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Phytoplankton communities and biomass size structure (fractionated chlorophyll “a”), along trophic gradients of the Basque coast (northern Spain)

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Abstract Eutrophication is a major threat to coastal ecosystems. Within Europe, the Water Framework Directive (WFD) has established the need of developing methods of assessment. Bulk chlorophyll “a” is used world-wide as an indicator of eutrophication. However, the size structure of the phytoplankton communities has not been investigated, in detail, in relation to eutrophication pressures. This study investigates the suitability of fractionated chlorophyll “a” (<10 and >10 μm), as an indicator of eutrophication. Along the Basque coast (northern Spain) several water bodies, ranging from offshore waters to the heads of the estuaries, were surveyed during 2008. Physico-chemical conditions and chlorophyll “a” showed a distinct spatial gradient. Trophic richness increased towards the middle and inner parts of the estuaries, where nutrient inputs, from natural or anthropogenic sources, together with the residence time of the water are usually higher. In summer, phytoplankton biomass and abundance decreased, generally, in offshore and coastal waters; in estuaries, they tended to increase. The chlorophyll distribution within the two size fractions was coherent with the phytoplankton taxonomic composition. In summer, the relative abundance of small-sized cells (diatoms and non-siliceous taxa) increased. The relationships between size-fractionated chlorophyll and physico-chemical variables differed, when comparing the offshore and coastal waters, with the estuaries. In the offshore and coastal waters, a strong seasonality was observed; in estuaries, an important spatial component was found. Fractionated chlorophyll provided complementary useful information on anthropogenic pressures. However, more research is necessary to utilise this variable as a tool for ecological status assessment, within the WFD.

Keywords Chlorophyll · Eutrophication · Indicator · Phytoplankton · Size · Water Framework Directive

Introduction

Primary producers play a very important role in maintaining the healthy structure and functioning of the marine coastal ecosystems, as they support all

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other trophic levels. Nutrients in adequate proportions and quantities are essential for these organisms. However, excessive anthropogenic nutrient inputs can result in an accumulation of extra biomass, which would have a negative impact on the ecosystems (European Commission 2005; Painting et al. 2007; Zaldívar et al. 2008). Many attributes are considered to be symptoms of negative impacts of nutrient enrichment. Some of these undesirable impacts include: the increased growth of different algae; the loss of submerged vegetation, due to shading; the development of hypoxic and anoxic conditions, due to decomposition of extra biomass; and changes in the community structure of benthic animals, due to oxygen deficiency or the presence of toxic phytoplankton species (Tett 1987; Bricker et al. 2003, 2008; Heisler et al. 2008). In addition, this process includes also alterations of the natural phytoplankton community composition; these may, in turn, change ecosystem food webs and nutrient cycling dynamics (Devlin et al. 2007a).

The European Directive 91/271/EEC, which concerns urban waste water treatment, defined eutrophication as ‘the enrichment of water by nutrients, especially compounds of nitrogen and/or phosphorus, causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned’ (European Commission 1991). Also, other European and international conventions, such as the Water Framework Directive (WFD; European Commission 2000) and the Common Procedure for the Identification of the Eutrophication Status of the OSPAR Maritime Area (OSPAR 2001) address eutrophication as a requirement, to assess water quality or a priority issue for water protection. Although the assessment of eutrophication is not the main objective of the WFD; this Directive has an implicit requirement to assess eutrophication caused by anthropogenic pressure, when classifying the ecological status of surface water bodies (Borja et al. 2006). One of the objectives of the WFD is to achieve, at least, ‘good ecological quality status’ for all water bodies, by 2015. The ecological quality status should be based on biological quality elements and supported by physico-chemical and hydromorphological elements (European Commission 2000). Water bodies that fail to achieve “good ecological quality status”, due to an adverse change in the phytoplankton quality element and, subsequently, indirect effects on the physico-chemical elements (i.e., oxygen and turbidity) and other biota (i.e., macroinvertebrates) as a result of anthropogenic nutrient enrichment can be considered to be ‘eutrophic’ (European Commission 2005).

Although the phytoplankton communities present many complexities, there are attributes that have been used widely as indicators of ecosystem function. Bulk measurements of biomass and abundance (CSTT 1994, 1997; European Commission 2000; OSPAR 2003), taxon diversity (Karydis and Tsirtsis 1996), seasonal succession (Belin et al. 1995; Gailhard et al. 2002) and indicator species (European Commission 2000; Edwards et al. 2001; OSPAR 2003; Paerl et al. 2003) are some of the most common phytoplankton attributes utilised in water quality assessment (Devlin et al. 2007a). Chlorophyll “a” concentration, as a proxy for phytoplankton biomass, is the most usual attribute because it represents a very simple and integrative measure of the phytoplankton community response to nutrient enrichment; at the same time, it is arguably the single most responsive indicator of N and P enrichment in marine systems (Harding 1994). Indicators of ecological quality based upon chlorophyll measurements have the advantage also of being time- and cost-efficient, which makes them suitable for application in extensive monitoring programmes. However, relevant information can be missed, if only bulk chlorophyll data are used in the assessment. The size distribution of the phytoplankton is a fundamental aspect of pelagic ecosystems that reflects the source and cycling of nutrients; it influences the pathways through which production is transferred to consumers. The conceptual models predict that most of the primary production in the communities dominated by small-sized phytoplankton is mineralised in situ; these communities present a high microbial activity and the nutrients are regenerated rapidly. In contrast, the communities dominated by large-sized phytoplankton can export their production directly to the mesozooplankton, to the sediments or to the adjacent waters. These communities require external nutrient inputs and produce an excess of biomass, which is not coupled completely with the grazing of the microheterotrophs (Tremblay and Legendre 1994; Legendre and Rassoulzadegan 1995, 1996; Tamigneaux et al. 1999). However, higher nutrient loading and the consequent increase in the amount of primary production available do not imply necessarily a linear and proportionate transfer in the pelagic food webs. Turner (2001) has reviewed the possible consequences of increased nutrient loading and changing nutrient ratios, to marine food webs, concluding that: eutrophication will be accompanied by greater carbon burial rates and larger hypoxic/anoxic zones; and fisheries production should not be anticipated, with eutrophication. Anthropogenic nutrient loads are characterised usually by low Si:N atomic ratios, which can produce modifications in the phytoplankton taxonomic composition and "cascade effects" within the pelagic food webs. Due to "bottom-up" processes, as well as complex predator–prey interactions, the reformed phytoplankton community is more likely to be composed of flagellated species (including noxious bloom-forming taxa) and smaller cells (Turner 2001).

Several studies have addressed size-fractionated chlorophyll biomass along the Basque coast (Orive et al. 2004; Seoane et al. 2006; Butrón et al. 2009). However, this research has focused upon only a few estuaries (Oka, Butrón and Nervión), out of the 12 estuaries present within this region: so far, no investigations have been published describing this attribute of the phytoplankton communities within offshore and coastal waters over the Basque shelf. In addition, although different approaches have been developed for the use of phytoplankton as an element for ecological quality assessment within the WFD (e.g., Nielsen et al. 2003; Borja et al. 2004, 2009a; Sagert et al. 2005; Loureiro et al. 2006; Devlin et al. 2007a; Domingues et al. 2008; Revilla et al. 2009), phytoplankton is one of the least studied elements, compared to other elements, such as the benthic macroinvertebrates (Borja et al. 2009b).

Hence, the aims of this contribution are: (i) to describe seasonal and spatial variability in the composition and biomass size structure (fractionated chlorophyll "a") of the phytoplankton communities within the offshore, coastal and estuarine waters of the Basque Country; (ii) to examine the relationships between phytoplankton and physico-chemical variables; and (iii) to investigate the suitability of fractionated chlorophyll "a" (<10 and >10 μm) as an indicator of eutrophication, together with its potential use within the WFD.

Materials and methods

The study area

The Basque coast is located in the southeastern Bay of Biscay (Fig. 1); it extends over 150 km and it is oriented E-W. The climate is temperate, oceanic, with moderate winters and warm summers. According to Köppen's classification, the area is associated with a Cfb climate (marine west coast-mild). The rivers are short in length and have high slopes. The Nervión, Oria and Bidasoa rivers have the highest flows (26–36 m^3 s^{-1}, annual mean) throughout the Basque coast. Under average conditions, river flows are at their minimum during summer and early autumn (Valencia et al. 2004). The Oka River has a low flow (3.6 m^3 s^{-1}, annual mean). The estuaries in this study are shallow ($\leq$10 m), except for the outer zone of the Nervión, which is 20–30 m in depth. The Oka and Oria estuaries present extensive intertidal flats whereas, in the Nervión and Bidasoa estuaries, the subtidal area is more important (Borja et al. 2004). Tides are semi-diurnal and tidal amplitudes vary between 1 m on neap tides, to more than 4.5 m on springs tides. As such, they can be considered as generally mesotidal systems, although they present some features which are characteristic of macrotidal estuaries (Hayes 1975). The adjacent coastal water bodies are euhaline and are exposed. A submarine outfall, which receives the treated sewage from a population of 300,000 inhabitants, discharges in the eastern coast (Fig. 1).

The present study draws on data from 2 offshore, 7 coastal and 13 estuarine stations (Fig. 1). The estuarine and coastal stations have been selected in order to characterise the systems receiving the major nutrient inputs, either from natural or anthropogenic origin, along the Basque coast. The offshore stations (lying in approx. 110 m water depth) are those least influenced by terrestrial nutrient inputs, in relation to their distance from land, 7.6 km (L-RF30) and 13.1 km (L-RF10) (Fig. 1).

