	0.00
Chaetognatha	0.00	3.33	0.00	0.00	0.00	2.22	0.00	2.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pholas dactylus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	3.33	0.00	0.00	4.44	0.00
Cerastoderma edule	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	1.11	2.22	0.00	2.22	22.22
Corbicula fluminea	2.22	0.00	0.00	0.00	0.00	1.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Scrobicularia plana	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	0.00	0.00	3.33	26.67	1.11	12.22
Nemertea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.89	1.11	3.33	2.22	0.00	1.11	6.67	0.00

Table S4. Average abundance (Av. Abund.) of the most relevant species over the sampling dates of samples in the polyhaline range. Species are listed in decreasing order according to their contribution to the average of the dissimilarity (Av. Diss.) between dates until 50% of the accumulated total similarity. Both impact and control areas are showed separately within their respective channel or shallow habitat.

Control channel

June vs July 2015	Jun	Jul	Average dissimilarity: 68.47		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Polydora hoplura	16.21	0	13.43	1.55	19.62
Bathyporeia pilosa	1.72	13.88	11.7	1.6	17.09
Cyathura carinata	15.37	11.15	9.82	1.17	14.34

June vs October 2015	Jun	Oct	Average dissimilarity: 66.47		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Polydora hoplura	16.21	0	16.33	1.66	24.54
Cyathura carinata	15.37	7.16	12.69	1.46	19.06
Streblospio shrubsolii	4.96	7.86	8.1	1.44	12.17

July vs October 2015	Jul	Oct	Average dissimilarity: 66.57		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Bathyporeia pilosa	13.88	5.77	11.46	1.59	20.76
Cyathura carinata	11.15	7.16	10.39	1.11	18.83
Lekanesphaera levii	9.2	0	9.76	1.56	17.67

October 2015 vs August 2016	Oct	Aug	Average dissimilarity: 66.52		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Bathyporeia pilosa	5.77	14.94	16.5	1.23	24.8
Cyathura carinata	7.16	13.67	12.53	1.32	18.84
Polydora hoplura	0	7.86	8.92	1.45	13.41

Dredging channel

June vs July 2015	Jun	Jul	Average dissimilarity: 38.06		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Bathyporeia pilosa	30.16	58.98	20.52	1.26	53.92

June vs October 2015	Jun	Oct	Average dissimilarity: 49.09		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Bathyporeia pilosa	30.16	18.03	19.65	1.26	40.03
Streblospio shrubsolii	13.64	6.98	8.44	1.55	17.19

July vs October 2015	Jul	Oct	Average dissimilarity: 54.88		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Bathyporeia pilosa	58.98	18.03	34.49	2.24	62.84
Streblospio shrubsolii	15.71	6.98	6.97	1.74	12.7

October 2015 vs August 2016	Oct	Aug	Average dissimilarity: 61.13		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Bathyporeia pilosa	18.03	4.16	18.25	1.16	29.85
Cyathura carinata	0	5.09	7.62	1.24	12.46
Alkmaria romijni	0	3.93	6.34	4.31	10.37

Table S4. (continued)

Control shallow

June vs July 2015	Jun	Jul	Average dissimilarity: 33.52		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Streblospio shrubsolii	89.23	61.9	18.83	1.24	56.18

June vs October 2015	Jun	Oct	Average dissimilarity: 33.71		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Streblospio shrubsolii	89.23	55.22	18.94	1.45	56.19

July vs October 2015	Jul	Oct	Average dissimilarity: 29.06		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Streblospio shrubsolii	61.9	55.22	15.77	1.35	54.27

October 2015 vs August 2016	Oct	Aug	Average dissimilarity: 46.41		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Streblospio shrubsolii	55.22	28.1	22.57	1.22	48.62
Alkmaria romijni	1.22	5.08	3.42	1.01	7.37

Dredging shallow

June vs July 2015	Jun	Jul	Average dissimilarity: 40.52		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Streblospio shrubsolii	26.82	17.64	6.7	1.77	16.54
Alkmaria romijni	8.19	9.23	3.56	1.91	8.78
Cyathura carinata	8.75	12.05	3.44	1.28	8.48
Melita palmata	2.28	5.24	3.4	1.3	8.38
Monocorophium acherusicum	3.6	1.49	2.27	1.12	5.6
Nemertino	0.86	3.29	1.94	1.49	4.8

