Estuaries and Coasts
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Multispecies Mortality Patterns of Commercial Bivalves in Relation to Estuarine Salinity Fluctuation

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Abstract Fluctuations in salinity may cause huge economic losses in estuaries with exploited commercial bivalves owing to their effect on mortality of these species. However, the same decrease in salinity does not affect all species in the same way, so it is interesting to study the effect of salinity from a multispecies standpoint. In the management of exploited bivalve beds, it is important to know the tolerance thresholds of the species, not only in cases of extremely low salinities but also over prolonged periods when salinities are low but not extreme. An analysis of mortality episodes of commercial bivalves in the Ulla River estuary (Galicia, NW Spain) from 1977 to 2009 revealed two mortality patterns related to how greatly the different species were affected. A mathematical model was designed to estimate salinity in the estuary based on weather conditions and tidal amplitude. By applying this model, it was possible to deduce the intensity and duration of the salinity decrease in the days prior to each mortality episode with the goal of relating these factors to mortality patterns. The two parameters found to be sufficient to explain the mortality observed were the minimum salinity at high tide and the number of consecutive days below a specific salinity threshold.

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Introduction

Estuaries are important ecosystems from an ecological standpoint. They are areas where seawater and fresh water mix, the latter containing terrigenous runoff. Therefore, estuaries are strongly influenced by regional weather, such as rainwater that has accumulated in the basin flowing into the estuary, the prevailing winds, and tidal amplitude. These forces affect the estuarine circulation and hence, the renovation or residence time of the water and its physico-chemical properties (Summer and Belaineh 2005; Kimmel et al. 2009). The fluctuations in environmental conditions which are characteristic of estuaries have an enormous impact on many different biogeochemical processes in the waters and sediments (Alber 2002; Kimmerer 2002a, b; Azevedo et al. 2008; Boyer et al. 2010) and on the biology of the species living there fully or partially (Powell et al. 2002; Anger et al. 2008; Hunt et al. 2009). In many cases, the species affected are those that support important fisheries in and around the estuaries. Hence, the fluctuations that typically occur in estuaries generally have huge economic repercussions (Soletchnik et al. 2007; Martín et al. 2008; La Peyre et al. 2009; Möller et al. 2009).

One of the most important economic and social activities in the estuaries of Galicia (NW Spain) is the exploitation of natural bivalve mollusc beds. The Ulla River in the inner Arousa ria (Fig. 1) has a natural bed called "Lombos do Ulla"—one of the most important shellfish beds in Galicia. Moreover, situated on the left bank of the estuary are 1,283 bivalve leases which are exploited and operated under government franchise. Covering an area of 1,134.7 ha

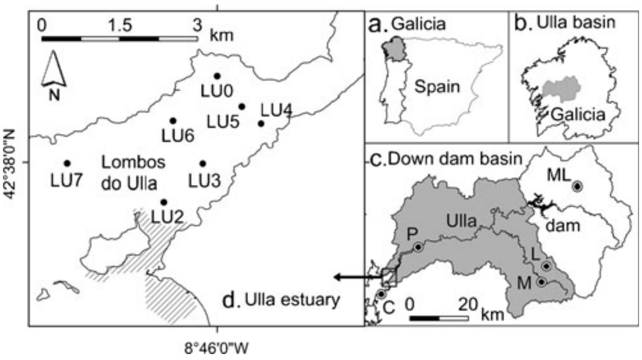

Fig. 1 Map of study area including Galicia (**a**), Ulla River Basin (**b**), portion of basin downstream from the dam (**c**) with meteorological station at Coron (**c**), Pontecesures (*P*), Mouriscade (*M*), Lalín (*L*), and Melide (*M*), and the Ulla estuary (**d**) with stations (*LU0–LU7*) where salinity was measured (Parada et al. 2007)

"Lombos do Ulla" generates enough resources to support the extractive effort of more than 300 shellfishers day^{-1}, 120 days year^{-1}, with a total gross annual income of 2.4 million euros (Parada et al. 2006). However, this economic resource is highly variable and depends largely on the connection between the characteristics of the estuary and the weather. One of the major causes of this variability is the mortality resulting from fluctuations in salinity due to increased river runoff from heavy rains. The last massive bivalve mortality episode caused by heavy rains was in autumn–winter 2000–2001, and it prevented these resources from being exploited for 1 year (Parada and Molares 2008).

The bivalve species that live in estuarine sand bank habitats such as the cockle *Cerastoderma edule*, pullet carpet shell *Venerupis senegalensis*, carpet shell clam *Tapes decussatus*, and Manila clam *Tapes philippinarum*, have adapted, to a greater or lesser degree, to fluctuations in salinity. Experiments on the resistance of some of these species to low salinity have, in most cases, been conducted in the laboratory (Russell and Petersen 1973; Kingston 1974; Rayment 2005). These studies show that the effect of salinity fluctuations on mortality depends not only on the absolute salinity but also on the conditions under which these fluctuations occur: duration of the low salinity levels, or the length of time the organisms are subjected to these low levels, fluctuation amplitude, and how fast the change occurs. In fact, one of the main problems with the laboratory studies is the adaptation period prior to the experiment (Ansell and Sivadas 1973; 1981; Anger et al. 2008). Moreover, in the natural environment, episodes of low salinity may last for long periods while there also may be short periods of low salinity alternating with high salinity, which recur owing to the influence of the tide.

