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# Classifying Ecological Quality and Integrity of Estuaries

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## Abstract

There is an increasing need in assessing ecological quality and integrity of estuaries and lagoons as transitional waters. This chapter shows the most recent efforts in assessing individual biological elements (from phytoplankton to fishes), together with the integrative tools developed in different geographical areas worldwide. However, reducing multifaceted information needed to describe complex ecosystems that are naturally stressed from multiple ecosystem elements to a single color or value is a substantial challenge to marine scientists, and requires the integration of different disciplines (chemists, engineers, biologists, ecologists, physicists, hydrologists, managers, etc.), to reach agreement on the final assignment of ecological status. Hence, in the future, emphasis needs to be directed at understanding the complexities of estuarine system functioning rather than simplifying, deconstructing and scaling down the system into smaller components. Indeed, the process of deconstructing an ecosystem for study and then reconstructing it to give a holistic and weighted assessment is by far the greatest challenge in areas where there are many activities, pressures and effects.

## Key Points

- To manage human pressures and impacts on transitional environments, legislation worldwide require methods to assess their ecological status, and here we revise the different methods.
- Although methods can address individual ecosystem components (e.g., phytoplankton, macroinvertebrates, fish), integrative methods to assess the status under an ecosystem approach are more common in recent times.
- Due to the natural variability of estuaries, sometimes disentangling the effects from human pressures and natural stress, can be difficult.
- We have reviewed methods to assess the status for phytoplankton, zooplankton, macroalgae, angiosperms, macroinvertebrates, and fish.
- Traditional and new methods (e.g., molecular-based methods) are reviewed.
- The integration of multiple components, in different geographical areas, is presented.

## Introduction

### Estuarine Management and the Need for Classifying Ecological Quality

Marine environments, in general, and transitional waters (estuaries and lagoons) in particular, are facing increasing and significant impacts, which include physical, hydrological and chemical transformation, habitat destruction, and biodiversity loss (Halpern *et al.*, 2007, 2008; United Nations, 2021a,b). The causes include land claim, dredging, pollution (sediment discharges, hazardous substances, eutrophication, etc.), unsustainable exploitation of estuarine resources (shellfishing, fishing, aggregate extraction, etc.), unmanaged tourism, introduction of alien species, and climate change with increases of sea level and sea water temperature (see Halpern *et al.*, 2007; United Nations, 2021a,b).

Being areas where rivers meet the sea, estuaries are highly variable environments that are also the focus of human activities (Wolanski and Elliott, 2016). These have modified the physical characteristics of estuaries through dredging, land claim, harbor and industrial development, as well as recreational and tourist development. The water quality of these environments is also affected by discharges of contaminants from domestic and industrial effluents, including plastics and microplastics (Wolanski and Elliott, 2016). Biological components have also been subject to human influence through commercial harvesting of certain species as well as the introduction of non-native species (either species that compete directly for resources or through the introduction of parasite and disease organisms) (Katsanevakis *et al.*, 2014; Anton *et al.*, 2019). Estuaries are also affected by human activities in the catchment such as water abstraction, as well as pollutants from agricultural and urban runoff, wastewater treatment effluent and industrial activities (Birk *et al.*, 2020).

To manage these pressures and impacts on transitional environments, legislative instruments approved worldwide (e.g., Clean Water Act (CWA) in USA or the Water Framework Directive (WFD) in Europe) address the need to assess their ecological or environmental status (Borja *et al.*, 2008a; Elliott *et al.*, 2022a). The concept of environmental or ecological status incorporates the structure, function and processes of marine ecosystems, bringing together natural physical, chemical, physiographic, hydrographic, geographic and climatic factors, and integrates these conditions with the anthropogenic impacts and human activities in the area concerned (Borja *et al.*, 2009b, 2010).

As commented upon in Borja *et al.* (2008a, 2012a) and Poikane *et al.* (2020), the above concept defines quality in an integrative way, by using several biological parameters (from phytoplankton to mammals) together with physico-chemical and pollution elements. Rogers *et al.* (2007) reviewed the selection of the ecosystem components, adding to the above-mentioned structural components other ecosystem attributes such as food web dynamics, species diversity, and the distribution of life histories; which are not direct biological properties but are functions of the entire ecosystem. These components are important because they provide information about the functioning and status of the ecosystem, and have been widely perceived as additional and potentially useful indicators of estuarine environmental status (Boerema and Meire, 2017).

