Title: Effects of freshwater input on the lower trophic levels of a temperate estuary: physical, physiological or trophic forcing?

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Abstract

The aim of this six year study was to assess whether freshwater inputs (rainfall and dam discharges) were acting as physical, physiological or trophic forcing factors on phytoplankton pigment concentrations and the dominant mysids of a temperate estuary (Guadalquivir estuary; SW, Spain). The effects of natural and human-controlled freshwater inputs modified the physicochemical conditions and consequently biological production (bottom up control). Nutrient (nitrogen hypernutrification), suspended particulate matter and allochthonous photosynthetic pigment importations linked to freshwater inputs from adjacent habitats were observed, as well as light-limited autochthonous primary production. Seasonal and/or spatial patterns were shown by all studied variables, including mysid species. Freshwater management effects on dominant mysids differed depending on the species' salinity tolerances (physiological forcing) and preferred prey availability (trophic forcing); moreover high inorganic matter content had a negative effect on the density of *Mesopodopsis slabberi* (physical forcing), which led to an increased detritivory/herbivory ratio (*Neomysis integer*/*M. slabberi* ratio). In conclusion, freshwater input appeared to affect estuarine lower trophic levels via a combination of different forcing mechanisms. Although several general patterns can be derived, the response of the system to freshwater input was not always univocal.

Keywords

Estuaries, Nutrients, SPM, hypernutrification, mysids, Guadalquivir estuary.

1. Introduction

Freshwater inputs play an important role in determining estuarine productivity, since riverine and rainfall inputs are the dominant nutrient source in estuaries (Correll and Ford 1982; Abril et al. 2002). The

Estuarine nutrient load input is directly related to land use: river catchments with intensive agriculture and large populations have enhanced nitrate and phosphate concentrations in their estuaries (Peierls et al. 1991; Balls 1994; Nixon 1995; Uncles et al. 1998). Although higher nutrient inputs to estuarine environments can lead to an increase in primary production, they may also cause a decrease in the oxygen water concentration and water quality, which negatively affects the estuarine aquatic inhabitants (Dame et al. 2000).

Increased soil erosion due to deforestation, overgrazing and other detrimental agricultural practices in river catchment areas have drastically increased sediment inputs to estuaries during the last decades (Wolanski et al. 2004). As limited light penetration reduces primary production (Cloern et al. 1983; Pennock 1985; Cloern 1987; Lehman 1992; Kocum et al. 2002), turbid estuaries are considered to be heterotrophic systems (Heip et al. 1995). Nevertheless, estuaries may show high concentrations of chlorophyll *a* in the maximal turbidity zone due to phytoplankton importation from more productive adjacent areas and/or estuarine phytoplankton species adaptation to light-limited conditions (Irigoin and Castel 1997).

High freshwater input may also influence estuarine biota by creating salinity gradients or by flushing suspended particulates and organisms (mainly small-size prey species) out of the estuary during flood events (Abril et al. 1999). Conversely, reduced freshwater flow may greatly increase the residence time of suspended matter and organisms within estuaries due to the prevalence of tides (Uncles et al. 2003). Indeed, plankton densities within estuaries are usually controlled by both abiotic (salinity, temperature, turbidity and current flushing, among others) and biotic (prey availability and food quality) factors (Mees et al. 1993; Koski et al. 1998; Gasparini et al. 1999; Burdloff et al. 2000; Kimmel and Roman 2004; Vilas et al. 2008).

Estuaries have large mesozooplankton populations of detritivorous consumers, which are food for zooplankton predators such as juvenile fishes and decapod crustaceans (Elliott et al. 2002; David et al. 2006; González-Ortegón et al. 2010a). Mysids (Crustacea: Mysidacea) play a key role in the food web of many estuaries as both consumers and prey (Mauchline 1980; Mees and Jones 1997; Baldó and Drake 2002; Elliott et al. 2002; Lobry et al. 2008). Although mysids are generally considered to be omnivorous, they show clear inter-specific trophic differences (Zagursky and Feller 1985; Gasparini and Castel 1999; Froneman 2001; Kouassi et al. 2006; Vilas et al. 2008). Thus, freshwater input-related changes in estuarine conditions, such as low primary productivity due to increased turbidity and high current velocity (Irigoin and Castel, 1997; Jassby et al. 2002), which favours detritivorous versus phytoplanktonic consumers, may lead to a higher detritivory/herbivory ratio (Scharler and Baird 2005).

Keeping the above considerations in mind, it is expected that freshwater input to estuaries mainly acts as a trophic forcer of the more tolerant estuarine species and as a physiological forcing (osmotic stress) for marine species. During floods, a physical forcing of freshwater input on all low trophic level components is also expected. In order to test the hypothesis that freshwater inputs to estuaries acts as a combination of physical, physiological or trophic forcing factors on their lower trophic levels, a six year field sampling programme was designed in a temperate estuary with regulated freshwater inflow.

2. Materials and methods

2.1. Study area

The Guadalquivir Estuary (SW Spain) is a well-mixed system with a longitudinal salinity gradient (Vannéy 1970). The tidal influence extends up to the Alcalá del Río dam (110 km from the river mouth) and the mean tidal range at spring tides is 2.5 m in the seaward area (Online Resource 1). From 1940 until today there was a significant transformation of its marshlands into irrigated agricultural lands. After the dam construction (1935), the mean annual freshwater discharge from this dam has been very irregular, varying between 3 hm³ month⁻¹ (in the 1994-1995 water year) and 1572 hm³ month⁻¹ (in 1935-1936), and usually reaching maximum levels in winter (730 hm³ month⁻¹) and minimums in summer (83 hm³ month⁻¹). During the last 30 years, the river discharges into the estuary have shown an average reduction of more than 50%; during droughts, the freshwater flow is almost completely controlled by the dam and is usually increasing from late spring to early summer and autumn to water the cultivation fields (Fernández-Delgado et al. 2007). The position of the estuarine mixing zone shows a tidal displacement of about 10 km at spring tides. The area situated seaward from an isohaline value of 5 ($\approx$ the last 32 km of the river course) acts as nursery grounds (Fernández-Delgado et al. 2007). Three sampling sites were selected in this area: Tarfia in the oligohaline estuarine region (mean salinity $\pm$ standard deviation: 5.4 $\pm$

3.5), La Esparraguera, in the mesohaline estuarine region (10.9 ± 6.7) and Bonanza ($36^{\circ}52'2''\text{N}$ - $6^{\circ}20'53''\text{W}$), in the polyhaline region (21.2 ± 9.8) situated at 32, 20 and 8 km from the river mouth, respectively.