Sampling strategy and stations grouping

In order to describe winter, spring, summer and autumn conditions, throughout 2008, surveys were undertaken on a quarterly basis (8 Jan–13 Feb; 5–22 May; 27 Aug–15 Sep; 20 Oct–5 Nov). In winter and

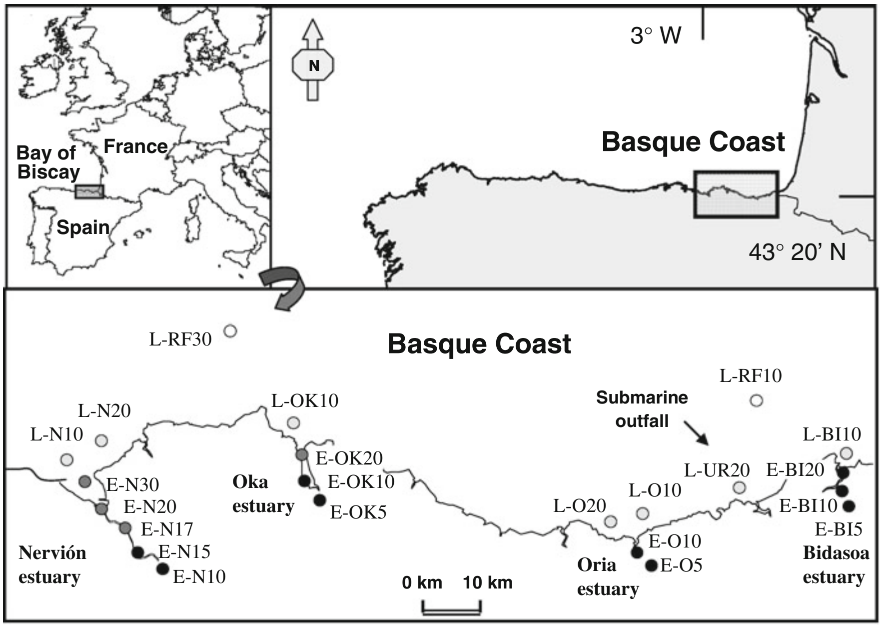


Fig. 1 Map of the Basque coast, showing the sampling stations. *Key*: *White circles*—offshore stations (L-RF30, L-RF10); *light-grey circles*—coastal stations (L-N10, L-N20, L-OK10, L-O10, L-O20, L-UR20, L-BI10); *deep-grey circles*—stations in euhaline-transitional waters (E-N30, E-N20, E-N17, E-OK20); and *black circles*—stations in transitional waters (E-N15, E-N10, E-OK5, E-OK10, E-O5, E-O10, E-BI5, E-BI10, E-BI20)

autumn, phytoplankton variables were not recorded at some of the estuarine stations (Supplementary Electronic Material): frequent rain events and high river flows characterised the winter and autumn seasons; these were not appropriate to obtain samples representative of the estuarine communities. Samples were collected in the surface waters (0–1 m), at fixed stations. The estuaries were surveyed, always, during high tide. In this way: interference of some of the measurements, by suspended sediments in the water column, was avoided; and tidal influence was minimised throughout the surveys, as the longitudinal distribution of the water masses can vary considerably within these estuaries, throughout the semidiurnal tidal cycle (Valencia et al. 2004).

The stations were grouped into four classes, to study the patterns of spatial variability (Fig. 1). Firstly, following Bald et al. (2005) the sampling stations were assigned to different salinity stretches. Subsequently, the stations assigned to the oligo-, meso- or polyhaline stretches of the estuaries were grouped, as representing transitional waters (TW). The stations ascribed to the euhaline stretches of the estuaries were grouped separately, being representative of euhaline-transitional waters (ETW). Likewise, the stations located within the coastal waters (CW) were differentiated from stations located in the offshore waters (OW). These 4 groups were further differentiated into two groups: the offshore and coastal stations (OW and CW); and by integrating all of the estuarine stations (ETW and TW). This approach resulted from 3 ANOVA tests, performed with the total and fractionated chlorophyll "a" data.

Physico-chemical conditions

Temperature, salinity and dissolved oxygen were measured in the field using a CTD (Seabird25), in the offshore and coastal waters and in the Nervión estuary. Within the remaining shallower estuaries, a Handheld Multiparameter Instrument (YSI556) was used. The Secchi disc depth (of disappearance) was measured as an indicator of water transparency. Water samples were collected using a clean bucket. Turbidity was measured directly in the water samples using a turbidimeter (2100 Turbidimeter, HACH; Loveland, Colorado, USA). Suspended solids (SS) were estimated as described in Clesceri et al. (1989), after filtration of the water through Whatman GF/C filters. Total Organic Carbon (TOC) was estimated in Non-Purgable Organic Carbon (NPOC) mode, with a TOC Analyzer (TOC-V CSH/CSN, Shimadzu Corporation, Kyoto, Japan) as described in Grasshoff et al. (1983) and recommended by the supplier. Total N, total P, ammonium, nitrate, nitrite, silicate and phosphate were measured using a Continuous-Flow Autoanalyzer

(Bran + Luebbe Autoanalyzer 3; Norderstedt, Germany), using the colorimetric methods described in Grasshoff et al. (1983). The concentration of dissolved inorganic nitrogen (DIN) was calculated as the sum of the ammonium, nitrate and nitrite concentrations.

Phytoplankton abundance and composition

Water samples were preserved with 1 ml of 25% glutaraldehyde, in 125 ml borosilicate bottles; these were maintained cold (4°C) and in the dark until their analysis, which were undertaken within 3 months after their collection. Standard methods were used for the identification and counting (inverted microscopy and Utermöhl). In order to calculate the percentage contribution of the major taxonomic groups, to total cell abundance, the groups that contributed less than 10% were excluded (euglenophytes, cyanobacteria and unidentified autotrophic coccoids). The summed contribution of the excluded taxa, at any station, was always $\leq$7%. Eight major groups were established: diatoms; heterokontophytes (chrysophyceans, dictyochophyceans and raphidophyceans); dinoflagellates; cryptophytes; haptophytes; chlorophytes; small flagellates; and unidentified cells (10 μm).

Total and size-fractionated chlorophyll “a”

Total chlorophyll “a” was measured by filtering a volume of water (0.2–2.5 l), until the filter (Whatman GF/F) showed colour. The chlorophyll due to the small-sized phytoplankton (<10 μm fraction) was estimated by performing a reverse filtration with a 10 μm-mesh (Revilla et al. 2002); subsequently, filtration (0.2–4.0 l) through a Whatman GF/F filter. The chlorophyll due to the large-sized phytoplankton (>10 μm fraction) was estimated by subtracting the concentration of the <10 μm fraction, from the total concentration. Pigments were extracted in 10 ml of 90% acetone, for 48 h, in dark and cold conditions. The absorbance of the extract was measured in a UV/VIS spectrophotometer (UV 2401PC Spectrophotometers, Shimadzu Corporation, Kyoto, Japan). Concentration was estimated according to the equations of Jeffrey and Humphrey (1975).

There is no general agreement concerning the threshold between small and large phytoplankton, which ranges usually between 1 and 20 μm (Tremblay and Legendre 1994). Several models of structure and ecological functioning of pelagic ecosystems use a threshold of 5 μm, instead of the more common pico-, nano- and microplankton (<2, 2–20 and >20 μm); this is because these models take into account trophic interactions and mesozooplankton generally do not graze efficiently particles <5 μm (e. g., Legendre and Rassoulzadegan 1995, 1996). Within the present study, filters of 5 μm were tried initially, but they were not suitable to perform the filtration, within a reasonable time, with some of the TW samples. Other authors have used also similar diameters as in the present study (i.e. 8 and 10 μm), to perform size-fractionated biomass studies undertaken in estuaries and river plumes (e.g., Butrón et al. 2009; Maguer et al. 2009).

Statistical analysis

The M-estimator of the location of Huber, together with the Median Absolute Deviation (MAD), was calculated for each of the station categories (OW, CW, ETW and TW), for: physico-chemical variables; chlorophyll “a” concentration (total and size-fractionated); and the abundance of general phytoplankton groups (diatoms, dinoflagellates and others). These estimators are more robust than the more commonly-used location and scale estimators (e.g., the average and the standard deviation), which are very sensitive to anomalous data (Huber 1981; Venables and Ripley 2002; García 2005). The estimators were run in R software.

Seasonal differences (spring versus summer), in the total and size-fractionated chlorophyll concentrations were analysed, on the basis of Repeated Measured ANOVA tests. In those cases where the difference was significant (α: 0.05), a Multiple Comparison Procedure (Tukey-test) was run. The winter and autumn data were not included in these statistical analyses, together with the analyses of the relationships between phytoplankton and physico-chemical variables, because the biological variables showed generally very low values and the data sets were incomplete for the estuaries (Supplementary Electronic Material).

Prior to the study of the relationships between the variables, an exploratory analysis of each variable was performed, to check variances and to test normality. Variables failing this test were log-transformed. Those variables that did not fit a log-normal distribution were treated with Box–Cox transformation, in Statgraphics Plus 5.0 software. Phytoplankton variables were related to physico-chemical variables on the basis of the Pearson correlation analysis. This analysis was used also to select an appropriate multivariate technique, between linear and unimodal models. Since the chlorophyll data did not show a unimodal response to the environmental variables and the data set did not present many zeros, Redundancy Analyses (RDA) was performed later.

Redundancy analyses were performed in CANOCO 4.5, to identify the environmental predictors that best explained the variance of chlorophyll “a”. Following each analysis, a plot representative of the model was obtained in CanoDraw 4.0 (Ter Braak and Similauer 2002). The RDA is a constrained linear ordination method. In constrained ordination, axes correspond to the directions of the greatest variability of the data set that can be explained by the environmental variables. When performing the RDA, chlorophyll “a” variables were included as dependent variables, whilst physico-chemical variables were included as independent variables. Finally, total phytoplankton abundance and abundance of diatoms, dinoflagellates and other groups were included as supplementary variables (these do not influence the ordination, although they are shown in the diagrams). The variables were transformed beforehand, as described above, to stabilise the variance and to make the distribution closer to normal. Dependent variables were centred and standardised, whilst the Monte Carlo permutation test (with manual forward selection procedure, under 1999 permutations) was used to select significant (α: 0.05) environmental variables.

Results

Physico-chemical conditions

General physico-chemical conditions in the surface waters are listed for different seasons and water categories in Table 1. Temperature showed a marked seasonality, with minima in winter (10–13°C) and maxima in summer (21–22°C). Salinity decreased from OW to TW. In autumn, the salinity reduced to values below 18 psu in ETW and below 2 psu in TW. Dissolved oxygen concentration ranged usually between 5 and 6 ml l^{-1}. The range of variation was larger within TW, from 4.0 ml l^{-1} in summer to 6.9 ml l^{-1} in autumn. The minimum in oxygen saturation was observed in summer within TW (76%); the maxima were found in spring, within OW and CW (106–108%). The Secchi disc depth decreased from OW to TW. The highest turbidity was observed always in TW. Turbidity was very low in CW, of similar magnitude as in OW. The concentration of SS presented low spatial variability during winter and spring, peaking in the estuaries during summer and autumn. TOC, total N and total P increased towards the estuaries. The maxima in total N were observed in winter, summer and autumn, within the estuaries. The minima in total N were found in spring and summer, within OW and CW (7–11 μM). The maxima in total P were observed in spring and summer, within ETW and TW, respectively. The minima in total P were found in summer and autumn, within OW and CW (0.4–0.5 μM).

The spatial and temporal variability in the concentration of dissolved inorganic nutrients is shown in Fig. 2. A strong spatial gradient was observed in DIN and silicate, increasing from OW to TW, inversely to the salinity. The concentration of phosphate was lower in the marine waters, than in the estuaries. However, phosphate did not present a strong relationship with salinity and some peaks could be observed in ETW. The maximum M-estimator values of DIN and silicate were observed during winter and autumn in TW (70–90 μM) (Fig. 2a, d). The phosphate maximum was found in spring in ETW (2.8 μM) (Fig. 2b). In OW and CW, the measured concentrations ranged from 1–18 μM DIN, 1–9 μM silicate and 0.1–1.5 μM phosphate (Supplementary Electronic Material).