This approach is intended to allow an assessment of the ecological status at the ecosystem level (“ecosystem-based approach” or “holistic approach” methodologies; Kirkfeldt, 2019) more effectively than can be done at a species or chemical level (i.e., quality objectives).

‘Ecosystem-based management’ emphasizes four common principles (Boesch, 2006; Elliott *et al.*, 2006), namely that effective management must: (1) be integrated among components of the ecosystem and resource uses and users; (2) lead to sustainable outcomes; (3) take precaution in avoiding deleterious actions, and (4) be adaptive in seeking more effective approaches based on experience.

Hence, an ecosystem-based approach should explicitly account for the interconnections within the estuarine ecosystem, recognizing the importance of interactions among many target species or key services and other non-target species; acknowledge interconnections among ecosystems, such as air, land, and sea, and integrate ecological, social, economic, and institutional perspectives, recognizing their strong interdependences (Boesch, 2006).

However, following Borja *et al.* (2009b), not all integrative tools currently available are able to respond to these requirements of the ecosystem-based approach. Hence, several well-established, integrative techniques, such as sediment quality triad (SQT), weight of evidence (WOE), and ecological risk assessment (ERA) (see Chapman, 2009) focus more on assessing pollution (at an eco-toxicological level) than assessing integrity of the ecosystem (Borja *et al.*, 2009b).

Hence, methods for classifying estuaries and lagoons according to anthropogenic stress include those centered on the primary community structural variables (abundance, species richness, and biomass) and derived community structural variables (such as diversity indices, abundance (A/S) and biomass (B/A) ratios, and evenness indices) (Gray and Elliott, 2009; Elliott *et al.*, 2022b). They also include functional analyses such as those involving feeding guilds (as in the infaunal trophic index (ITI), by Word, 1990) and their responses to elevated organic levels (as in the AZTI marine biotic index (AMBI) by Borja *et al.* (2000, 2019), and the benthic quality index (BQI) by Rosenberg *et al.* (2004), among others). For example, detritus and deposit-feeding dominance are reflected in any assessment of trophic analysis.

As indicated in Gray and Elliott (2009), there are well-defined numerical methods which aim to detect and reflect stress in benthic communities, and Elliott *et al.* (2022a) for fish communities, although these methods are applicable to most macrobiotic communities. For example, species–abundance–biomass (SAB) curves (Pearson and Rosenberg, 1978), abundance–biomass comparisons (ABCs, Warwick, 1986), AMBI (Borja *et al.*, 2000), and diversity indices. (e.g., Rosenberg *et al.*, 2004; Borja *et al.*, 2007). Given the estuarine quality paradox (see this concept in the next section), the main problem is that some of these methods detect naturally as well as anthropogenically stressed areas, thereby decreasing the ability of detecting and maximizing the signal (anthropogenic change)-to-noise (natural variability) ratio.

Finally, although successful, the recent advances in assessing estuarine and lagoonal quality are probably only a first step and many challenges remain to be addressed in the future, including the development of reliable methods to integrate multiple physico-chemical and biological elements into a single evaluation of estuarine system condition (Borja *et al.*, 2008a, 2009a, 2012a), as well as methods using environmental DNA (Pawlowski *et al.*, 2018; Franco *et al.*, 2022). This integration should be made by using different elements, different media, and results from different locations within the same estuarine water body to evaluate spatial distribution of status of these components. The challenge is not only to integrate indicators for single ecosystem elements, but also to include measures of ecosystem structure, function, and processes. Hence, the ecological integrity of an estuary or a lagoon should be evaluated using all information available, including as many biological ecosystem elements as is reasonable, and using an ecosystem-based assessment approach (Borja *et al.*, 2008a, 2009e, 2010). This chapter focuses on this challenging issue, as an overview of the current situation worldwide, updating the information made available in Borja *et al.* (2012a).

