Empirical modelling of seston quality based on environmental factors in a mussel culture area (NW Iberian upwelling system)

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Abstract

We analyze the spatial and temporal variability of seston parameters at four locations in the Ría de Ares-Betanzos (NW Spain) and throughout five years. Seston content was higher in the inner part of the ría and during winter, while seston quality was better in the outer part of the ría with maximum values during summer, showing a marked relationship with water circulation. Inter-annual differences were detected only in the organic content of seston –which was not always well-correlated with chlorophyll *a*- and at some locations. Seston quality was the variable that showed the strongest relation with meteorological factors and the only that showed to be consistent at the four sites within the embayment. This fact let us to develop an empirical model that explains the spatial-temporal variability of seston quality in terms of wind stress and river discharge.

Keywords: Modelling; seston quality; coastal upwelling; river flows; seston; aquaculture; spatial-variability; seasonal-patterns.

1. INTRODUCTION

Coastal upwelling regions are unique niches for marine communities that feed on suspended particles (Isla et al. 2010). Among these regions, the Galician coast (NW Spain) is a top site for mussel culture of *Mytilus galloprovincialis* on hanging ropes. The high yield of mussel culture in the large coastal embayments that occupy the Galician coast, known as “rías”, has been attributed to their particular combination of fertilization by upwelling and intricate coastline that guarantees protection to mussel rafts against rough weather conditions (Figueiras et al. 2002a, Villegas-Ríos et al. 2011). In these highly productive embayments, suspension-feeders are exposed to dramatic changes in the numbers, size and nutritional value of suspended particles, which are often related with environmental forcings (Hawkins et al. 1996).

Seston accessible to marine filter feeders includes a range of organic particles with varying nutritional value, from living phytoplankton to detritus of different origins and silt (Navarro et al. 2009). In the Galician Rías, phytoplankton biomass estimated from chlorophyll concentration explains <40% of the particulate organic matter (Navarro et al. 1996, Figueiras et al. 2002a). These embayments are considered as low-seston environments where total particulate matter (TPM) is usually less than 3 mg l⁻¹, and chlorophyll-a (Chla) concentration is less than 5 µg l⁻¹. The feeding process in bivalves in low-seston environments is expected to be less complex (Duarte et al. 2010a); selective ingestion processes and, therefore, pseudofaeces are not produced due to the particular conditions of the seston (Figueiras et al. 2002a, Fernández-Reiriz et al. 2007, Filgueira et al. 2009, 2010).

Historically, seston quality has been assessed measuring its total organic matter, organic carbon, organic nitrogen and/or chlorophyll-a content. Other biochemical parameters such as the protein, lipid and carbohydrate content have been suggested to assess seston

quality too (Widdows et al. 1979, Navarro et al. 1993, Navarro & Thompson 1995, Sarà et al. 1998, Sarà & Pusceddu 2008). Many other works define it as the ratio between organic and total particulate matter (denoted as f) (Navarro et al. 1991, 1996, Iglesias et al. 1996, Babarro et al. 2000, Wong & Cheung 2001, 2003, Velasco & Navarro 2002, Fernández-Reiriz et al. 2007, Helson & Gardner 2007, Irisarri et al. 2014, 2015). Hawkins et al. (2002) concluded that the bivalve absorption efficiency is independent of the composition of the diet, being related to the organic content of ingested matter (OCI) that, in absence of selective ingestion processes, is **equivalent to seston quality, defined as the proportion of organic matter, $f = \text{POM}/\text{TPM}$. This ratio will be used as a proxy to the nutritional value of seston throughout the manuscript.**

In this work, we have considered the quantity of seston available to suspension feeders in terms of phytoplankton ($\text{Chl}a$), total particulate matter (TPM) which is the sum of the particulate organic matter (POM, including phytoplankton) and particulate inorganic matter (PIM) and seston quality in terms of f (POM/TPM). The relationship between environmental forcings and seston is a highly demanded task within the framework of the Ecosystem Approach to Marine Aquaculture (EAA) (Byron et al. 2011). This approach looks for the comprehensive integrated management of human activities based on the best available scientific knowledge about the ecosystem and its dynamics (Cranford et al. 2012). In this context, our study integrates an extensive database of both relevant seston parameters recorded weekly during 5 years in four sampling sites and simultaneous data of wind and river discharge in the Ría de Ares-Betanzos.

The main motivation of this study is to look for and to establish relationships between seston parameters and meteorological forcing agents within the Ría de Ares-Betanzos. Results were divided in two specific goals: (1) to describe and characterize the content and quality of seston for a better understanding of the coastal embayment of the Ría de

Ares-Betanzos and (2) to develop an empirical model that reveals and assesses the intimal connexion between seston and both winds and river discharge in the ría. The main innovative value of this work is the use of such large amount of data to describe both the spatial and the temporal variability of seston in different cultivation areas of this embayment and the establishment for first time, to the best our knowledge, an empirical model able to explain seston behavior based on key meteorological variables. This approach is interesting for the development of seston quality predictive models, which are demanded for a correct ecosystem-management.