Considered both the ecology and the management of fishery and hydraulic resources, it is of utmost interest to determine the effect of the different types of salinity fluctuations in the estuary. At the same time, given the multispecies nature of the fishery, it would also be interesting to approach the study of mortality on a global level to understand the resistance to the different typologies of low salinity periods in all of the species.

These kinds of studies require highly complex systems that can reproduce the characteristics of natural salinity fluctuations in the lab. An additional way to approach the problem is to conduct long-term studies that record the evolution of salinity levels parallel to the population dynamics of the species being harvested. Unfortunately, these studies are costly and very few research projects last long enough to record all the characteristics of periods with low salinity and mortalities. However, since these are species of great economic importance, some episodes of mortality have been recorded in the technical reports of the fishery administration. These reports, however, are seldom published. The papers that do get published usually refer to out-of-the-ordinary mortality episodes and include general data on certain environmental conditions or they describe the existing conditions just after the start of an episode.

The goal of the present study is to present the thresholds and the evolution of the decrease in salinity in the natural environment that may be related to the different multispecies mortality patterns of commercial bivalves. Owing to the lack of salinity records coinciding with mortality episodes prior to 2002, it was necessary to establish a second goal, namely to design a model that would allow us to reconstruct salinity in the estuary based on other environmental variables with long-term time series. Using this reconstructed time series of salinity and the identification of the critical conditions defined in the main objective of this paper, we studied the frequency of the critical periods due to decreases in salinity in the field.

Materials and Methods

Study Area

The Ulla River, with a length of 132 km and a mean flow of 79 m^3 s^{-1} (Prego et al. 2008), is the largest of the rivers running into the Galician estuaries. The flow of the river is regulated by a hydroelectric dam located 74 km from the mouth of the river with a capacity of 297 hm^3. The SW facing Ulla River basin covers an area of 2,804 km². In terms of climate, Rodríguez (1982) makes the distinction between a pluviometric coastal region and a pluviometric interior region. The former includes the northern slope and the whole estuary, while the interior region largely coincides with the southern slope. The estuary of the Ulla River, which covers the area between the mouth of the river in the inner Arousa ria and the limit of tidal influence, is

7 km in length. Only in the final 3.7-km upstream stretch of the estuary is there a deposit of sandy sediments where the bivalve mollusc bed "Lombos do Ulla" (Fig. 1) is found.

Mortality Episodes

Mortality records were taken from two sources: (1) technical reports from the Autonomous Government of Galicia (AGC) and (2) data from the monitoring program on the Lombos do Ulla conducted by the AGC. The unpublished technical reports were used for mortality episodes prior to 2005. Most of these reports base the quantification of mortality on a count of valves joined together (Strasser et al. 2001) taken after the mortality episode had ended. After the occurrence of extreme freshwater freshets, which even caused flooding in waterfront towns, some reports, however, referred to the "total" or "practically total" mortality of the species studied, as they did not find enough specimens to count. The data gathered by monitoring the bed began in 2005 by measuring mortality in the different species kept in cages. For each species, a steel structure consisting of five plastic cages each 1,000 cm² in area and 20 cm deep, filled with sediment from the estuary, was submerged in the water. Fifteen specimens of the species corresponding to the confinement structure were placed in each cage ($N=75$). The specimens were replaced by others at monthly intervals and the mortality rate was calculated by averaging the number of survivors from the five cages. The mortality rate was calculated as an instant rate adjusted to 30 days (Z_{30}) and later converted to a finite rate (M), expressed as a percentage (Krebs 1998).

Mortality episodes were classified into four synoptic categories: "severe mortality," "moderate mortality," "morbidity," and "no mortality." The "severe mortality" category included all episodes in which mortality rates of ≥50% for *C. edule* and/or *V. senegalensis* and ≥15% for *T. decussatus* and/or *T. philippinarum* were recorded. Also included in this category were episodes with mortalities described as "total mortality" or "almost total mortality" in the technical reports reviewed. Episodes were considered to be of "moderate mortality" in cases reflecting a mortality of <50% for *C. edule* and 15% for *T. decussatus* and *T. philippinarum*, although very high mortalities might be found for *V. senegalensis* since these mortalities are considered normal in shallow estuaries during the autumn and winter seasons (Sebe et al. 2001). The category called "morbidity" referred to cases of specimens with swollen flesh as a result of the presence of fresh water but without mortalities in any of the species. The technical reports of episodes in which no mortalities were found after the rainy period, but where there was no mention of specimens with swollen flesh, were classified as "no mortality." Table 1 shows the 17 cases studied and their respective category assignments.