### The Estuarine Quality Paradox and Environmental Homeostasis

The ecological components of estuaries have long been known to follow a well-defined set of characteristics – for example, estuaries are characterized by having a few dominant species of stress-tolerant, euryoecious, small-form (low individual biomass), short-lived organisms which occur in high abundances and are tolerant of organic-rich areas such as those found in high depositional areas, e.g. intertidal estuarine mudflats (Elliott and Whitfield, 2011). In such habitats, intraspecific competition is more likely than interspecific competition especially where competition between the dominant detritivores and deposit feeders is dictated by food supply whereas competition between suspension feeders is dictated by space (e.g., McLusky and Elliott, 2004). The forms are more likely to be r-strategists rather than k-strategists (Gray and Elliott, 2009). These typical estuarine species are tolerant of high variability in environmental master factors; for example, tubificid oligochaetes and certain nereid polychaetes are not stressed by variable salinity (Dauvin and Ruellet, 2009). Salinity tolerance becomes the over-riding determinant of distributions in estuaries and as such has led to classification schemes such as the Venice Classification and the Remane’s Curve (Whitfield *et al.*, 2012; Smyth and Elliott, 2016).

Hence, estuaries and lagoons have long been regarded as environmentally naturally stressed areas because of the high degree of variability in their physico-chemical characteristics. In particular, most environmental variables such as temperature, salinity, dissolved oxygen (DO), turbidity, and bed sediment composition vary widely along spatial and temporal variables. However, it is now accepted that an estuary or transitional water body is only stressed if the organisms are not adapted to that variability (Elliott and Quintino, 2019); organisms that are adapted are in a preferential position, thus making the conditions a subsidy rather than a stress. Accordingly, the true estuarine biota is adapted to such changes, thus ensuring they are naturally stress tolerant and hence resilient to change. This then suggests that they can tolerate the variable conditions without showing adverse effects, and

**Table 1** Conceptual basis and assumptions inherent in macrobenthic impact studies (modified and expanded from Warwick, 1986; McManus and Pauly, 1990).

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A. Natural State
1) A natural macrobenthic assemblage either tends towards or is in an equilibrium state;
2) Under non-impacted conditions, there are well-defined relationships (which therefore may be modeled) between faunal and environmental (abiotic) variables;
3) In approaching the normal equilibrium state, the biomass becomes dominated by a few species characterized by low abundance but large individual size and weight;
4) Numerical dominance is of species with moderately small individuals, this produces among the species a more even distribution of abundance than biomass;
5) The species are predominantly K-selected strategists;
B. Moderate Pollution
6) With moderate pollution (stress), the larger (biomass) dominants are eliminated, thus producing a greater similarity in evenness in terms of abundance and biomass;
7) Also with moderate pollution, diversity may increase temporarily through the influx of transition species;
C. Severe Pollution
8) Under severe pollution or disturbance, communities become numerically dominated by a few species with very small individuals;
9) Those small individuals are often of opportunist, pollution-tolerant species which have r-selected strategies;
10) Under severe pollution, any large species that remain will contribute proportionally more to the total biomass relative to their abundance than will the numerical dominants;
11) Thus under severe pollution, the biomass may be more evenly distributed among species than is abundance;
12) However, under severe pollution, species with large individuals may be so rare as to be not taken with normal sampling;
13) The change in assemblage structure with increasing disturbance is predictable, follows the conceptual models and is amenable to modeling and significance testing;
D. Recovery
14) Opportunists are inherently poor competitors and may thus be out-competed by transition species and K-strategists if conditions improve;
McManus and Pauly (1990) also consider that under normal conditions:
1) The biomass-dominants will approach a state of equilibrium with available resources;
2) The smaller species are out of equilibrium with available resources;
3) The abundances of the smaller species are subject to more stochastically controlled variation than the larger species.

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communities and environmental characteristics reflecting high variability can absorb stresses – the so-called ‘environmental homeostasis’ (Elliott and Quintino, 2007). Under these conditions, many species are absent (e.g., stenohaline marine forms which cannot tolerate widely varying salinities) and so only stress-tolerant species are found (Whitfield *et al.*, 2012). Hence, stress for one species, by causing it to be absent, becomes a subsidy (i.e., a benefit) for another species which can then capitalize on the lack of interspecific competitors and thus be more successful. Odum (1985) first discussed the stress–subsidy continuum and indicated that one organism’s stress (adverse effect) is another organism’s subsidy (benefit).