Phytoplankton abundance and composition

The spatial and seasonal variability of the three general phytoplankton groups (diatoms, dinoflagellates and other groups) is shown in Fig. 3. During the winter and autumn surveys, the community was dominated by other groups (non-siliceous taxa, usually of small size), with relatively low abundance values (Fig. 3a, d). In spring, the abundance of diatoms increased (Fig. 3b). Diatoms and small non-siliceous taxa presented low spatial variability and

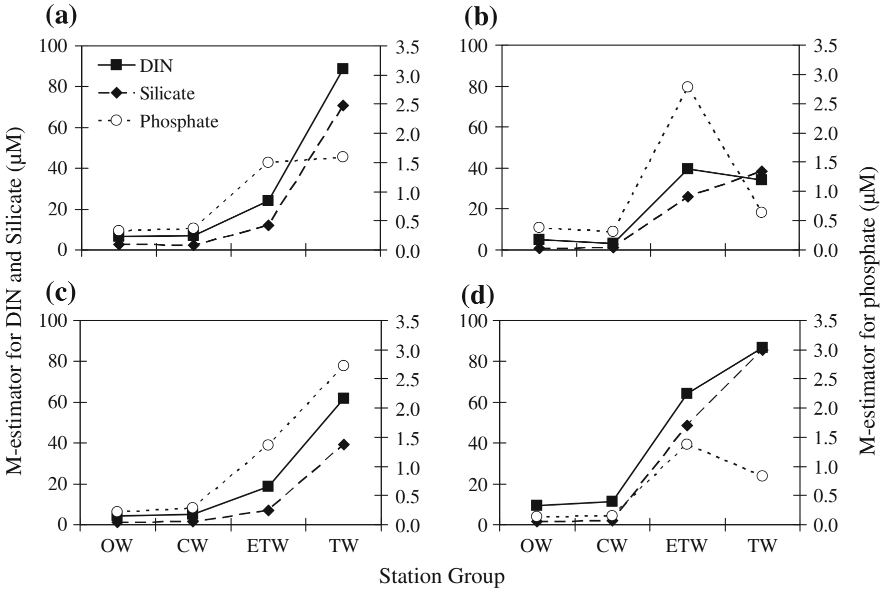
Table 1 The M-estimator of location of Huber with MAD (Median Absolute Deviation), for some physico-chemical variables measured in surface waters, during winter, spring, summer and autumn

		Temperature (°C)		Salinity (psu)		DO conc. (ml l^{-1})		DO sat. (%)		Secchi disc (m)		Turbidity (NTU)		SS (mg l^{-1})		TOC (µM)		Total N (µM)		Total P (µM)	
		Huber	MAD	Huber	MAD	Huber	MAD	Huber	MAD	Huber	MAD	Huber	MAD	Huber	MAD	Huber	MAD	Huber	MAD	Huber	MAD
Winter	OW	12.7	0.2	35.7	0.0	5.7	0.1	96	0.7	9.0	3.0	0.7	0.2	7.4	3.3	101	15	22	7	0.7	0.3
	CW	12.5	0.1	35.5	0.3	5.8	0.1	97	2.8	8.1	2.2	0.4	0.3	6.1	4.8	90	9	21	3	0.7	0.1
	ETW	12.0	0.2	33.4	1.5	5.7	0.1	93	2.0	2.7	1.5	1.9	2.1	9.5	2.5	107	6	47	19	2.5	1.2
	TW	10.2	1.6	11.3	11.6	6.0	0.4	84	2.2	0.9	0.7	4.6	2.8	7.7	4.0	205	27	138	89	3.4	2.0
Spring	OW	15.3	0.0	35.4	0.1	6.1	0.0	108	0.7	13.0	1.5	0.4	0.4	11.2	1.5	65	3	11	2	0.8	0.0
	CW	15.6	0.7	35.3	0.1	6.0	0.2	106	3.0	10.3	1.5	0.2	0.2	6.3	3.9	63	15	7	2	0.7	0.1
	ETW	15.8	0.2	33.1	1.2	5.7	0.5	102	10.4	2.3	1.1	2.2	1.8	8.4	1.1	95	24	71	76	4.0	3.5
	TW	16.4	1.0	23.4	12.1	6.0	0.7	99	5.9	1.3	0.7	3.4	2.6	9.8	8.7	95	27	57	43	1.3	0.7
Summer	OW	21.7	0.1	35.3	0.2	5.1	0.0	102	0.7	14.5	8.2	0.5	0.1	4.0	2.4	85	9	9	2	0.4	0.0
	CW	22.0	0.7	35.2	0.2	5.2	0.1	103	1.5	9.6	4.4	0.6	0.3	3.6	0.7	79	2	10	2	0.5	0.1
	ETW	20.9	0.2	33.8	0.7	4.9	0.2	97	4.4	2.9	1.5	2.1	0.4	14.4	3.2	90	13	32	13	2.0	1.2
	TW	21.9	0.7	22.7	8.5	4.0	0.3	76	11.1	1.6	0.7	2.8	1.8	11.9	4.8	142	49	114	101	4.1	2.6
Autumn	OW	17.7	1.1	35.3	0.1	5.4	0.1	100	0.2	14.5	3.7	0.6	0.8	12.6	2.2	86	17	17	1	0.4	0.1
	CW	16.4	1.1	35.0	0.2	5.5	0.1	99	1.3	7.7	3.0	0.1	0.0	11.5	0.6	77	1	20	4	0.4	0.0
	ETW	13.1	1.6	17.3	19.2	6.2	1.3	94	3.8	1.1	0.7	12.0	10.2	20.5	7.7	213	142	87	70	2.2	1.6
	TW	11.8	0.7	1.8	1.9	6.9	1.2	95	13.3	0.6	0.1	14.5	13.5	14.6	7.5	194	72	111	22	1.8	0.7

Key: OW-offshore waters ($n = 2$); CW-coastal waters ($n = 7$); ETW-euhaline-transitional waters ($n = 4$); and TW-transitional waters ($n = 9$)

DO conc. dissolved oxygen concentration, *DO sat.* dissolved oxygen saturation, *SS* suspended solids, *TOC* total organic carbon

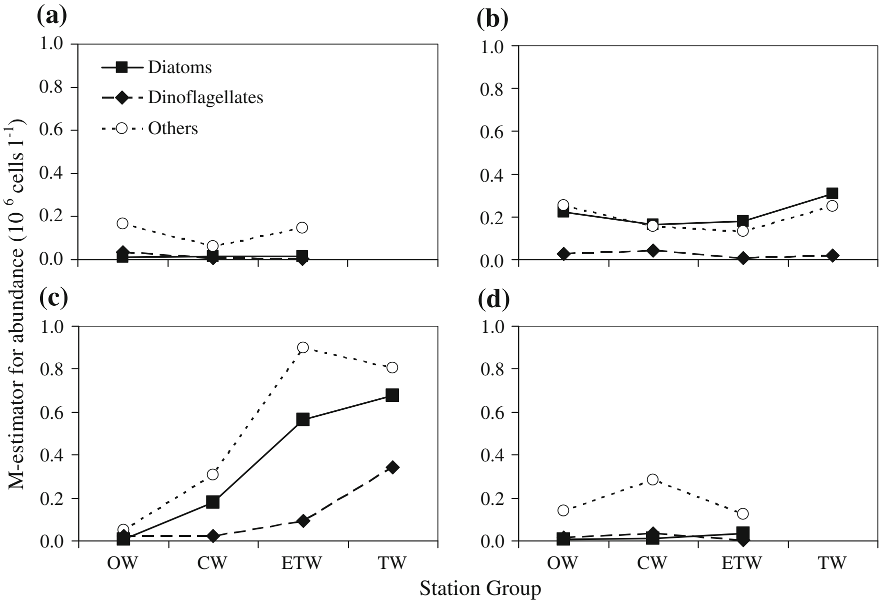
Fig. 2 The M-estimator for the concentration of dissolved inorganic nitrogen (DIN), phosphate and silicate in: (**a**) winter; (**b**) spring; (**c**) summer; and (**d**) autumn. *Key*: OW—offshore waters; CW—coastal waters; ETW—euhaline-transitional waters; and TW—transitional waters



the summed M-estimator values ranged over approximately 0.3–0.6 • 10^6 cells l^{-1}. In summer, a strong spatial gradient was observed in cell abundance, due to a substantial increment in diatoms, dinoflagellates and other groups within the ETW and TW stations (Fig. 3c). The small non-siliceous taxa presented the highest cell abundance, followed by the diatoms and, then, the dinoflagellates. In summer, the total abundance (M-estimator) was >1 • 10^6 cells l^{-1} for the estuarine waters, ~0.5 • 10^6 cells l^{-1} for the coastal waters and ~ 0.1•10^6 cells l^{-1} for the offshore waters.

The spatial and seasonal variation in the phytoplankton community composition has been studied in more detail for the bloom seasons (spring and summer). At each sampling station, the percentage contribution of the major phytoplankton groups is shown in Fig. 4. Composition at the species or genus level can be consulted in the Supplementary Electronic Material.

Fig. 3 The M-estimator for the abundance of diatoms, dinoflagellates and other phytoplankton taxa (others) in: (**a**) winter; (**b**) spring; (**c**) summer; and (**d**) autumn. *Key*: OW—offshore waters; CW—coastal waters; ETW—euhaline-transitional waters; and TW—transitional waters



In OW and CW, spring peaks were caused by many species of chain-forming diatoms (*Chaetoceros* spp., *Leptocylindrus danicus* and *Thalassiosira* spp.), together with solitary diatoms (*Chaetoceros* sp.) and small non-siliceous cells (Fig. 4a, c, e, g, i; Supplementary Electronic Material). Dinoflagellates were only occasionally important; for example, at the L-BI10 station, the community was dominated by several species of diatoms, together with the dinoflagellate *Scrippsiella* spp. (>20 μm cell diameter) (Fig. 4g; Supplementary Electronic Material).

In summer, phytoplankton abundance tended to decrease within OW and most of the CW stations. However, some CW stations presented high densities of small cells (Fig. 3c; Supplementary Electronic Material). The maximum was recorded near the submarine outfall station (L-UR20), with $2.4 \bullet 10^6$ cells l^{-1}. This community was composed mainly of narrow chain-forming diatoms (*Pseudo-nitzschia delicatissima* complex), cryptophytes of the genus *Plagioselmis*, chlorophytes of the genera *Tetraselmis* and *Pyramimonas* and small flagellates (Fig. 4j; Supplementary Electronic Material). The contribution of the diatoms was very low at the OW stations (Fig. 4j).

