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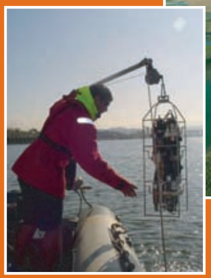

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A new method for phytoplankton quality assessment in the Basque estuaries (northern Spain), within the European Water Framework Directive

Marta Revilla*, **Javier Franco, Maialen Garmendia, Ángel Borja**

Abstract

Since 2002, a modified method from Ifremer has been applied to the Basque estuaries for phytoplankton quality assessment, within the context of the European Water Framework Directive (WFD). This method has built upon a tool for the Shellfish Waters Directive, presenting some limitations for the WFD; the latter is focused upon ecological quality, with the former on human health. In 2009, during the second phase of the WFD intercalibration, a new method was agreed for transitional waters in northern Spain, between experts from the regional governments of the Asturias, Cantabria and the Basque Country. The present study compares the previously-applied method, with the new method, in the estuaries of the Basque Country. It checks the consistency of both methods with eutrophication risk of these marine systems evaluated by expert judgment and historical data analysis.

Firstly, geomorphological, hydrographical conditions and anthropogenic pressures are described for the main 12 Basque estuaries. Secondly, the new method is explained in detail, including sampling and analytical methodologies, the metrics involved, reference conditions and class boundaries. Subsequently, the new method is applied to several case-studies within the Basque estuaries. The resulting ecological quality classification is discussed in terms of hydrography, wastewater management and nutrient pollution. Finally, the results of the classification are compared, between both the previous and the new methods.

The new method was based upon indicators of biomass (chlorophyll) and bloom frequency (single taxa counts). This method resulted more effective at discriminating water bodies with different eutrophication degrees, than the previous method. However, other aspects of the phytoplankton (i.e. composition) still need investigation in order to completely fulfil the WFD requirements.

Key words: Water Framework Directive, Intercalibration, Transitional Waters, Estuaries, Phytoplankton, Eutrophication

Introduction

The European WFD (Directive 2000/60/EC) considers phytoplankton composition, abundance and biomass amongst the biological indicators for the classification of the ecological quality status (European Commission, 2000). Also, frequency and intensity of phytoplankton blooms are mentioned in the normative definitions for high, good and moderate ecological status, in coastal and transitional waters. However, in practice, few Member States (MS) have proposed an integrative tool including all the parameters (e.g. the UK, Devlin *et al*., 2009). The majority of MS have established only the measurement of chlorophyll "a" as a proxy for phytoplankton biomass (Carstensen and Henriksen, 2009; Henriksen *et al*., 2009). Although chlorophyll-based metrics are cost-effective, the results must be treated with caution, given that the pico- and nanoplankton can be underestimated (Domingues *et al*., 2008; Garmendia *et al*., 2010).

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The first method used to evaluate the quality status of the phytoplankton element in the Basque marine environment (Borja *et al*., 2004) was based upon that of Ifremer (Vincent *et al*., 2002). The phytoplankton was evaluated in the coastal (CW) and transitional waters (TW), over five-year periods, by using four indicators: (i) chlorophyll; (ii) cell abundance of toxic species for human health; (iii) cell abundance of harmful species for fauna and flora; and (iv) total cell abundance. At station level, the phytoplankton status was classified by the worst indicator. In order to obtain the status at water body level, the qualitative result of each station (high, good, moderate, poor or bad status) was substituted by an equivalent value; then, a weighted average was performed, taking into account the area represented by each sampling station (*see* Table 4, in Borja *et al*., 2009a). However, this method did not provide any information on reference conditions and ecological quality ratios, which is a requirement of the WFD (European Commission, 2000). Furthermore, this methodology had to be revised, as the metrics based upon chlorophyll and harmful species showed little discrimination between water bodies exposed to high nutrient inputs and lowly-polluted water bodies in the Basque coast.

The first phase of the intercalibration amongst MS in the Northeast Atlantic Eco-region dealt with CW (Carletti and Heiskanen, 2009).

In order to be more in accordance with the classification tools used by other MS, the method for phytoplankton assessment in the Basque CW was modified. In this regard, the new methodology adopted was based upon chlorophyll biomass and bloom frequency of any single taxa (Revilla *et al*., 2008b; 2009b).

During the second phase of the intercalibration, a new method was developed for the assessment of the phytoplankton element in the Basque TW, following a comparable approach to that of the CW. In a preliminary study, a metric based upon chlorophyll biomass was proposed for the Basque TW (Revilla *et al*., 2008a). The euhaline zone was distinguished from the rest of the estuary as a different environment, independently of the typologies. However, as a minimum requirement for a method comparable to that applied in CW, it was still necessary to develop a metric based upon bloom frequency; also, to integrate both metrics (biomass and bloom frequency) into a single assessment tool for estuaries. The intercalibration exercise undertaken in 2009 by the northern Spanish regions (Asturias, Cantabria and the Basque Country) dealt with these last two issues; as a result, a more complete tool was agreed for phytoplankton assessment in the Cantabrian shelf estuaries (Revilla *et al*., 2009a).

The present study describes, in detail, the new tool for phytoplankton quality assessment in the Basque estuaries. This study compares also the suitability of the previous method (Ifremer-modified) (Vincent *et al*., 2002; Borja *et al*., 2004), against the new method (Revilla *et al*., 2009a). The ultimate goal was to ensure the consistency of the new method with the eutrophication risk in the Basque estuaries; this was evaluated by expert judgment and historical data analysis. In order to undertake this approach, the anthropogenic pressure (sewage discharge) was considered. Also, the hydrographical and physico-chemical conditions that influence importantly on the phytoplankton responses (i.e. flushing time, tidal exchange, turbidity and nutrients) were evaluated.

Geomorphological and hydrological aspects

The Basque coast is contained within the Northeast Atlantic Eco-region and extends along 150 km, approximately, of the eastern Cantabrian shelf (Figure 1).

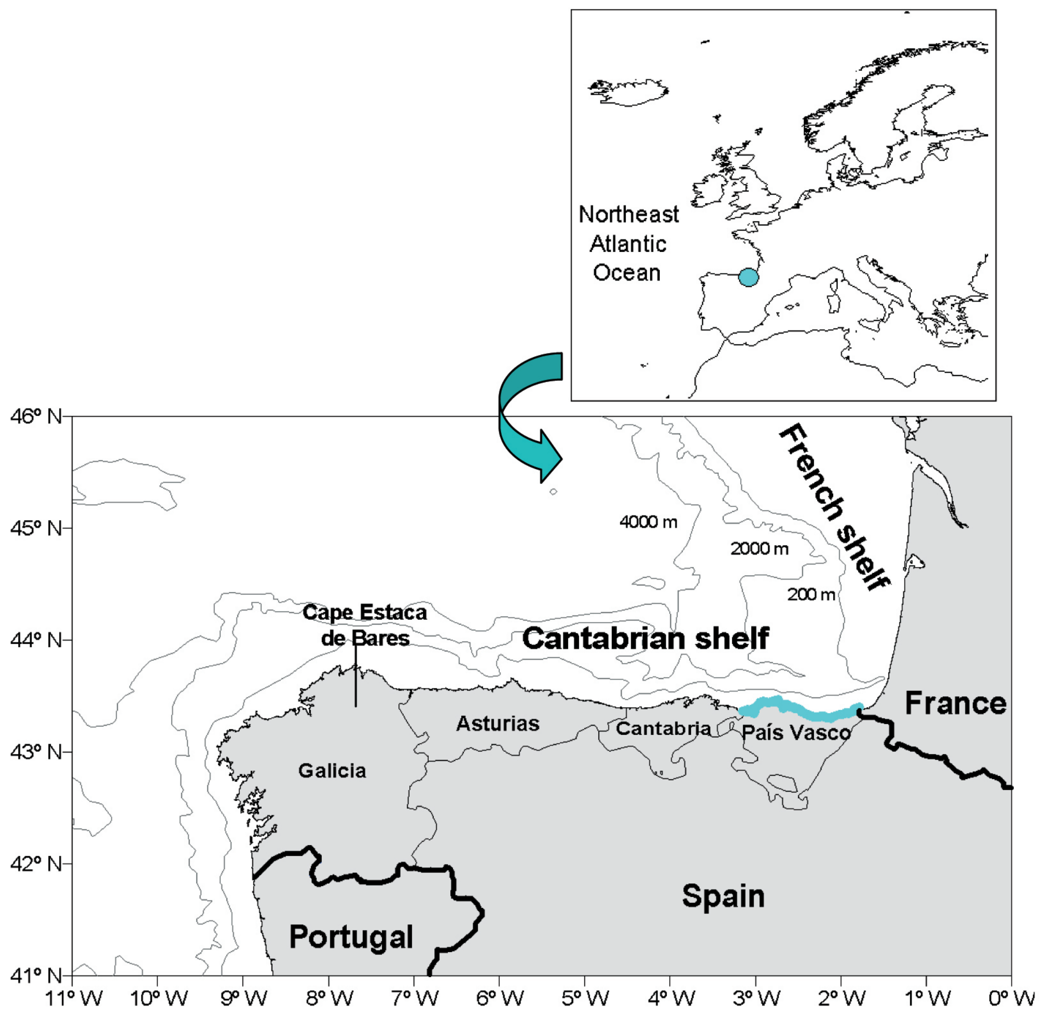

Figure 1. Location of the Basque coast (País Vasco) within the context of the Cantabrian shelf and the Northeast Atlantic Ocean.