Estuaries and their biota and habitats are classified extensively according to the response to anthropogenic stress, especially the way in which the biota respond at the individual, population, and community levels of biological organization. In many cases, this relates to the structure of those biological elements, especially community structure. These features have been shown for the estuarine fish (Breine *et al.*, 2007; Whitfield *et al.*, 2022) and macrobenthos (Gray and Elliott, 2009; Table 1); the latter of which under anthropogenic stress is characterized by small organisms, r-strategists and the replacement of k-strategists, high abundances of few organically tolerant species. The low diversity of small organisms with the potential to produce high biomasses serve as prey for fishes at high tide and birds at low tide in intertidal waterbodies (Dauvin and Desroy, 2005). In addition, they have a high turnover and biological productivity (as shown by an increase in the production to average biomass ratio, P/B) and a dominance by oligochaetes and polychaetes which tolerate adverse environmental conditions such as low oxygen and low and variable salinity. It is emphasized that these are expected characteristics of many stressed ecosystems not only pertaining to the marine and estuarine environment (Odum, 1969, 1985) (Table 2).

These features also apply to the floral community (e.g., Wilkinson *et al.*, 2007) wherein polluted estuarine and lagoonal areas, especially those influenced by organic discharges, sewage runoff and industrial effluent, become dominated by opportunistic green algae that occasionally form mats. Despite this, large concentrations of ephemeral green filamentous algae are naturally occurring in transitional water bodies, which often have large nutrient inputs and retain these nutrients (Wilkinson *et al.*, 1995; Romero *et al.*, 2018). Estuaries naturally show the transition from a highly diverse marine flora, with many red and brown macroalgae as well as green macroalgae in the lower regions, to an upper estuarine algal flora dominated by the Chlorophyceae. Under high organic and nutrient loading these features produce macroalgal mats, with the latter often displacing seagrass beds (de Jonge and Elliott, 2002).

Such an estuarine stress is detected not only at the community level but also at the physiological level of biological organization (Franco *et al.*, 2022; Elliott *et al.*, 2022a). Methods such as scope for growth (SFG) have long been used to indicate natural and anthropogenic stress in marine and estuarine areas (e.g., Widdows and Johnson, 1988; Mazik *et al.*, 2013) and Navarro (1988) and Guerin and Stickle (1992) indicate the way in which salinity stress, through natural freshwater inputs, reduces energetic budgets.

**Table 2** Trends expected in stressed ecosystems and the estuarine features associated with the applied topics summarized by Odum (1985).