2.2. Data sets

Monthly samplings were conducted from December 2002 to February 2009 (6 annual cycles), at each new moon, from a boat anchored on the East bank of the Guadalquivir estuary; except between January and April of 2007 due to technical problems. Mysid density was estimated by passive hauls carried out with three 10 m-long nets (mesh size: 1 mm; net opening: 2.5 m width and 3 m depth) working in parallel during the first 2 h of the diurnal flood and ebb tides (for more details see González-Ortegón et al. 2010b).

Water temperature ($^{\circ}\text{C}$) and salinity (Refractometer ATAGO S/Mill) were measured in the field at the start of each haul and water samples collected in black glass bottles for estimation of the following variables: turbidity (NTU, Nephelometric Turbidity Units), suspended particulate matter (SPM), nutrient concentrations (nitrate+nitrite, ammonium, phosphate and silicate) and photosynthetic pigment concentration (chlorophyll a and phaeopigments). SPM concentrations were expressed as dry weight after water filtration through preweighted glass-fibre filters (Whatman GF/F) and drying at 80°C for 48 h. Organic matter lost on ignition (OM) was determined after baking the filters for 4 h at 500°C , and inorganic matter concentration (IM) estimated by subtracting organic matter from SPM. To analyse nutrients, water was filtered through Whatman GF/F glass fibre filters under low vacuum, frozen and determined by colorimetric methods, using a TRAACS 800 autoanalyser. Analysis of photosynthetic pigments was conducted by filtering samples through Whatman GF/F glass fibre filters ($0.7\ \mu$ pore-size), freezing until assayed, extracting in 90% v/v acetone and measuring chlorophyll a (Chl a) with a Turner Design 10-AU fluorometer, and phaeopigments (Phaeo) after acidification with 0.1M HCl, following procedure of Yentsch & Menzel (1963). The Chl a/SPM ratio was used as an index of potential primary production in the estuary (Irigoin and Castel 1997).

Daily dam freshwater discharges ($\text{m}^3\ \text{s}^{-1}$) were provided by the Confederación Hidrográfica del Guadalquivir, and daily rainfall (mm) records for the study period obtained from the meteorological station of El Palacio (National Park of Doñana). As a measure of the riverine inflow, freshwater volumes discharged from the dam to the estuary during the week (V_7) or month (V_{30}) were estimated by adding daily freshwater flow ($\text{m}^3\ \text{s}^{-1}$) during those periods; local rainfall during the week (R_7) or month (R_{30}) were similarly estimated before each sampling date. Associations between Chl a concentration and mysid densities with local rainfall and/or dam discharges could be interpreted as physiological (osmotic stress, increased turbidity) or physical (flushing organisms out of the estuary) forcings by freshwater inputs on these biological parameters.

2.3. Data analysis

As the focus of this study was the identification of the freshwater input effects on the rest of the variables recorded, dam discharges and rainfalls were always considered as independent variables. Individual correlation between freshwater inputs and the rest of the variables was ascertained by cross-correlation analysis using three months as the maximum number of time lags between the variables. Possible interactions among dependent variables were assessed by Principal Component Analyses (PCA). The spatio-temporal variability of estuarine conditions was assessed by carrying out a first PCA on all available samples. In order to facilitate the analyses of temporal variability, spatial variability was eliminated by averaging discrete data into monthly means and a second PCA was applied on this new data set. When necessary, data were log-transformed (concentrations and densities) or arcsin square root-transformed (percentages) prior to analyses. The mix of measurement scales of variables was overcome by standardization of data (subtracting the mean and dividing by the standard deviation). Although independent (freshwater inputs) and dependent variables with high co-linearity (OM and IM with SPM) were excluded from the PCA analyses, they were a posteriori introduced as supplementary variables in the PCA plot to visualize their covariation with the rest of variables. Results of PCA analyses were also summarized by providing the correlation coefficients of each variable with the first four or five (eigenvalues > 1) PCs. The role of freshwater inputs on spatial and temporal variations of dependent variables was statistically assessed by a stepwise search of available independent variables (V_7 , V_{30} , R_7 and R_{30}) using the BVSTEP algorithm in BEST analysis. Significance of the correlation coefficients obtained was ascertained by random permutation tests. PCA and BEST analyses and permutation tests were carried out using the computer software PRIMER v6 package (Clarke and Gorley, 2006).

Stepwise least-squares multiple regression was further used to explore the relationship between all environmental variables and photosynthetic pigment concentrations, and between environmental variables (nutrient concentrations excluded) and mysid densities (individuals 10^5 m^{-3}). Prior to their inclusion in regression analyses, turbidity, freshwater volume, rainfall, photosynthetic pigments and density data were $\log(x+1)$ transformed. Only variables with $P < 0.01$ were included in the final model. While the mysid *Neomysis integer* is an estuarine species with strong osmoregulatory capacity, *Rhopalophthalmus tartessicus* and *Mesopodopsis slabberi* are marine species with a moderate tolerance to low salinities and winter migration to the sea (Vilas et al. 2006, 2008). In order to discriminate osmotic stress from other physiological/trophic effects of freshwater input, a further multiple regression analysis for the two marine mysids was carried out using samples with water temperature $> 15^\circ \text{C}$ (both species), and salinity > 9 and > 18 for *M. slabberi* and *R. tartessicus*, respectively. Similarly, for Chl *a* concentration, in addition to the global analysis, a multiple regression analysis was carried out at each sampling site separately. Multiple regression and cross-correlation analyses carried out using the computer software packet STATGRAPHICS Plus 5.0.