June vs October 2015	Jun	Oct	Average dissimilarity: 39.33		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Streblospio shrubsolii	26.82	23.57	5.39	1.35	13.71
Cyathura carinata	8.75	8.9	3.59	1.76	9.13
Capitella capitata	0.86	4.89	3.45	1.79	8.78
Alkmaria romijni	8.19	6.27	3.03	1.69	7.71
Cerastoderma edule	0.86	4.82	2.97	1.26	7.54
Monocorophium acherusicum	3.6	0	2.72	1.27	6.93

July vs October 2015	Jul	Oct	Average dissimilarity: 43.72		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Cyathura carinata	12.05	8.9	4.79	1.17	10.96
Streblospio shrubsolii	17.64	23.57	4.76	1.28	10.88
Melita palmata	5.24	0	3.66	1.19	8.37
Cerastoderma edule	0.86	4.82	3.09	1.28	7.07
Capitella capitata	1.72	4.89	3.03	1.44	6.92
Alkmaria romijni	9.23	6.27	2.93	1.16	6.7

October 2015 vs August 2016	Oct	Aug	Average dissimilarity: 51.39		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Alkmaria romijni	6.27	53.2	24.33	6.72	47.34
Streblospio shrubsolii	23.57	43.49	10.15	2.12	19.74

Table S5. Multivariate and Univariate PERMANOVA results in the studied area based on the Euclidian similarity matrix of the heavy metal concentrations. *p estimation obtained by Monte Carlo sampling.

	df	MS	Pseudo-F	p	Unique perms
Multivariate					
Impact vs Control	1	121.14	157.46	0.0001	9834
Time	1	23.041	29.951	0.0001	9843
Impact vs Control x Time	1	18.324	23.819	0.0001	9825
Res	23	0.76931			
Total	26				
MCI8					
Impact vs Control	1	121.14	157.46	0.0001	9834
Time	1	23.041	29.951	0.0001	9843
Impact vs Control x Time	1	18.324	23.819	0.0001	9825
Res	23	0.76931			
Total	26				
As	df	MS	Pseudo-F	P(perm)	perms
Impact vs Control	1	36.05	23.582	0.0001	9858
Time	1	25.14	25.57	0.0694*	3
Dates(Time)	1	0.91497	0.59852	0.4651	9817
Impact vs Control x Time	1	0.23444	0.15336	0.6945	9853
Res	22	1.5287			
Total	26				
Cd					
Impact vs Control	1	1.06E-05	4.18E-03	0.9515	9831
Time	1	5.65E-03	1.9322	0.367*	3
Dates(Time)	1	2.97E-03	1.1712	0.2881	9829
Impact vs Control x Time	1	1.25E-03	0.49284	0.491	9839

Table S5. (continued)

Res	22	2.54E-03			
Total	26				
Co					
Impact vs Control	1	10.525	22.258	0.0002	9865
Time	1	5.2484	11.099	0.0023	9833
Impact vs Control x Time	1	5.5604	11.758	0.0019	9822
Res	23	0.47289			
Total	26				
Cr					
Impact vs Control	1	2158.9	135.03	0.0001	9834
Time	1	225.19	9.5891	0.1663*	3
Dates(Time)	1	24.421	1.5275	0.2366	9845
Impact vs Control x Time	1	318.01	19.89	0.0004	9816
Res	22	15.988			
Total	26				
Cu					
Impact vs Control	1	2467	176.48	0.0001	9845
Time	1	126.46	3.7984	0.2735*	3
Dates(Time)	1	35.708	2.5545	0.1252	9840
Impact vs Control x Time	1	295.18	21.117	0.0002	9849
Res	22	13.978			
Total	26				
Ni					
Impact vs Control	1	398.22	140.16	0.0001	9836
Time	1	44.208	13.055	0.1373*	3
Dates(Time)	1	3.4544	1.2159	0.2772	9816
Impact vs Control x Time	1	89.431	31.477	0.0001	9809
Res	22	2.8411			

Table S5. (continued)