<i>Feature</i>	<i>Odum (1985)</i>		<i>Estuarine feature</i>
Energetics	1. Community respiration increases	Yes, in general:	Higher respiration in larger populations of small organisms and organic rich sediments; possibly with osmoregulatory stress caused by salinity change
	2. P/R (production/respiration) becomes unbalanced	Unknown	Possibly due to higher respiration caused by salinity stress
	3. P/B and R/B (maintenance:biomass structure) increase	Yes, in general:	Higher P/B in smaller and shorter lived organisms, e.g. dominance by oligochaetes and small polychaetes; high turnover organisms
	4. Importance of auxiliary energy increases	Depends on meaning:	Increase in allochthonous energy input as well as relatively high autochthonous production
	5. Exported or unused primary production increases	Depends on meaning:	Export of material to adjacent sea areas but also import from catchment
Nutrient cycling	6. Nutrient turnover increases	Yes, but:	Because of physical characteristics - high nutrient inputs and use
	7. Horizontal transport increases and vertical cycling of nutrients decreases (cycling index decreases)	Partly the case:	Both horizontal and vertical cycling is high, depending on flushing characteristics and residence time; importance of material movement from pelagic to benthic system
	8. Nutrient loss increases	Yes, but:	because of the physical characteristics - high nutrient loss through flushing and export through predators
Community structure	9. Proportion of r-strategists increases	Yes:	High abundances of few, short-lived stress-tolerant species
	10. Size of organisms decreases	Yes:	High abundances of small organisms dominant in benthos; low megafaunal populations
	11. Lifespans of organisms decreases	Yes, in general:	On average, benthic and planktonic community composed of short-lived organisms; planktonic organisms adapted to prevent flushing of populations
	12. Food chains shorten because of reduced energy flow at higher trophic levels and/or greater sensitivity of predators to stress	Not necessarily:	Food chains can be very short (macrophytes-herbivorous ducks) but also very long because of the opportunistic nature of many predators; while marine predators (stenohaline marine fishes) may be reduced there are many other fish and bird predators
	13. Species diversity decreases and dominance increases; if original diversity is low, the reverse may occur; at the ecosystem level, redundancy of parallel processes theoretically declines	Yes (first part); unknown (second part):	Classic estuarine community in all components of few species; exacerbated with distance landward in the estuary; competition between species may be less than competition within species
General system-level trends	14. Ecosystem becomes more open (i.e. input and output environments become more important as internal cycling is reduced)	Not necessarily so;	Internal cycling is important even though nutrients and organic matter are delivered from external sources
	15. Autogenic successional trends reverse (succession reverts to earlier stages)	Unknown:	
	16. Efficiency of resource use decreases	Not necessarily so:	While there may be an excess of organic resources, leading to export, much is used within the system to support high predator populations
	17. Parasitism and other negative interactions increase, and mutualism and other positive interactions decrease	Not shown:	
	18. Functional properties (e.g. community metabolism) are more robust (homeostatic-resistant to stressors) than are species composition and other structural properties	Yes:	Ability of the system to withstand stressor-effects without adverse impacts

Therefore, although estuaries and lagoons are exposed to high degrees of anthropogenic stress, they are also naturally stressed, highly variable ecosystems. The difficulty of separating these causes of change (natural and anthropogenic stress) in estuaries in relation to the usual structural features in estuaries has given rise to the estuarine quality paradox (Dauvin, 2007; Elliott and Quintino, 2007; Dauvin and Ruellet, 2009; Elliott and Quintino, 2019). This can be defined as: “the characteristic by which the dominant estuarine faunal and floral community is adapted to and reflects high spatial and temporal variability in naturally highly stressed areas but the natural community has features very similar to those found in anthropogenically-stressed areas thus making it difficult to detect anthropogenically-induced stress in estuaries. Furthermore, as estuaries are naturally organically-rich areas then the biota has similarities to anthropogenically-organic rich areas. Because of this, there is the danger that any indices which are based on those features and used to plan environmental improvements are flawed.”

The main difficulty posed by the estuarine quality paradox is that any technique classifying areas using the characteristics shown by both natural estuarine and unnatural anthropogenic features will erroneously show estuaries to be stressed. Hence, there is a difficulty in using these techniques in estuaries for detecting and separating anthropogenic stress from natural stress.

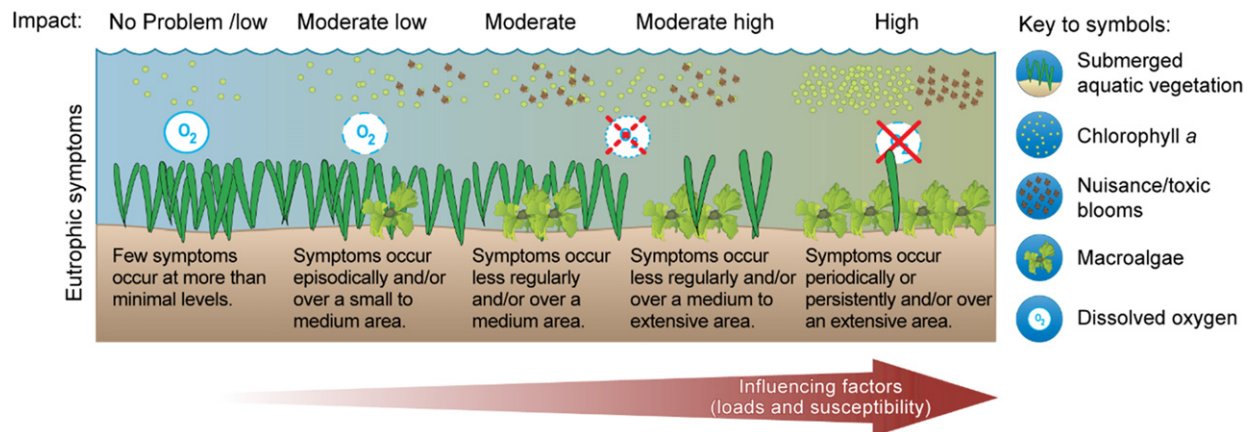
## Classifying Biological Quality Elements