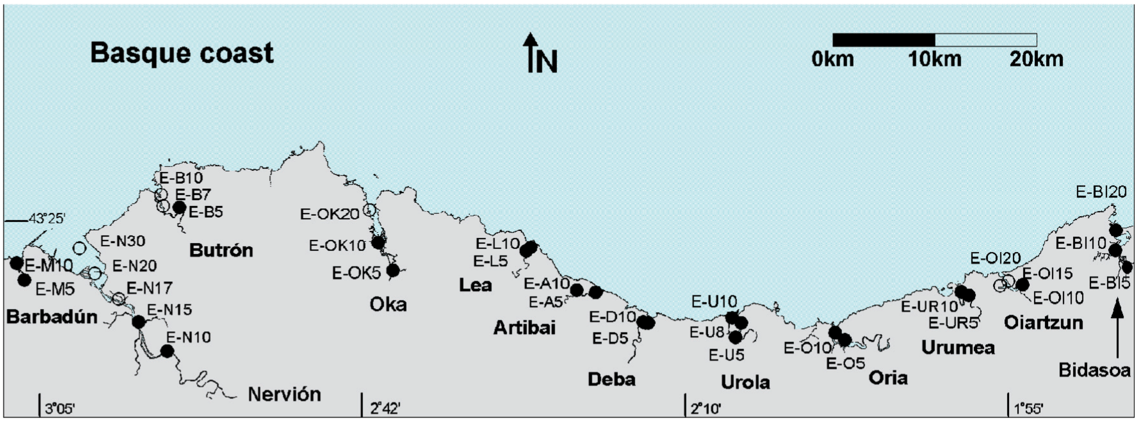

Figure 2. Sampling stations for water quality monitoring in the Basque estuaries. Stations are represented by: (i) open circles in euhaline waters; and (ii) solid circles in oligo-, meso- or polyhaline waters.

The Basque coast shares the general features of the Cantabrian littoral, which extends along 1500 km in northern Spain, from the River Mera in the proximities of Cape Estaca de Bares, to the River Bidasoa on the border with France (Figure 1). The Cantabrian littoral is exposed and mostly formed by cliffs of calcareous rocks, with small beaches and many short rivers that flow to the shelf, mainly through estuaries. As described in Prego *et al*. (2008), the rivers of the Cantabrian Fluvial System have small basin areas, ranging from 37 to <2,600 km^2 (except to Nalón River in Asturias, with 4,893 km^2). Average annual water flows are low; they vary from 2 to 109 m^3 s^{-1}. The Cantabrian drainage basins are occupied, to a large extent, by forest and agricultural land. Industries are situated mostly on the eastern part of the coast, where the population density is higher. The coastal zone is characterised by a typical oceanic climate, with a rainy period (November-May) and a dry season (June-October). The suspended solids load from rivers is relatively modest and diffuse (Prego *et al*., 2008).

The annual contribution of nutrients from the Cantabrian basin, to the Bay of Biscay (500-600 m^3 s^{-1} of freshwater), may be estimated to be: 1.0 x 10^9 mol of N (dissolved inorganic nitrogen); 0.062 x 10^9 mol of phosphate; and 1.2 x 10^9 mol of silicate. The Nalón River (Asturias) followed by the Nervión River (Basque Country) are the main sources. Cantabrian fluxes are very low in value, in comparison with those of the French basins, where the Loire River annually transports 6.4 x 10^9 mol of N, 0.11 x 10^9 mol of phosphate and 1.2 x 10^9 mol of silicate. In addition, the nutrient fluxes are distributed between several small rivers along the coast, whilst no extensive coastal plumes are formed. Therefore, coastal fertilisation related to continental waters could be considered negligible, affecting only estuarine zones (Díez *et al*., 2000).

There are 12 main estuaries within the Basque Country (Figure 2), although some additional small estuaries (<0.5 km^2) are present. Since 1994, the water quality of these systems has been monitored by the Littoral Water Quality Monitoring and Control Network of the Basque Water Agency (Borja *et al.,* 2009a).

A detailed review of the hydrography of the Basque estuaries has been provided by Valencia *et al.* (2004) and is summarized here. The Basque estuaries are essentially drowned river valleys. Most of the systems can be considered as shallow: water depths are only large within the outer reaches of the Nervión and the Oiartzun

Table 1. Main geomorphological and hydrological characteristics of the Basque estuaries (from Borja *et al.*, 2006).

Estuary	Catchment area (km^2)	River flow (m^3 s^{-1})	Estuary length (km)	Estuary depth (m)	Estuary volume[a] (10^6 m^3)	Intertidal area (%)	Residence time (days)
Barbadún	129	2.9	4.4	5	1.6	69	0.01
Nervión	1799	36.0	22.0	30	402.1	28	224
Butrón	172	4.7	8.0	10	2.2	78	0.04
Oka	183	3.6	12.5	10	12.9	86	63
Lea	99	1.8	2.0	5	1.0	65	0.04
Artibai	104	2.5	3.5	10	2.2	34	0.001
Deba	530	14.0	5.5	5	2.9	54	0.04
Urola	342	8.0	5.7	10	2.5	53	0.17
Oria	882	26.0	11.1	10	3.1	84	0.25
Urumea	272	17	7.7	10	6.8	36	0.33
Oiartzun	86	4.8	5.5	20	7.3	19	35
Bidasoa	700	29.0	11.1	10	45.8	18	1.46

[a] Mean estuary volume, for 2.5 m tidal height

estuary (20-30 m). Intertidal zones have been occupied by human settlements since the 18th Century; nonetheless, the intertidal area is important in some estuaries (Table 1).

River flow and tides are the main factors conditioning the dynamics of the Basque estuaries. Freshets occur relatively frequently throughout the year; in turn, they have a considerable influence on estuarine hydrology, chemistry and biology. The Nervión, Oria and Bidasoa rivers show the highest annual flows (Table 1). Consequently, their estuaries would receive the highest nutrient input from natural sources. Tides over the area are semidiurnal and tidal amplitudes vary between 1 m on neap tides, to more than 4.5 m on spring tides; as such, they can be considered as generally mesotidal systems, although they present some features of macrotidal estuaries. The distribution of water masses can vary greatly between low and high tidal conditions; the fortnightly cycle (springs-neaps) has also a distinct influence. The displacement of water masses throughout a tidal cycle can be identified by the changes in salinity at a specific location, with oligohaline or mesohaline waters at low tide and polyhaline, or even euhaline, waters at high tide. The haline stratification in these estuaries is affected also considerably by the tidal cycles. Within the shallow systems, stratification is a semi-permanent feature only in the innermost part of these systems. Tidal currents are sufficiently high to break down the stability of the water column; as such, mixing and stratification alternate throughout a tidal cycle. In contrast, in deep estuaries, such as the Nervión and the Oiartzun, a permanent salt wedge is present within the bottom layers, with salinities being normally higher than 30 psu.

Water residence time varies between the Basque estuaries (Table 1). Phytoplankton communities require the water residence time to be higher than the time for biomass duplication, in order to overcome the physical advection losses (Ketchum, 1954). *In-situ* duplication times measured in phytoplankton communities of the Oka estuary range from 0.23 – 8.7 d (Revilla, 2001). Therefore, the chance for phytoplankton biomass accumulation is low in many Basque estuaries. The residence time is greater than a day only in the Nervión, Oka, Oiartzun and Bidasoa estuaries. As such, high nutrient loads in these systems could cause undesirable eutrophication symptoms. The estuaries where residence time ranges between 0.1 of and a day (Urola, Oria and Urumea) are less susceptible to eutrophication problems. The very short residence time (<0.1 d) that the Barbadún, Butrón, Lea, Artibai and Deba present precludes the accumulation of phytoplankton biomass, with the exception of the inner zones of these estuaries at periods of low river flow.