Within the estuaries, during summer, the relative abundance of the non-siliceous species tended to increase and the dinoflagellates became more important, with the exception of the Oka estuary, where diatoms dominated during both seasons (Fig. 4). The blooming species in the estuaries consisted of small solitary diatoms of the genus *Cyclotella* and unidentified centric diatoms; larger solitary diatoms of the genus *Chaetoceros* and pennales 10–50 μm in length; the dinoflagellate *Kryptoperidinium foliaceum* and other dinoflagellates of the genus *Heterocapsa* and *Pfiesteria*-like; and cryptophytes of the genera *Hemiselmis*, *Plagioselmis* and *Teleaulax* (Supplementary Electronic Material). The maximum in TW was recorded in the Oka estuary, related to a bloom of *Cyclotella* ($67 \bullet 10^6$ cells l^{-1}). Within this estuary, the chain-forming diatom *Thalassiosira guillardii* peaked also in spring ($9.2 \bullet 10^6$ cells l^{-1}) (Supplementary Electronic Material). The Nervión estuary presented also high phytoplankton abundance in summer (up to $3.9 \bullet 10^6$ cells l^{-1}); in this case, the community was dominated by non-siliceous taxa (Fig. 4b; Supplementary Electronic Material).

Total and size-fractionated chlorophyll "a"

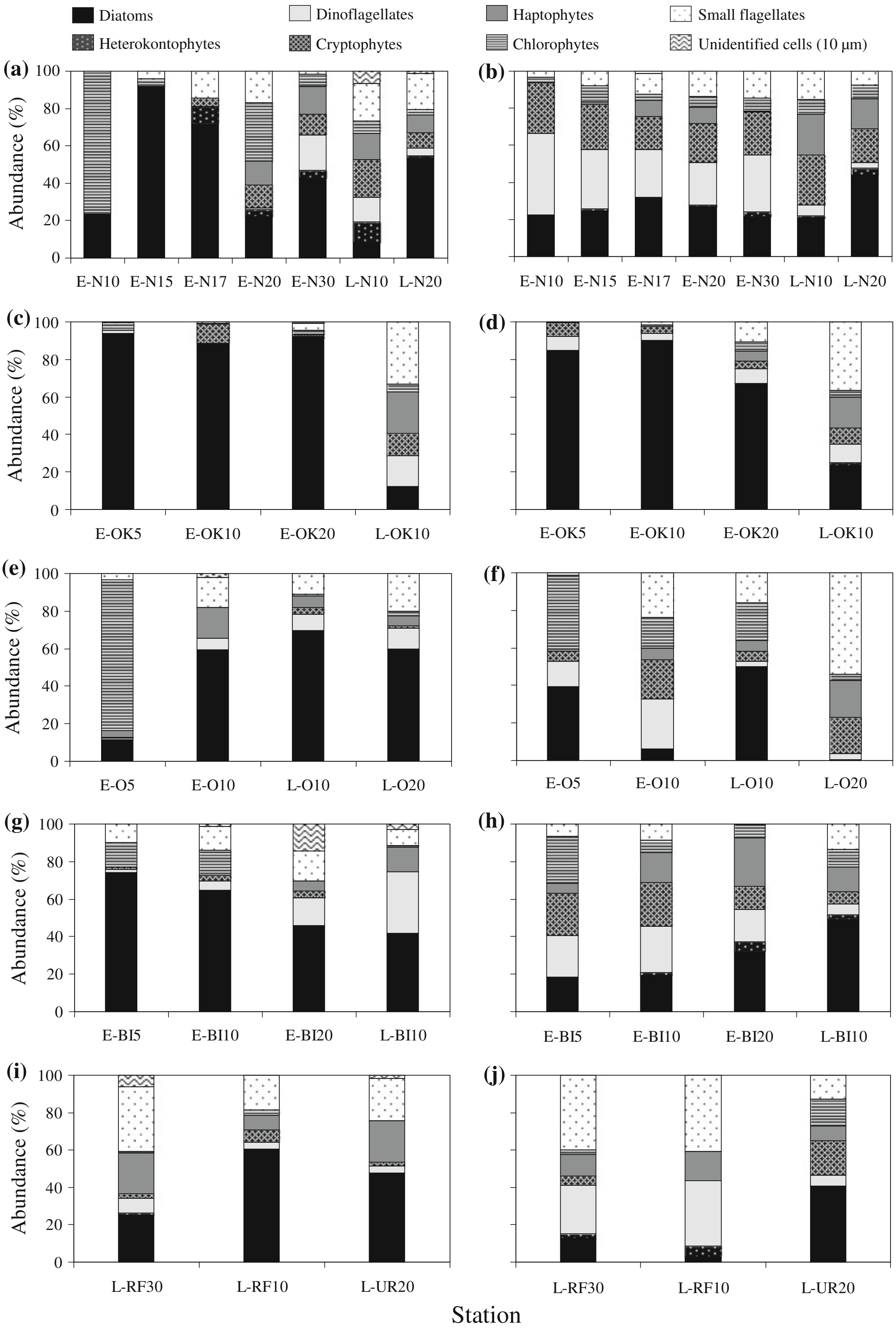
During winter and autumn, the M-estimator for the total chlorophyll and for both size-fractions presented low values and low spatial variability (Fig. 5a, d). Total chlorophyll varied between 0.5 and 1.8 μg l^{-1} and the size-fractions ranged over 0.1–1.0 μg l^{-1}. In spring, a peak of 3.8 μg l^{-1} was found in TW, due mainly to the large-size fraction (Fig. 5b). In summer, the spatial variability was more pronounced. From the marine waters to the estuaries, a progressive increase was observed in the total chlorophyll and in both size-fractions (Fig. 5c). Total chlorophyll varied between 0.2 and 5.6 μg l^{-1}, in summer (Fig 5c).

The contribution of the large-size fraction to the total chlorophyll was important in OW and CW stations, during winter and spring (Fig. 5a, b). In contrast, the small-size fraction contributed relatively more in these waters during summer and autumn (Fig. 5c, d). Within ETW, the small-size fraction contributed always ≥50% to the total concentration. In TW, the contribution of both size fractions was similar in winter and autumn (Fig. 5 a, d); during the blooming seasons, the contribution of the large-size fraction was relatively more important (Fig. 5b, c).

Significant statistical differences ($p < 0.05$) in chlorophyll concentration, between spring and summer, are shown in Table 2 for the marine waters (OW and CW) and the estuaries. Within the marine waters, total chlorophyll "a" was significantly higher in spring (1.4 μg l^{-1}), than in summer (0.6 μg l^{-1}); the large-size fraction showed also significantly higher concentration in spring (1.0 μg l^{-1}), than in summer (0.1 μg l^{-1}). In the estuaries, the small-size fraction increased between spring (0.8 μg l^{-1}) and summer (1.6 μg l^{-1}).

Relationships between phytoplankton and physico-chemical variables

In the offshore and coastal waters, the total and the large-size fraction (>10 μm) chlorophyll showed significant (α: 0.05), positive, correlations with P nutrients (Table 3). The large-size fraction was also correlated negatively with temperature, and correlated positively with dissolved oxygen concentration and nitrite. The total and the small-size fraction (<10 μm) chlorophyll were correlated, inversely, with Secchi disc depth and salinity (Table 3).


Diatoms
Heterokontophytes
Dinoflagellates
Cryptophytes
Haptophytes
Chlorophytes
Small flagellates
Unidentified cells (10 μm)
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
Abundance (%)
0
20
40
60
80
100
E-N10
E-N15
E-N17
E-N20
E-N30
L-N10
L-N20
E-OK5
E-OK10
E-OK20
L-OK10
E-O5
E-O10
L-O10
L-O20
E-BI5
E-BI10
E-BI20
L-BI10
L-RF30
L-RF10
L-UR20
Station

◀ **Fig. 4** The abundance of the main phytoplankton groups, as a percentage of the total phytoplankton abundance. For each river basin (*X*-axes), the stations are ordered from the inner part of the estuaries, to the coastal zone. *Key*: Nervión river basin in spring (**a**) and summer (**b**); Oka river basin in spring (**c**) and summer (**d**); Oria river basin in spring (**e**) and summer (**f**); Bidasoa river basin in spring (**g**) and summer (**h**); and the offshore stations and the submarine outfall station in spring (**i**) and summer (**j**)

Within the estuaries, a higher number of significant correlations were observed (Table 4). The total and the large-size fraction chlorophyll were correlated, positively, with some nutrients (nitrite, ammonium, total P) and with TOC. The small-size fraction was correlated positively with N, P and Si nutrients, TOC and temperature; it was correlated negatively with oxygen. The chlorophyll variables were also correlated negatively with Secchi disc depth; they were correlated positively with turbidity and suspended solids (Table 4).

The RDA for the offshore and coastal stations retained only Secchi disc depth and total P (α: 0.05) (Fig. 6). These variables explained 54.3% of the variance in all of the chlorophyll data ($p = 0.001$). The small-size fraction was related negatively to Secchi disc depth; other structural parameters of the community, such as the abundance of the major phytoplankton groups (diatoms, dinoflagellates and others), followed the same pattern. The large-size fraction chlorophyll was related positively to total P. Total chlorophyll was related also positively to total P and related negatively to Secchi disc depth; however,

Table 2 Differences in the total and the size-fractionated chlorophyll “a” concentration between spring and summer

Chlorophyll “a” concentration	Offshore and coastal waters	Estuaries
Total	Spring > Summer	Spring ~ Summer
	$F = 6.608$; $p = 0.033$	$F = 0.396$; $p = 0.541$
Small-size fraction	Spring ~ Summer	Spring < Summer
	$F = 0.437$; $p = 0.527$	$F = 21.492$; $p < 0.001$
Large-size fraction	Spring > Summer	Spring ~ Summer
	$F = 8.006$; $p = 0.022$	$F = 0.002$; $p = 0.968$

Results of an ANOVA test are showed for two water categories: (i) offshore and coastal waters and (ii) estuaries

Fig. 5 The M-estimator for the concentration of total and size-fractionated chlorophyll “a” in: (**a**) winter; (**b**) spring; (**c**) summer; and (**d**) autumn. *Key*: OW—offshore waters; CW—coastal waters; ETW—euhaline-transitional waters; and TW—transitional waters

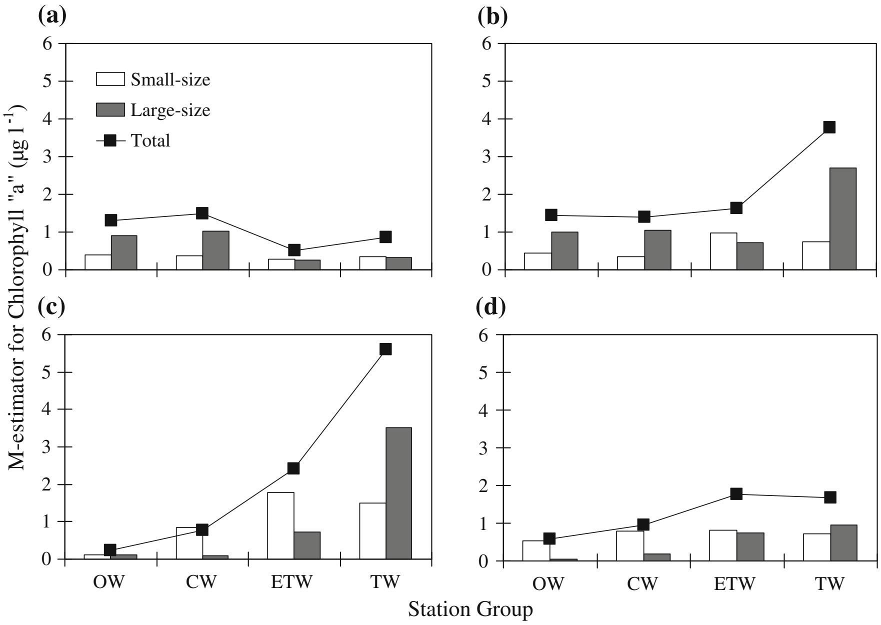


Table 3 Significant correlations (α: 0.05) between chlorophyll "a" and physico-chemical variables in offshore and coastal waters

Chlorophyll "a"	Physico-chemical variables	*r*	*p*
Total	Salinity	−0.537	0.022
	Secchi disc	−0.536	0.022
	Phosphate	0.495	0.037
	Total P	0.595	0.009
Small size-fraction	Salinity	−0.653	0.003
	Secchi disc	−0.516	0.029
Large-size fraction	Temperature	−0.538	0.021
	DO conc.	0.527	0.025
	Nitrite	0.672	0.002
	Phosphate	0.506	0.032
	Total P	0.751	0.000

Only spring and summer data have been included. The Pearson coefficient (*r*) and the probability (*p*) are showed

DO conc. dissolved oxygen concentration

these relationships were weaker than in the case of the fractionated chlorophyll. In addition, although the RDA did not retain temperature (α: 0.05), the stations sampled in spring were separated clearly from the stations sampled in summer (Fig. 6).