2. MATERIALS AND METHODS

2.1 Study site

The Galician coast is at the northern limit of the eastern boundary upwelling system of the North Atlantic. Coastal winds in this area describe a seasonal cycle characterized by upwelling favourable north-easterly winds from March-April to September-October and downwelling favourable south-westerly winds the rest of the year (Wooster et al. 1976, Torres et al. 2003). During the upwelling season, upwelling events occur with a 1–2 weeks periodicity (Alvarez-Salgado et al. 1993). The Ría de Ares-Betanzos is the largest of the six embayments located in the northern Galician coast, between Cape Fisterra and Cape Prior (NW Iberian Peninsula; Fig. 1), with a surface area of 72 km², a volume of 0.75 km³ and a maximum length of 19 km. It has two main branches: Ares, the estuary of river Eume, and Betanzos, the estuary of river Mandeo. In the outer part, the two branches converge into a confluence zone that is freely connected to the adjacent shelf through a mouth that is 40 m deep and 4 km wide. In fact, the confluence zone can be considered as an extension of the adjacent shelf that is affected by the intensity, persistence and direction of coastal winds (Bode & Varela 1998, Villegas-Ríos et al. 2011). This study is based on the data collected in four locations within this

Ría (Fig. 1): Miranda and Lorbé in the northern and southern outer part, respectively, and Redes and Arnela in the northern and southern inner part, respectively.

2.2 Seston

Seston concentration and quality were monitored weekly during 5 years (January 2007 to December 2011) at the four locations. The quality and quantity of seston was determined as follows. Total particulate matter (TPM; mg L^{-1}) and the constituent organic (POM; mg L^{-1}) and inorganic (PIM; mg L^{-1}) concentrations were gravimetrically determined. Seston samples were filtered onto pre-ashed ($450\text{ }^{\circ}\text{C}$ for 4 h) and pre-weighed Whatman GF/F filters and rinsed with isotonic ammonium formate (0.5 M) to remove salts and prevent lysing of living algal cells. TPM was determined as the weight increment after drying the filters to constant weight at $110\text{ }^{\circ}\text{C}$. Filters were then ashed at $450\text{ }^{\circ}\text{C}$ in a muffle furnace to determine the content of PIM. Particulate organic matter corresponded to the difference between the total dry matter weight and the ash weight. Filters were weighed with an accuracy of 0.001 mg using an electronic microbalance (Sartorius M3P, M3P-000V001). Seston quality was expressed as $f = \text{POM} / \text{TPM}$ to account for the relative organic content by weight.

Two one-liter seawater samples were weekly collected at each location and filtered through 25-mm Whatman using GFF filters ($0.7\text{ }\mu\text{m}$) for determination of Chl*a* concentrations. All filters were frozen at $-20\text{ }^{\circ}\text{C}$ to facilitate cellular lysis and enhance chlorophyll extraction. Pigments were extracted using 5 ml of 90% acetone as a solvent, and left in the dark for 12 h. The solution was then centrifuged at 4500 rpm at $10\text{ }^{\circ}\text{C}$ for 10 min to isolate the chlorophyll extract from the filter residues. Chl*a* was quantified using a Perkin-Elmer Lambda 35 UV/VIS spectrophotometer and the concentration was calculated following Jeffrey and Humphrey (1975): Chl-*a*

$$=(11.85 (E_{664} - E_{750}) - 1.54 (E_{647} - E_{750}) - 0.08 (E_{630} - E_{750})v) / V$$
, where E_{750} , E_{664} , E_{647} and E_{630} are the absorbances at 750, 664, 647 and 630 nm respectively; v is the volume of acetone used in the extraction (ml); and V is the volume of filtered seawater (ml). Three replicas were obtained from each seawater sample. The final weekly chl a values at each location were obtained averaging the six replicas.

2.3 Environmental factors

Shelf winds

Shelf winds were obtained at 6 hours intervals from the Seawatch buoy of the Spanish Agency Puertos del Estado off Cape Vilano ([http:// www.puertos.es](http://www.puertos.es)). Gaps of less than 24 hours were interpolated linearly. For gaps of more than 24 hours, the time series were reconstructed from FNMOC model data obtained in the nearest location available (off Cape Fisterra) using General Additive Models (GAM). The goodness of fit of the GAM was around 70% of deviance explained. Reconstructed data represented 17% of the time series. Then, daily wind values were obtained by applying an 8th order Chebyshev type I low-pass filter with cut-off frequency of $8*(F_s/2)/R$, where F_s is the sampling interval and R is the rate at which we resampled our data.

River discharge

The flow of river Mandeo, Q_M , was taken from gauge station nº 464 at Irixoa, administered by the Galician Agency Augas de Galicia. The Horton's Law (Strahler 1963) was applied to estimate flow at the river mouth (total drainage basin: 456.97 km²) from the flow at the gauge station (gauged drainage basin: 248.21 km²). The flow of the river Eume, Q_E , is a combination of regulated and natural flows. Daily volumes of the Eume reservoir, which controls 80% of its drainage basin, were provided by the managing company ENDESA S.A. Assuming that the retention constant for the drainage basin of river Eume is the same than for the river Mandeo, the natural

component of the flow of the river Eume was calculated again from the Horton's Law considering the area not controlled by the reservoir (96.04 km²). Both time series have a daily sampling interval.