3. Results

3.1. Freshwater input

During this six year study, annual rainfall can be considered standard (concerning the last 30 years) whereas the riverine freshwater input to the estuary was only moderate (Figure 1a). As a considerable interannual variation in mean rainfall and the dam's freshwater discharge was observed, three different hydrological periods had been identified (Figure 1b): a wetter hydrological period (WHP), which corresponds to water years with mean to high rainfalls and dam discharges (November 2003-August 2004); a drier hydrological period (DHP), in which after a very dry water year the mean rainfall was not accompanied by freshwater inputs from the dam (September 2004-August 2005); and a more turbid hydrological period (THP), with mean rainfalls followed by strong and sudden freshwater discharges but with a moderate total discharge volume that prevented the introduced SPM from being exported to the sea (September 2007-February 2009).

When the average seasonal variations in the riverine inflow during the last 30 years and those during this study (Figure 1c) are compared, we can see that there were lower winter riverine inflows during DHP and THP, and a certain temporal mismatch during WHP (lower winter discharges but higher discharges in autumn and spring). In contrast, the seasonal rainfall pattern showed a certain temporal mismatch during the first and third hydrological periods (Figure 1c): increased mean rainfall was observed in early autumn (October) and spring (May) during WHP and THP respectively (Figure 1c).

3.2. Physicochemical parameters

Environmental conditions were always very stable during the warmest season (high mean salinity, low/moderate mean turbidity and SPM linked to low freshwater inputs). Contrastingly, notable differences were observed from early autumn to late spring depending on the hydrological periods (Figures 2a-e and Online Resource 2a-e): low water salinity, moderate turbidity and SPM during WHP; high salinity, low turbidity and SPM during DHP; and high salinity, turbidity and SPM during THP (Figures 1b and 2d, e). Only 17% of the estuarine SPM concentration was organic matter (mean OM concentration of 0.06 g l^{-1}). This percentage (POM) followed an inverse seasonal trend to those of SPM and turbidity, with minimum values in winter (13%) and maximum values in summer (21%) (Figures 2f and Online Resource 2f).

On each sampling date, the water temperature was almost homogeneous throughout the studied stretch, whereas salinity decreased gradually upstream (Figures 2b, c and Online Resource 3a, b). In contrast, maximal turbidity and SPM were observed at the most landward sampling site and minimum ones in the seaward zone (Figures 2d, e and Online Resource 3c, d). Nevertheless, the water mass with maximum SPM/turbidity usually migrated downstream during winter and upstream in summer due to the respective increase and decrease in freshwater inputs in these seasons. This seasonal migration was more limited during DHP (Figure 2d, e). IM content displayed a similar spatial pattern to that of SPM, while mean POM decreased (19.3% to 12.7%) downstream (Figures 2f and Online Resource 3f).

3.3. Nutrients, Chlorophyll *a* and Phaeopigments

Nitrates+nitrites were the predominant nutrients in the estuary (on average, 269.8 μM vs 4.4 μM , 3.0 μM and 99.5 μM for ammonium, phosphates and silicates, respectively) (Figures 2g-j and Online Resource 2g-j). They showed a clear, regular seasonal pattern, with maximum concentrations in winter to early spring and minimum concentrations in summer to early autumn. However, since their concentrations tended to peak simultaneously or just after rainfall, an additional maximum was observed in the early autumn (October-November 2003) of WHP (Figures 2a, g and Online Resource 2a, g). Seasonal patterns of ammonium and phosphate concentrations displayed remarkable interannual differences: minimum phosphate concentrations tended to occur when there were maximum freshwater discharges, whereas maximum ammonium levels did when there was strong rainfall (Figures 2h, i and Online Resource 2h, i). In contrast, silicate concentrations did not display a clear seasonal pattern, although minimum values occurred during periods of low freshwater inputs (Online Resource 2j). Spatially, a decreasing downstream nutrient concentration was generally observed in the estuary (Online Resource 3g-j), except for ammonium (Online Resource 3h).

The mean Chl *a* concentration was 3.2 $\mu\text{g l}^{-1}$ and ranged between 20.2 $\mu\text{g l}^{-1}$ in March 2003 and 0.4 $\mu\text{g l}^{-1}$ in April 2004 (Figure 2l). The lowest annual Chl *a* concentration occurred in winter, and two annual maxima were usually recorded in spring and summer. Nevertheless, no peak of Chl *a* was observed during THP (Online Resource 2l). Although the highest mean Chl *a* concentration was recorded at the most landward sampling site (Online Resource 3l), on 45% of the sampling dates the maximum Chl *a* values were observed at the intermediate or most seaward sampling sites (Figure 2l). Moreover, a higher mean Chl *a*/SPM ratio was generally observed at the most seaward sampling site (20.7 $\mu\text{g g}^{-1}$), but this ratio was relatively low at all sampling sites during THP (Figure 2k and Online Resource 3k).

Since the phaeopigment concentration (2.7 $\mu\text{g l}^{-1}$ on average) peaked when there was high freshwater input, lower concentrations were observed in summer and higher values in winter and spring (Online Resource 2m). On average an increasing phaeopigment concentration was observed upstream, but on 37% of the sampling dates (mainly during WHP and THP) the maximal concentration occurred at the intermediate sampling site (Figure 2m and Online Resource 3m).

3.4. Mysid densities

The three dominant mysid species (> 99% of the total mysid density) showed large spatiotemporal changes in density within the estuary. Namely, *Mesopodopsis slabberi* and *Rhopalophthalmus tartessicus* densities showed minimum values in winter and two or three annual density peaks between spring and early autumn. Even though these peaks occurred for both species when freshwater inputs were low, a clear pattern linked to the hydrological periods was only observed for *M. slabberi* (Online Resources 2n, p): the highest and lowest monthly densities of this species were recorded during the driest (DHP) and the most turbid (THP) hydrological period respectively. In contrast, *Neomysis integer* showed high densities after the strongest freshwater inputs in WHP and THP, and very low density throughout DHP, except for a sudden peak in May 2007 after a moderate increase in the freshwater discharge from the dam (Figure 2o).