Recordings and Estimation of Salinity Values

From December 2002 to January 2006, a WTW multiparametric sounder was used to measure the salinity at seven stations 1 m above the bottom during the two daily high tides

Table 1 Characterization of mortality episodes documented in this study

Date	*Cerastoderma edule*	*Venerupis senegalensis*	*Tapes decussatus*	*Tapes philippinarum*	Type	Identifier
28/02/77	T	T	T	T	SM	Sev0277
22/12/78	T	T	T	T	SM	Sev1278
31/12/81	PT	PT	PT	PT	SM	Sev1281
23/10/87	T	T	T	T	SM	Sev1087
28/12/89	90%	99%	10%	0%	SM	Sev1289
14/01/91	0%	80%	0%	0%	MM	Mod0191
04/01/94	0%	0%	0%	0%	MB	Morb0194
12/01/94	17%	87%	0%	0%	MM	Mod0194
19/01/96	60%	96%	5%	43%	SM	Sev0196
27/04/00	0%	80%	0%	0%	MM	Mod0400
07/12/00	0%	95	0%	0%	MM	Mod1200
27/11/02	0%	0%	0%	0%	NM	NoMort1102
16/01/03	PT	PT	PT	PT	SM	Sev0103
29/03/06	71%	50%	45%	78%	SM	Sev0306
25/11/06	0%	10%	2%	5%	MB	Mod1106
07/03/07	33%	97%	2%	6%	MM	Mod0307
05/02/09	30%	30%	7%	14%	MM	Mod0209

Finite mortality rate (%) is specified for each species: *C. edule*, *V. senegalensis*, *T. decussatus*, and *T. philippinarum*. Extreme mortality cases assessed as either total mortality (T) or practically total mortality (PT). Type includes cases of severe mortality (SM), moderate mortality (MM), morbidity (MB), or no mortality (NM)

(Fig. 1). Salinity recorded during high tides was used as a reference since this value was considered to be more critical than the low tide salinity. Salinity data recorded every 10 min in the estuary (see Online Resource 1) demonstrate how entering seawater can displace fresh water from the estuary bed and reduce the presence of near 0 salinities to just a few hours. In estuaries with semidiurnal tidal cycles, low salinities during two consecutive high tides always imply exposure to low salinities for 24 h. Details regarding the methodology used in the recordings and the data are available in Parada et al. (2007). From these data, the series from station LU6 was selected as most representative of the estuary, given that its mean and SE were most similar to those from the seven stations as a whole (Table 2).

Owing to the lack of daily salinity records during mortality episodes prior to 2002, it was necessary to carry out an indirect estimation of these values. Data from the first year of salinity records at LU6 (December 2002–November 2003) were used to develop an empirical model which, on the basis of other environmental variables with long-term time records, would allow us to reconstruct a daily time salinity series in bottom waters. Mean salinity of the bottom water of the two daily high tides was used as the model's dependent variable. Due to the seasonality of rainfall in the Ulla basin (Rodríguez 1982), the salinity data used were limited to those from the spring and winter months. The independent variables used in the model were net rainfall or runoff in the Ulla River basin, wind direction and force, and tidal amplitude. These three variables are commonly used in models that estimate the influence of different parameters on the salinity of estuaries (Powell et al. 2002; Wang et al. 2008; Marshall et al. 2009; Yuk and Aoki 2009).

To prevent any possible distortion that the activity of the hydroelectric dam might exert on the relationship between the model's variables, we used only data corresponding to the days on which the absolute value of the difference between the entry and exit of the water to the reservoir did not exceed the 95 percentile of the daily records of these parameters between 2002 and 2006, provided by the autonomous institution "Augas de Galicia." The value of this percentile was 2,963 dm^3.

Table 2 Mean and SE of salinities recorded during all high tides from December 2002–January 2006 in Ulla estuary and at all seven stations (Parada et al. 2007)

Station	Mean	SE
LU0	31.2	0.3
LU2	33.2	0.2
LU3	32.8	0.2
LU4	31.9	0.3
LU5	31.8	0.3
LU6	32.5	0.2
LU7	33.7	0.2
All	32.4	0.1

Net Precipitation (Runoff)

Net precipitation was calculated using the Spanish adaptation of the Runoff Curve Number Method (US Soil Conservation Service 1972) carried out by Témez (1978) and proposed as the standard methodology by the Spanish Ministry of Public Works (MOPU 1990). Calculations were made from the precipitation recorded at the two meteorology stations of Meteogalicia (Meteorology Institute of the AGC) representative of the rainfall regimes in the Ulla River basin (Rodríguez 1982). Corón station is near the mouth of the river at an altitude of 14 m and in this paper it is considered representative of the coastal sub-basin. Mouriscade station is 83 km from the estuary at an altitude of 500 m. The data related to this station are assumed to represent the interior sub-basin (Fig. 1). For the 1961–1977 period, before the meteorological station at Corón came into operation, we used precipitation data from Pontecesures, in the Ulla estuary. Similarly, for the 1961–2000 period, prior to the existence of Mouriscade station, we used precipitation data from the National Institute of Meteorology in Lalín, replaced in 1983 by the station in Melide, both of which are in the interior sub-basin (Fig. 1).