2.4 Statistical analysis

An assortment of statistical tools was used for a straightforward interpretation and comparison of the data. First, exploratory analysis was conducted to describe the environmental factors and their seasonal patterns. Then, spatial variability in the time series of seston concentration and quality (f) was evaluated comparing trends between locations by means of a non-parametric test for dependent data. Annual-seston time series were classified by means of a cluster algorithm for functional data. Finally, Generalized Mixed Additive Model (GAMM) were run to model f from meteorological factors (coastal wind and river discharge).

Exploratory analysis of meteorological factors

In order to explore the seasonal variability of the meteorological factors, we analysed their distribution by seasons. Seasons were defined as: spring (from 22 March to 21 June), summer (from 22 June to 21 September), autumn (from 22 September to 21 December) and winter (from 22 December to 21 March). The seasonal structures of river discharge and wind speed were fitted by kernel density estimation using Silverman's rule of thumbs to select the optimal bandwidth (Silverman 1986). To analyse wind direction we applied the kernel circular density estimator using least squares cross-validation to select the optimal bandwidth (Oliveira et al. 2013b). Finally, the relationship between wind direction and speed was analysed by circular-linear kernel regression, using least squares cross-validation to select the optimal bandwidth

169 too (Oliveira et al. 2013b). These analysis were conducted with the NPCirc package of
 170 R (Oliveira et al. 2013a, R Core Team 2013).

171 *Comparison of trends in seston concentration and quality*

172 For each location, we estimated the temporal trends of seston content and quality by
 173 kernel regression for dependent data, using least squares cross-validation for bandwidth
 174 selection (Chu & Marron 1991, Francisco-Fernández & Vilar-Fernández 2001).
 175 Comparison between locations was conducted using the non-parametric test for curves
 176 with dependent errors proposed by Vilar-Fernández & González-Manteiga (2004).
 177 These authors used a Cramer-von-Mises type statistic to compare K non parametric
 178 regression curves $(m_1, \dots, m_K)$ from samples $\{Y_k(t); k=1, \dots, K\}$, where:

$$Y_k(t) = m_k(t) + e_k(t) \quad (1)$$

179 In our case, the functions m_k , refer to the estimated trends ($K=4$) and e_k are the random
 180 errors which have a time-dependent structure (ARMA(p,q)). Both trend estimation and
 181 their corresponding comparison were implemented with the PLRModels package of R
 182 (Aneiros-Pérez & López-Cheda 2014).

183 *Cluster analysis for functional data*

184 In functional data analysis (FDA) (Ferraty & Vieu 2006) we assume that a high
 185 dimensional vector represents a set of discrete observations of a continuous function.
 186 This method replaces the sampled functions (discrete observations) by functional
 187 representations (curves). In our case, each curve represents the evolution of a variable
 188 thorough a year at each location. FDA allows working with irregular sampling intervals
 189 and missing values, which are two of the commonest drawbacks of field data from
 190 monitoring networks.

In this work, functional representations were constructed using B-splines basis functions, where both the optimal number of basis and the penalization parameter were estimated by generalized cross validation (GCV). Once we have obtained the functional representation, we applied a k-means algorithm for functional data to search for inter-annual and spatial variability in seston concentration and quality. This analysis was conducted with the `fda.usc` package of R (Febrero-Bande & Oviedo de la Fuente 2012).

Generalized mixed additive model (GAMM)

The relationship between seston quality (f) and environmental factors (Eume and Mandeo freshwater discharges (Q_E and Q_M , respectively), wind intensity (w) and wind direction (θ)) at each location was modelled using generalized additive mixed models (Wood 2006a, Zuur et al. 2009) with quasi-binomial family and logit link function, given that the response (f) is a proportion. GAMM models were chosen since the residuals of the fixed effects models (GAM) did not fulfil the normality assumption (see Appendix II). The use of GAMM instead of GAM models lets us to consider the spatial variability in the response without adding new parameters.

Model selection was performed in two stages: first, we considered a pure additive model and looked for interactions between environmental factors and location. Once these interactions were discarded, the interactions between environmental factors were incorporated. The variables and high order interactions involved in the model were selected using the shrinkage procedure proposed by Marra & Wood (2011). These procedures provided the following model:

$$\begin{aligned} \log it(E[f_{ik}]) = & \alpha_k + g_1(w_i) + g_2(\theta_i) + g_3(\log(Q_{E,i} + 1), \log(Q_{M,i} + 1)) \\ & + g_4(\log(Q_{E,i} + 1), w_i, \theta_i) + g_5(\log(Q_{M,i} + 1), w_i, \theta_i) + s_{ik} \end{aligned} \quad (2)$$

where f_{ik} is the seston quality at time i and location k ; α_k is the intercept for each location; $g_{1...5}$ are the environmental-factors (covariates) smooth functions. g_1 and g_2 , are

the functions for speed and wind direction represented using thin plate penalized regression splines (TPRS) and cyclic cubic regression splines (CCRS), respectively. g_3 , g_4 and g_5 represent the interactions between covariates. They were estimated using scale-invariant tensor product smoothers (Wood 2006b): g_3 is a tensor product of TPRS, while g_4 and g_5 are tensor products of TPRS (for river discharges and wind intensity) and CCRS (for wind direction); s_{ik} are the location random effects assumed independent and identically distributed $N(0, \sigma_s^2)$. River discharges were log-transformed to reduce over-dispersion. Model fitting was conducted with the *mgcv* package of R (Wood 2006a).