Total	26				
Pb					
Impact vs Control	1	718.04	101.73	0.0001	9831
Time	1	42.568	1.0848	0.4812*	3
Dates(Time)	1	43.265	6.1299	0.0244	9829
Impact vs Control x Time	1	82.062	11.627	0.0028	9847
Res	22	7.0581			
Total	26				
Zn					
Impact vs Control	1	5059.2	116.38	0.0001	9831
Time	1	479.61	5.4621	0.2302*	3
Dates(Time)	1	93.349	2.1474	0.1535	9837
Impact vs Control x Time	1	440.41	10.131	0.0042	9840
Res	22	43.471			
Total	26				
V					
Impact vs Control	1	3627.7	124.86	0.0001	9843
Time	1	952.25	30.754	0.0791*	3
Dates(Time)	1	31.202	1.0739	0.315	9837
Impact vs Control x Time	1	414.1	14.252	0.0013	9839
Res	22	29.055			
Total	26				

Table S6. Average abundance (Av. Abund.) of the most relevant species of the Impact vs Control areas over all sampling dates. Species are listed in decreasing order according to their contribution to the average of the dissimilarity (Av. Diss.) between areas until 50% of the accumulated total similarity.

Impact vs Controls Jun 2015	I	C	Average dissimilarity: 44,52		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Apseudopsis latreillii	21.96	35.48	2.43	2.38	5.46
Magelona papillicornis	16.02	7.66	1.78	1.57	4
Hyala vitrea	7.07	7.9	1.56	1.54	3.5
Sternaspis scutata	7.33	13.02	1.55	2.8	3.49
Spiophanes kroyeri	11.39	2.47	1.52	1.62	3.41
Eudorella truncatula	17.11	9.2	1.5	2.31	3.37
Sigambra parva	11.94	3.84	1.44	1.86	3.23
Medicorophium runcicorne	7.58	5.55	1.05	3.26	2.35
Capitella capitata	7.49	9.88	1.04	1.26	2.33
Medicorophium aculeatum	4.3	2.15	1.01	0.77	2.28
Nephtys hombergii	11.21	6.73	0.8	0.97	1.8
Iphinoe tenella	4	0	0.79	2.21	1.76
Bodotria scorpioides	4.55	0	0.75	1.17	1.69
Glycera tesselata	5.76	1.65	0.75	1.8	1.69
Nassarius incrassatus	3.84	0	0.72	3.86	1.61
Microspio mecznikowianus	7.95	4.47	0.7	1.47	1.57
Upogebia tipica	3.8	0	0.69	5.3	1.56
Nemertino	13.34	9.67	0.69	1.64	1.56
Aonides oxicephala	8.19	4.79	0.67	1.65	1.49
Oestergrenia digitata	4.32	4.56	0.65	1.67	1.47
Cheirocratus sundevalli	1.72	3.29	0.59	1.26	1.34

Impact vs Controls July 2015	I	C	Average dissimilarity: 36,66		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Apseudopsis latreillii	30.66	38.92	2.02	1.23	5.5
Magelona papillicornis	13.67	7.77	1.38	1.61	3.77
Spiophanes kroyeri	7.95	1.47	1.34	2.72	3.66
Hyala vitrea	8.25	11.48	1.3	1.57	3.53
Nephtys hombergii	11.37	5.71	1.24	1.44	3.39
Lumbrineris latreilli	15.08	12.2	1.13	1.46	3.09
Eudorella truncatula	9.69	4.61	1.06	1.93	2.88
Sigambra parva	8.27	3.27	1.03	1.84	2.82
Medicorophium runcicorne	7.24	2.33	1.01	1.36	2.75

Table S6. (continued)

Glycera tesselata	5.49	1.29	0.89	2.67	2.42
Spisula subtruncata	4.55	0	0.86	0.69	2.36
Corbula gibba	11.19	7.11	0.86	1.49	2.34
Mysia undata	3.8	0	0.79	5.04	2.16
Phoronida	5.79	2.21	0.78	1.37	2.14
Sternaspis scutata	7.28	10.2	0.75	1.24	2.05
Nucula hanleyi	8.48	6.59	0.72	1.55	1.96
Turritella turbona	4.2	0.86	0.71	2.52	1.94
Urothoe grimaldii	3.33	0	0.69	1.32	1.88