For the estuaries, more variables were retained by the RDA (Fig. 7). Phosphate, total P, TOC, suspended solids and salinity explained 74.1% of the variance in all of the chlorophyll data ($p = 0.0005$). However, silicate and N nutrients, together with the variables related to light availability (turbidity and Secchi disc), which showed significant Pearson correlations with the chlorophyll variables (Table 4), were not retained by the RDA. In the RDA, the total and the large-size fraction chlorophyll were associated with suspended solids concentration and, to a lesser extent, TOC. The small-size fraction chlorophyll was related positively to TOC and P nutrients; it was related inversely to salinity (Fig. 7).

Discussion

Physico-chemical conditions and eutrophication risk in the Basque coast

The Basque coast has been conditioned strongly, historically, by different anthropogenic pressures. Reclamation of estuarine areas, for agricultural purposes and subsequent occupation of those areas for urban, industrial and port developments have provoked a dramatic reduction in size and the degradation of water quality in the estuaries (Cearreta

Table 4 Significant correlations (α: 0.05) between chlorophyll "a" and physico-chemical variables in estuaries

Chlorophyll "a"	Physico-chemical variables	*r*	*p*
Total	Secchi disc	−0.551	0.004
	Turbidity	0.466	0.016
	SS	0.581	0.002
	TOC	0.581	0.002
	Ammonium	0.511	0.008
	Nitrite	0.552	0.004
	DIN	0.444	0.023
	Total P	0.419	0.033
Small-size fraction	Temperature	0.529	0.006
	Salinity	−0.440	0.025
	DO conc.	−0.421	0.032
	DO sat.	−0.443	0.023
	Secchi disc	−0.413	0.036
	Turbidity	0.579	0.002
	SS	0.485	0.012
	TOC	0.661	0.000
	Ammonium	0.716	0.000
	Nitrite	0.696	0.000
	Nitrate	0.561	0.003
	DIN	0.613	0.001
	Silicate	0.618	0.001
	Phosphate	0.735	0.000
	Total N	0.695	0.000
	Total P	0.778	0.000
Large-size fraction	Salinity	−0.483	0.013
	Secchi disc	−0.604	0.001
	Turbidity	0.511	0.008
	SS	0.468	0.016
	TOC	0.522	0.006
	Ammonium	0.408	0.039
	Nitrite	0.443	0.023
	Total P	0.422	0.032

Only spring and summer data have been included. The Pearson coefficient (*r*) and the probability (*p*) are showed

SS Suspended solids, *TOC* total organic carbon, *DIN* dissolved inorganic nitrogen, *DO conc.* dissolved oxygen concentration, *DO sat.* dissolved oxygen saturation

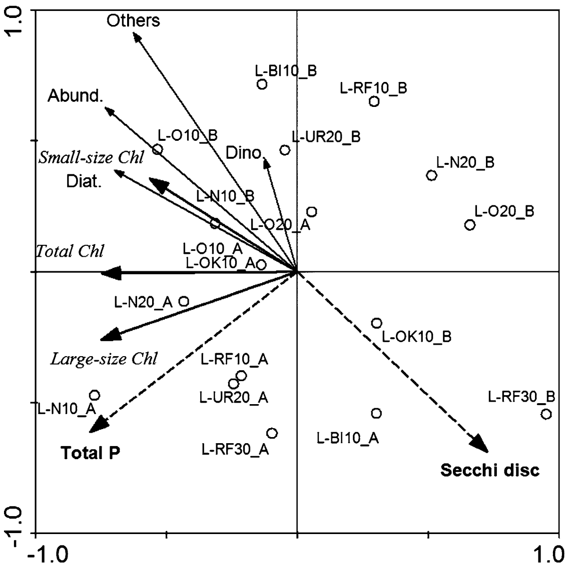


Fig. 6 Redundancy Analysis for the offshore and coastal waters. *Key*: In station labels, _A—spring and _B—summer; *dashed arrows* represent significant physico-chemical variables (α: 0.05); *large solid arrows* represent chlorophyll "a" variables; *small solid arrows* represent supplementary variables, where Abund.—total phytoplankton abundance, Diat.—diatom abundance, Dino.—dinoflagellate abundance; and Others—other taxa abundance

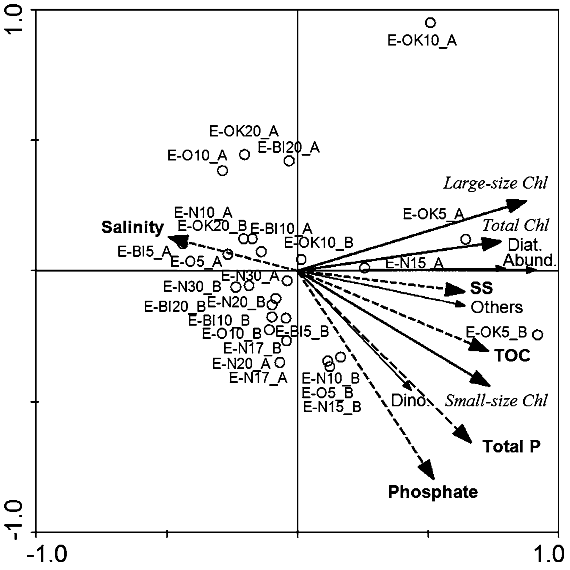


Fig. 7 Redundancy Analysis for the estuaries. *Key*: In station labels, _A—spring and _B—summer; *dashed arrows* represent significant physico-chemical variables (α: 0.05), where SS—suspended solids; and TOC—total organic carbon; *large solid arrows* represent chlorophyll "a" variables; *small solid arrows* represent supplementary variables, where Abund.—total phytoplankton abundance, Diat.—diatom abundance, Dino.—dinoflagellate abundance; and Others—other taxa abundance

et al. 2004; Franco et al. 2004). This situation began to improve during the last decade, due to the closure of many industries and the implementation of sewerage schemes (García-Barcina et al. 2006; Borja et al. 2009a). The spatial patterns observed in oxygen and optical conditions (Secchi disc depth and turbidity) could reflect the high anthropogenic pressures that some of the estuaries have suffered. In the offshore and coastal waters, the oxygen and optical conditions were within the range described, typically, for surface waters in the Bay of Biscay (Valencia et al. 2004). However, the oxygen saturation values in the estuaries, during summer, deviated from the reference conditions (i.e., those typical of non-impacted ecosystems), if they are compared to the quality standards derived by Bald et al. (2005) for the Basque estuaries (98.57% for the euhaline zone and 81.57% for the oligohaline zone). Also, the Secchi disc depth in TW was below the reference conditions established by Bald et al. (2005) (2.0 m). Oxygen saturation was lower in the transitional waters, related probably to: (i) nutrient and organic matter inputs that these estuaries still receive; (ii) the hydromorphological modifications, resulting in higher retention times of bottom waters within the inner part of these estuaries (Borja et al. 2006; Painting et al. 2007); (iii) higher oxygen demand produced by the bottom sediments (reduced and enriched in organic matter); and (iv) low diffusion of oxygen to the bottom waters, due to stratification, as described by Valencia and Franco (2004). Optical properties showed also the same spatial pattern, since the inputs increase directly the turbidity and decrease the transparency, in response to the supply of particulate matter (Fries et al. 2007; Bilotta and Brazier 2008; Devlin et al. 2008) and, indirectly, through the enhancement of the phytoplankton growth.

Although nutrient inputs to the Basque coast have reduced dramatically in recent years, some of the estuaries still receive discharges. In terms of water quality, according to Bald et al. (2005), ammonium and phosphate concentrations measured within ETW and TW showed important deviations from the reference conditions. The treated sewage from a population of about 800,000 inhabitants is discharged into the middle part of the Nervión estuary. Biological treatment has reduced the ammonium loads to the estuary by up to 70% (García-Barcina et al. 2006). However, it is possible still that some nutrient-rich effluents are discharged sporadically by the wastewater treatment plant (Butrón et al. 2009). The Oka estuary supports a much lower population and has not suffered such strong industrialisation. However, it presents usually very high concentrations of ammonium and phosphate over its upper and middle parts, which result from wastewater discharges at the head of the estuary (Franco 1994; Revilla et al. 2000, 2002).

Apart from the WFD, other international policies have addressed physico-chemical variables as assessment parameters of eutrophication. One of the most holistic assessment methods is that proposed by the OSPAR Common Procedure, for the identification of eutrophication status. If the dissolved oxygen levels along the Basque coast are compared to the quality standards described by Best et al. (2007), the estuaries could suffer only some impairment in summer (TW would be at "good status", but lying close to the boundary between "good" and "moderate status"). For the winter DIN concentrations, the Basque offshore and coastal waters did not show any deviation from the reference conditions proposed by Devlin et al. (2007b) (13 μM for coastal waters), whereas the Basque estuaries showed a marked deviation from the reference conditions within the TW zone (20 μM for transitional waters, Devlin et al. (2007b)).

It may be concluded that the physico-chemical variables measured along the Basque coast, during this study, did not indicate eutrophication within the offshore and coastal waters. However, based upon the water oxygen, transparency and nutrient conditions, in general, the inner and middle parts of some of the estuaries could present a risk of eutrophication, at least during the summer season.