Anthropogenic pressures in the Basque estuaries

The Basque Country has an area of 723,271 Ha. Although intensive land uses only occupy the 8% of the area, the majority of these pressures are located on the coastal zone of the territory (Basque Government, 2008). Therefore, some estuaries have suffered from important impacts, with population density and industry concentration being the most relevant driving factors (Borja *et al*., 2006). Among them, the Nervión, Urumea and Oiartzun support the highest density of population within their watersheds (2,000 – 3,600 hab km^{-2}), followed by the Bidasoa (1,000 hab km^{-2}). The population density in the other estuaries ranges from 100 – 300 hab km^{-2}. The highest concentration of industry is found at the basins of the Nervión, Oiartzun and Bidasoa; in others, such as the Urumea, Oria and Artibai, there is a moderate industry concentration. In addition, the Nervión and the Oiartzun have large commercial ports, whilst the Barbadún estuary supports a petrol refinery. Taking all these pressures into account, historically the most impacted estuaries in terms of geomorphology, as well as water and sediment pollution, have been the Nervión and the Oiartzun estuaries.

Regarding nutrient pollution, although wastewater treatment has improved during the last decade (García-Barcina *et al*., 2006; Borja *et al*., 2009a) some estuaries still receive significant discharges. In a recent report, Borja *et al*. (2009b) evaluated the risk of eutrophication along the Basque coast, in the context of the Urban Waste Water Treatment Directive, UWWTD (European Commission, 1991). The main criteria for risk assessment were the efficiency of sewage treatment, the water residence time and the physico-chemical and biological quality. The estuaries identified as sensitive zones (i.e. under high risk of eutrophication) were the Butrón, Oka, Artibai and Oiartzun. All of them received sewage discharges and presented high concentration of nutrients (N and P) and organic matter, together with low oxygen saturation and alterations in the benthic communities. In the case of the Oiartzun estuary, the eutrophication risk was attributed to the direct discharge of organic matter, as the phytoplankton response in terms of chlorophyll was not appreciable. The estuaries classified as less sensitive zones (i.e. under low-no risk of eutrophication) were the Deba and Oria. In these estuaries the anthropogenic nutrient inputs were very low and no undesirable effects on the ecosystems were observed; these systems presented also low water residence time (<1 d). Finally, showing intermediate conditions, the Barbadún, Nervión, Lea, Urola, Urumea and Bidasoa were identified as normal zones (i.e. under low-moderate risk of eutrophication).

Description of the new methodology for phytoplankton status assessment

Water body types

The coastal and transitional water bodies that have been identified for the application of the WFD in the Basque Country encompass four types. CW belong to the NEA1 Type, which are defined as exposed and shallow, euhaline fully-mixed waters. TW consist of 14 water bodies, from 12 estuaries. As described in Table 2, by using the optional factors of the WFD, the Basque TW can be classified into one of three different types: (i) Type 8, Atlantic intertidal river-dominated estuaries; (ii) Type 9, Atlantic intertidal sea-dominated estuaries (with extensive intertidal areas); and (iii) Type 10, Atlantic subtidal sea-dominated estuaries (Borja *et al*., 2004; BOE, 2008).

Phytoplankton metrics

In a similar way as for CW, the assessment of the phytoplankton element in TW employs two sub-metrics. The first one is based

Table 2. Water body types determined for the Basque transitional waters (modified from Borja *et al*., 2004).

Variables	Typologies		
	Type 8	**Type 9**	**Type 10**
Definition	Atlantic intertidal river-dominated estuaries	Atlantic intertidal sea-dominated estuaries (with extensive intertidal flats)	Atlantic subtidal estuaries
Locations	Estuaries of Deba and Urumea	Estuaries of Barbadún, Butrón, Oka (inner and outer water bodies), Lea, Artibai, Urola and Oria	Estuaries of Nervión (inner and outer water bodies), Oiartzun and Bidasoa
Salinity	5-30 psu	18- >30 psu	18- >30 psu
Tidal range	1-3 m	1-3 m	1-3 m
Depth	<30 m	<30 m	<30 m
Current velocity	50-150 cm s-1	50-150 cm s-1	50-150 cm s-1
Wave exposure	Sheltered	Sheltered	Sheltered, very sheltered
Mixing	Semi-permanent stratification	Semi-permanent stratification	Permanent stratification
Residence time	<1 day	<1 day - months	>1 day - months
Substrata	Mixed sediments	Mixed sediments	Mixed sediments
Intertidal area	<50%	>50%	<50%

upon chlorophyll "a" concentration, as an indicator of the phytoplankton biomass; the second is based upon single taxa cell counts, as an indicator of the phytoplankton blooms.

TW differ in hydrographical and ecological characteristics from CW, and therefore, reference conditions should be also different. In the Basque estuaries it is not possible to select sampling stations for reference conditions (i.e. with no, or only very minor anthropogenic disturbance) because all of the estuaries have been impacted historically by human activities (Borja *et al*., 2006). Moreover, the Basque Country has no pre-industrial historical data (Borja *et al*., 2004). Therefore, in order to set reference conditions and class boundaries, data analysis (physico-chemical and phytoplankton variables, measured from 1995 to present) and expert judgement were used (Revilla *et al*., 2008a; 2009a).

Biomass indicator

Samples for determining the chlorophyll "a" concentration (Chl-a) are taken within the surface waters (0 m depth), using a Niskin bottle or a clean bucket. Stations are sampled quarterly to record winter, spring, summer and autumn conditions. Samples are collected both at high and at low tide. In the deep estuaries (Nervión and Oiartzun), Chl-a is measured by means of CTD (Seabird25) fluorescence; the CTD is calibrated regularly with natural samples. In shallow estuaries, water samples are filtered through Whatman GF/C filters. Immediately, pigments are extracted in 10 ml of 90% acetone, for 24-48 hours, under dark and cold (4°C) conditions. The absorbance of the extract is read in a UV/VIS spectrophotometer. Chl-a concentration is estimated on the basis of the equations of Jeffrey and Humphrey (1975).

As an indicator of phytoplankton biomass, the 90th percentile of the Chl-a is calculated over the complete data set of a 6-year period (n= 48). In order to apply the biomass indicator in TW, the sampling stations are split into two groups, based upon salinity: (i) stations that belong to the euhaline zone; and (ii) stations that belong to the oligo-, meso- or polyhaline zones (Figure 2). The methodology for assigning the sampling stations to salinity stretches in estuaries is described, in detail, in Bald (2005) and Bald *et al*. (2005). In TW, reference conditions and class boundaries for chlorophyll differ with salinity, as indicated in Table 3.

Bloom indicator

Samples for phytoplankton counts and taxonomical identification are taken at surface (0 m depth) using a Niskin bottle or a clean bucket. Samples are collected only at high tide. As a minimum requirement, two sampling efforts per year are undertaken (spring and summer) at each estuarine station. Samples are preserved with 1 ml of 25% glutaraldehyde in 125 ml borosilicate bottles, and maintained cold (4ºC) and in the dark until their analysis; this is completed within three months after collection. Standard methods are used for identification

Table 3. Reference condition and class boundaries for the metric based upon phytoplankton biomass (90th percentile of Chl-a). Values underlined were established through the first phase of the European intercalibration exercise. Key: CW- Coastal Waters; TW- Transitional Waters. From Revilla *et al.* (2008a).

Water category	Salinity stretch	Reference condition (μg L^{-1})	High/ Good (μg L^{-1})	Good/ Moderate (μg L^{-1})	Moderate/ Poor (μg L^{-1})	Poor/ Bad (μg L^{-1})
CW	Euhaline	<u>2.33</u>	<u>3.5</u>	<u>7.0</u>	10.5	14.0
TW	Euhaline	2.67	4.0	8.0	12.0	16.0
TW	Oligo/Meso/Polyhaline	5.33	8.0	12.0	16.0	32.0

Table 4. Reference condition, class boundary values and the Ecological Quality Ratio (EQR) scale for the metric based upon bloom frequency (%) in coastal and transitional waters of the Basque Country. Values underlined were established through the first phase of the European intercalibration exercise for the CW.