Impact vs Controls Oct 2015	I	C	Average dissimilarity: 31,49		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Apseudopsis latreillii	48.98	71.95	4.28	1.36	13.59
Capitella capitata	3.93	13.35	1.71	2.32	5.43
Nephtys hombergii	11.6	6.33	0.96	1.14	3.06
Hemilepton nitidum	6.05	10.69	0.95	1.36	3.03
Upogebia tipica	11.94	10.46	0.88	1.23	2.79
Eudorella truncatula	3.93	8.32	0.87	1.42	2.78
Hyala vitrea	6.24	10.08	0.86	1.32	2.75
Lumbrineris latreilli	13.12	15.29	0.86	1.54	2.74
Sternaspis scutata	10.13	11.22	0.8	1.46	2.54
Phoronida	4.54	0.43	0.74	2.6	2.35
Corbula gibba	10.64	6.79	0.7	2.05	2.24
Upogebia deltaura	2.35	6.03	0.67	1.66	2.14
Turritella turbona	6.74	3.27	0.65	1.38	2.07
Chamelea gallina	3.44	0	0.63	2.64	1.99
Neanthes fucata	4.28	0.86	0.62	1.81	1.98

Impact vs Controls March 2016	I	C	Average dissimilarity: 44,00		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Hemilepton nitidum	12.64	0.61	2.92	1.22	6.63
Upogebia tipica	12.84	1.67	2.88	1.34	6.53
Apseudopsis latreillii	21.73	28.68	2.59	1.78	5.88
Magelona papillicornis	10.99	7.1	1.98	2.06	4.49
Capitella capitata	6.44	13.48	1.82	1.53	4.14
Terebellides stroemii	0	6.71	1.55	5.04	3.51
Medicorophium runcicorne	7.61	1.9	1.33	2.12	3.01
Corbula gibba	8.16	3.43	1.1	1.91	2.51
Hyala vitrea	6.9	9.75	1.01	1.26	2.29
Amphiura chiajei	6.31	2.04	0.99	2.4	2.25
Urothoe grimaldii	4.7	0.43	0.99	3.92	2.24
Aonides oxicephala	11.19	7.2	0.94	1.97	2.14
Aricidea catherinae	1.72	5.1	0.87	1.67	1.98
Lagis koreni	3.65	0	0.84	15.35	1.91

Table S6. (continued)

Eudorella truncatula	9.06	6.91	0.78	1.37	1.78

Impact vs Controls August 2016	I	C	Average dissimilarity: 42,01		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Apseudopsis latreillii	21.5	19.9	2.07	1.24	4.92
Corbula gibba	17.95	8.56	2.06	1.45	4.9
Magelona papillicornis	13.67	5.92	1.66	1.63	3.95
Aonides oxicephala	12.16	5.2	1.42	1.73	3.37
Nephtys hombergii	13.82	8.19	1.15	1.32	2.73
Eudorella truncatula	8.47	3.4	1.14	1.75	2.72
Spiophanes kroyeri	5.83	0.43	1.08	1.53	2.57
Hyala vitrea	10.01	11.45	1.05	1.46	2.5
Photis longicaudata	5.26	0.43	1.03	3.7	2.44
Lumbrineris latreilli	15.44	14.86	0.98	1.42	2.34
Medicorophium aculeatum	3.44	1.67	0.93	0.77	2.21
Glycera tesselata	8.07	3.47	0.91	1.4	2.16
Urothoe grimaldii	4.39	0.43	0.87	1.39	2.07
Phoronida	5.07	1.04	0.87	1.73	2.06
Thracia phaseolina	4.13	0	0.75	0.69	1.79
Nucula hanleyi	8.2	6.67	0.73	2.1	1.75
Phaxas pellucidus	3.65	0	0.73	1.36	1.75
Tellina cf. compressa	3.72	0	0.71	1.07	1.69
Capitella capitata	12.9	15.92	0.69	1.2	1.65
Cossura soyeri	2.94	0	0.68	1.33	1.62

Table S7. Average abundance (Av. Abund.) of the most relevant species over the sampling dates of samples in the control areas. Species are listed in decreasing order according to their contribution to the average of the dissimilarity (Av. Diss.) between areas until 50% of the accumulated total similarity.