On average, the density of *M. slabberi* and *R. tartessicus* decreased gradually upstream. Nevertheless, maximum monthly densities of both species were sometimes recorded at the intermediate sampling site, generally in summer of DHP, when the warmest, clearest and most saline water conditions occurred in the studied stretch (Figures 2n, p and Online Resource 3n, p). Contrastingly, the density of *N. integer* increased gradually upstream during DHP, or had an almost constant density throughout the studied area during WHP. However, when the strongest freshwater inputs of WHP and THP caused extremely high turbidity and low salinity in the estuary, maximum densities of *N. integer* usually occurred at the two seaward sampling sites (Figure 2o and Online Resource 3o).

3.5. Linking freshwater inputs to environmental and biological patterns

When all available samples were included in the PCA, temperature related (seasonal) and salinity related (spatial) segregations of samples were respectively observed along the bottom left-top right and bottom right-top left diagonals of the PC1 versus PC2 plot. That is, samples from summer (high temperature and low freshwater inputs) and winter (low temperature and high freshwater input) were respectively clustered at the top right and bottom left corners of the plot. Samples from the most landward sampling site (lower salinity and higher nutrient concentrations) appeared at the top left corner of the plot, and those from the most seaward sampling site (higher salinity and lower nutrient concentrations) at the bottom right corner (Figure 3a, b). Results of the BEST analysis (selected combination of variables: V_{30} and R_{30} or V_7 and R_{30} ; $p = 0.20$; $p < 0.01$) and the correlation analysis of freshwater input variables with

the first four PCs (Table 1) indicated that freshwater inputs significantly contributed to explaining the variability of the environmental and biological conditions observed in the estuary. Although rainfall and dam freshwater discharges were usually associated with increases in the SPM/turbidity and nutrient inputs to the estuary (negative correlations of these variables with PC1), the freshwater input also seemed to dilute the nutrient and *Chl a* concentrations (inverse sign of the correlation coefficients between dependent and independent variables with the PC2, PC3 or PC4).

When spatial variability was eliminated, on the PC1 versus PC2 plot, samples from summer (at the right) and winter (at the left) were segregated along the first PC1 irrespective of their hydrological period (Figure 4a). In contrast, winter and spring samples of WHP and DHP appeared partially segregated from those of THP along the second PC2. According to the orientation of the freshwater inputs/salinity and SPM/turbidity correlation vectors, freshwater inputs were not always associated with an increase in SPM and turbidity during WHP and DHP, whereas they usually were during THP. Similarly, high densities of the mysids *R. tartessicus* and *M. slabberi* were associated with low freshwater inputs (high salinity), high temperatures and high POM, whereas high densities of *N. integer* were linked to high SPM, turbidity and rainfalls (Figure 4b). The results of the BEST analysis again indicated that the combination of V_{30} and R_{30} ($\rho = 0.379$) or V_7 and R_{30} ($\rho = 0.376$) significantly ($p < 0.01$) contributed to explaining the estuarine variability of the environmental and biological conditions. Furthermore, when the Euclidean distances between each pair of correlative samples were represented versus time, the most extreme multivariate changes usually occurred associated with freshwater inputs. However, as indicated by the different trajectory of these changes on PCA plot (Figure 4a, c), a different ecosystem response occurred depending on the season (C_1 and C_2) and/or hydrological period (C_1 , C_3 and C_4).

Results of correlation analyses (Table 2) again highlight the negative correlation between freshwater inputs and salinity, POM and the density of marine mysids; and the positive correlation between some of the freshwater input variables and SPM, turbidity, nutrients, *Chl a* and Phaeo concentrations and density of the estuarine mysid *N. integer*. The only exception was the negative correlation between phosphate concentrations and V_{30} , which suggests that the dam discharges have a dilution effect on this nutrient. Sometimes, there was a one to two month delay between rainfall and the environmental/biological response.

When the entire study area was included in the multiple regressions between the *Chl a* concentration and the environmental and nutrient variables, temperature and the percentage of organic matter were the variables selected by the model with positive and negative coefficients respectively. At the two most seaward sampling sites, temperature was the only variable with a significant positive effect on the *Chl a* concentration. At the most landward sampling site, the only variable selected by the model was the V_7 , which suggests an external *Chl a* input from upstream riverine habitats (Table 3).

When all available samples were included in the multiple regressions, *M. slabberi* density was positively related to water temperature, salinity and POM but negatively to V_{30} and IM; when only samples under physiologically suitable conditions for mysids were analyzed, the *M. slabberi* density was positively related to the *Chl a* concentration and negatively to the IM and R_{30} (Table 2). Using all available samples, the *R. tartessicus* density was positively related to water salinity and *M. slabberi* density; if only samples under physiologically suitable conditions for mysids were used, the *R. tartessicus* density was also positively related to the *M. slabberi* density and OM but negatively to salinity (Table 3). The density of the estuarine mysid *N. integer* was positively related to that of *M. slabberi*, R_{30} and turbidity, but negatively to salinity and potential primary production (*Chl a*/SPM).

4. Discussion

In temperate estuaries, physicochemical and biotic conditions usually display clear temperature-dependent seasonal patterns (Drake and Arias 1991; Childers et al. 2006; Barbosa et al. 2010). Thus, the effects of freshwater inputs on the estuarine biota may depend on their seasonal timing. Furthermore, as freshwater requirements for human activities within the estuarine drainage basin often do not correspond to the natural patterns of freshwater inputs, the irregularity of rainfall in temperate zones may mean that the river inflow is almost completely controlled by dams for a long time. The estuary becomes then dominated by tidal currents, the water residence time increases, and therefore the effects of freshwater inputs may change from year to year (Childers et al. 2006; Barbosa et al. 2010). Indeed, due to interannual changes in rainfall and the subsequent different freshwater management, three clearly different hydrological periods have been observed during this study. The occurrence of both (seasonal and interannual) sources of variability implies that, although some general patterns can be established, the assessment of the freshwater input effects on the estuary may sometimes require a separate analysis of each situation. A good example to illustrate this is the temporal trend of SPM within each hydrological period (Figure 2d, e). Increased freshwater inputs between autumn and spring led to higher SPM and

turbidity in the three hydrological periods, but these increases were larger and more persistent during THP. Different responses to freshwater inputs can also be observed within each hydrological period depending on the season. For example, in 2003 the strong freshwater input in early autumn caused a reduction in density of the two marine mysid species, whereas that of winter did not because both species had already migrated to the sea (C_1 and C_2 in Figure 4b).