The overall biological and ecological status of estuaries can be defined according to the status of its constituent components, often termed biological quality elements (as in the European WFD, i.e., phytoplankton, zooplankton, macroalgae, macroinvertebrates, angiosperms, or fishes). These are, in turn, supported by several master-factors such as the physico-chemical elements of temperature, salinity, nutrients, dissolved oxygen, suspended solids, and turbidity (Gray and Elliott, 2009). These elements, together with geomorphological, hydrological and tide characteristics, determine the type of estuary (Harrison, 2004; Wolanski and Elliott, 2016), and, to some extent, the biological communities that the transitional waters can support. Hence, they have been used, under some legislation, as key elements in assessing ecological quality linked to oxygen (Best *et al.*, 2007a), nutrients (Devlin *et al.*, 2007), or suspended solids (Bilotta and Brazier, 2008; Devlin *et al.*, 2008; Druine *et al.*, 2018).

Most of these variables are related to eutrophication, and normally are studied in the same context as phytoplankton or macroalgae (Loureiro *et al.*, 2006; Azevedo *et al.*, 2008; Giordani *et al.*, 2009). However, some legislation such as the WFD seeks for independent assessments. This led to the proposal for methods to assess physico-chemical elements alone (Borja *et al.*, 2004b; Bald *et al.*, 2005), although the concept of ecohidrology emphasizes the inherent influence of the physical system in structuring the biotic communities and influences the individuals (Wolanski and Elliott, 2016).

## Phytoplankton

The effects of nutrient enrichment of estuarine water bodies firstly relies on the uptake by and stimulation of phytoplankton growth. Excessive amounts of nutrients may, under the right conditions, cause overgrowth of phytoplankton leading to low dissolved oxygen conditions as the bloom dies and the biomass decays, as well as reduced water transparency which may lead to losses of seagrasses (de Jonge and Elliott, 2002) (Fig. 1). Additionally, nutrient additions may cause changes in natural nutrient ratios and/or speciation leading to blooms or increased numbers of opportunistic species, many of which are harmful or toxic (e.g., *Aureococcus anophagefferens*: Glibert *et al.*, 2007; *Nitzschia pungens*: Rabalais *et al.*, 1996; *Lepidodinium chlorophorum*: Serre-Fredj *et al.*, 2021).



**Fig. 1** Progression of eutrophication and impact evaluation starting with increased primary production reflected as increased phytoplankton biomass (Chl-a) and macroalgal abundance leading to low dissolved oxygen, losses of seagrasses and changes in community composition to include nuisance and toxic blooms. From: Bricker *et al.*, 2007.

Because of their direct link and sensitivity to nutrient loading, phytoplankton growth is considered a direct effect (e.g., OSPAR, 2002; WFD methods in Birk *et al.*, 2012) or a primary symptom (e.g., assessment of estuarine trophic status method in Bricker *et al.*, 2003), indicative of the start of eutrophication as shown by a suite of signs and symptoms (de Jonge and Elliott, 2002). As such, phytoplankton is a good indicator for nutrient-related impacts. Typically, measurements of chlorophyll *a* (Chl-*a*) are used to represent phytoplankton biomass in estuarine systems. Other measures that are used to evaluate the status of phytoplankton populations include the abundance and species composition of the phytoplankton community and changes in the frequency and duration of blooms (Birk *et al.*, 2012).