Jun vs Jul 2015	Jun	Jul	Average dissimilarity: 41,20		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Apseudopsis latreillii	21.96	30.66	2.01	2.02	4.87
Magelona papillicornis	16.02	13.67	1.45	1.55	3.53
Hyala vitrea	7.07	8.25	1.45	1.63	3.52
Eudorella truncatula	17.11	9.69	1.25	2.3	3.04
Sternaspis scutata	7.33	7.28	1.2	1.76	2.92
Nephtys hombergii	11.21	11.37	1	1.55	2.44
Phoronida	0	5.79	0.96	2.49	2.32
Medicorophium runcicorne	7.58	7.24	0.95	1.51	2.3
Sigambra parva	11.94	8.27	0.83	1.76	2.02
Spisula subtruncata	2.72	4.55	0.83	0.89	2.02
Lumbrineris latreilli	14.2	15.08	0.8	1.58	1.94
Medicorophium aculeatum	4.3	0	0.8	0.66	1.93
Capitella capitata	7.49	7.88	0.79	1.57	1.92
Spiophanes kroyeri	11.39	7.95	0.79	0.98	1.91
Hemilepton nitidum	0	4.35	0.72	2.15	1.75
Iphinoe tenella	4	0	0.69	2.18	1.67
Bodotria scorpioides	4.55	0	0.67	1.12	1.63
Aonides oxicephala	8.19	4.07	0.64	2.05	1.56
Oestergrenia digitata	4.32	2.35	0.64	1.2	1.54
Nemertino	13.34	9.87	0.63	1.55	1.53
Nassarius incrassatus	3.84	2.43	0.62	3.32	1.5
Ampelisca diadema	6.14	3.94	0.61	1.26	1.48
Nucula hanleyi	5.49	8.48	0.61	1.48	1.48

Jun vs Oct 2015	Jun	Oct	Average dissimilarity: 45,93		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Apseudopsis latreillii	21.96	48.98	4.41	1.46	9.61
Eudorella truncatula	17.11	3.93	2.22	3.48	4.84
Magelona papillicornis	16.02	9.55	1.53	1.75	3.32
Upogebia tipica	3.8	11.94	1.39	3.4	3.03
Sternaspis scutata	7.33	10.13	1.27	1.54	2.76
Hyala vitrea	7.07	6.24	1.26	1.5	2.74
Spiophanes kroyeri	11.39	4.36	1.09	1.07	2.37
Medicorophium runcicorne	7.58	2.35	1.08	1.84	2.34
Hemilepton nitidum	0	6.05	1.02	2.92	2.21
Capitella capitata	7.49	3.93	1	1.65	2.18

Table S7. (continued)

Sigambra parva	11.94	6.35	0.95	1.65	2.07
Nephtys hombergii	11.21	11.6	0.92	1.2	2.01
Nemertino	13.34	8.01	0.9	2.19	1.95
Medicorophium aculeatum	4.3	0	0.81	0.67	1.76
Lumbrineris latreilli	14.2	13.12	0.79	1.67	1.71
Nucula hanleyi	5.49	9.99	0.78	2.89	1.69
Phoronida	0	4.54	0.76	3.19	1.66
Neanthes fucata	0	4.28	0.72	2.77	1.57
Iphinoe tenella	4	0	0.7	2.21	1.52

Jul vs Oct 2015	Jul	Oct	Average dissimilarity: 34,28		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Apseudopsis latreillii	30.66	48.98	3.89	1.47	11.34
Upogebia tipica	4.07	11.94	1.45	2.09	4.24
Lumbrineris latreilli	15.08	13.12	1.15	1.38	3.34
Hyala vitrea	8.25	6.24	1.13	2.38	3.29
Eudorella truncatula	9.69	3.93	1.02	1.45	2.99
Nephtys hombergii	11.37	11.6	0.99	1.35	2.9
Magelona papillicornis	13.67	9.55	0.94	1.83	2.73
Medicorophium runcicorne	7.24	2.35	0.92	1.48	2.68
Aonides oxicephala	4.07	9.1	0.91	2.21	2.64
Sternaspis scutata	7.28	10.13	0.9	1.38	2.63
Spisula subtruncata	4.55	0.86	0.81	0.82	2.37
Capitella capitata	7.88	3.93	0.73	1.2	2.14
Spiophanes kroyeri	7.95	4.36	0.71	1.13	2.07
Mysia undata	3.8	0	0.68	4.66	1.98
Abra nitida	3.21	3.14	0.56	1.52	1.63
Ampelisca diadema	3.94	3.21	0.55	1.72	1.59