Freshwater inputs as physical forcing of the estuarine environment

Freshwater inputs normally determine the extent of the sea water intrusion, and the level and residence time of nutrients within estuaries. As in other estuaries (Irigoien and Castel 1997; Grange et al. 2000; Verity 2002; Chicharo et al. 2000), a nutrient input linked to freshwater inflows seems to occur in the Guadalquivir estuary. Indeed, the seasonal pattern of the estuarine inorganic nitrogen concentration usually matched the timing of local agricultural fertilization practices (winter and early spring); however, strong rainfall events may cause additional peaks of the inorganic nitrogen concentration, as in early autumn of 2003 (Figures 2g). In contrast, because urban sewage from towns can be another important source of phosphate (Sewell 1982; Boyes and Elliott 2006), the location of populous towns along the main course of the Guadalquivir estuary may have obscured the seasonal phosphate input linked to rainfall. Furthermore, dam discharges seem to have a dilution effect on estuarine phosphates. Similarly, nutrient dilution by tidal currents may explain the spatial nutrient patterns observed in the Guadalquivir estuary and other temperate estuaries (Chicharo et al. 2000; Bate et al. 2002); a dilution effect which was more evident in the driest years (Figure 2g).

Under non-nutrient limiting conditions, light availability is often the predominant factor in determining Chl *a* variations in estuarine and coastal waters (Wofsy 1983; Pennock 1985; Barbosa et al. 2010). In the most turbid estuaries, light limitation reduces primary production and increases Chl *a* degradation products (Phaeo) (Wofsy 1983; Kromkamp et al. 1995; Irigoien and Castel 1997). Since the SPM threshold of phytoplankton photosynthesis limitation (50 mg l^{-1} according to Cloern 1987) is usually exceeded in the Guadalquivir estuary (Figure 2e), light-limited primary production is expected there. The exception was the most seaward and less turbid stretch, where typical spring blooms ($\text{Chl } a > 10 \mu\text{g l}^{-1}$) sometimes occur (Figure 2l). Nevertheless, as found in other temperate estuaries (Cloern 1987; Alpine and Cloern 1988; Cole et al. 1992; Irigoien and Castel 1997; Bate et al. 2002), the highest mean concentration of chlorophyll *a* was found at the most landward and turbid zone, mainly during WHP (Online Resource 3l). Furthermore, freshwater inputs sometimes increased the estuarine concentrations of Chl *a* and SPM simultaneously (Figure 2e, l), which suggests importation of Chl *a* from waters with higher primary production (upstream water reservoirs or irrigation channels of adjacent agricultural fields where $159 \text{ Chl } a \mu\text{g l}^{-1}$ were recorded).

PCA and regression analyses also point to this double (autochthonous/allochthonous) source of Chl *a* in the estuary. In the PCAs there was a positive covariance between the Chl *a* concentration and temperature, and a negative covariance with SPM, turbidity and ammonium concentration (Table 1), which suggest autochthonous primary production. In contrast, the positive correlation between the Chl *a* concentration and dam discharges (Table 2) points to an allochthonous input from adjacent habitats. Similarly, multiple regression analyses suggested a Chl *a* importation linked to rainfall, at the most landward and turbid sampling site, and autochthonous Chl *a* production linked to high temperature, at the two less turbid sampling sites (Table 3), where the environmental conditions in the warm period (strong solar irradiation and low turbidity because low freshwater inputs) favour primary production. However, during THP (light-limited conditions) the expected spring/summer peak of autochthonous Chl *a* was not observed (Figure 2l).

When large dam discharges or strong rainfall occur in the estuary, small organisms can be pushed downstream or even flushed out of the system (Wooldridge 1986; Chicharo et al. 2006). In fact, together with salinity, rainfall or dam discharges were selected by the multiple regression models carried out on *N. integer* and *M. slabberi* densities (Table 3), which suggests an additional physical forcing of mysids by freshwater inflows. Due to their different spatial distributions, strong freshwater inflows led to an increased *N. integer* density within the study stretch (downstream drift), whereas *M. slabberi* population was partially flushed out of the estuary by downstream currents (early autumn of 2003 in Figure 2n). Furthermore, as previously found for *Mesopodopsis africana* (Carrasco et al. 2007), high IM seemed to have a negative effect on the *M. slabberi* density (Table 3), particularly evident during spring/summer 2008 (Figure 2n). This supports the hypothesis that a high IM concentration could reduce the health of mysids and their prey.

Freshwater inputs as trophic forcing of the lower trophic levels

Nutrient concentrations significantly exceed the upper range of the half-saturation constants that limits phytoplankton growth (Fisher et al., 1988), which suggests that primary production was not nutrient-limited in this system. Moreover, according to the shallowness and non-water stratification of the estuary, primary production is not likely to be controlled by depth (Wofsy 1983). However, high phytoplankton ingestion by the aquatic inhabitants of the estuary could have contributed to the low mean Chl *a* concentration observed (Sautour et al., 2000). In effect, phytoplankton is an important item in the diet of zooplanktonic estuarine species, as the species of the genus *Mesopodopsis* (Webb et al. 1987; Froneman 2001; David et al. 2006). Contrastingly, phytoplankton was negligible in the diet of the more carnivorous *R. tartessicus* and the opportunistic omnivore *N. integer*, which mainly feed on mesozooplankton (as *M. slabberi* juveniles), and the latter on members of the detrital-microbial loop also (Fockedey and Mees 1999; Vilas et al. 2008). These interspecific differences in food habits of the studied mysids were supported by the multiple regression results (Table 3). Under suitable physiological conditions, *M. slabberi* was the only mysid favoured by phytoplankton availability, whereas both *R. tartessicus* and *N. integer* showed an increased density when the density of *M. slabberi* also increased. Furthermore, the organic matter concentration and turbidity seemed to have a positive effect on *R. tartessicus* and *N. integer* respectively, which could be related to a shift in their diets to prey of lower quality during seasons of low resources (Vilas et al. 2008).