	Reference condition	High/ Good	Good/ Moderate	Moderate/ Poor	Poor/ Bad
Metric value (% Bloom)	16.7	20	39	69	89
EQR	1.00	0.84	0.43	0.24	0.19

Table 5. Ecological Quality Ratio (EQR) scale for the metric based upon phytoplankton biomass (90th percentile of Chl-a) in each water category and salinity zone. Values underlined were established through the first phase of the European intercalibration exercise. Key: CW- Coastal Waters; TW- Transitional Waters. From Revilla et al. (2008a).

Water category	Salinity stretch	Reference condition EQR	High/ Good EQR	Good/ Moderate EQR	Moderate/ Poor EQR	Poor/ Bad EQR
CW	Euhaline	1.00	0.67	0.33	0.22	0.17
TW	Euhaline	1.00	0.67	0.33	0.22	0.17
TW	Oligo/Meso/Polyhaline	1.00	0.67	0.44	0.33	0.17

and counting (inverted microscopy and Utermöhl). A taxa list is reviewed regularly, to update and standardize names (e.g. Revilla *et al*., 2009b).

In the Basque TW, the sub-metric based upon phytoplankton abundance is similar to that used in CW, which was described in Revilla *et al*. (2008b; 2009b). Thus, it is derived by the percentage of samples, at each station, where any single taxa exceed a threshold of 750,000 cells L^{-1}, for a 6-year period. For a complete period, n=12. Salinity stretches are not considered when calculating the bloom sub-metric. The reference condition and class boundary values are indicated in Table 4.

Determination of the Ecological Quality Ratio

The WFD requires the results of the assessment to be expressed following the Ecological Quality Ratio (EQR) approach. The EQR expresses the relationship between the reference and the observed conditions; its numerical value lies between 0 and 1. At high status, the EQR lies close to 1; at bad status, the EQR lies close to 0.

For the biomass metric, the EQR scale was derived by dividing the reference by the class boundary values (Table 5). The reference and boundary values were established by data analysis and expert judgement. Some of these parameters had been established for the Basque CW, during the first phase of the European intercalibration exercises in the Northeast Atlantic Eco-region (European Commission, 2008). Through those exercises, the reference had been set at 2.33 µg L^{-1} (Table 3), by using historical data from coastal and offshore stations in the eastern Cantabrian Sea considered under no risk of eutrophication; the High/Good and Good/Moderate boundaries had been established assuming some degree of deviation (50% from the reference to the first class boundary and 100% from the first to the second class boundary). The boundaries between the worse status classes (Moderate/Poor and Poor/Bad) do not require to be intercalibrated; those were established for the Basque CW by assuming a constant increment of 3.5 µg L^{-1}, which is similar to the increment from the first (High/Good) to the second (Good/Moderate) class boundary.

In euhaline TW the chlorophyll reference and boundary values were set, intentionally, only slightly higher than in CW (Table 3). Consequently, the EQR scale was similar to that in CW (Table 5). This decision was made by assuming that, under no anthropogenic pressure, physico-chemical conditions and phytoplankton communities should be very similar in the Basque CW and euhaline TW, as these estuaries are generally subject to a strong tidal exchange at their outer reaches. In contrast, for the oligo-, meso- or polyhaline TW, a much higher reference condition and boundary values were established; this was to allow for the natural nutrient loads (Table 3). The relative increments among the status classes in the oligo/meso/polyhaline waters were, in some cases, lower when compared to those allowed in the euhaline waters (Table 3). It resulted in different EQRs at the Good/Moderate and Moderate/Poor boundaries (Table 5). It was taken into account that in the oligo-, meso- or polyhaline stretches of the estuaries, lower increments in Chl-a above the reference could have stronger effects on the ecosystems, as these salinity zones are usually under more stressful conditions (e.g. lower oxygen concentrations, higher turbidity and more frequent variations in salinity). The Poor/Bad class boundary was adjusted to result in a similar EQR for all the water categories.

In the case of the bloom metric, the EQR scale for TW (Table 4) was similar to that proposed for CW during the first phase of the intercalibration (*see* Table 2.4.11, in Carletti and Heiskanen, 2009).

Integrating results from different metrics

The use of multimetric tools combining various phytoplankton metrics is appropriate for water quality assessment; each metric carries its own information and, although a consistency is expected, different metrics (e.g. chlorophyll, abundance and diversity) can show different degree of response to eutrophication (Spatharis and Tsirtsis, 2010). The method proposed here to integrate the phytoplankton status, at station level, involves assigning a score to the quality status indicated by each sub-metric. The scores are 1.00 for high status, 0.80 for good status, 0.60 for moderate status, 0.30 for poor status and 0.00 for bad status. Then, an arithmetic average is calculated with the scores; this average will be the final EQR. The final phytoplankton quality status is classified on the basis of the final EQR, as shown in Table 6.

Table 6. Integration of the quality status of the phytoplankton element in coastal and transitional waters of the Basque Country. The final Ecological Quality Ratio (EQR) is calculated by averaging the scores of the sub-metrics.

Final EQR	Final quality status	Divergence with reference conditions	Disturbance in the biota or in the physico-chemical quality
1.00	High	No divergence	No disturbance
≥ 0.80 and <1.00	Good	Slight divergence	No disturbance
≥ 0.60 and < 0.80	Moderate	Moderate divergence	Significant disturbance
≥ 0.30 and < 0.60	Poor	Important divergence	Significant disturbance
≥ 0.00 and < 0.30	Bad	Severe divergence	Significant disturbance

Table 7. Representativeness of each sampling station in the total surface of the Basque transitional water bodies. The surface ratio is indicated in brackets. For station locations, see Figure 2.

Water body	Sampling station		
Barbadún estuary	E-M10 (0.94)	E-M5 (0.06)	-
Outer Nervión estuary	E-N30 (0.80)	E-N20 (0.20)	-
Inner Nervión estuary	E-N17 (0.31)	E-N15 (0.31)	E-N10 (0.38)
Butrón estuary	E-B10 (0.68)	E-B7 (0.16)	E-B5 (0.16)
Outer Oka estuary	E-OK20 (0.55)	E-OK10 (0.45)	-
Inner Oka estuary	E-OK5 (1)	-	-
Lea estuary	E-L10 (0.90)	E-L5 (0.10)	-
Artibai estuary	E-A10 (0.85)	E-A5 (0.15)	-
Deba estuary	E-D10 (0.46)	E-D5 (0.54)	-
Urola estuary	E-U10 (0.66)	E-U8 (0.22)	E-U5 (0.12)
Oria estuary	E-O10 (0.37)	E-O5 (0.63)	-
Urumea estuary	E-UR10 (0.64)	E-UR5 (0.36)	-
Oiartzun estuary	E-OI20 (0.37)	E-OI15 (0.15)	E-OI10 (0.48)
Bidasoa estuary	E-BI20 (0.45)	E-BI10 (0.22)	E-BI5 (0.33)

Following this method, the final phytoplankton status is classified as high only if both scores equal to 1.00, which implies that both sub-metrics, biomass and bloom frequency, indicate high status. For other metric combinations, this method is somewhat biased towards conservative values. For example, if the resulting integrated status is good (i.e., the average of the scores lies between 0.80 and 1.00), there are three possible combinations for the sub-metrics: (i) good-good; (ii) high-good; and (iii) high-moderate. The second and third combinations can be found in a water body with frequent blooms of small-sized phytoplankton species. The biomass metric will indicate high status, whereas the bloom metric will indicate good or moderate status. In these cases, although the phytoplankton community shows divergence with reference conditions (i.e. accelerated growth of some populations) there is little risk for eutrophication problems associated with biomass excess. In this example, the final status classification copes with the normative definitions of the WFD for the phytoplankton element; this involves not only the divergence with reference condition, but also the resulting undesirable disturbance to the balance of organisms present in the water body or to the physico-chemical quality of the water (European Commission, 2000).

The previous assessment method in the Basque waters (Ifremer-modified) classified the final phytoplankton status by the worst status found among the indicators (Borja *et al*., 2004). The Ifremer-modified method was built upon a tool for the Shellfish Waters Directive (European Commission, 2003). Classification by the worst indicator is a meaningful approach for a Shellfish Directive, with the purpose of protecting public health. However, from an ecological standpoint, such a method would not discriminate between different environmental situations, e.g. high-biomass blooms and low-biomass blooms. Other authors have proposed also integrative methods that take into account the contribution of several metrics, for assessing phytoplankton status (Devlin *et al*., 2007; Spatharis and Tsirtsis, 2010) or eutrophication (Giordani *et al*., 2009).