3. RESULTS

3.1 Environmental factors

Shelf winds

The rose of shelf winds (Fig. 2a) shows that the predominant direction was along the NE-SW axis. North-Easterlies (NE) were more common (40%) than South-Westerlies (SW) (33%) during the study period. South-Easterlies (SE) and North-Westerlies (NW) were much less frequent (8% and 18%, respectively). The most frequent wind speed was 5–10 m s⁻¹ and the most intense, 15–20 m s⁻¹, were reached only with SW winds.

The density estimators of wind speed (Fig. 2b) show that all seasons followed a similar distribution; although the most intense winds were recorded during winter. The density estimators of wind direction (Fig. 2c) point out the prevalence of NE winds throughout the year, except during the winter when SW and NE winds had the same prevalence. The interaction between wind speed and direction (Fig. 2d) reveals that NE winds were the most intense for all seasons, except during winter when SW winds acquired the same intensity as NE winds.

239 *River discharge*

240 The long-term average flow of River Eume ($15.4 \text{ m}^3 \text{ s}^{-1}$) was significantly higher than
 241 River Mandeo ($13.4 \text{ m}^3 \text{ s}^{-1}$; Wilcoxon test, $p\text{-value} < 2 \times 10^{-16}$). The long-term
 242 variability of river discharges was higher at River Mandeo (coefficient of variation, c.v.
 243 149 %) than at River Eume (c.v. 102 %). Time series of both rivers showed a marked
 244 seasonal pattern with higher discharges during winter and spring (Fig. 3a). River flows
 245 did not exceed $23 \text{ m}^3 \text{ s}^{-1}$ during the summer months and, although they exceeded
 246 occasionally $150 \text{ m}^3 \text{ s}^{-1}$, 95% of the time during winter they were $< 75 \text{ m}^3 \text{ s}^{-1}$.
 247 Figures 3b and 3c highlight the seasonal pattern described above but also provide
 248 information about the inter-annual variability. It is remarkable the increase of both river
 249 volumes from March to May 2008 and during June 2010. The seasonal density
 250 estimators of river flows (Figs. 3d & e) show that during summer barely had dispersion
 251 and their discharges were concentrated around 5 and $2.5 \text{ m}^3 \text{ s}^{-1}$, for rivers Eume and
 252 Mandeo, respectively. During the spring and autumn both rivers discharged similar
 253 volumes with median values of $9 \text{ m}^3 \text{ s}^{-1}$ for River Eume (both spring and autumn) and
 254 $8.5 \text{ m}^3 \text{ s}^{-1}$ (spring) and $4 \text{ m}^3 \text{ s}^{-1}$ (autumn) for River Mandeo. During winter the median
 255 values increased for both rivers: $25 \text{ m}^3 \text{ s}^{-1}$ for River Eume and $17 \text{ m}^3 \text{ s}^{-1}$ for River
 256 Mandeo.

257 **3.2 Spatial, seasonal and inter-annual variability of seston**

258 The long-term mean (standard deviation) values for TPM, POM, PIM, Chla and f in the
 259 Ría de Ares-Betanzos were 1.72 (1.65), 0.61 (0.42), 1.13 (1.42) mg L^{-1} , 1.81 (1.55) μg
 260 L^{-1} and 0.46 (0.22), respectively. Table 1 and the raw time series (Appendix I) show
 261 their spatial and seasonal variability.

262 *Spatial variability*

Seston content and quality differences within the ría were evaluated comparing the trends of the main seston variables throughout the five years between locations (Fig. 1). We found that the inner and the outer part of the ría followed different trends (p-values < 0.01): locations at the inner side (Redes and Arnela) presented higher seston content (TPM, POM and PIM; Figs. 4a, b & c) than the outer locations (Miranda and Lorbé). However, we only observed significant differences between the northern and southern shores for POM, which was higher in Redes than in Arnela (Fig. 4b). Seston quality (f ; Fig. 4d) followed an opposite spatial pattern to seston content, with higher values in the outer side of the ría, although we only found significant differences between Lorbé, which recorded the highest values, and the inner side (p-values < 0.001).

Seasonal patterns and inter-annual variability

The cluster analysis for functional data found more spatial than inter-annual variability in the seasonal patterns of seston content and quality, confirming the differences between the inner and the outer side of the embayment. Inter-annual variability was detected only for POM. Results are summarized in Figure 5 and Table 2.

The classification of TPM and PIM into two groups (Figs. 5a & c; Table 2) shows that both variables followed the same seasonal pattern at the four locations with higher values in the inner (red line; Redes and Arnela) than in the outer (black line; Miranda and Lorbé) side of the ría. Both seasonal patterns indicate that TPM and PIM increase in winter and decrease in summer.

Classification on three groups was necessary to identify the main seasonal patterns of POM. Inter-annual variability was detected at Arnela, Lorbé and Miranda, which exhibited a different seasonal pattern during 2008. This pattern of POM was characterized by a large summer peak, which was also observed at Arnela in 2010 (Table 2, green; Fig. 5b). Redes had a common seasonal pattern along the five years

with slightly higher POM values in winter and summer than in spring and autumn (red line; Figure 5b). This pattern was similar to the one obtained at the other three locations (black line; Fig. 5b) but with higher content of POM.

f (Fig. 5d) followed a well-defined and common seasonal pattern at all locations, with high (low) quality during the summer (winter) months. Outer locations (Miranda and Lorbé; red line) had higher seston quality than inner locations (Redes and Arnela; black line) along the year.