An extremely light-limited autochthonous primary production and a greater accumulation of phaeopigments within algal cells would be expected during THP (Welschmeyer and Lorenzen 1985). Indeed, moderate Chl *a* concentrations and higher Phaeo and IM concentrations were observed in the estuary from December 2007 onwards (Figures 2l, m). That is, the increase in nonliving/living particle ratio may have decreased the availability (low Chl *a*), quality (high Phaeo) and catchability of food. Food characteristics that play a key role in determining mesozooplankton growth and reproduction (Durbin et al. 1983; Koski et al. 1998; Gasparini et al. 1999; Burdloff et al. 2000; Guisande et al. 2000). Thus, the observed decrease in *Mesopodopsis slabberi* abundance (González-Ortegón et al. 2010b) and the increased density of the more detritivorous *N. integer* during THP were also expected (Table 3). A similar increase in the detritivory/herbivory ratio in estuaries has been previously associated with the lack of frequent freshwater inflow and the consequent renewal of the nutrient pool (Scharler and Baird 2005). This change in the *N. integer*/*M. slabberi* ratio could lead to temporal changes in the estuarine food web (González-Ortegón et al. 2010b).

Freshwater inputs as physiological forcing of the lower trophic levels

Freshwater inputs are essential for preserving the estuarine salinity gradient (seawater dilution), which is one of the most crucial spatial gradients within estuaries (McLusky 1993). PCA, correlation and regression analyses confirmed that salinity has a significant positive effect on the two marine mysid species (*R. tartessicus* and *M. slabberi*) and a negative effect on the estuarine species *N. integer* (Tables 1, 2 and 3). Nevertheless, under salinity conditions above physiological thresholds, the salinity effect on marine mysids was negligible (*M. slabberi*) or even negative (*R. tartessicus*), indicating that only physiologically low salinities acted as a critical factor for less euryhaline mysids (McKenney 1994; Webb et al. 1997; Vilas et al. 2009). In fact, *M. slabberi* and *R. tartessicus* populations showed more upstream distributions during DHP than during WHP (Online Resource 3 n, p). In contrast, the high euryhalinity of *N. integer* does not explain its estuarine distribution associated to oligohaline waters (Bernat et al. 1994; Mees et al. 1995; Mouny et al. 1998), and it has been suggested that *N. integer* inhabits more stressful oligohaline zones to avoid competition with other mysid species (Vilas et al. 2006 and 2009). Thus, the low density values shown by this species during DHP (low freshwater inflows) must be related to an upstream shift of the species associated with the upstream shift of the saline gradient (Online Resource 3c, o).

As in most temperate estuaries (Pennock 1985; Goosen et al. 1995; Irigoien and Castel 1997; Heip et al. 1995; Boyes and Elliott 2006), the environmental characteristics of the Guadalquivir estuary suggest that heterotrophic processes are probably dominant. Hypoxic conditions currently occur in the less saline stretch of the estuary (Ruiz, personal communication) due to a low capacity to assimilate the excess organic matter (Heip and Herman 1995; Cloern 2001; O'Higgins and Wilson 2005). This facilitates proliferations of bacteria and other heterotrophs in the oligohaline zone (Crump and Baross 1996; Vilas et al. 2008). However, changes in the river flow may cause changes in nutrient concentrations and stoichiometry and phytoplankton dynamics (Morais et al. 2009). At the start of THP, the strong and sudden freshwater discharges caused an extreme and generalised increase in SPM that may have reinforced the estuarine heterotrophy. In fact, the highest ammonium concentrations were recorded during THP in the oligohaline region (Figures 2h and Online Resource 3h), which suggests an ammonium input from its anoxic/hypoxic bottom. The reinforcement of the anoxic condition in this zone (low

photosynthetic activity and increased oxygen demand) could have caused the observed shift towards more saline waters of the most tolerant species, as with the mysid *N. integer* (González-Ortegón et al. 2010b).

Effects of freshwater inputs on the neighbouring coast

The excess of nutrient inputs (hypertrophication) to estuaries is usually linked to human activities within their drainage basin (Cloern 1987; Peierls et al. 1991; Nixon 1995; Cruzado et al. 2002; Verity 2002; Boyes and Elliott 2006). When nutrient concentrations of the Guadalquivir estuary were compared with those from other estuaries (Kromkamp et al. 1995; Kennish 2001; Iriarte and Purdie 2004; Lillebø et al. 2005; Azevedo 2006), a nitrogen hypertrophication similar to that of the Westerschelde estuary (Kromkamp et al. 1995) was observed. However, under the current light-limited conditions (high SPM), the estuary can receive large nutrient inputs without eutrophication occurring (Irigoin and Castel 1997). The exportation of these excessive nutrients to more transparent neighbouring coastal waters generates phytoplankton blooms on the shelf (Navarro and Ruiz 2006; Prieto et al. 2009). The associated increase in the coastal productivity may favour the survival of marine larvae and the local adult population of commercial species (Chícharo et al. 2001; Lloret et al. 2001, 2004). Currently, the northeastern shelf of the Gulf of Cadiz acts as a spawning and nursery habitat for marine fish (Baldó et al. 2006). In moderation, nutrient inputs from estuaries to the adjacent coasts may therefore be beneficial. However, hypoxia in coastal waters is predicted to increase in frequency and intensity in next years due to the combined effect of eutrophication and increased temperature (Vaquer-Sunyer and Duarte 2008). Thus, even if the current hypertrophication of this estuary may benefit primary production on the adjacent coasts, in the long term excesses of agricultural fertilizers could become a threat to them.