In studies focused on the relationship between the salinity of estuaries and precipitation, salinity recorded on a specific day often correlates better with the precipitation recorded several days previously than on the same day (Chen et al. 2000; Ostrander et al. 2008; Yuk and Aoki 2009). To determine the possible existence and magnitude of such a lag in the Ulla estuary, a correlation analysis was performed, applying different lags to the data from Mouriscade station.

To calculate the value of the initial abstraction required to estimate the net precipitation, we used a geographic information system which included the entry of data related to permeability, slope, and soil use (MOPU 1990) distributed by SITGA and available at http://sitga.xunta.es/cartografia/. Owing to the presence of the reservoir, only the downstream area of the basin was taken into account. In view of a possible lag between the daily runoff and the salinity recorded in the estuary, a correlation analysis was performed between the former and the runoff obtained for each day, as well as the amount accumulated between 1 and 7 days.

Wind Direction and Force

In order to include the relationship between wind and salinity in the estuary, daily records of the prevailing wind direction and force from Corón station were used. The influence of the wind was considered to be dependent upon its speed, provided the direction was appropriate (Chase 1975). Hence, these records were subjected to different possible corrections, with a positive value being assigned to wind speeds blowing from any direction between N and SE, which facilitates the renewal of seawater in the estuary and a negative value being given to winds blowing between S and NW direction, and a value of 0 to the wind speed from one or several directions in the event that they should have no effect on salinity. The correction that was finally applied was the one that maximized the correlation between the records of wind and salinity.

Tidal Amplitude

In order to numerically implement the effect of tidal amplitude on salinity (Nuttle et al. 2000), a correction was applied to the net precipitation. The correction consisted of dividing the precipitation on the days with tidal amplitude above a certain threshold by a specific constant. The combination that was selected was the constant and the threshold that maximized the correlation between corrected net precipitation and salinity.

Development and Validation of the Salinity Model

Using the salinity data from December 2002–November 2003 as the dependent variable and, as independent variables, net precipitation and wind speed corrected, respectively, with tidal amplitude and wind direction, as specified above, a multiple regression analysis was performed using the MINITAB program. The possibility of multi-colinearity was evaluated using the variance inflation factor (VIF) as a diagnostic tool (Graybill and Iyer 1994). To reduce the residuals of the model, salinities estimated by linear regression were transformed by sigmoid fitting with observed salinities. The values resulting from the transformation were tempered with a range 2 mobile medium. The log transformation used, with the help of the Origin program, was

$$y = \mathrm{A}_2 + \left[(\mathrm{A}_1 - \mathrm{A}_2)/\left(1 + (x - x_0)^P\right)\right]$$

where x is the salinity estimated by the regression model. In this equation, A_1 and A_2 represent the minimum and maximum values for which y, is asymptotic; x_0 is the value of x for which y takes on a value that is equal to half of the amplitude of the curve and P is a power.

The salinity values resulting from the model were validated with a series of data from the same station (LU6) from December 2003–January 2006. Model fits of the data sets used for calibration and validation were compared by means of different statistical tests (correlation coefficient (R), mean bias error (MBE), mean absolute error (MAE), root mean squared error (RMSE), index of agreement (d) [Willmott et al. 1985]).

Relationship Between Mortality Episodes and Salinity Evolution

For each mortality episode, the changes in salinity were examined in the days prior to the episode. A low salinity period was defined as consecutive days on which salinity was <30. Assuming that the influence of the water characteristics on these organisms depends not only on critical values being exceeded but also on the length of time they are subjected to these critical values (Ansell et al. 1981), each low salinity period was characterized using seven parameters: duration in days (ND), minimum salinity (MIN) within this period, maximum number of consecutive days with salinity levels of <5, <10, <15, <20, and <25, respectively. The values of these parameters were centered and standardized at a mean of 0 and variance of 1 (Jager and Looman 1987) according to the following equation:

$$\mathrm{x}'_{\mathrm{ki}} = (\mathrm{x}_{\mathrm{ki}} - \mathrm{X})/\sigma_{\mathrm{x}}$$

where x_{ki} is the value of variable k in episode i, X the mean, and σ_x the SD of the value of the variable in all the episodes.

A cluster analysis of the kinds of mortality episodes and a principal component analysis (PCA) were then carried out. The classification analysis was conducted using two different procedures based on Euclidean distances: Ward's method, widely used in synoptic climatology (Yarnal 1993) and a UPGMA analysis, with the help of the PAST program (http://folk.uio.no/ohammer/past/).

The mean and standard deviation of each of the seven parameters were used to characterize the different groups of mortality episodes identified in the classification analysis. The significance of the groups defined was corroborated with a Mann–Whitney U test.

After characterizing the salinity changes prior to the mortality events, the salinity time series was used to identify the low salinity periods. Once these periods were identified, we quantified for each year the number of periods liable to cause severe or moderate mortalities. Similarly, every 5 years, we calculated the number of years in which there was at least one low salinity period liable to produce a mortality event.