Chl a followed a common seasonal pattern in Arnela, Lorbé and Miranda. Inter-annual variability was only observed at Miranda, which in 2011 followed the same pattern as Redes. This pattern is characterized by two peaks in spring and autumn. Chl a concentration is higher in Redes than in the other locations, except during summer.

The results of the correlations between f and Chl a and between POM and Chl a are shown in Table 3. For both cases the correlation coefficients were positive and significant at all locations. The correlation between variables was low ($R < 0.4$) at all locations but at Lorbé (southern outer). The minimum correlation was found at Redes (northern inner).

3.3 Seston quality in terms of meteorological factors

The aim of this section is explaining the spatial and temporal variability of the seston variables (TPM, Chl a , POM, PIM, and f) in terms of wind and river discharge. We obtained that f is the variable that shows the strongest relationship with the meteorological factors and the only that presents a consistent behavior at the four sites. From these results, we decided to focus only on the model developed for this variable.

The model

The selected model involved the effect of location (Table 4; parametric coefficients), the effect of meteorological factors, and the interactions between them (Table 4; smooth terms). The fitted model reproduces the observed seasonal pattern, which is common to the four locations. From a quantitative point of view, the parametric coefficients confirm once again that Lorbé (outer southern shore) was the location with the highest seston quality followed by Miranda (outer northern), Arnela (inner southern) and Redes (inner northern). Moreover, our model states that seston quality was mainly driven by: (1) wind direction and speed (despite the effect of the later was barely significant); (2) the joint effect of rivers Eume and Mandeo discharge; and (3) the interaction of each river with wind direction and speed.

Model checking (Appendix II) confirms the normality of the errors and therefore that we have used an adequate model fit. The variability of seston quality is successfully explained with the selected model (adjusted $R^2=0.57$) and the observed and fitted time series of seston quality at each location (Appendix III) confirm this goodness of fit.

Interpretation of the model

An overall interpretation of the high order interactions detected by our model can be seen in Appendices IV and V. The former shows the joint effect of wind direction and speed on seston quality under some benchmark conditions for river discharges, and the latter shows the joint effect of Eume and Mandeo discharges under some benchmark conditions for wind direction and speed. For a clearer and easier interpretation of results, we choose particular cases —from the Appendixes— as the most representative scenarios in our study area: (1) upwelling/downwelling conditions with low/moderate/high river discharges to evaluate the effect of wind speed on seston quality (Fig. 6); and (2) upwelling/downwelling conditions with light/moderate/strong winds to evaluate the effect of river discharges on seston quality (Figs 7 and 8).

Upwelling (downwelling) conditions were defined on basis of winds with 45° (225°) direction from North. Low, moderate and high river discharges were defined as the median values during summer, spring/autumn and winter, respectively (section 3.2). Light, moderate and strong winds were defined as speeds of 2, 6 and 10 m s^{-1} . Scenario 1 corresponds with upwelling/downwelling episodes during summer, autumn-spring and winter, respectively. Conversely, scenario 2 is not associated with any season (since distribution of wind speeds does not follow a seasonal pattern) but with particular events.

Upwelling events had a positive effect on seston quality during spring, summer and autumn, i.e. with low and moderate river discharges (Figs. 6a & b) but not especially during winter (Fig. 6c). During summer, seston quality increased with wind speed. However, spring and autumn upwelling episodes with speeds $> 6 \text{ m s}^{-1}$ do not have positive effects on seston quality.

Contrarily, during downwelling events, seston quality rapidly decreased with wind speed during summer, spring and autumn (Figs. 6d & e). The more intense the winds, the lower the seston quality. During winter, seston quality remained low and barely affected by wind speed, whatever under upwelling or downwelling conditions (Figs. 6c & f).

The effects of river discharge on seston quality under upwelling conditions are presented in Figure 7. During light and moderate upwelling events, the increase of River Eume discharge (Figs. 7a & b) had a positive effect on seston quality but only until a certain threshold, which was higher with light winds ($\sim 15 \text{ m}^3 \text{ s}^{-1}$) than with moderate winds ($\sim 10 \text{ m}^3 \text{ s}^{-1}$). From these thresholds, seston quality started to decrease. During strong upwelling events (Fig. 7c), seston quality increased with river flows up to $\sim 20 \text{ m}^3 \text{ s}^{-1}$ but it kept constant thereafter.

Maximum values for seston quality were obtained with practically absence of River Mandeo discharge ($< 2 \text{ m}^3 \text{ s}^{-1}$) but contrarily to River Eume, a slight increase of River Mandeo flow (up to $10 \text{ m}^3 \text{ s}^{-1}$) had a prompt and negative effect on seston quality, which was more pronounced with more intense winds (Figs. 7d, e & f).

The effects of river discharges on seston quality under downwelling conditions are represented in Figure 8. Effects of rivers on seston quality with light winds (Figs. 8a & d) was analogous that under upwelling conditions, although in this case, a lower River Eume discharge (Fig. 8a) is needed to decrease the seston quality (upwelling: $15 \text{ m}^3 \text{ s}^{-1}$ vs. downwelling: $10 \text{ m}^3 \text{ s}^{-1}$). Seston quality variability under moderate (Figs. 8b & e) and strong (Figs. 8c & f) downwelling events were not significant.