Conclusions

The results of this study suggest that natural and human-controlled freshwater inputs to the Guadalquivir estuary currently play a significant role in determining the physicochemical conditions and therefore the biological production within the estuary (bottom up control). Moreover, natural irregularity in rainfall combined with freshwater requirements for certain human activities have caused the development of different hydrological periods, in which the estuary's response to freshwater inputs is not always the same and therefore cannot be easily predicted. Nutrient, SPM and allochthonous photosynthetic pigment importation linked to freshwater (rainfall/riverine) inputs was observed, as well as changes in the mysid populations which were directly or indirectly (via physicochemical water conditions) related to freshwater management. Despite the high nutrient concentrations (mainly nitrogen hypertrophication due to cropland runoff), the high turbidity suggests that there is light-limited primary production (physical forcing) in the estuary, at least in the oligohaline region, and that the autochthonous primary production is constrained to the most seaward and less turbid reaches. The effects of freshwater inputs on mysids varied depending on their specific salinity tolerances and trophic features. The populations of marine mysids *M. slabberi* and *R. tarteassicus* only showed high densities at salinities above physiological thresholds (physiological forcing), while the more euryhaline and detritivorous *N. integer* mainly showed high densities in less saline but more turbid waters (higher SPM, trophic forcing). In contrast, under physiologically suitable conditions for mysids, the availability of preferred preys (*M. slabberi* density for *N. integer* and *R. tarteassicus*; Chl *a* for *M. slabberi*) affected the densities of the dominant mysid species (trophic forcing). Moreover, low *M. slabberi* densities were observed under conditions of high inorganic matter probably due to particle impact injuries on this small mysid (physical forcing) and/or diminished food quality (trophic forcing). This led to an increased detritivory/herbivory ratio and a consequent transitory alteration of the estuarine food web (González-Ortegón et al. 2010). In summary, as previously proposed for other estuaries, the effects of estuarine freshwater inputs on the lower trophic levels seem to respond to a combination of several (physical, physiological and trophic) forcing mechanisms.

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Figure captions

Fig. 1 Dam freshwater discharge and rainfall in the Guadalquivir estuary: annual discharge and rainfall during last 30 years, and mean values of freshwater discharge (dotted lines) and rainfall (dashed lines) during last 30 years and the study period (a); daily freshwater discharge and rainfall during the study period (b); and seasonal freshwater discharge and rainfall during the study period (vertical bars) and during last 30 years (full circles) for each hydrological period (c). Vertical dotted lines delimitate the first wetter hydrological period (WHP), the second driest hydrological period (DHP) and the third more turbid hydrological period (THP).

Fig. 2 For each sampling site (● Bonanza, ▲ Esparraguera and ■ Tarfia) and date, mean values of rainfall and dam's discharges (a), water temperature (b), salinity (c), turbidity (d), SPM (suspended particulate matter) (e), POM (percentage of organic matter) (f), nutrient (nitrate+nitrite, ammonium, phosphate and silicate) concentrations (g, h, i, j), Chl *a* (chlorophyll *a* concentration)/SPM (k), Chl *a* (l), Phaeo (phaeopigment concentration) (m) and mysid densities (n, o, p) in the Guadalquivir Estuary from December 2002 to February 2009. F, February; M, May; A, August; N, November. Vertical dotted lines delimitate the first wetter hydrological period (WHP), the second driest hydrological period (DHP) and the third more turbid hydrological period (THP).

Fig. 3 Results of the Principal components analysis (PCA) carried out on discrete samples of the Guadalquivir estuary: PC1 and PC2 ordination diagrams showing the projection of samples (a), and the correlation circle and correlation vectors of included and supplementary (variables labelled in pink) with the PCs (b). T water temperature, S salinity, Tu turbidity, Chl *a* Chlorophyll *a* concentration, Phaeo Phaeopigments, IM suspended inorganic matter, OM suspended organic matter, SPM suspended particulate matter, Vi and Ri freshwater inflow from the dam and rainfall

during the previous i days, respectively, Si Silicate, Am Ammonium, Phos Phosphate, N+N Nitrate + Nitrite, Ni *Neomysis integer*, Rt *Rhopalophthalmus tartessicus*, Ms *Mesopodopsis slabberi*.

Fig. 4 Results of the Principal components analysis (PCA) carried out on monthly mean samples of the Guadalquivir estuary: ordination diagrams showing the projection of samples (a), the correlation circle ($r = 1$) and vectors of included and supplementary (variables labelled in pink) with the PC1 and PC2 (b). Besides, Euclidean distances between successive samples, dam's freshwater discharge and rainfall versus time (c). Variables abbreviations see Figure 6., C_i the greatest multivariate changes between successive samples.

Online Resource 1. Map of the Guadalquivir estuary (SW Spain) showing the location of the sampling sites.

Online Resource 2 For each hydrological period, monthly mean values (+S.E.) of rainfall and dam's discharges (a), water temperature (b), salinity (c), turbidity (d), SPM (e), POM (f), nutrient (nitrate+nitrite, ammonium, phosphate and silicate) concentrations (g, h, i, j), Chl *a* /SPM (k), Chl *a* (l), Phaeo (m) and mysid densities (n, o, p) in the Guadalquivir Estuary from December 2002 to February 2009. J...D, January...December. See the caption of Figure 3 for explanation of abbreviations.

Online Resource 3 For each sampling site and hydrological period, mean values (+S.E) of water temperature (a), salinity (b), turbidity (c), SPM (d), IM (inorganic matter concentration) (e), POM (f), nutrient (nitrate+nitrite, ammonium, phosphate and silicate) concentrations (g, h, I, j), Chl *a* /SPM (k), Chl *a* (l), Phaeo (m) and mysid densities (n, o, p) at 32, 20 and 8 kilometres from mouth of the Guadalquivir Estuary from December 2002 to February 2009. See the caption of Figure 3 for explanation of abbreviations.