Oct 2015 vs Mar 2016	Oct	Mar	Average dissimilarity: 38,99		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Apseudopsis latreillii	48.98	21.73	5.47	1.54	14.02
Hemilepton nitidum	6.05	12.64	2.07	1.58	5.31
Upogebia tipica	11.94	12.84	1.71	2.61	4.38
Magelona papillicornis	9.55	10.99	1.5	1.73	3.86
Capitella capitata	3.93	6.44	1.07	1.24	2.74
Eudorella truncatula	3.93	9.06	1.03	1.48	2.65
Sternaspis scutata	10.13	5.56	1.03	1.2	2.65
Medicorophium runcicorne	2.35	7.61	1	2.2	2.56
Lumbrineris latreilli	13.12	14.05	0.93	1.42	2.38
Nephtys hombergii	11.6	8.79	0.9	1.2	2.31
Sigambra parva	6.35	3.44	0.74	1.49	1.89
Chamelea gallina	3.44	0	0.66	2.56	1.7
Neanthes fucata	4.28	0.86	0.66	1.76	1.7
Nemertino	8.01	5.8	0.63	1.42	1.62
Leucothoe incisa	4.7	3.14	0.57	2.29	1.46

Mar 2016 vs Aug 2016	Mar	Aug	Average dissimilarity: 43,87

Table S7. (continued)

Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Upogebia tipica	12.84	0.86	2.34	1.37	5.33
Hemilepton nitidum	12.64	3.65	2.17	1.12	4.96
Apseudopsis latreillii	21.73	21.5	2.02	1.14	4.61
Corbula gibba	8.16	17.95	1.77	1.34	4.03
Magelona papillicornis	10.99	13.67	1.56	1.56	3.56
Capitella capitata	6.44	12.9	1.39	1.39	3.17
Nephtys hombergii	8.79	13.82	0.97	1.18	2.21
Photis longicaudata	0	5.26	0.95	8.51	2.17
Aricidea catherinae	1.72	6.75	0.94	1.72	2.15
Hyala vitrea	6.9	10.01	0.93	1.7	2.12
Medicorophium runcicorne	7.61	3.44	0.91	1.46	2.07
Lumbrineris latreilli	14.05	15.44	0.86	1.49	1.96
Venus casina	1.22	5.37	0.78	1.97	1.78
Sigambra parva	3.44	6.85	0.78	1.49	1.78
Turritella turbona	4.16	0	0.77	5.02	1.75
Thracia phaseolina	0.86	4.13	0.73	0.83	1.65
Medicorophium aculeatum	0	3.44	0.71	0.67	1.61
Spiophanes kroyeri	6.48	5.83	0.7	4.35	1.6
Spisula subtruncata	1.72	3.33	0.67	1.23	1.53

Table S8. Average abundance (Av. Abund.) of the most relevant species over the sampling dates of samples in the disposal area. Species are listed in decreasing order according to their contribution to the average of the dissimilarity (Av. Diss.) between areas until 50% of the accumulated total similarity.

Jun vs Jul 2015	Jun	Jul	Average dissimilarity: 30,25		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Apseudopsis latreillii	35.48	38.92	1.55	1.23	5.12
Hyala vitrea	7.9	11.48	1.43	1.18	4.71
Upogebia tipica	0	5.06	1.28	4.39	4.22
Eudorella truncatula	9.2	4.61	1.16	1.55	3.85
Capitella capitata	9.88	8.02	0.92	1.25	3.05
Ampelisca diadema	5.28	2.51	0.9	2.3	2.97
Hemilepton nitidum	0	3.53	0.89	1.72	2.94
Sternaspis scutata	13.02	10.2	0.86	1.49	2.84
Medicorophium runcicorne	5.55	2.33	0.8	1.96	2.66
Kurtiella bidentata	0	2.82	0.7	1.35	2.33
Magelona papillicornis	7.66	7.77	0.69	1.23	2.28
Microspio mecznikowianus	4.47	2.35	0.69	1.32	2.28
Nucula hanleyi	4.41	6.59	0.68	1.4	2.24
Turritella turbona	2.92	0.86	0.67	1.36	2.2
Cheirocratus sundevalli	3.29	2.64	0.65	1.36	2.16
Nemertino	9.67	7.44	0.64	1.49	2.11
Aonides oxicephala	4.79	2.51	0.62	1.26	2.06