Integrating data from all stations, within a water body

Having derived the final EQR and quality status for each station, using both sub-metrics (Table 6), the phytoplankton status of the whole water body is calculated. This is undertaken by considering the surface area represented by each of the sampling stations. Subsequently, a weighting average is performed. For the phytoplankton element, this method implies multiplying the final EQR, by the corresponding surface ratio (Table 7) at each station. The phytoplankton EQR at the water body level is obtained as the sum of the surface-weighted EQRs. The quality status at the water body level corresponds to the obtained EQR, as indicated in Table 6.

Complementary information

Physico-chemical data are recorded to support the interpretation of the phytoplankton status results. The sampling strategy is similar to that of Chl-a (surface waters; quarterly sampling; high and low tide conditions). Temperature, salinity and dissolved oxygen are measured in the field, using a CTD (Seabird25) in the deep estuaries. In the shallow estuaries, a Handheld Multiparameter Instrument (YSI556) is used. The Secchi disc depth is obtained as an estimator of water transparency. Turbidity is directly measured in water samples by a turbidimeter (2100 Turbidimeter, HACH; Loveland, Colorado, USA). Suspended solids are estimated as described in Clesceri *et al*. (1989), after filtration of the water through Whatman GF/C filters. Ammonia, nitrate, nitrite, silicate, phosphate, total N and total P are measured by a Continuous-Flow Autoanalyzer (Bran + Luebbe Autoanalyzer 3; Norderstedt, Germany), using the colorimetric methods described in Grasshoff *et al*. (1983). Total organic carbon (TOC) is estimated in Non Purgable Organic Carbon (NPOC) mode, using a TOC Analyzer (TOC-V CSH/CSN, Shimadzu Corporation, Kyoto, Japan) as described in Grasshoff *et al*. (1983) and recommended by the supplier.

Case-Study: the assessment of the 2003-2008 period

In order to check the feasibility of the new methodology, the phytoplankton quality in the 12 Basque estuaries has been assessed for a recent 6-year period (2003-2008); the data set was provided by the Basque Monitoring Network (Figure 2).

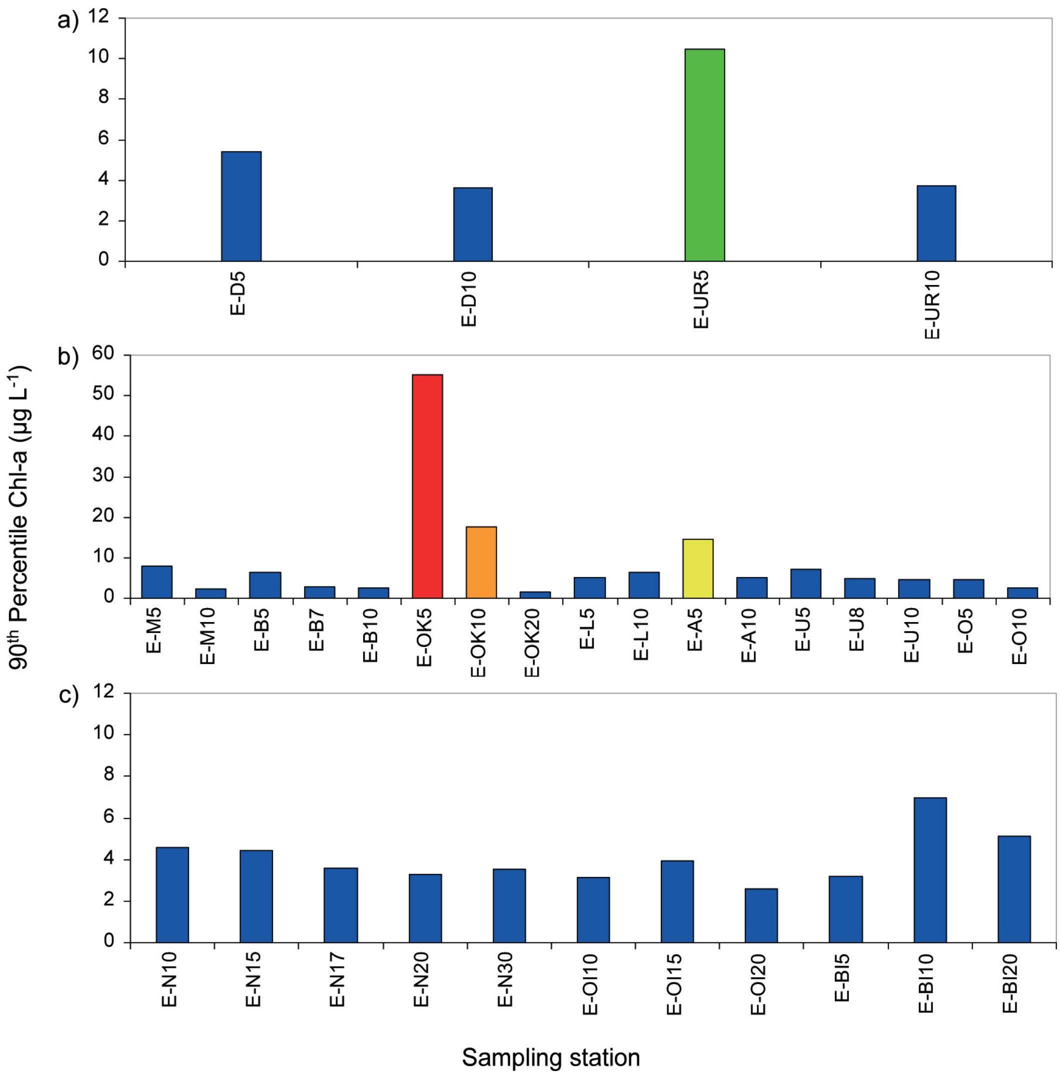

Figure 3. The value of the biomass indicator in the Basque transitional waters for the three different types: a) Type 8, river-dominated estuaries; b) Type 9, sea-dominated estuaries with extensive intertidal flats; and c) Type 10, sea-dominated estuaries with large subtidal areas. The colours at each station indicate the ecological quality: blue- high status; green- good status; yellow- moderate status; orange- poor status; and red- bad status.

Biomass indicator applied to the Basque estuaries

The 90th percentile of Chl-a ranged from 1.5 – 55.2 µg L^{-1} (Figure 3). The biomass metric showed, in general, low values (<8 µg L^{-1}). It resulted in most of the stations classified in high or good status. Undesirable quality status was found only in the Oka and Artibai estuaries. The sites with quality equal or worse than moderate were in the inner and middle zones of Type 9 estuaries (i.e. sea-dominated estuaries, with extensive intertidal flats).

The biomass metric was found to be useful in identifying some impacted zones and nutrient pollution gradients in the Basque estuaries. The Oka estuary receives, at its head, the discharge of raw urban wastewater; consequently, the concentration of inorganic nutrients and organic matter is very high at the E-OK5 station (Annex 1, physico-chemical conditions). This estuary presents a distinct longitudinal gradient in physico-chemical conditions and microbial rates. At the inner zone, high rates of primary production, bacterial production and microplankton respiration have been reported. However, at the outer euhaline zone, which is exposed to strong tidal exchange, the physico-chemical conditions and microbial rates are very similar to the adjacent non-polluted coastal waters (Revilla *et al*., 2000; Revilla, 2001). Similarly, both the Oka and Artibai estuaries have been classified recently as sensitive zones, within the context of the UWWTD (Borja *et al*., 2009b).

On the other hand, the Chl-a metric presented little variation between the stations and classified most of them in high status. However, if the nutrients indicating anthropogenic pressure (phosphate and ammonia) are considered, the Basque TW cannot be defined as pristine (Borja *et al*., 2009b). Furthermore, light conditions are generally suitable in the Basque estuaries to permit phytoplankton growth: turbidity is low, with the exception of a few stations or specific hydrological conditions (Annex 1), and most of the estuaries are shallow (Table 1). Water residence time