4. DISCUSSION

Variability of seston

Seston can be incorporated into any pelagic coastal ecosystem from both allochthonous and autochthonous sources. Allochthonous sources include particles transported by the sea and freshwater flows that enter the study ecosystem as well as by resuspension from the sediments (Navarro & Iglesias 1993, Zúñiga et al. 2014). Simultaneously, these water flows transport dissolved organic and inorganic nutrients, which are the substrate for the growth and accumulation of autochthonous plankton communities. Seston content can vary on a scale of hours, days and/or year-to-year. These changes occur not only in the concentration of suspended particles, but also in their size and nutritional status (Anderson & Meyer 1986, Berg & Newell 1986, Velasco & Navarro 2002a), which is particularly important in the absorption of energy consumed by filter feeders (Figueiras et al. 2002a, Froján et al. 2014).

Our results reveal that seston seasonal patterns and magnitude depend on the location within the Ría de Ares-Betanzos. Seston concentrations (as TPM and PIM) were higher

in the inner part of the ría and during winter than in summer, which emphasize the influence of river discharges. Regarding POM and Chl*a*, the innermost location in the northern coast (Redes) recorded significantly higher concentrations than the others. Our results also revealed that only some anomalous year (2008 and 2010) have a different seasonal pattern at some locations and specifically for POM. The anomalously high summer maximum of POM coincided with rainy springs during these years (section 3.1) and with an elevated number of closures of the exploitation of mussel rafts due to the presence of dinoflagellates in the study area (unpublished data). These results reinforce the conclusions of Álvarez-Salgado et al. (2011), which assured that in this ría a rainy spring will produce extensive closures in summer.

Chl*a* has been often used as a proxy for seston quality in mussel growth models (e.g. Filgueira et al., 2011; Larsen et al., 2014) since monitoring of this variable is straightforward and inexpensive. However in areas with low phytoplankton concentrations non-phytoplankton organic matter may also be an important part of the diet (Handå et al. 2011, Maar et al. 2008). The low Chl*a* concentrations recorded in the Ría de Ares-Betanzos and the relatively weak correlations observed between POM and Chl*a*, support the use of *f* instead of Chl*a* as a seston quality measure. The limitations of Chl*a* as proxy food for blue mussels were also demonstrated by using dynamic energy budget (DEB) models (Rosland et al. 2009).

Seston quality has been used in several recent works related with mussel growth and physiology in the field (e.g. Duarte et al. 2012, 2010, 2008, Filgueira et al. 2002, Fernández-Reiriz et al. 2007, Irisarri et al. 2014, 2015, Velasco and Navarro 2002, Wong and Cheung 2003, 2001, Zuñiga et al., 2013). Irisarri et al. (2014) and Wong and Cheung (2001) reported positive correlations between *f* and the SFG (scope for growth) of mussels; Figueiras et al., (2002) and Irisarri et al., (2015) reported positive

correlations between the absorption efficiencies of mussels and f . Moreover, Cubillo et al. (2012) and Irisarri et al. (2015) found greater growth rates during seasons with high seston quality in our study area. All these works validate the reliability of the ratio between organic and total particular matter (f) as an indicator of favourable conditions for mussel growth, as established in the seminal papers of Bayne et al. (1988), Navarro et al. (1994) and Hawkins et al. (1998).

In our study area the variability of seston quality was more affected by the variability of its inorganic rather than its organic content. It has a marked seasonal behaviour with their maximum values during summer upwelling conditions and minimum levels during winter periods. Seston quality varies opposite to seston abundance and best-quality seston is present in the outer part of the ría. This pattern is in agreement with the fertilization of the ría by nutrient-rich upwelled Eastern North Atlantic Central (ENACW) waters in their outer part, and the variability of river discharges throughout the year.

Seston quality and environment

Model outputs confirm that seston quality is better in the outer than in the inner part of the ría. Moreover, it resulted to be better modelled by the interaction of rivers and winds rather than by these factors individually. Coupling of both factors was previously claimed by Álvarez-Salgado et al. (2011) to explain the closures of mussels farm due to harmful algal bloom episodes. More recently, Duarte et al. (2014) developed a 3-D numerical circulation model that showed that both under upwelling and downwelling conditions over the adjacent shelf, the residual circulation of the Ría de Ares-Betanzos remained positive with a strong influence from river discharge and a positive feedback from wind. However, they concluded that it is hard to generalize on the relative weight of these two potentially important mechanisms that change as a function of their

respective magnitudes. The empirical model developed in this paper, **explains** the relative weight of these two factors affect seston quality within the ría.

Our empirical model suggests that high river flows tend to decrease seston quality and that coastal upwelling conditions always tend to benefit it. In this sense, the balance between nutrient fluxes and flushing time, both controlled by continental and shelf water flows, seem to be the key to modulate the seston quality within the ría. Our model let us to establish some scenarios in which: (1) wind speed can have negative effects on seston quality; and (2) certain river discharges can produce positive effects on seston quality.