Results

Salinity Model

A 1-day lag in the precipitation data between Mouriscade and Corón correlated best with the salinity records (Online Resource 2). Therefore, the precipitation that had a higher correlation with the daily salinity recorded in the estuary resulted from the sum of the precipitation in Corón that day and the precipitation in Mouriscade the day before ($r^2=-0.624$; $p<0.01$). The best correlation between salinity and net precipitation or runoff ($r^2=-0.705$; $p<0.01$) was calculated from the rainfall recorded at the two stations at a 1 day lag and accumulated over the 6 days prior to the salinity record (Online Resource 2). The correlation between net precipitation and salinity had a maximum value when the former was divided by 2.76 on the days when tidal amplitude was ≥3.1 m ($r^2=-0.709$; $p<0.01$). The correlation between the salinity of the estuary and the wind regime was maximal ($r^2=0.576$; $p<0.01$) when negative values were assigned to the speeds of wind from any N to SE direction; positive values were assigned to winds from the W or NW, and a value of 0 to S or SW winds (Online Resource 3).

Wind speed (corrected for direction) and precipitation (corrected for tidal amplitude) explained 66.7% of the variance in the salinity records (see multiple regression results in Online Resource 4) (VIF=1.069), with this percentage increasing to 75% when a log transformation was applied to the salinities estimated by the regression (Online Resources 5 and 6). After log transformation, the residual analysis showed an acceptable fit between estimated and observed values, although the distribution was not normal (Shapiro–Wilk=77.47; $p=0.001$) which reflected an underestimation of the salinity of two to four units (Online Resource 5). The indices used to evaluate the calibration and validation of the model (Online Resource 7) showed a better fit after the logistic correction of the linear model. The final fit between the observed and estimated values was acceptable, with a deviation of 2.4 salinity units (RMSE) in the case of calibration (Online Resource 7, Fig. 2a) and 2.7 units with a slight underestimation (MBE=−1.93), in the case of validation (Online Resource 7, Fig. 2b) in keeping with the distribution of the residuals. In both calibration and validation, agreement between the observed and estimated values was very good ($d>0.99$).

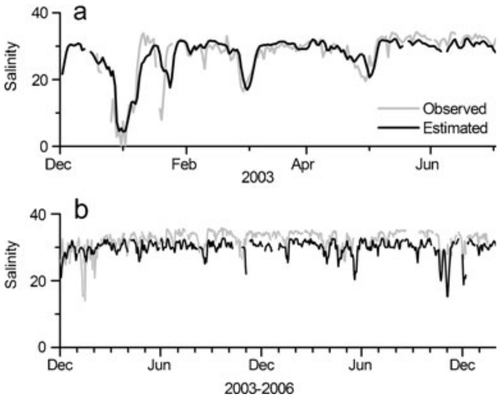

Fig. 2 Fit of salinity estimation model. **a** Comparison of estimated values with those used to design the model (winter–spring 2002–2003); **b** validation with data series up to January 2006

Analysis of Low Salinity Periods Prior to Mortality Events

By applying the model, it was possible to estimate the mean daily salinity during the high tide that was not included in the salinity monitoring period in the estuary. Despite the variability in the changes in salinity in the days prior to the mortality episodes (Fig. 3), most of the severe mortality episodes were preceded by sharp declines in salinity to values close to 0, which persisted for several days. Although there were some cases, such as Sev1281 or Sev0103, in which 0 values were not reached, salinity remained around 10 for prolonged periods of time. In cases of moderate mortalities, two patterns of salinity change may be indicated. In some cases, salinity stayed >10 over a long time, while in cases such as Mod0191, Mod0194, and Mod0400, where salinity was close to or <10, it remained

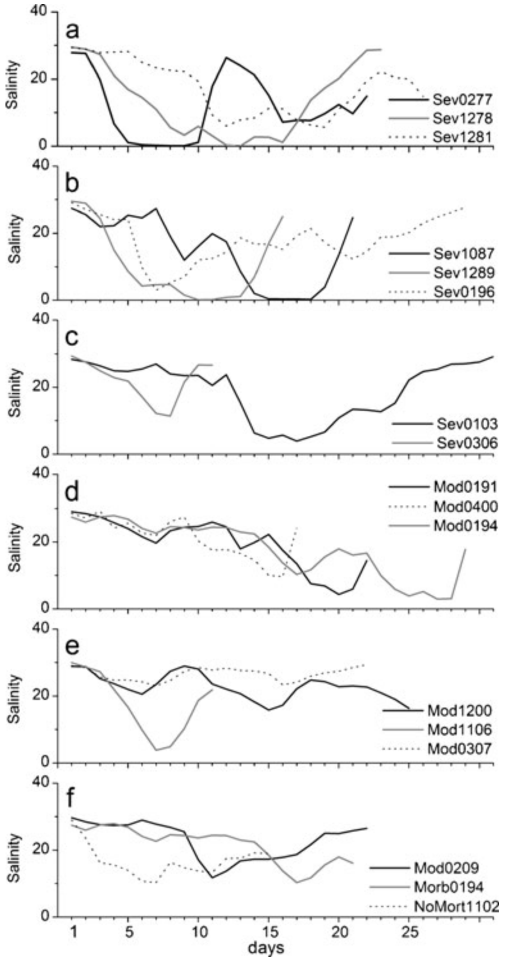

Fig. 3 Salinity estimated for bottom waters at high tide on days corresponding to each mortality episode recorded. *Different lines* pertain to each episode according to identifiers in Table 1

at this value and slowly decreased. Table 3 shows the values of the parameters that defined each low salinity period.