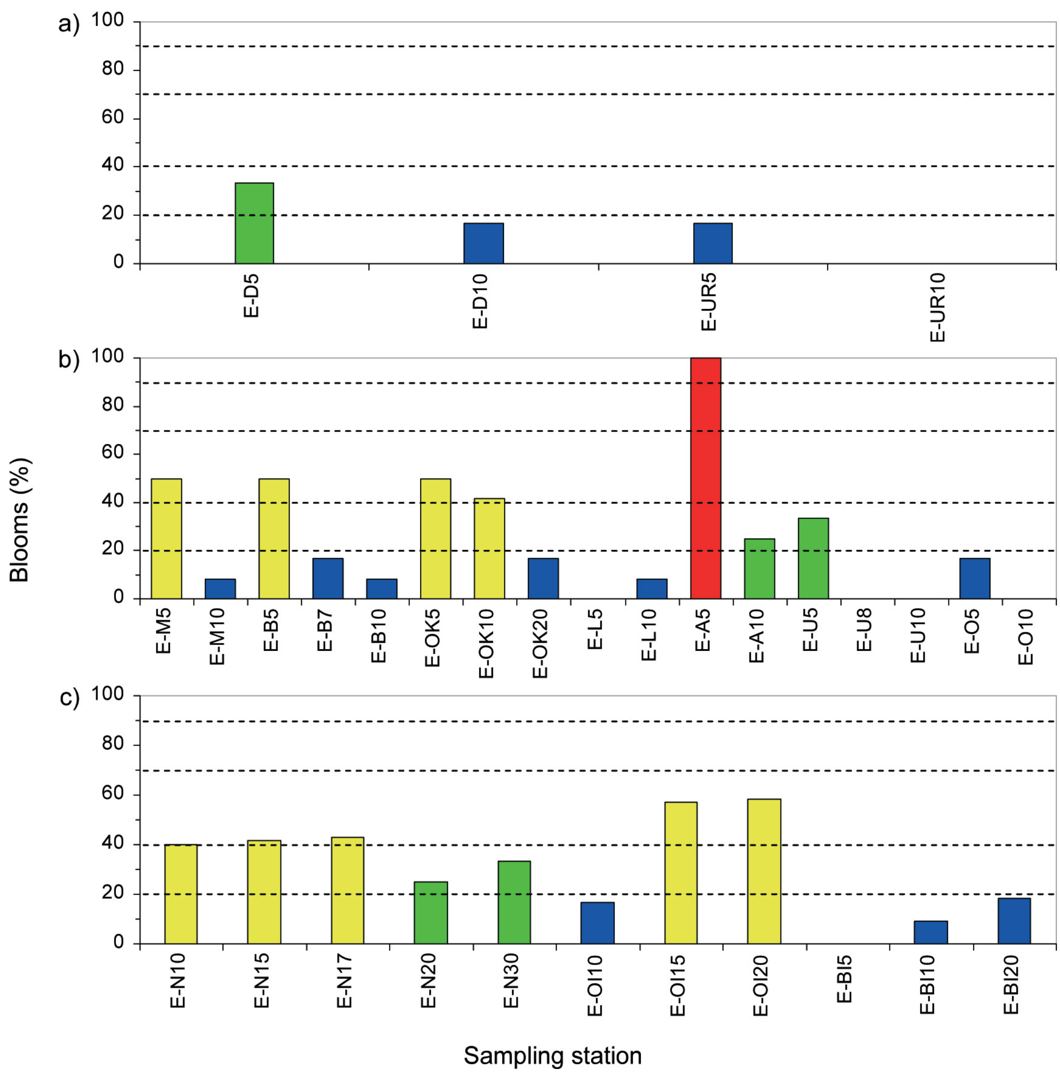

Figure 4. The value of the bloom indicator in the Basque transitional waters for the three different types: a) Type 8, river-dominated estuaries; b) Type 9, sea-dominated estuaries with extensive intertidal flats; and c) Type 10, sea-dominated estuaries with large subtidal areas. The colours at each station indicate the ecological quality; blue- high status; green- good status; yellow- moderate status; and red- bad status. Dotted lines indicate the class boundaries.

could prevent biomass accumulation in some of them, but not in the inner zones during periods of low river flow (particularly, in summer). Therefore, the biomass indicator alone appears to be insufficient to establish an accurate phytoplankton status. It must be noted that the dominant species in some of the Basque estuaries are of small cell size. For example, blooms of small diatoms and cryptophytes occur recurrently in the Nervión and the Oiartzun estuaries, during spring and summer (Borja *et al*., 2007; 2008). These blooms, which may be related to anthropogenic pressure, would not be detected by the biomass metric.

Bloom indicator applied to the Basque estuaries

The bloom frequency ranged from 0 – 100% (Figure 4). Fewer stations were classified in high status, if compared to the biomass indicator.

River-dominated estuaries presented high or good quality at all locations (Figure 4a). Sampling stations in moderate or worse status were found generally in the inner and middle zones of the sea-dominated estuaries (Figures 4b, c). This reflects an important influence of water residence time, together with nutrient availability, on the frequency of the blooms. During periods of low river flow, blooms would be favoured at the head of the estuaries, where nutrient concentrations are higher than at the outer zones (Annex 1). A particular case was the Oiartzun estuary, where the bloom-metric at the outer zone indicated worse quality (E-OI20, moderate status) than at the head of the estuary (E-OI10, high status). This was probably due to the high nutrient inputs, from anthropogenic sources, at the E-OI15 station, which can affect the downstream-located E-OI20 station.

At the euhaline stations, the status obtained by the bloom indicator was related to a large extent to the anthropogenic nutrient pressure (Figure 5). Also, other factors (water residence time or turbidity) could have modulated the bloom frequency at these stations. With

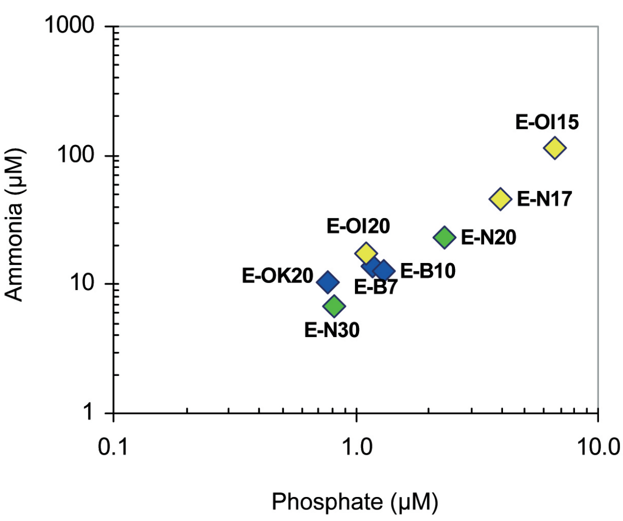

Figure 5. The status measured by the bloom indicator at the euhaline stations of the Basque estuaries, in relation to the average ammonia and phosphate concentrations, during the 2003-2008 period. The colours at each station indicate the ecological quality: blue- high status; green- good status; and yellow- moderate status. For station locations, see Figure 2.

the lowest nutrient concentrations (~1 µM phosphate and ~10 µM ammonia), the euhaline waters of the Oka (E-OK20) and the Butrón estuary (E-B7 and E-B10) were classified as being of high status (Figure 5). Although nutrient concentrations were higher than those assumed for a high physico-chemical status (*see* Bald *et al*., 2005), the water residence time of these zones is low; this could have reduced the chance for blooming. As was explained previously, tidal exchange is strong over the euhaline stretch of the Oka estuary. The Butrón estuary presents a very low water residence time (<0.1 d), as shown in Table 1. In contrast, the Nervión and the Oiartzun estuaries have residence times >30 d. Within the Nervión estuary, the status was good at the outer zone (E-N30 and E-N20) and moderate at the middle zone (E-N17), following the nutrient gradient (Figure 5). Within the Oiartzun estuary, the status was moderate at both stations (E-OI15 and E-OI20), although nutrient concentrations were considerably higher at E-OI15. However, turbidity could be one factor precluding blooms becoming more frequent at the most polluted station, in the Oiartzun estuary (Annex 1).

The status obtained by the bloom indicator was consistent with reported deficiencies in the wastewater management of the river basins. The Butrón, Oka, Artibai and Oiartzun estuaries had been identified as sensitive zones for eutrophication risk, within the context of the UWWTD (Borja *et al*., 2009b). Similarly, the indicator based upon bloom frequency detected undesirable quality (equal or worse than moderate) at many stations in these estuaries (Figures 4b, c). In the estuaries classified as less sensitive zones (Deba and Oria), as well as in some of the estuaries classified as normal zones (Lea, Urola, Urumea and Bidasoa) (Borja *et al*., 2009b), all stations were classified as being of high or good status (Figures 4a, b). In other estuaries classified as normal zones (Barbadún and Nervión), the bloom indicator assigned moderate status to the inner part (E-M5, E-N10, E-N15 and E-N17) and high or good status to the outer part (E-M10, E-N20 and E-N30).

Comparison of results between two different methods

The new method described here for the assessment of the phytoplankton status in the Basque TW has been compared to a previous approach (Borja *et al*., 2004; 2009a), firstly, by a Pearson correlation analysis (Figure 6). Equivalence values (previous method) and final EQRs (new method) have been compared after multiplying by the corresponding surface ratio at each station. Results from both methods correlate significantly, both at station and water body levels (Figures 6a, b).