Seasonal and spatial variability in the phytoplankton taxonomic composition

Different factors controlling the dynamics of different phytoplankton taxa could explain the variability patterns observed in phytoplankton composition, along the Basque coast. Offshore and coastal waters, in general, showed the typical seasonal cycle observed in the southern Bay of Biscay, with maximum phytoplankton abundance in spring and minimum abundance in summer (Fernández and Bode 1992; Varela 1996). As there is not any nutrient limitation during winter and early spring (Valencia et al. 2004; Revilla et al. 2009), light availability would be the triggering factor for the diatom spring bloom along the Basque coast. On the other hand, there is increasing evidence supporting the theory of trophic control in the oceans, which considers blooms as events generated by a failure of the microzooplankton grazers to contain phytoplankton production (Zarauz et al. 2009). During the summer stratification period, nutrient concentrations in the water layer above the thermocline are comparable to those found in oligotrophic areas (Valencia et al. 2004); this would lead to a summer minimum in phytoplankton abundance. However, some coastal stations showed relatively high phytoplankton abundance in summer. This pattern was particularly noticeable at the L-UR20 station, where a potentially domoic acid producer of the *Pseudo-nitzschia delicatissima* complex was the most abundant species. This fast-growing species has been associated widely with anthropogenic nutrient pulses, such as large coastal runoff or discharge events (Smith et al. 1990; Liefer et al. 2009). The nutrient pulses from the submarine outfall, together with the optimal light and temperature conditions in summer, could be the main causes of the bloom observed at this station.

Within the estuaries, a different pattern was observed, with higher phytoplankton densities in summer. Butrón et al. (2009) have described also summer maxima within small estuaries in the Bay of Biscay, explaining them as the result of an increase in the water residence-time and the lowering of turbidity. Nutrients could also play an important role. Silicate inputs are mainly fluvial, unlike those of phosphate and ammonium. Therefore, during dry periods, Si could be a limiting factor within the lower parts of the estuaries (Butrón et al. 2009). This relationship would explain the relative increase in the abundance of dinoflagellates and other non-siliceous species in summer. Overall, this was not the case for the Oka estuary, which was dominated by diatoms, both in spring and summer. The collapse of the diatom spring bloom is a very common feature in temperate estuaries, due to Si depletion (Fisher et al. 1992; Kocum et al. 2002; Domingues et al. 2005). The Si depletion promotes usually a shift from diatom-dominated communities, to flagellate-dominated communities (Conley et al. 1993; Sommer

1994). The key factor supporting the diatom population in the Oka estuary, in summer, seems to be the continuous availability of Si within this estuary (Trigueros and Orive 2001). The sources are probably both nutrient recycling and freshwater discharges. It has been hypothesised that the mixed nature of the macrotidal systems makes the silic acid, originating from dissolution of the settled frustules in summer, readily available in the water column (Del Amo et al. 1997). In addition, the wastewater treatment plant at the head of the Oka estuary is another additional Si source (Franco 1994). *Cyclotella* sp. was the species with the highest cell density within the Oka estuary. This small diatom (5–10 μm) is capable of tolerating a wide range of salinities and temperatures, as well as high levels of organic matter (Trigueros et al. 2000). Apart from Si availability, the selective control that the zooplankton grazing exerts could be another factor influencing the dominance of the diatoms; they might not be an optimal prey, due to the presence of siliceous setae (Haigh et al. 1992).

Seasonal and spatial variability in chlorophyll biomass and size-structure

In the offshore and coastal waters, the results of the fractionated chlorophyll “a” have indicated that in winter and spring the large-sized phytoplankton (>10 μm) constituted a high percentage of the total chlorophyll. In view of the taxonomic information, large diatoms were the major contributors to total phytoplankton biomass. In spring, light availability, the relatively turbulent water-column that assures nutrient inputs and reduces sedimentation, the weak coupling between grazers and large-sized phytoplankton, are all favourable factors for blooms of large diatoms (Margalef 1978; Legendre 1990; Bode and Fernández 1992; Maguer et al. 2009; Zarauz et al. 2009). In the present study, although the small-sized phytoplankton contributed little to the total chlorophyll in spring, the abundance of small non-siliceous taxa was relatively high. As the survey took place in mid-spring (5–22, May), the community composition was reflecting, probably, the succession towards the thermal stratification period. During summer, the contribution of the small-sized phytoplankton (diatoms and/or non-siliceous taxa) was higher in OW and CW. Other investigators have observed a higher contribution of the small-size chlorophyll fractions (<2 μm, <5 μm), when total phytoplankton biomass is at low levels in coastal systems, such as: in the Gulf of St Lawrence during summer stratification (Tamigneaux et al. 1999); in the Ría de Vigo during winter mixing (Cermeño et al. 2006); and in the central Cantabrian Sea during early winter and autumn (Calvo-Díaz et al. 2008). Physiological processes have been suggested as controlling the size structure of the phytoplankton communities, under specific environmental conditions. It may be anticipated that smaller cells compete better than larger cells for nutrients and light acquisition, under unfavourable conditions, due to their surface-to-volume ratio (Fogg 1991; Finkel et al. 2004). However, under favourable conditions for growth, larger cells (in particular, diatoms) may have a higher photosynthetic efficiency (Cermeño et al. 2005).

During summer, the thermal stratification and consequent oligotrophy above the nutricline could explain the chlorophyll minima observed in the surface OW; this has been described previously for temperate coastal seas and, in particular, for the southern Bay of Biscay (e.g., Serret et al. 1999; Valencia et al. 2004; Calvo-Díaz et al. 2008). In ETW and at some of the coastal stations, nutrient depletion is less likely to occur, due to anthropogenic inputs and summer storms; these activate the export of nutrients from the rivers and estuaries, to the coastal waters (Revilla et al. 2009). The location of the chlorophyll maxima in the oligo- and mesohaline regions of the Basque estuaries has been described in previously studies (Orive et al. 2004; Seoane et al. 2005; Butrón et al. 2009). The shallowness of these estuaries and the higher nutrient concentrations and water residence times within their middle and inner parts appear to be responsible for this axial chlorophyll distribution, during periods of low river runoff (usually the summer season). These hydrological conditions are characteristic of the Nervión, Oka and Bidasoa estuaries. In contrast, the Oria estuary has shorter water residence times, related to its geomorphology; this makes it more of a supplier of nutrients to the coast, than an in situ production area (Valencia et al. 2004).

In the ETW, the small-size fraction constituted a relatively high percentage of the total chlorophyll, especially in summer. In contrast, in TW, the large-size fraction contributed importantly to the total chlorophyll. Previous studies conducted in the Nervión and

Oka estuaries have shown a similar spatial pattern (Revilla et al. 2002; Butrón et al. 2009). The seasonal shift in ETW, from a community with an important contribution of the large-sized phytoplankton in spring, towards a community dominated by small phytoplankton in summer has been observed also by Revilla et al. (2002), within the Oka estuary. Elsewhere, in the eutrophic system of the Thames (U.K.) plume, Weston et al. (2008) observed a high contribution of the large-size fraction (>5 μm) to the total chlorophyll; this fraction responded also to nutrient enrichment in incubation experiments, but not the small-size fraction (<5 μm). These authors explained the suppression of the small-size fraction growth as being due to the high rates of microzooplankton grazing. The balance between small and large cells in estuaries might be determined also, by: (i) the inputs of exogenous nutrients, from their watersheds that would favour new-production based-systems; (ii) the strength of grazing by suspension-feeders, which typically select larger cells; (iii) the temperature-driven differences in growth rates, between picoplankton and larger cells (Ning et al. 2000). All these factors could explain the spatial and temporal dynamics observed in the fractionated chlorophyll within the Basque estuaries.

Relationships between phytoplankton and physico-chemical variables

Within the offshore and coastal waters, the temporal variability in the phytoplankton communities (biomass and composition) was linked with nutrient availability. Total chlorophyll increased with P-nutrients and decreased with salinity and water transparency. Salinity and water transparency can indicate episodic freshwater inputs, which would have a fertilisation effect. Also, the biomass of the large-size fraction increased with P-nutrients; this increment took place in spring. In the Basque coastal waters, the annual cycle of dissolved nutrients in the upper layers is related to the thermal cycle and the succession of homogeneity together with stratification conditions. Further, riverine inputs modulate the nutrient cycle, together with the pulses of phytoplankton production (Valencia et al. 2004). During winter, silicate and nitrate concentrations can be high, due to the mixing conditions in the water column and the freshwater inputs to the continental shelf. The onset of the late-winter and spring blooms, which are composed usually of large diatoms at these latitudes, is expected to cause a high demand of the inorganic nutrient forms of Si and N (Glé et al. 2008). In this respect, the Redfield ratios measured in spring indicate a potential limitation, firstly by Si and, secondly, by N (Supplementary Electronic Material). Therefore, the results of the statistical analysis should not be interpreted as a direct cause-effect relationship, between P and phytoplankton biomass, but as a seasonal covariance of these two variables. The diatoms and small non-siliceous taxa (others) were not associated with any particular season. In contrast, the dinoflagellates were associated with the samples collected in summer. The Redfield ratios indicated that, at this time, the first potentially limiting nutrient was again the Si, then P. These conditions would enable the growth of dinoflagellates, as high N:P ratios are favourable for slow-growing species (Skjodal 1993) and the Si potential limitation would favour the growth of non-siliceous species.

In estuaries, correlation and RDA analyses have indicated a strong spatial gradient marked by salinity, where phytoplankton biomass and abundance increased in the same direction as turbidity, nutrients, TOC and suspended solids concentration. This axial pattern is not uncommon in other estuaries, elsewhere (Butrón et al. 2009). However, along the Basque coast it is enforced by the anthropogenic pressures which some of the estuaries still support, since the highest chlorophyll and abundance values were found at the head of the Oka estuary; this is the water body with the strongest nutrient inputs of anthropogenic origin.

Suitability of fractionated chlorophyll, as an indicator of eutrophication within the WFD

When using phytoplankton as a biological quality element, in assessing the ecological status within the WFD, some authors have expressed their concerns. Hence, the setting of reference conditions for water quality, the sampling frequency and the use of chlorophyll “a” as a proxy of phytoplankton biomass and abundance have been discussed elsewhere (Domingues et al. 2008). Chlorophyll “a” should be used with care as an alternative for phytoplankton abundance and biomass, especially when pico- and nanophytoplankton are important components of the community. In order to counteract these limitations, the use of combined microscopy techniques (Utermöhl and fluorescence), together with biovolume measurements, have been proposed (Domingues et al. 2008). However, a high degree of expertise is needed to obtain good-quality information on phytoplankton taxonomic composition and biovolume; this makes difficult the comparison between results obtained by different Member States, as data sets can be laboratory-dependent.

In turn, some successful methodologies for assessing phytoplankton quality status within the WFD in coastal areas have been described (Nielsen et al. 2003; Borja et al. 2004, 2009a; Sagert et al. 2005; Devlin et al. 2007a; Revilla et al. 2009). Moreover, the use of phytoplankton indicators within the Marine Strategy Directive has been discussed (McQuatters-Gollop et al. 2009). However, there is a lack of methodologies for the WFD within transitional waters (Borja et al., 2004; Zaldívar et al. 2008; Giordani et al. 2009). Most of the methods used in assessing the status of phytoplankton in estuaries use chlorophyll as a primary indicator (Bricker et al. 2003; USEPA 2006); other investigators have suggested alternative approaches, with taxonomic indices based upon body size or size spectra (Mouillot et al. 2006). However, for phytoplankton, even though there is evidence of variation in phytoplankton size structure as a function of environmental conditions, there is no methodological standardisation yet for its application as an ecological status classification tool (Mouillot et al. 2006; Zaldívar et al. 2008).