Jun vs Oct 2015	Jun	Oct	Average dissimilarity: 36,15		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Apseudopsis latreillii	35.48	71.95	7.46	4.53	20.64
Hemilepton nitidum	0	10.69	2.17	2.57	5.99
Upogebia tipica	0	10.46	2.11	1.81	5.83
Aonides oxicephala	4.79	11.28	1.33	1.99	3.68
Hyala vitrea	7.9	10.08	1.1	1.33	3.03
Nucula hanleyi	4.41	9.66	1.07	2.07	2.95
Leucothoe incisa	1.47	6.4	1.01	2.21	2.8
Capitella capitata	9.88	13.35	0.91	1.61	2.52
Ampelisca diadema	5.28	1.18	0.9	1.84	2.49
Sternaspis scutata	13.02	11.22	0.63	1.61	1.75

Jul vs Oct 2015	Jul	Oct	Average dissimilarity: 33,27		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%

Table S8. (continued)

Apseudopsis latreillii	38.92	71.95	7.05	3.84	21.18
Aonides oxicephala	2.51	11.28	1.86	3.05	5.6
Hemilepton nitidum	3.53	10.69	1.53	1.63	4.61
Upogebia tipica	5.06	10.46	1.32	1.29	3.98
Capitella capitata	8.02	13.35	1.26	2.02	3.8
Leucothoe incisa	1.9	6.4	0.95	2.16	2.86
Hyala vitrea	11.48	10.08	0.9	1.19	2.71
Eudorella truncatula	4.61	8.32	0.84	1.57	2.52
Upogebia deltaura	2.26	6.03	0.79	1.65	2.38
Nucula hanleyi	6.59	9.66	0.74	1.44	2.23

Oct 2015 vs Mar 2016	Oct	Mar	Average dissimilarity: 37,58		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Apseudopsis latreillii	71.95	28.68	9.29	5.73	24.71
Hemilepton nitidum	10.69	0.61	2.14	2.34	5.69
Upogebia tipica	10.46	1.67	2.03	1.6	5.39
Upogebia deltaura	6.03	0	1.28	4.04	3.42
Sternaspis scutata	11.22	5.39	1.27	1.73	3.37
Leucothoe incisa	6.4	1.35	1.09	2.02	2.9
Hyala vitrea	10.08	9.75	1.01	1.39	2.68
Terebellides stroemii	2.21	6.71	0.97	2.04	2.59

Mar 2016 vs Aug 2016	Mar	Aug	Average dissimilarity: 31,96		
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Apseudopsis latreillii	28.68	19.9	2.67	1.41	8.34
Hyala vitrea	9.75	11.45	1.48	1.37	4.62
Corbula gibba	3.43	8.56	1.47	1.8	4.59
Terebellides stroemii	6.71	1.65	1.45	2.13	4.52
Eudorella truncatula	6.91	3.4	1.05	1.17	3.3
Kurtiella bidentata	0.61	4.15	1.05	1.31	3.27
Spiophanes kroyeri	3.82	0.43	0.97	1.4	3.02
Lumbrineris latreilli	12.96	14.86	0.93	1.44	2.9
Capitella capitata	13.48	15.92	0.92	1.22	2.88
Ampelisca diadema	2.76	5.71	0.82	1.4	2.58
Magelona papillicornis	7.1	5.92	0.81	1.14	2.54
Venus casina	0.43	3.08	0.77	1.7	2.41
Spiochaetopterus costarum	0.43	3.12	0.77	1.73	2.4
Upogebia tipica	1.67	1.92	0.76	0.8	2.39
Turritella turbona	2.82	0.43	0.75	1.34	2.33

Table S9. Carbon and nitrogen stable isotope signatures of primary producers in Guadalquivir and others close estuaries extracted from the literature. POM (Particulate organic matter), SOM (sediment organic matter).