Our results suggest that seston quality improves monotonically with wind intensity but only during summer. During spring and autumn, seston quality improves up to a wind intensity of 6 m s^{-1} and, then it declines, and during winter, seston quality does not depend on the intensity of upwelling events. During summer, river flows are low and the dominant source of nutrients is the upwelled ENACW transported into the ría by the enhanced bottom ingoing flow (Villegas-Ríos et al. 2011). At the same time, haline stratification created by the river discharges does not allow upwelled waters to crop up at the surface, in such a way that nutrients are gently injected in the surface layer across the pycnocline and efficiently utilized by the phytoplankton communities of the surface ría. On the contrary, during spring and autumn, river flows are much higher; under this situation the pycnocline is so intense that the exchange of nutrients with the bottom layer is severely reduced and surface waters are flushed out faster than the time required for an efficient utilization of the nutrients introduced in the ría. This is likely the reason behind the decline of seston quality at costal wind speeds larger than 6 m s^{-1} .

During downwelling events, seston quality rapidly declines with wind speed in summer, spring and autumn (Figs. 6d & e). In these situations nutrient-poor shelf surface waters

push to enter into the ría and slow down the positive circulation pattern (Duarte et al. 2014). Although the flushing time of the ría decreases in these hydrographic conditions, which favour an efficient utilization of nutrients, nutrient flows are so low under this situations that phytoplankton growth becomes substrate limited and, therefore, seston quality declines.

Model outputs also demonstrate that both rivers have different effects on seston quality. Under upwelling conditions, the discharge of River Eume (placed at the northern shore) has a positive impact on seston quality up to a certain threshold. On the contrary, the discharge of River Mandeo (placed at the southern shore) has a negative effect on seston quality whatever the intensity of winds is. Nutrients transported by upwelled waters and river flows should contribute to increase seston quality if phytoplankton has enough time to growth within the ría. However, when the discharge of River Eume exceeds $15 \text{ m}^3 \text{ s}^{-1}$ (for light winds) or $10 \text{ m}^3 \text{ s}^{-1}$ (for moderate winds) the flushing time of the surface layer decreases to the point that nutrients cannot be efficiently utilized and, therefore, the seston quality decreases. Under very intense upwelling conditions, although the river discharge increases, the seston quality remains constant due to the high amount of nutrients transported by upwelling that are able to erode the pycnocline, transporting nutrients from the bottom to the surface layer and decreasing its flushing time. Finally, it is remarkable the high seston quality estimated under downwelling conditions with light winds and low river discharges (Fig. 8d). When downwelling events occur during summer, the ría is plenty of nutrients transported by previous upwelling events. Moreover, downwelling conditions tend to reverse the positive circulation, increasing therefore the flushing time. In that situation, phytoplankton has enough time to growth within the ría and seston quality increases.

The reasons why River Mandeo affects negatively the seston quality are unclear. Despite its lower flow, River Mandeo could have a larger effect on the flushing time of the ría than River Eume because of its influence on the cyclonic gyre developed in the inner and central parts of the ría, which contribute to decrease the flushing time of the surface layer, but could be disrupted by the presence of River Mandeo. It should also be taken into account that the flow of River Eume is regulated by a dam whereas River Mandeo flows naturally into the ría.

Conclusions

The spatial and temporal characterization of seston parameters (POM, PIM, TPM, Chl_a) in the Ría de Ares-Betanzos allowed us to establish the continental or marine origin of seston and its dynamics. Seston quality varied opposite to seston abundance. Seston content was higher in the inner part of the ría and during winter, while the best-quality seston was present in the outer part of the ría with maximum values during summer, showing a marked relationship with water circulation. The variability of seston quality was more affected by the variability of its inorganic rather than organic content and was the variable that showed the strongest relation with meteorological factors and the only that showed to be consistent at the four sites. This robust dependence enabled the development of a model to explain the dynamics of our environment and the relative weight of each forcing. Our model settles the basis for future development of predictive models, which are highly demanded to achieve a sustainable management of the ecosystem.

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List of figures

Figure 1 Ría de Ares-Betanzos. Data were collected at four locations: Miranda (M) and Lorbé (L) in the outer part (north and south, respectively) and Redes (R) and Arnela (A) at the inner part (north and south, respectively).

Figure 2 Rose of shelf winds that covers the period from 2007 to 2011 (a). Probability density of shelf wind speeds (b) and directions (c) by seasons. Seasonal relationship between wind speed and direction (d). Winds are defined as where they come from.

Figure 3 Discharge of rivers Eume and Mandeo from 2007 to 2011 (a). Seasonal comparison of annual-river discharges for Eume (b) and Mandeo (c) rivers from 2007 to 2011. Probability density of Eume (d) and Mandeo (e) discharges by seasons.

Figure 4 Seston trends: TPM (total particulate matter) (a), POM (particulate organic matter) (b), PIM (particulate inorganic matter) (c), f (seston quality) (d) and Chla (chlorophyll content) (e) from 2007 to 2011 at each location. Outer/inner locations are denoted by broken/continuous lines and northern/southern by grey/black lines.

Figure 5 Cluster analyses of seston variables. Seasonal patterns of TPM (a), POM (b), PIM (c), f (d) and Chla (e) time series. The significantly different seasonal patterns were marked with bold line. See Table 2 to identify which locations are identified with each line.