Therefore, there is a need for the development of indicators based upon phytoplankton attributes that are ecologically-relevant, time and cost-efficient, reproducible and suitable for comparison. In this study, size-fractionated chlorophyll is addressed as a potential tool. The partition of the bulk chlorophyll in the <10 and >10 μm size fractions reflected, well, the spatial and temporal dynamics of the community composition, between systems of different nutrient richness along the Basque coast. In some situations, under anthropogenic pressure conditions, fractionated chlorophyll would provide useful information, as total chlorophyll may not reflect any anomaly. For example, in the case of the submarine outfall station (L-UR20), during summer, the phytoplankton abundance showed unusual numbers; however, it did not cause a response in the total chlorophyll concentration, due to the small cellular size of the phytoplankton taxa involved. Nevertheless, the need for more studies should be highlighted, comparing systems under different pressure level, using a more intensive sampling frequency; likewise, applying experimental approaches (such as, nutrient addition bioassays), in order to establish reference conditions and suitable indicators, based upon fractionated chlorophyll.

Conclusions

Overall, a spatial gradient in the fractionated chlorophyll "a" (<10 and >10 μm) was observed, with generally lower concentrations in offshore and coastal marine waters and higher within the estuaries. However, the spatial and temporal patterns of variation, as well as their relationships with the environmental variables, differed between the two size fractions. Seasonality was the main factor explaining the variability in phytoplankton biomass and composition in the marine zone, controlling the changes in the chlorophyll concentration of the large-size fraction. In estuaries, total chlorophyll and the large-size fraction presented a strong spatial gradient, increasing from the outer euhaline zone, to the inner transitional waters; the small-size fraction varied more seasonally, increasing during the warmest season. In transitional waters (oligo-, meso- or polyhaline stretches of the estuaries), the supply of nutrients all year round induced the dominance of the large-size fraction, during spring and summer, as light and water residence times were favourable for growth in these seasons.

Although further research is needed, fractionated chlorophyll "a" is a useful attribute of the phytoplanktonic community, for ecological quality assessment; it is a simple indicator of the community composition and a rapid indicator of the way the community responds to nutrient inputs. Summarising, it could facilitate the understanding of the structure and functioning of phytoplankton communities during different seasons and at different trophic states.

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References

Bald J, Borja A, Muxica I, Franco J, Valencia V (2005) Assessing reference conditions and physico-chemical status according to the European Water Framework Directive: a case-study from the Basque Country (Northern Spain). Mar Pollut Bull 50:1508–1522

Belin C, Beliaeff B, Raffin B, Rabia M, Ibanez F (1995) Phytoplankton time-series data of the French Phytoplankton Monitoring Network: toxic and dominant species. In: Lassus P, Arzul G, Erard Le Denn E, Gentien P, Marcaillou Le Baut C (eds) Harmful marine algal blooms. Paris, France

Best MA, Wither AW, Coates S (2007) Dissolved oxygen as a physico-chemical supporting element in Water Framework Directive. Mar Pollut Bull 55:53–64

Bilotta GS, Brazier RE (2008) Understanding the influence of suspended solids on water quality and aquatic biota. Water Res 42:2849–2861

Bode A, Fernández E (1992) Influence of water-column stability on phytoplankton size and biomass succession patterns in the central Cantabrian Sea (Bay of Biscay). J Plankton Res 14:885–902

Borja A, Franco J, Valencia V, Bald J, Muxika I, Belzunce MJ, Solaun O (2004) Implementation of the European Water Framework Directive from the Basque Country (northern Spain): a methodological approach. Mar Pollut Bull 48:209–218

Borja A, Galparsoro I, Solaun O, Muxika I, Tello EM, Uriarte A, Valencia V (2006) The European Water Framework Directive and the DPSIR, a methodological approach on assessing the risk of failing to achieve the good ecological status. Estuar Coast Shelf Sci 66:84–96

Borja A, Bald J, Franco J, Larreta J, Muxika I, Revilla M, Rodríguez JG, Solaun O, Uriarte A, Valencia V (2009a) Using multiple ecosystem components in assessing ecological status in Spanish (Basque Country) Atlantic marine waters. Mar Pollut Bull 59:54–64

Borja A, Ranasinghe A, Weisberg SB (2009b) Assessing ecological integrity in marine waters, using multiple indices and ecosystem components: challenges for the future. Mar Pollut Bull 59:1–4

Bricker SB, Ferreira JG, Simas T (2003) An integrated methodology for assessment of estuarine trophic status. Ecol Model 169:39–60

Bricker SB, Longstaff B, Dennison W, Jones A, Boicourt K, Wicks C, Woerner J (2008) Effects of nutrient enrichment in the nation's estuaries: a decade of change. Harmful Algae 8:21–32

Butrón A, Iriarte A, Madariaga I (2009) Size-fractionated phytoplankton biomass, primary production and respiration in the Nervión-Ibaizabal estuary: a comparison with other nearshore coastal and estuarine ecosystems from the Bay of Biscay. Cont Shelf Res 29:1088–1102

Calvo-Díaz A, Morán XAG, Suárez LA (2008) Seasonality of picophytoplankton chlorophyll *a* and biomass in the central Cantabrian Sea, southern Bay of Biscay. J Mar Syst 72:271–281

Cearreta A, Irabien MJ, Pascual A (2004) Human activities along the Basque coast during the last two centuries: geological perspective of recent anthropogenic impact on the coast and its environmental consequences. In: Borja Á, Collins M (eds) Oceanography and Marine Environment of the Basque Country. Elsevier Oceanography Series, vol 70. Elsevier, Amsterdam, pp 27–50

Cermeño P, Estévez-Blanco P, Marañón E, Fernández E (2005) Maximum photosynthetic efficiency of size-fractionated phytoplankton assessed by 14C-uptake and fast repetition rate fluorometry. Limnol Oceanogr 50:1438–1446

Cermeño P, Marañón E, Pérez V, Serret P, Fernández E, Castro CG (2006) Phytoplankton size structure and primary production in a highly dynamic coastal ecosystem (Ría de Vigo, NW-Spain): seasonal and short-time scale variability. Estuar Coast Shelf Sci 67:251–266

Clesceri LS, Greenberg AE, Rhodes Trussell R (eds) (1989) Standard methods for the examination of water and wastewater, 17th edn. Port City Press, Baltimore

Conley DJ, Schelske CL, Stoermeer EF (1993) Modification of the biogeochemical cycle of silica with eutrophication. Mar Ecol Prog Ser 101:179–192

CSTT (1994) Comprehensive studies for the purposes of Article 6 of DIR 91/271 EEC, the Urban Waste Water Treatment Directive. Published for the Comprehensive Studies Task Team of Group Coordinating Sea Disposal Monitoring by the Forth River Purification Board, Edinburgh

CSTT (1997) Comprehensive studies for the purposes of Article 6 and 8.5 of DIR 91/271 EEC, the Urban Waste Water Treatment Directive, 2nd edn. Published for the Comprehensive Studies Task Team of Group Coordinating Sea Disposal Monitoring by the Department of the Environment for Northern Ireland, the Environment Agency, 2002 Scottish Environmental Protection Agency and the Water Services Association, Edinburgh

Del Amo Y, Quéguiner B, Tréguer P, Breton H, Lampert L (1997) Impacts of high-nitrate freshwater inputs on macrotidal ecosystems. II. Specific role of the silic acid pump in the year round dominance of diatoms in the Bay of Brest (France). Mar Ecol Prog Ser 161:225–237

Devlin M, Best M, Coates D, Bresnan E, O'Boyle S, Park R, Silke J, Cusack C, Skeats J (2007a) Establishing boundary classes for the classification of UK marine waters using phytoplankton communities. Mar Pollut Bull 55:91–103

Devlin M, Painting S, Best M (2007b) Setting nutrients thresholds to support an ecological assessment based on nutrient enrichment, potential primary production and undesirable disturbance. Mar Pollut Bull 55:65–73

Devlin M, Barry J, Mills DK, Gowen RJ, Foden J, Sivyer D, Tett P (2008) Relationships between suspended particulate material, light attenuation and Secchi disc depth in UK marine waters. Estuar Coast Shelf Sci 79:429–439

Domingues RB, Barbosa A, Galvão H (2005) Nutrients, light and phytoplankton succession in a temperate estuary (the Guadiana, south-western Iberia). Estuar Coast Shelf Sci 64:249–260

Domingues RB, Barbosa A, Galvão H (2008) Constraints on the use of phytoplankton as a biological quality element within the Water Framework Directive in Portuguese waters. Mar Pollut Bull 56:1389–1395

Edwards M, John AWG, Johns DG, Reid PC (2001) Case-history and persistence of the non-indigenous diatom *Coscinodiscus wailesii* in the north-east Atlantic. J Mar Biol Assoc UK 81:207–211

European Commission (1991) Directive 91/271/EEC of the European Parliament and of the Council of 21 May 1991 concerning urban waste water treatment

European Commission (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy

European Commission (2005) WFD CIS towards a guidance document on eutrophication assessment in the context of European water policies. Meeting of Water Directors, London, 28 November 2005

Fernández E, Bode A (1992) Succession of phytoplankton assemblages in relation to hydrography in the southern Bay of Biscay: a multivariate approach. Sci Mar 58:191–205

Finkel ZV, Irwin AJ, Schofield O (2004) Resource limitation alters the 3/4 size scaling of metabolic rates in phytoplankton. Mar Ecol Prog Ser 273:269–279

Fisher TR, Peele ER, Ammerman JW, Harding LW Jr (1992) Nutrient limitation of phytoplankton in Chesapeake Bay. Mar Ecol Prog Ser 82:51–63

Fogg GE (1991) The phytoplanktonic ways of life. New Phytol 118:191–232

Franco J (1994) Variabilidad espacio-temporal de la biomasa y producción del fitoplancton en el estuario de Urdaibai. Dissertation, University of the Basque Country, Spain

Franco J, Borja A, Valencia V (2004) Overall assessment: human impacts and quality status. In: Borja Á, Collins M (eds) Oceanography and Marine Environment of the Basque Country. Elsevier Oceanography Series, vol 70. Elsevier, Amsterdam, pp 581–597

Fries JS, Noble RT, Paerl HW, Characklis GW (2007) Particle suspensions and their regions of effect in the Neuse River Estuary: Implications for water quality monitoring. Estuaries Coasts 30:359–364

Gailhard I, Gros PH, Durbec JP, Beliaeff B, Belin C, Nezan E, Lassus P (2002) Variability patterns of microphytoplankton communities along the French coasts. Mar Ecol Prog Ser 242:39–50