Estuaries		Fresh water POM	Brackish water POM	Marine water POM	Microphytobenthos	SOM	Phytoplankton	References
Guadalquivir	$\delta^{13}C$		-26		-20.5	-24		1
	$\delta^{15}N$		6.37		9.5	10.9		
	$\delta^{13}C$	-26.5	-26	-20	-22			2
	$\delta^{15}N$	-0.5	2.5	9				
Tagus	$\delta^{13}C$		-22.7		-20.8	-24.1		3
	$\delta^{15}N$		10.8		14.3	16.2		
	$\delta^{13}C$	-24.8	-23.5	-21.3	-17.6	-20		4
	$\delta^{15}N$		3		6	6		
Minho	$\delta^{13}C$	-27.8	-27.4	-21.5	-24	-28	-38.5	5-6
	$\delta^{15}N$	4.9	5.3	5.2	7.3	0.2	5	
Lima	$\delta^{13}C$			-21.8				6
	$\delta^{15}N$			5.7				
Mira	$\delta^{13}C$		-23.3			-19.4		3
	$\delta^{15}N$		8.9			5.8		
Gironde	$\delta^{13}C$	-27.7	-26.7	-22.1	-23.9	-25.5	-34.5	7
	$\delta^{15}N$	5.8	6.3	9.7	9.1	5.3		
Charente	$\delta^{13}C$	-29.2	-25.3		-16.2			8
	$\delta^{15}N$							
Mondego	$\delta^{13}C$			-22.5	-14			9
	$\delta^{15}N$			6	6			

Table S10. Processing method, number of analysis and number of organism included in each analysis for each species.

Species	Processing method	Weigh (mg)	Analysis	Number per replies
Engraulis encrasicolus large	Individual	0.3	5	1
Engraulis encrasicolus medium	Individual	0.3	5	10
Engraulis encrasicolus small	Pool	0.3	5	> 10
Pomatoschistus sp.	Pool	0.3	5	5
Palaemon sp.	Pool	0.3	5	> 7
Synidotea laticauda	Pool	0.3	5	> 7
Neomysis integer	Pool	0.3	5	Pool
Mesopodopsis slabberi	Pool	0.3	5	Pool
Corbicula fluminea	Individual	0.3	5	1
Copepods	Pool	0.3	5	Pool
Oligohaline vegetal matter	Pool	1	5	Pool
SOM	Sediment	15	3	1

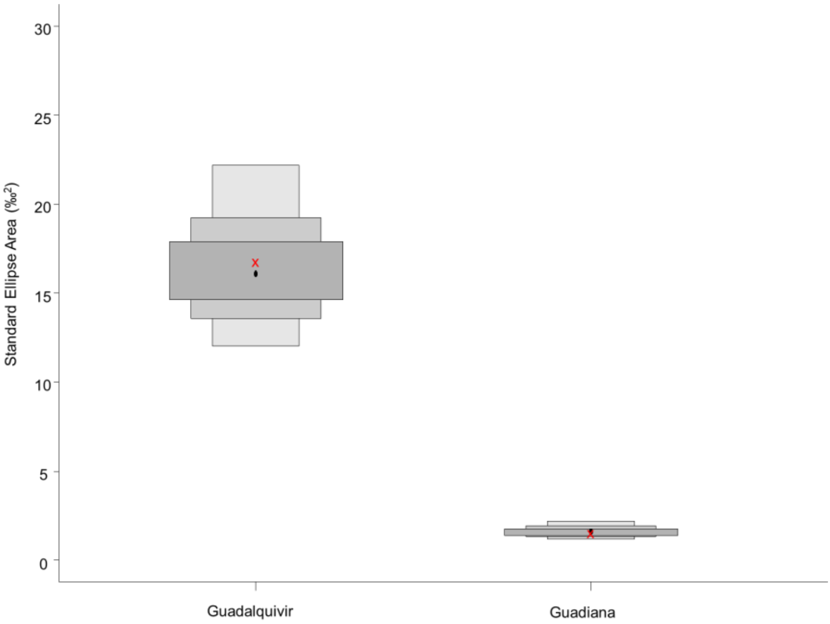

Figure S1. Density plots showing the credibility intervals of the Bayesian standard ellipse areas (SEA_B). Black circles are the SEA_B modes, and boxes indicate the 50%, 75% and 95% credible intervals. Red crosses are the true population values.

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