Figure 3

Table 1. Pearson's correlation coefficients of correlation between environmental and biological variables, and the principal components (eigenvalues > 1) of PCAs carried out on Guadalquivir estuary data

Variable	All samples (n = 379)				Monthly mean samples (n =142)				
	PC ₁	PC ₂	PC ₃	PC ₄	PC ₁	PC ₂	PC ₃	PC ₄	PC ₅
Var Exp (%)	(36)	(15)	(12)	(7)	(33)	(16)	(13)	(9)	(7)
Temperature	0.57	0.50	0.26	n.s.	0.82	n.s.	n.s.	-0.21	n.s.
Salinity	0.83	-0.46	n.s.	n.s.	0.69	-0.59	n.s.	n.s.	n.s.
Turbidity	-0.75	-0.31	n.s.	0.14	-0.82	-0.45	n.s.	n.s.	n.s.
SPM	-0.48	-0.50	0.41	n.s.	-0.57	-0.66	0.25	-0.20	n.s.
IM	-0.49	-0.49	0.41	n.s.	-0.58	-0.65	0.27	-0.19	n.s.
OM	-0.29	-0.47	0.36	0.16	-0.40	-0.71	0.20	-0.22	n.s.
POM	0.77	0.11	-0.14	0.16	0.58	-0.49	n.s.	-0.26	-0.20
Chl a	n.s.	0.31	0.78	0.19	0.28	n.s.	0.82	0.32	n.s.
Phaeo	-0.38	-0.14	0.52	0.30	-0.47	-0.29	0.70	0.21	n.s.
Ammonium	-0.15	-0.60	-0.32	0.26	-0.46	-0.29	-0.39	0.19	-0.34
NO₃ + NO₂	-0.74	0.22	-0.21	0.16	-0.59	0.18	-0.19	0.50	0.40
Phosphate	-0.24	0.46	-0.21	0.41	n.s.	n.s.	-0.27	0.56	-0.67
Silicate	-0.65	0.57	n.s.	-0.13	-0.23	0.65	n.s.	-0.47	-0.43
<i>M.slabberi</i>	0.75	0.24	n.s.	0.32	0.90	n.s.	n.s.	0.18	n.s.
<i>N. integer</i>	-0.56	n.s.	-0.34	0.24	-0.45	-0.27	-0.39	-0.18	n.s.
<i>R. tartessicus</i>	0.74	-0.30	n.s.	0.28	0.69	-0.26	-0.27	0.16	n.s.
V ₇	-0.23	0.14	n.s.	-0.23	-0.74	0.18	n.s.	n.s.	-n.s.
V ₃₀	-0.24	n.s.	n.s.	-0.29	-0.55	0.38	n.s.	n.s.	-0.21
R ₇	-0.40	n.s.	-0.13	n.s.	-0.30	0.28	n.s.	-0.33	n.s.
R ₃₀	-0.55	-0.24	-0.11	n.s.	-0.32	0.48	n.s.	n.s.	n.s.

Bold number p<0.01, normal number p<0.05, n.s. not significant

Highlighted in bold, variables included in PCAs, in bracket percent of variation explained by each PC

Table 2. Maximal associations (Pearson's correlation coefficient) between freshwater inputs (rainfalls and dam's discharges) and monthly mean values of environmental and biological variables in the Guadalquivir estuary.

	R ₇	R ₃₀	V ₇	V ₃₀
Temperature	-0.48*	-0.64•	n.s.	n.s.
Salinity	-0.63•	-0.65•	-0.67•	-0.55•
Turbidity	0.28•	0.49•	n.s.	n.s.
Suspended Particulate Matter	0.27**	0.30•	0.26*	n.s.
Inorganic Matter	0.28**	0.29•	n.s.	n.s.
Organic Matter	n.s.	n.s.	-0.36 **	n.s.
Percentage of Organic Matter	-0.42•	-0.49•	-0.38•	-0.23•
Ammonium	n.s.	0.29•	n.s.	n.s.
Nitrate + Nitrite	0.42**	0.54**	0.30•	n.s.
Phosphate	n.s.	n.s.	n.s.	-0.37•
Silicate	0.44•	0.32•	0.45•	0.42•
Chlorophyll a concentration	n.s.	n.s.	0.41•	0.36•
Phaeopigments	0.23•	0.38•	n.s.	n.s.
<i>M. slabberi</i>	-0.51•	-0.63•	-0.24•	n.s.
<i>N. integer</i>	0.31**	0.46**	n.s.	0.24•
<i>R. tartessicus</i>	-0.51•	-0.53•	-0.35•	n.s.

Bold number p<0.01, normal number p<0.05, n.s. not significant.

0(•), 1(*) and 2(**) sampling dates after which the dependent variable showed maximal correlation with freshwater input variables. V_i and R_i dam's freshwater discharge and rainfall during the previous i days

Table 3. Partial results of least-squares multiple regressions of Chlorophyll a concentration and mysid density (X) with environmental variables in the Guadalquivir estuary.

	Regression equation	r^2 (n)
<i>Chl a</i> concentration		
All samples	$X = 0.62 + 0.02 T - 0.35 POM$	12 (376)
32 km from sea	$X = 0.19 + 0.29 V_7$	16 (114)
20 km from sea	$X = 0.09 + 0.02 T$	28 (126)
8 km from sea	$X = 0.08 + 0.02 T$	22 (122)
<i>Mysid</i> species		
<i>Mesopodopsis slabberi</i>		
All samples	$X = 2.76 + 0.11 T + 0.04 S - 0.84 V_{30} - 1.56 IM + 1.31 POM$	62 (377)
$S > 9$; $T > 15^\circ C$	$X = 6.26 - 4.28 IM - 0.17 R_{30} + 0.56 Chl a$	42 (158)
<i>Rhopalophthalmus tartessicus</i>		
All samples	$X = -1.33 + 0.18 S + 0.29 Ms$	61 (380)
$S > 18$; $T > 15^\circ C$	$X = 3.20 + 0.48 Ms + 7.87 OM - 0.03 S$	42 (69)
<i>Neomysis integer</i>		
All samples	$X = 0.67 R_{30} + 0.39 Ms - 0.07 S - 1.06 Chl a/SPM + 0.70 Tu$	44 (379)

Prior to the analysis, all variable were logged-transformed (except water temperature and salinity) and only variable with $P \leq 0.01$ were included in the model

T water temperature, S salinity, Tu turbidity, $Chl a$ Chlorophyll a concentration, IM suspended inorganic matter, OM suspended organic matter, POM percent of suspended organic matter, $Chl a/SPM$ chlorophyll a concentration/ suspended particulate matter, Ms *Mesopodopsis slabberi*, Vi and R_i , freshwater inflow from the dam and rainfall during the previous i days, respectively

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Aquatic Sciences

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