Classification of the mortality episodes proposed in this paper was in keeping with the parameters used to define low salinity periods. Hence, both the classification analyses (Online Resource 8) and the PCA (Fig. 4) divided the mortality episodes into two groups: M and S. Group M included all the episodes of moderate mortality except one (Mod0194), along with the episode with no mortality (NoMort1102), the morbidity episode (Morb0194), and the severe mortality event that occurred in March 2006 (Sev0306). This group was found to be directly related to the MIN value (positive side of component 2 of the PCA) and indirectly related to the other parameters. They are mortality episodes preceded by short-term low salinity periods and with the highest minimum salinity. Group S included all the episodes of severe mortality (except the one in March 2006) and one moderate mortality event (Mod0194). This group was divided into two subgroups (Sa and Sb), which were more evident in the UPGMA and PCA analyses. Group Sa included the episodes of severe mortality that occurred in December 1981, January 1996 and 2003 (Sev1281, Sev0196, and Sev0103) and the episode of moderate mortality in January 1994 (Mod0194). This group was directly related to the total duration of the low salinity periods (ND) and the number of consecutive days with a salinity of <25 (positive side of component 2). Therefore, they may be considered mortalities preceded by moderate salinities maintained over a long period of time. In contrast, group Sb was directly related to the number of consecutive days with a salinityof <10 and 5 (negative side of component 2), grouping together the mortality episodes preceded by the lowest salinities.

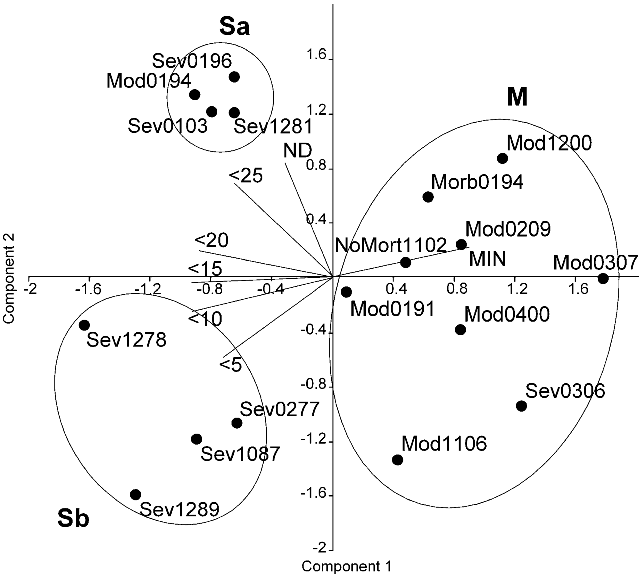

Fig. 4 PCA Analysis of mortality episodes. Components 1 and 2 account for 62.66% and 24.23% of the variance, respectively. *M*, *S*, *Sa*, and *Sb* groups resulting from classification analyses. Labels match the identifiers of mortality episodes and parameters defining low salinity events in Tables 1 and 3

Table 3 Values of variables that define low salinity periods (salinity, <30) prior to each mortality episode: number of days (ND); minimum salinity (MIN); maximum number of consecutive days with salinity of <5, <10, <15, <20, and <25 (<5, <10, <15, <20, and <25)

Mortality episode	ND	MIN	<5	<10	<15	<20	<25
Sev0277	21	0.1	6	7	7	9	10
Sev1278	23	0	6	10	13	15	18
Sev1281	26	5.6	0	4	11	13	21
Sev1087	15	0.2	6	7	8	13	14
Sev1289	16	0.1	8	10	11	12	13
Mod0191	22	4.3	1	4	6	7	11
Morb0194	21	10.3	0	0	3	7	16
Mod0194	29	2.9	2	6	6	15	24
Sev0196	29	2.9	1	4	7	12	24
Mod0400	17	9.6	0	1	3	6	8
Mod1200	25	15.8	0	0	0	3	15
NoMort1102	15	10.3	0	0	3	13	14
Sev0103	31	3.9	1	6	10	12	19
Sev0306	11	11.4	0	0	2	3	7
Mod1106	11	3.8	2	3	4	6	8
Mod0307	22	23.3	0	0	0	0	5
Mod0209	22	11.7	0	0	2	8	9

The moderate mortality episodes (group M) were characterized by salinities of <10 for 1 day or salinities of <15 for three consecutive days while the mean value for severe mortality episodes (group S) was 7 and 9 days under 10 and 15, respectively. In both cases, the differences between the two types of mortality episodes were significant ($p<0.001$). The mean value of minimum salinity achieved in the group of moderate mortality episodes (M) was 11.2, whereas in the group of severe mortalities (S) it was 2 ($p<0.01$). The mean number of consecutive days with salinities of <20 was 6 for moderate mortalities and 13 for severe mortalities ($p<0.01$). However, the duration of the period of low salinity (ND) was not significantly different between the two groups. The mean duration of the low salinity periods (ND), the average MIN value reached, and the mean number of consecutive days with a salinity of <25 were greater in group Sa than in Sb ($p<0.05$). Similarly, the mean number of consecutive days of <5 and 10 was greater in the episodes pertaining to group Sb ($p<0.05$) (Table 4 and Online Resource 9).