The quality status results of both methods, at metric, station and water body levels, are detailed in Annex 2.

The previous Chl-a metric (Ind. 1) classified all stations as being of high or good status. The stations in good status were E-OK5, E-OK10 and E-A5. In contrast, these stations were classified by the new Chl-a metric as being of bad, poor and moderate status, respectively (Annex 2). If the anthropogenic pressures discussed above are taken into account, the new biomass indicator was more accurate than that adopted previously.

The previous method utilised two metrics based upon harmful phytoplankton species. The Ind. 2 (human health) classified all of the stations as in high status. The Ind. 3 (flora and fauna) classified most of the stations as in high or good status; it only detected

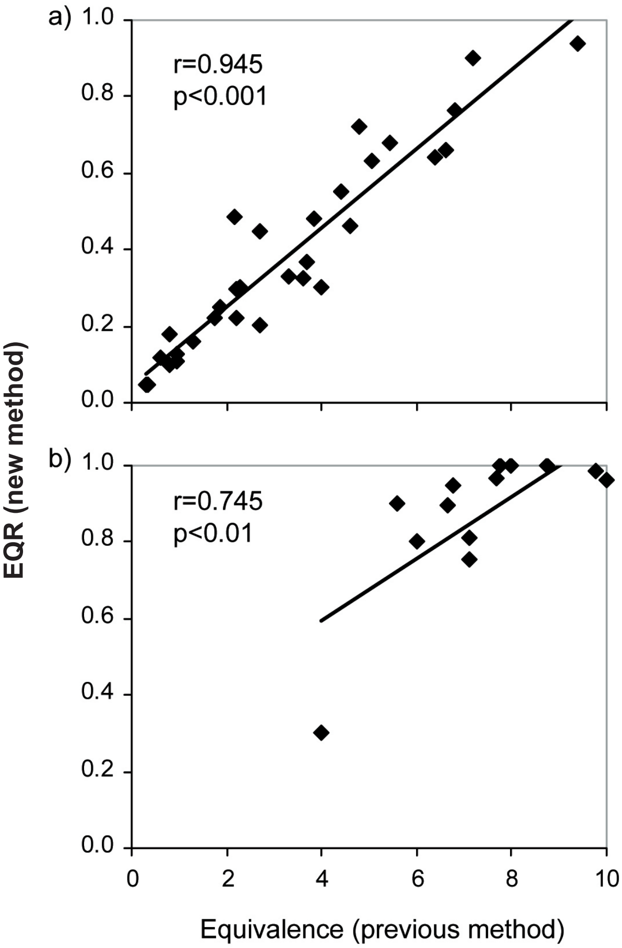

Figure 6. Comparison of the final Ecological Quality Ratio (EQR), obtained by the new method, and the Equivalence value, obtained by the previous method: a) at each station; and b) for each water body. All values have been multiplied by the surface ratio at each station, then summed for each water body. For surface ratios, see Table 7. The linear adjustment, together with the Pearson correlation coefficients (r) and probability (p) are shown.

moderate status at two stations, in the Oka and Butrón (Annex 2). The new method does not include any metric based upon harmful phytoplankton, following the recent recommendations of the Northeast Atlantic Geographical Intercalibration Group (NEA-GIG phytoplankton sub-group meeting, Edinburgh, November 2009) (http://circa.europa.eu/Members/irc/jrc/jrc_eewai/library?l=/coastal_nea_gig/neagig_phytoplankton/phytoplankton_november&vm=detailed&sb=Title).

In the previous method, the bloom-metric (Ind. 4) was based upon the total cell counts of the phytoplankton community, with a threshold of 10^6 cells L^{-1} for considering a bloom event. By applying the Ind. 4, several stations were classified as in moderate, poor or bad status; as such, Ind. 4 determined the final status classification at station level (Annex 2). In the new method, the bloom-metric utilised the abundance of any single taxa, with a threshold of 750,000 cells L^{-1}. Results from both metrics coincided in many cases (18 of 32 stations); in the remaining cases, the new bloom-metric resulted in higher quality values (Annex 2).

When the results were integrated at the water body level, the previous and new methods coincided in 4, out of the 14, cases (Annex 2). The classification of the Oria estuary was consistent in both methods as high status; the Butrón and the Artibai in good status; and the inner Oka in poor status. Slight differences were found in 5 other water bodies; these were classified in good status by one of the methods, and in high status by the other (Barbadún, Lea, Urola, Urumea and Bidasoa).

Important differences (with implications for management) were observed in 5, out of the 14, water bodies. Using the previous method, the inner and outer Nervión were classified in moderate status; by the new method, these were classified in good status (Annex 2). In the Nervión estuary, the wastewater treatment has improved considerably over recent years; consequently, this estuary has been classified as a normal zone in the context of the UWWTD (Borja *et al*., 2009b). In this regard, the Nervión estuary presents a marked positive trend, due to the reduction in nutrient and organic matter discharges; this has produced a decrease in nutrient concentrations, an increase in water transparency, as well as an increase in dissolved oxygen (from anoxic or hypoxic situations to well-oxygenated bottom layers) (García-Barcina *et al*., 2006). This has led to a progressive biological recovery, with benthic recolonisation permitting demersal fishes to feed and reproduce in the intermediate and inner part of the estuary (Borja *et al*., 2009a). Therefore, a general improvement may be expected also for the phytoplankton element in the Nervión estuary.

Similarly, when changing from the previous to the new method, the Deba and Oiartzun estuaries changed from moderate to good status. In contrast, the outer Oka worsened, from good to moderate status, with the new method (Annex 2). In the Deba estuary, the previous method probably underestimated the phytoplankton quality; this is because this estuary is a river-dominated system with low residence time (0.04 d), which involves a very low risk of eutrophication. The Oka estuary receives large nutrient inputs from anthropogenic sources at its head, which causes a longitudinal gradient of eutrophication (Revilla *et al*., 2000; Garmendia *et al*., 2010). The outer Oka water body comprises the mouth (E-OK20) and the middle reaches (E-OK10) of the estuary. The mouth does not present eutrophication symptoms due to the strong tidal exchange in this zone. However, the middle zone can be impacted, due to the increase in the water residence time and the influence of the upstream inputs. Therefore, the status obtained by the new method in the Oka estuary was more consistent with the anthropogenic pressure. In the report of Borja *et al*. (2009b), on sensitive zones, several estuaries (Butrón, Oka, Artibai and Oiartzun) were classified under high risk of eutrophication. By the new method, only the Oka estuary presented undesirable phytoplankton status, at water body level. However, the report by Borja *et al*. (2009b) dealt with the UWWTD; as such, it evaluated not only the risk for phytoplankton biomass excess but also, the direct discharge of organic matter from anthropogenic sources.

Conclusions and recommendations

The new methodology presented here, for the ecological quality assessment of the phytoplankton in transitional waters of the Basque

Country, classified the majority of the water bodies as in high or good status. The inner and outer zones of the Oka estuary were classified as in poor and moderate status, respectively. The results of the phytoplankton assessment were reasonably consistent with the anthropogenic pressures, as well as the hydrological conditions in the Basque TW. Although nutrient concentrations were much higher than reference conditions, water residence time is generally low in most of the Basque estuaries; this prevents them from an excessive accumulation of phytoplankton biomass. The new method described in this study relies upon two indicators: biomass and frequency of blooms. As such, it integrates two fundamental aspects of phytoplankton response to anthropogenic pressure. Further studies addressing phytoplankton composition metrics are recommended. The methodologies that employ phytoplankton composition for ecological quality status assessment are scarce (e.g. Devlin *et al*., 2007; 2009; Jaanus *et al*., 2009). At this point, this aspect has not been considered in the estuaries of the Cantabrian coast. Cross-system comparison, between water bodies under different nutrient pressure, and information on other estuaries under non-impacted conditions could assist in filling this gap in the methodology for phytoplankton quality assessment.

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Annex 1. Average physico-chemical conditions in surface waters of the Basque estuaries, during the 2003-2008 period. Key: Temp- temperature; Sal- salinity; DO- dissolved oxygen; Secchi- Secchi disc depth; Turb- turbidity; SS- suspended solids; DIN- dissolved inorganic nitrogen; TN- total nitrogen; TP- total phosphorus; and TOC- total organic carbon.