Figure 6 Effect of wind speed on seston quality (f) under upwelling and downwelling conditions with summer (a & d), spring/autumn (b & e) and winter (c & f) typical river discharges. Dashed lined indicate 95% confidence intervals.

Figure 7 Effect of Eume and Mandeo rivers discharge on seston quality (f) under low (a & d), moderate (b & e) and strong (c & f) upwelling conditions.

Figure 8 Effect of Eume and Mandeo rivers discharge on seston quality (f) under low (a & d), moderate (b & e) and strong (c & f) downwelling conditions.

748 **Figure 1**

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Figure 2

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Table 1 Long-term means and standard deviations of seston content and quality depending on the location (Arnela, Lorbé, Miranda and Redes). TPM (‘total particulate matter’; mg l^{-1}), POM (‘particulate organic matter’; mg l^{-1}), PIM (‘particulate inorganic matter’; mg l^{-1}), seston quality ratio (f) and Chla, (chlorophyll content; $\mu\text{g l}^{-1}$).

	TPM	POM	PIM	f	Chla
Arnela	2.0 (1.8)	0.6 (0.5)	1.4 (1.6)	0.4 (0.2)	1.7 (1.3)
Lorbé	0.8 (0.6)	0.5 (0.4)	0.4 (0.5)	0.6 (0.2)	1.4 (1.2)
Miranda	1.3 (1.0)	0.5 (0.3)	0.7 (0.9)	0.5 (0.2)	1.6 (1.3)
Redes	2.7 (2.0)	0.8 (0.3)	1.9 (1.8)	0.4 (0.2)	2.6 (2.0)

Table 2 Results of cluster analysis for functional data. Two significantly different seasonal-patterns (red-black) were enough to explain satisfactory the variability of TPM, PIM, f and Chla between locations (Arnela, Lorbé, Miranda and Redes) during five years (07, 08, 09, 10 and 11). For POM it was necessary to established three groups (red-black-green) to a proper classification. Colours correspond with black, red and green seasonal-patterns observed in Figure 5.

	TPM					POM					PIM					f					Chla				
	07	08	09	10	11	07	08	09	10	11	07	08	09	10	11	07	08	09	10	11	07	08	09	10	11
Arnela	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Lorbé	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Miranda	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Redes	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

Table 3 Correlation coefficients between seston quality (f) and chlorophyll content ($Chla$) and between POM and $Chla$ at each location. p-value < 0.001 (***), p-value < 0.01 (**), p-value < 0.05 (*), p-value < 0.1 (.)

	f-$Chla$		POM-$Chla$	
Arnela	0.298	***	0.369	***
Lorbé	0.478	***	0.753	***
Miranda	0.288	***	0.222	***
Redes	0.105	*	0.165	**

Table 4: GAMM model parameters (Adjusted $R^2 = 0.57$). Effect of the location (parametric coefficients): intercept, standard deviation (std), error, the test statistical value (t-value) and significance level (p-value) for each location. Environmental forcings (w : wind intensity; θ : wind direction; Q_E : Eume river discharge and Q_M : Mandeo river discharge) and its interactions (smooth terms): estimated degrees of freedom (edf), reference degrees of freedom (ref df), Snedecor's F statistical value (F) and significance level. (p-value). p-value < 0.001 (***), p-value < 0.01 (**), p-value < 0.05 (*).

Parametric coefficients					
	intercept	Std error	t-value	p-value	
Arnela	-0.378	0.039	-9.659	<2e-16	***
Lorbé	0.262	0.055	11.614	<2e-16	***
Miranda	0.011	0.055	7.091	2.63e-12	***
Redes	-0.504	0.056	-2.256	0.024	*
Smooth terms					
	edf	ref df	F	p-value	
w	0.556	9.000	0.125	0.003	**
θ	6.756	8.000	5.291	1.23e-10	***
$\log(Q_E+1)*\log(Q_M+1)$	6.013	24.000	1.831	7.92e-14	***
$\log(Q_E+1)*w*\theta$	40.855	91.000	1.544	<2e-16	***
$\log(Q_M+1)*w*\theta$	24.811	76.000	0.899	5.03e-11	***

APPENDIXES

Appendix I Seston time series: total particulate matter, TPM (a); total organic matter, POM (b); total inorganic matter, PIM (c); seston quality; f (d) and chlorophyll content, Chl-a (e) from 2007 to 2011 at each location. Outer/inner locations are denoted by broken/continuous lines and northern/southern by grey/black lines.

49 **Appendix II** Q-Q plots (a, b) that compare the probability distribution of the deviance
50 residuals of the model and the theoretical values (by plotting their quantiles against each
51 other). Histograms of deviance residuals (c, d) obtained for GAM model (c) and
52 forGAMMmodel (d) fits of seston quality. Note that the residuals of the GAM (c) are
53 not normal and this assumption is fulfilled in the GAMM model (d).

56 **Appendix III** Observed and model-fitted temporal series of seston quality at Arnela (a),
 57 Lorbé (b), Miranda (c) and Redes (d).

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60 **Appendix IV** Joint effect of wind speed and direction on seston quality (f) under spring

61 (a), summer (b), autumn (c) and winter (d) conditions.

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- 64 **Appendix V** Joint effect of river discharge on seston quality (f) under light (a, b)
- 65 moderate (c, d) and strong (e, f) upwelling (UP) and downwelling (DW) conditions.