Several methods have been developed to evaluate the status of phytoplankton in coastal and estuarine water bodies for use as an indicator of nutrient-related water-quality impairment (Table 3). The methods include different metrics, some use only Chl-*a* concentration, e.g., Trophic State Index (TRIX), Environmental Protection Agency National Coastal Assessment (EPA NCA) and Institut Français pour l'Exploration de la Mer (IFREMER), whereas others combine additional characteristics such as duration and spatial coverage of bloom concentrations (e.g., the ASSETS program in Bricker *et al.*, 2003), or weighting factors that represent the relative contribution to overall water quality, e.g., transitional water quality index (TWQI) and lake water quality index (LWQI) (Table 3). This highlights that the Chl-*a* indicators, while able to stand alone, are typically part of larger multivariable indices designed to accurately evaluate overall eutrophic conditions. This reflects the fact that they indicate the first signs of nutrient-related problems, whereas other indirect problems such as low dissolved oxygen and losses of seagrasses indicate more significant nutrient-related impairment.

Additionally, although phytoplankton (i.e., as shown by the Chl-*a* concentration) may increase with increased nutrient additions in some types of systems, in others, biomass of the macroalgal component (e.g., *Ulva* spp.) will increase, rather than phytoplankton. It is important to recognize these situations (i.e., lagoons; Nobre *et al.*, 2005) and, as an integrative approach, to consider including macroalgal biomass as a complementary primary eutrophication indicator just as other indicators (e.g., dissolved oxygen and losses of seagrasses) are included in indices of overall eutrophic condition and are a required variable within many mandated monitoring programs (e.g., WFD, Oslo Paris Convention for the Protection of the North Sea (OSPAR), and EPA NCA) in order to track the more severe problems associated with nutrient enrichment. Hence, it is necessary to examine phytoplankton indicators to see how well they reflect the water quality of estuarine and coastal/transitional systems, at the same time understanding that they typically are combined with other indicators to give a more complete assessment of eutrophication.

### Phytoplankton indices

*Chl-a concentrations and reference conditions.* The simplest of the phytoplankton indicators uses only the concentration of Chl-*a* as a measure of phytoplankton biomass. Although most of the methods listed in Table 3 include Chl-*a* concentration, there are different ways of determining the status of Chl-*a* dependent upon the time frame and spatial scales of sampling, the statistical measure used to determine the representative concentration (e.g., mean annual, index period mean, and/or maximum), and the reference concentration and scale that determines the final status.

Although all the Chl-*a* indices are included in a multiparameter index, TRIX is the only one for which the Chl-*a* indicator cannot stand alone because it is integrated with three other variables that make up the index (Table 3).

The EPA NCA uses comparison of samples from an annual index period (June – October) to reference conditions determined from national studies to determine the rating of poor, fair, or good (where poor  $> 20 \mu\text{g l}^{-1}$ , fair  $5\text{--}20 \mu\text{g l}^{-1}$ , and good  $0\text{--}5 \mu\text{g l}^{-1}$ ). The samples are taken once per year based on a random statistical design and provide 90% confidence in the rating for a region (USEPA, 2001a). However, the sampling design does not allow the EPA NCA to make determinations for individual water bodies.

The TWQI/LWQI method uses nonlinear functions to transform annual average Chl-*a* concentrations from sites representative of the system into a quality value (0 = worst, 100 = best) which is then multiplied by a weighting factor (here, 15% of total water quality is attributed to Chl-*a*) that accounts for the relative contribution to the overall index (Giordani *et al.*, 2009). The Chl-*a* quality value scores range from optimal conditions of  $< 6 \mu\text{g l}^{-1}$ , for a quality value of 100, to a low-quality value of 0 at a concentration of  $30 \mu\text{g l}^{-1}$  or greater. These ranges are consistent with conditions in Mediterranean lagoons and continental estuaries (Giordani *et al.*, 2009).

The Helsinki Convention (HELCOM) uses HEAT (HELCOM Eutrophication Assessment Tool) as mean summertime concentrations of samples that are spatially representative of a water body, combined with reference values that are basin specific and are determined from historical data, empirical modeling, or state-of-the-art hydrodynamic or ecological modeling for pristine conditions. The boundary between good and moderate status, as required by the WFD, is the reference concentration + 50%, which is equal to an ecological quality ratio (EQR, *sensu* the WFD; see Borja, 2005) of 0.67. The threshold between good and moderate EQR is used to determine the extent of deviation of the sample EQR from the reference EQR and from good status (Andersen and Laamanen, 2009).