García A (ed) (2005) Métodos avanzados de estadística aplicada. Métodos Robustos y de Remuestreo. Madrid, Spain

García-Barcina JM, González-Oreja JA, De la Sota A (2006) Assessing the improvement of the Bilbao estuary water quality in response to pollution abatement measures. Water Res 40:951–960

Giordani G, Zaldívar JM, Viaroli P (2009) Simple tools for assessing water quality and trophic status in transitional water ecosystems. Ecol Indic 9:982–991

Glé C, Del Amo Y, Sautour B, Laborde P, Chardy P (2008) Variability of nutrients and phytoplankton primary production in a shallow macrotidal coastal ecosystem (Arcachon Bay, France). Estuar Coast Shelf Sci 76:642–656

Grasshoff K, Ehrhardt M, Kremling K (1983) Methods of seawater analysis, 2nd edn. Verlag Chemie, Weinheim, Germany

Haigh R, Taylor FJR, Sutherland TF (1992) Phytoplankton ecology of the Sechelt Inlet, a fjord system on the British Columbia coast. I. General features of the nano- and microplankton. Mar Ecol Prog Ser 89:117–134

Harding L (1994) Long term trends in the distribution of phytoplankton in Chesapeake Bay: roles of light, nutrients and streamflow. Mar Ecol Prog Ser 104:267–291

Hayes MO (1975) Morphology of sand accumulation in estuaries; an introduction to the symposium. In: Cronin LE (ed) Estuarine research, 2nd edn. Academic Press, New York

Heisler J, Glibert PM, Burkholder JM, Anderson DM, Cochlan W, Dennison WC, Dortch Q, Gobler CJ, Heil CA, Humphries E, Lewitus A, Magnien R, Marshall HG, Sellner K, Stockwell DA, Stoecker DK, Suddleson M (2008) Eutrophication and harmful algal blooms: a scientific consensus. Harmful Algae 8:3–13

Huber PJ (1981) Robust statistics. Wiley, New York

Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanz 167:191–194

Karydis M, Tsirtsis G (1996) Ecological indices: a biometric approach for assessing eutrophication levels in the marine environment. Sci Total Environ 186:209–219

Kocum E, Underwood GJC, Nedwell DB (2002) Simultaneous measurement of phytoplankton primary production, nutrient and light availability along a turbid, eutrophic UK east coast estuary (the Colne Estuary). Mar Ecol Prog Ser 231:1–12

Legendre L (1990) The significance of microalgal blooms for fisheries and for export of particulate organic carbon in oceans. J Plankton Res 12:681–699

Legendre L, Rassoulzadegan F (1995) Plankton and nutrient dynamics in marine waters. Ophelia 41:153–172

Legendre L, Rassoulzadegan F (1996) Food-web mediated export of biogenic carbon in oceans: hydrodynamic control. Mar Ecol Prog Ser 145:179–193

Liefer JD, Macintyre HL, Novoveská L, William LS, Dorsey CP (2009) Temporal and spatial variability in *Pseudo-nitzschia* spp. in Alabama coastal waters: a "hot spot" linked to submarine groundwater discharge? Harmful algae 8:706–714

Loureiro S, Newton A, Icely J (2006) Boundary conditions for the European Water Framework Directive in the Ria Formosa lagoon, Portugal (physico-chemical and phytoplankton quality elements). Estuar Coast Shelf Sci 67:382–398

Maguer JF, L'Helguen S, Waeles M, Morin P, Riso R, Caradec J (2009) Size-fractionated phytoplankton biomass and nitrogen uptake in response to high nutrient load in the North Biscay Bay in spring. Cont Shelf Res 29:1103–1110

Margalef R (1978) Life-forms of phytoplankton as survival alternatives in an unstable environment. Oceanol Acta 1:494–509

McQuatters-Gollop A, Gilbert AJ, Mee LD, Vermaat JE, Artioli Y, Humborg C, Wulff F (2009) How well do ecosystem indicators communicate the effects of anthropogenic eutrophication? Estuar Coast Shelf Sci 82: 583–596

Mouillot D, Spatharis S, Reizopoulo S, Laugier T, Sabetta L, Basset A, Do Chi T (2006) Alternatives to taxonomic-based approaches to assess changes in transitional water communities. Aquat Conserv Mar Freshwat Ecosyst 16:469–482

Nielsen K, Somod B, Ellegaard C, Krause-Jensen D (2003) Assessing reference conditions according to the European Water Framework Directive using modelling and analysis of historical data: an example from Randers Fjord, Denmark. Ambio 32:287–294

Ning X, Cloern JE, Cole BE (2000) Spatial and temporal variability of picocyanobacteria *Synechococcus* sp. in San Francisco Bay. Limnol Oceanogr 45:695–702

Orive E, Franco J, Madariaga I, Revilla M (2004) Bacterioplankton and phytoplankton communities. In: Borja Á, Collins M (eds) Oceanography and Marine Environment of the Basque Country. Elsevier Oceanography Series, vol 70. Elsevier, Amsterdam, pp 367–393

OSPAR (2001) Draft common assessment criteria and their application within the comprehensive procedure of the common procedure. Meeting of the Eutrophication Task Group, London, 9–11 October 2001

OSPAR (2003) OSPAR integrated report 2003 on the eutrophication status of the OSPAR maritime area based upon the first application of the comprehensive procedure, London

Paerl HW, Valdes LM, Pinckney JL, Piehler MF, Dyble J, Moisander PH (2003) Phytoplankton photopigments as indicators of estuarine and coastal eutrophication. Bioscience 53:953–964

Painting SJ, Devlin MJ, Malcolm SJ, Parker ER, Mills DK, Mills C, Tett P, Wither A, Burt J, Jones R, Winpenny K (2007) Assessing the impact of nutrient enrichment in estuaries: susceptibility to eutrophication. Mar Pollut Bull 55:74–90

Revilla M, Iriarte A, Madariaga I, Orive E (2000) Bacterial and phytoplankton dynamics along a trophic gradient in a shallow temperate estuary. Estuar Coast Shelf Sci 50: 297–313

Revilla M, Ansotegui A, Iriarte A, Madariaga I, Orive E, Sarobe A, Trigueros JM (2002) Microplankton metabolism along a trophic gradient in a shallow temperate estuary. Estuaries 25:6–18

Revilla M, Franco J, Bald J, Borja Á, Laza A, Seoane S, Valencia V (2009) The assessment of the phytoplankton ecological status in the Basque coast (northern Spain) according to the European Water Framework Directive. J Sea Res 61:60–67

Sagert S, Krause Jensen D, Henriksen P, Rieling T, Schubert H (2005) Integrated ecological assessment of Danish Baltic sea coastal areas by means of phytoplankton and macrophytobenthos. Estuar Coast Shelf Sci 63:109–118

Seoane S, Laza A, Urrutxurtu I, Orive E (2005) Phytoplankton assemblages and their dominant pigments in the Nervion River estuary. Hydrobiologia 549:1–13

Seoane S, Laza A, Orive E (2006) Monitoring phytoplankton assemblages in estuarine waters: the application of pigment analysis and microscopy to size-fractionated samples. Estuar Coast Shelf Sci 67:343–354

Serret P, Fernández E, Sostres JA, Anadón R (1999) Seasonal compensation of microbial production and respiration in a temperate sea. Mar Ecol Prog Ser 187:43–57

Skjodal HR (1993) Eutrophication and algal growth in the North Sea. In: Della Croce NFR (ed) Proceedings from: Symposium Mediterranean Seas (200), Istuto Science Ambientala Marine Santa Margherita Ligure Genova, Italy, September 1991

Smith JC, Cormier R, Worms J, Bird CJ, Quilliam MA, Pocklington R, Angus R, Hanic L (1990) Toxic blooms of the domoic acid containing diatom *Nitzschia pungens* in the Cardigan River, Prince Edward Island. In: Graneli E, Sundstrom B, Edler L, Anderson DM (eds) Toxic marine phytoplankton. Elsevier, New York

Sommer U (1994) Are marine diatoms favoured by high Si:N ratios? Mar Ecol Prog Ser 115:309–315

Tamigneaux E, Legendre L, Klein B, Mingelbier M (1999) Seasonal dynamics and potential fate of size-fractionated phytoplankton in a temperate nearshore environment (Western Gulf of St Lawrence, Canada). Estuar Coast Shelf Sci 48:253–269

Ter Braak CJF, Similauer P (2002) CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Microcomputer Power, Ithaca, New York

Tett P (1987) The ecophysiology of exceptional blooms. Rapport et Proces-verbaux des Reunions. Conseil international pour l'Exploration de la Mer 187:47–60

Tremblay JE, Legendre L (1994) A model for the size-fractionated biomass and production of marine phytoplankton. Limnol Oceanogr 39:2004–2014

Trigueros JM, Orive E (2001) Seasonal variations of diatoms and dinoflagellates in a shallow, temperate estuary, with emphasis on neritic assemblages. Hydrobiologia 444:119–133

Trigueros JM, Ansotegui A, Orive E, Nó ML (2000) Morphology and distribution of two brackish diatoms, (Bacillariophyceae): *Cyclotella atomus* Hustedt and *Thalassiosira guillardii* Hasle in the estuary of Urdaibai (Northern Spain). Nove Hedwigia 70:431–450

Turner RE (2001) Some effects of eutrophication on pelagic and demersal marine food webs. In: Rabalais NN, Turner RE (eds) Coastal and estuarine studies: costal hypoxia: consequences for living resources and ecosystems. American Geophysical Union, pp 371–398

USEPA (2006) Indicator development for estuaries. United States Environmental Protection Agency, 138 pp

Valencia V, Franco J (2004) Main characteristics of the water masses. In: Borja Á, Collins M (eds) Oceanography and Marine Environment of the Basque Country. Elsevier Oceanography Series, vol 70. Elsevier, Amsterdam, pp 198–232

Valencia V, Franco J, Borja Á, Fontán A (2004) Hydrography of the southeastern Bay of Biscay. In: Borja Á, Collins M (eds) Oceanography and Marine Environment of the Basque Country. Elsevier Oceanography Series, vol 70. Elsevier, Amsterdam, pp 160–194

Varela M (1996) Phytoplankton ecology in the Bay of Biscay. Sci Mar 60:45–53

Venables WN, Ripley BD (2002) Modern applied statistics with S, 4th edn. Springer-Verlag, Berlin

Weston K, Greenwood N, Fernand L, Pearce DJ, Sivyer DB (2008) Environmental controls on phytoplankton community composition in the Thames plume, U.K. J Sea Res 60:262–270

Zaldívar JM, Cardoso AC, Viaroli P, Newton A, de Wit R, Ibanez C, Reizopoulou S, Somma F, Razinkovas A, Basset A, Holmer M, Murray N (2008) Eutrophication in transitional waters: an overview. Transitional Waters Monogr 1:1–78

Zarauz L, Irigoien X, Fernandes JA (2009) Changes in plankton size structure and composition, during the generation of a phytoplankton bloom, in the central Cantabrian sea. J Plankton Res 31:193–207