Water body	Station	Temp (ºC)	Sal (psu)	DO mL $^{L-1}$	DO (%)	Secchi (m)	Turb (NTU)	SS mg L^{-1}	Ammonia µM	Nitrate µM	Nitrite µM	DIN µM	Silicate µM	Phosphate µM	TN µM	TP µM	TOC µM
Barbadún	E-M5	14.3	6.8	6.1	87	0.7	29	25	11	55	1.8	67	73	1.4	111	2.7	428
	E-M10	15.2	22.4	5.4	88	1.1	22	12	9	33	0.9	42	34	0.9	79	2.1	409
Inner Nervión	E-N10	15.1	14.3	4.9	71	0.8	7	6	11	59	2.2	73	67	1.5	111	3.3	378
	E-N15	15.4	18.7	5.2	80	0.8	9	10	24	76	3.4	104	58	2.9	159	9.1	485
	E-N17	15.6	23.4	5.3	85	1.1	7	8	46	61	2.4	109	48	4.0	121	5.8	378
Outer Nervión	E-N20	15.8	30.3	5.3	94	2.0	6	9	23	37	1.4	62	29	2.3	88	3.9	417
	E-N30	16.0	33.8	5.8	104	3.7	2	6	7	14	0.6	21	11	0.8	42	1.7	264
Butrón	E-B5	15.4	11.8	5.2	78	0.6	19	15	19	56	2.9	78	82	2.5	134	5.1	507
	E-B7	15.7	25.5	5.0	81	1.0	13	13	14	26	1.6	41	33	1.2	73	2.6	407
	E-B10	15.8	24.7	4.8	80	1.1	10	9	13	31	1.9	46	36	1.3	65	2.1	401
Inner Oka	E-OK5	14.9	3.6	5.5	78	0.5	30	27	90	42	2.7	135	110	4.0	213	8.1	405
Outer Oka	E-OK10	15.6	22.0	4.9	80	0.8	13	10	24	22	2.0	48	45	1.0	81	2.3	505
	E-OK20	16.0	31.0	5.3	93	1.3	5	6	10	10	0.4	20	14	0.8	54	1.9	471
Lea	E-L5	15.1	14.2	5.7	87	0.8	6	6	8	29	1.2	38	54	0.9	69	1.9	538
	E-L10	15.2	19.4	5.4	84	1.2	4	5	10	25	1.3	36	42	0.9	64	2.1	442
Artibai	E-A5	14.6	3.7	5.0	69	0.6	10	7	31	49	2.3	82	81	1.9	155	4.6	460
	E-A10	15.3	19.6	4.5	70	1.0	8	7	14	31	1.5	47	42	1.0	78	2.5	478
Deba	E-D5	15.2	5.0	4.9	72	0.6	9	7	32	65	7.2	104	85	2.9	160	5.6	388
	E-D10	15.1	13.4	4.9	76	0.9	7	5	20	54	6.0	80	63	1.9	127	4.0	414
Urola	E-U5	15.1	4.9	6.2	89	0.8	7	10	10	72	1.8	84	63	1.8	125	3.3	369
	E-U8	15.3	14.8	5.5	84	0.8	8	9	12	52	1.4	66	46	1.5	109	3.1	579
	E-U10	15.3	17.6	5.4	85	1.2	6	6	11	44	1.4	56	40	1.3	113	3.4	684
Oria	E-O5	15.5	14.9	5.1	79	0.9	7	6	12	37	2.6	52	40	1.5	96	3.0	413
	E-O10	15.9	22.0	5.5	89	1.4	5	5	9	24	1.8	35	28	1.0	69	2.3	456
Urumea	E-UR5	14.4	7.2	5.4	79	0.8	4	5	6	26	1.0	34	84	0.8	67	1.8	352
	E-UR10	15.6	20.6	5.3	86	1.3	3	5	7	16	0.9	24	45	0.7	55	1.7	372
Oiartzun	E-OI10	18.3	32.0	4.8	87	1.4	14	14	13	34	1.1	48	44	1.6	78	3.2	480
	E-OI15	17.4	32.0	3.0	54	0.8	9	9	115	40	8.7	164	31	6.6	287	11.9	720
	E-OI20	17.9	31.7	4.9	89	2.0	7	7	17	27	1.3	46	28	1.1	83	2.5	484
Bidasoa	E-BI5	14.8	4.1	6.2	87	1.6	3	3	8	33	0.8	42	77	0.9	69	2.0	240
	E-BI10	16.0	14.5	4.7	74	0.8	12	18	36	31	1.5	69	61	2.4	153	5.8	502
	E-BI20	16.4	26.9	5.3	90	2.0	2	4	12	14	0.8	27	25	1.0	58	1.8	467

Annex 2. Phytoplankton status classification in the Basque estuaries during the 2003-2008 period, by the new method (this study) and by the previous method (Borja et al., 2004). Key: Ind. 1, chlorophyll; Ind. 2, toxic species for human health; Ind. 3, harmful species for fauna and flora; Ind. 4, total phytoplankton cells; H- High status; G- Good status; M- Moderate status; P- Poor status; and B- Bad status. For explanation of water body types, see Table 2.

Basque estuaries				Biomass indicator (new method)		Bloom indicator (new method)		Classification (new method)		Classification (previous method)					
Type	Water body	Station	Stretch	Status	Score	Status	Score	Station	Water body	Ind. 1	Ind. 2	Ind. 3	Ind. 4	Station	Water body
9	Barbadún	E-M5	Oligohaline	H	1.0	M	0.6	G	G	H	H	H	M	M	H
		E-M10	Polyhaline	H	1.0	H	1.0	H		H	H	H	H	H	
10	Inner Nervión	E-N10	Oligohaline	H	1.0	M	0.6	G	G	H	H	H	M	M	M
		E-N15	Polyhaline	H	1.0	M	0.6	G		H	H	H	M	M	
		E-N17	Euhaline	H	1.0	M	0.6	G		H	H	H	M	M	
10	Outer Nervión	E-N20	Euhaline	H	1.0	G	0.8	G	G	H	H	G	P	P	M
		E-N30	Euhaline	H	1.0	G	0.8	G		H	H	G	M	M	
9	Butrón	E-B5	Mesohaline	H	1.0	M	0.6	G	G	H	H	M	M	M	G
		E-B7	Euhaline	H	1.0	H	1.0	H		H	H	H	G	G	
		E-B10	Euhaline	H	1.0	H	1.0	H		H	H	H	G	G	
9	Inner Oka	E-OK5	Oligohaline	B	0.0	M	0.6	P	P	G	H	M	P	P	P
9	Outer Oka	E-OK10	Polyhaline	P	0.3	M	0.6	P	M	G	H	G	M	M	G
		E-OK20	Euhaline	H	1.0	H	1.0	H		H	H	H	G	G	
9	Lea	E-L5	Mesohaline	H	1.0	H	1.0	H	H	H	H	H	G	G	G
		E-L10	Polyhaline	H	1.0	H	1.0	H		H	H	H	G	G	
9	Artibai	E-A5	Oligohaline	M	0.6	B	0.0	P	G	G	H	H	B	B	G
		E-A10	Polyhaline	H	1.0	G	0.8	G		H	H	H	G	G	
8	Deba	E-D5	Oligohaline	H	1.0	G	0.8	G	G	H	H	H	P	P	M
		E-D10	Mesohaline	H	1.0	H	1.0	H		H	H	H	H	H	
9	Urola	E-U5	Oligohaline	H	1.0	G	0.8	G	G	H	H	G	G	G	H
		E-U8	Mesohaline	H	1.0	H	1.0	H		H	H	H	H	H	
		E-U10	Polyhaline	H	1.0	H	1.0	H		H	H	H	H	H	
9	Oria	E-O5	Mesohaline	H	1.0	H	1.0	H	H	H	H	H	G	G	H
		E-O10	Polyhaline	H	1.0	H	1.0	H		H	H	H	H	H	
8	Urumea	E-UR5	Oligohaline	G	0.8	H	1.0	G	G	H	H	H	H	H	H
		E-UR10	Mesohaline	H	1.0	H	1.0	H		H	H	H	H	H	
10	Oiartzun	E-OI10	Polyhaline	H	1.0	H	1.0	H	G	H	H	H	G	G	M
		E-OI15	Euhaline	H	1.0	M	0.6	G		H	H	H	P	P	
		E-OI20	Euhaline	H	1.0	M	0.6	G		H	H	H	M	M	
10	Bidasoa	E-BI5	Oligohaline	H	1.0	H	1.0	H	H	H	H	H	H	H	G
		E-BI10	Mesohaline	H	1.0	H	1.0	H		H	H	H	G	G	
		E-BI20	Polyhaline	H	1.0	H	1.0	H		H	H	H	M	M	

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