Disregarding the decreases in salinity that might be attributed to the activity of the hydroelectric dam (not included in the model), since 1961 the number of possible declines in salinity related to meteorological causes that might cause mortalities, has dwindled over the intervening 50 years, with fewer years showing possible events in the

Table 4 Mean value and 95% confidence intervals (L1 and L2) of variables that define periods of low salinity prior to each group of mortality episodes identified by classification analyses and PCA

Group M	ND	MIN	<5	<10	<15	<20	<25
Mean	18	11.2	0	1	3	6	18
L1	22	15.1	1	2	4	8	22
L2	15	7.3	0	0	1	3	15
Group S							
Mean	24	2.0	4	7	9	13	24
L1	28	3.5	6	8	11	14	28
L2	20	0.4	2	5	7	11	20
Group Sa							
Mean	29	3.8	1	5	9	13	29
L1	31	5.1	2	6	11	14	31
L2	27	2.6	0	4	6	12	27
Group Sb							
Mean	19	0.1	7	9	10	12	19
L1	23	0.2	8	10	13	15	23
L2	15	0.0	6	7	7	10	15

ND number of days, *MIN* minimum salinity, *<5*, *<10*, *<15*, *<20*, and *<25* maximum number of consecutive days with salinity of <5, <10, <15, <20, and <25, respectively

last 15 years (Fig. 5a, b). Moreover, the number of years exhibiting possible severe mortality events every 5 years has tended to decrease and in the last 15 years the conditions have been such that mortalities have occurred in only 1 or 2 years out of every 5 (Fig. 5c, d).

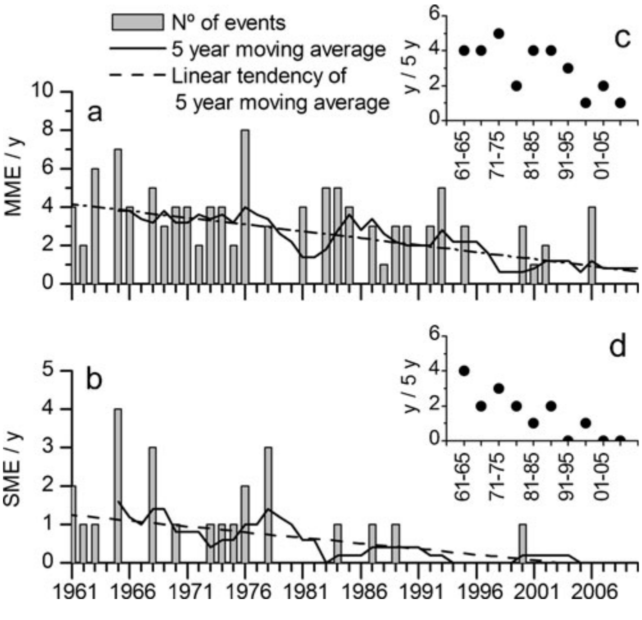

Fig. 5 Expected mortality events in Ulla estuary from 1961 to 2009. **a** Number of moderate mortality events per year (*MME year*$^{-1}$); **b** number of severe mortality events per year (*SME year*$^{-1}$); **c** number of years per 5-year period with at least one possible event of moderate; or (**d**) severe mortality

Discussion

The model designed to estimate salinity in this paper had an acceptable fit to the values used in the validation. Salinity estimates also showed a good fit to the records of Landín and Guerrero (1978) at a point very close to station LU6, used as a reference for the construction of the model (salinities of 19.1 and 17.5, respectively).

The parameters used to characterize the period of low salinity proved to be in keeping with the results of the classification and organization of the mortality episodes. Only one severe mortality episode (Sev0306) was classified with the moderate mortality episodes and one moderate mortality episode (Mod0194) was included with those of severe mortality. In the former case, the regulation of the hydroelectric dam (not included in the model) may have caused a greater decrease in salinity than expected in the model, which only takes into account the weather and the tide. In the 2 weeks prior to this mortality episode, the capacity of the dam went from 75% to 56% (http://augasdegalicia.xunta.es/). Since 1999, comparable releases of water have only taken place on two occasions (December 2000–January 2001 and November 2006), after severe mortalities were recorded, which means that they were unrelated. In contrast, the changes in salinity prior to the moderate mortality episode Mod0194, may not, in fact, have caused severe mortalities since the decrease was very small (Fig. 3). A slight decrease in salinity may produce lower mortalities, given that populations have more time to adapt and turbidity may be lower, as opposed to the sharp decreases originating from strong flooding (Kimmerer 2002a, b; Azevedo et al. 2008). Cases such as the severe mortality in March 2006 (Sev0306) show that the model could be used to detect the influence of the release of large volumes of water from the reservoir at times when the weather-related decrease in salinity was not critical.

This study has demonstrated the long-term versatility of this approach since it allows determination of possible critical levels of salinity not only for one particular species but also for several species at a time. One of these critical levels was a bottom salinity of 11.2 during high tide. This was the mean value corresponding to cases of moderate mortalities, i.e., those with perhaps high mortalities of *V. senegalensis*, but mortalities of <50% for *C. edule* and <15% for *T. decussatus* and *T. philippinarum*. Russell and Peterson (1973) set the lower limit for *C. edule* in an aquarium at 12.5 while Rayment (2005) reported a lower limit of 18 for *V. senegalensis*. The critical level of salinity for *T. decussatus* and *T. philippinarum* (severe mortality) had mean values of <2 (3.8 in subgroup Sa). In keeping with this, Le Treut (1986, in Shafee and Daoudi 1991) indicated that the resistance of *T. decussatus* to occasional decreases in salinity was <6.

However, the decrease in salinity affects the mortality of bivalves not only through the occurrence of low values but also through moderate values that are maintained over longer periods. Salinities of <15 for a mean period of three consecutive days were enough to cause mortalities in *C. edule* and *V. senegalensis* (moderate mortality typology), whereas an average of 9 days was needed to cause mortalities in *T. decussatus* and *T. philippinarum* as well (severe mortality typology). In keeping with our results, Rygg (1970) reported mortalities of 100% in *C. edule* when subjected to salinities of 13 for 3 days. Landín and Guerrero (1978), on the other hand, considered "dangerous salinity" levels, from the standpoint of commercial bivalves, to be values of ≤15 for 1 week or more. Similarly, salinity values of <5 for an average of four consecutive days led to near-total mortality of *C. edule* and *V. senegalensis* (severe mortality typology) and substantial mortalities of *T. decussatus* and *T. philippinarum*. The classification of subgroups Sa and Sb in cases of severe mortality would suggest that, even without reaching extremely low salinities (<5 for >2 days), severe mortalities may occur if the duration of salinities of <30 or 25 is long enough (around 30 days). In this sense, Le Treut (1986, in Shafee and Daoudi 1991) found that *T. decussatus* was not able to withstand prolonged periods with salinities of <20.

For low salinity periods with the same characteristics, the ND is also a key factor. Grouping no mortality (NoMort1102) and morbidity (Morb0194) episodes together with moderate mortality events may be justified in terms of the duration of the low salinity period. Although a salinity of <15 for 3 days would lead us to expect, in these two cases, a moderate mortality, these were the episodes preceded by the shortest low salinity period under these conditions (Table 3). Another similar period was the one preceding the mortality event Sev0306. However, as discussed above, the decrease in salinity was probably more extreme than was estimated by the model owing to the release of water from the reservoir.

The results obtained suggest that mortality episodes occur when the weather causes such a strong decrease in salinity that even the contribution of seawater during high tide cannot make up for the decline. The opposite situation is depicted in Online Resource 1, in which no mortalities were recorded. This is probably due to the fact that these organisms are able to resist occasional decreases in salinity during low tide, provided that, during high tide, the salinity returns to tolerable levels. Hence, the model used to reconstruct salinity suggests that the contribution of fresh water may be less critical when it coincides with conditions conducive to the renewal of seawater in the estuary: winds between N and SE and large tidal amplitudes.

In estuaries whose river basin is regulated by hydroelectric plants, alteration of the flood regime may cause decreases in salinity greater than would be expected from precipitation. During seasons with heavy rainfall, regulating the release of water from the reservoirs in accordance with the tidal regime may reduce its effect on the survival of commercial bivalve beds. In practice, the release of large volumes of water generally coincides with neap tides to reduce the risk of floods in the estuaries. However, it was under these conditions when events such as the severe mortality of March 2006 occurred (Sev0306). On the other hand, if the release of water takes place gradually, the survival of bivalves may be increased, such as in the moderate mortality event of January 1994 (Mod0194) or the one shown in Online Resource 1, as long as the thresholds limits are not exceeded in terms of salinity and duration.

The relationships between the different typologies of mortality defined in this paper, salinity changes, and the relationship between the latter and the weather, highlight the potential vulnerability of the bivalves studied to predicted changes in climate. The frequency of low salinity episodes may affect the frequency of mortality events and the recovery of populations after these episodes (Wang et al. 2008; La Peyre et al. 2009).

The trend toward fewer recent low salinity events found in this study, would lead us to expect an increase in the density of the bivalves, but it might also promote density-dependent phenomena, such as intra- and inter-specific competition (André et al. 1993; Montaudouin and Bachelet 1996), and increase the prevalence of pathogens (Lafferty 2004) which may in turn compensate for the density of their populations. Low salinity periods have often been suggested as a way to maintain healthy populations of commercial bivalves, given the effect on the dynamics of the disease organisms (La Peyre et al. 2009). Thus, salinity may affect bivalve mortality directly or in synergy with other factors, which highlights the need for multi-disciplinary research to address the problems from a multi-factorial standpoint (Kimmerer 2002b; Ringwood and Keppler 2002; Wang et al. 2008).

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