The approach ASSETS uses the 90th percentile of annual values for Chl-*a* combined with the spatial coverage of high values and the frequency of occurrence of blooms to determine the Chl-*a* condition within each salinity zone (tidal fresh 0–0.5 salinity, mixing zone 0.5–25 salinity, and seawater zone  $> 25$ ) within a system. The ratings for each zone are combined as an area weighted sum to determine the final Chl-*a* rating for the system (high, moderate, and low; Bricker *et al.*, 1999, (2003,2007)).

The Chl-*a* assessment under WFD guidance and the OSPAR Comprehensive Procedure (OSPAR COMPP) are similar in that they both use mean summertime/growing season concentrations for samples that are spatially representative of the water body, and the maximum summertime Chl-*a* concentration is also used (OSPAR, 2002; ECOSTAT, 2003). In some cases, under the WFD guidance, the 90th percentile of annual Chl-*a* measured values are also used (i.e., Revilla *et al.*, 2009). Both the WFD guidance and OSPAR COMPP require development of reference conditions for comparison to measured values from reference sites or historical



**Table 3** Methods to evaluate the status of phytoplankton in coastal and estuarine water bodies.

Method/ Approach	Country/Region using method	Biomass				Community composition	Abundance	Indicators in Overall Eutrophication Index
		Chl-a Thresholds and Ranges (ug/l)	Sample Timeframe	Statistical measure	Other characteristics			
EPA NCA <sup>a</sup>	US	Poor > 20 Fair 5–20 Good 0–5 lower for sensitive systems	Index period (June - Oct)	concentration, % of coastal area in poor, fair and good condition based on probabilistic sampling design for 90% conf in areal result		No	Chl-a, water clarity, DO, DIP, DIN	
TRIX <sup>b</sup>	EU	no thresholds, integrated with other index variables		concentration		No	Chl-a, DO, DIN, TP	
TWQI /LWQI <sup>c</sup>	EU	Good QV100 = 6 Bad QV0 = 30	annual	Chl concentration mean annual or seasonal modified by weighting factor		No	Chl-a, seagrasses, macroalgae, DO, DIN, DIP	
HEAT <sup>d</sup>	Baltic	Deviation from ref EQR < 6.7 No dev from ref EQR > 0.67	summer (June - Sept)	mean summer concentration	increases in concentration, frequency and duration	indicator spp	X Chl-a, phytoplankton, nutrients, water transparency, SAV, DO, benthic invertebrates, summertime bloom intensity index	
ASSETS <sup>e</sup> (Eutrophic Condition component only)	US, EU, Asia, Australia	High > 20 Mod 5–20 Low 0–5 lower for sensitive systems	annual	90th percentile Chl concentration of annual data	spatial coverage, frequency occurrence	Nuisance and toxic bloom occurrence, frequency, duration	Chl-a, macroalgae, DO, seagrasses, nuisance/toxic blooms	
WFD <sup>f</sup>	EU	Cantabrian coast: Bad > 14 Poor 10.5–14 Moderate 7–10.5 Good 3.5–7 High 0–3.5	summer	summer Chl concentration mean, max and sometimes 90th percentile annual data	increases in concentration, frequency and duration	indicator spp	X Chl-a, phytoplankton, macroalgae, microphytobenthos, seagrasses, DO, nutrients, algal toxins	
OSPAR COMPP <sup>g</sup>	EU	Good QV100 = 6 Bad QV0 = 30	growing season	growing season Chl concentration mean, max	increases in concentration, frequency and duration	indicator spp	X Chl-a, phytoplankton, macroalgae, microphytobenthos, seagrasses, DO, nutrients, algal toxins	

IFREMER <sup>h</sup> (lagoons)	France	> 30 Red 10–30 Orange 7–10 Yellow 5–7 Green 0–5 Blue	annual	mean annual Chl concentration	phytoplankton abundance of < 2 μm, > 2 μm	X	Chl-a, phytoplankton counts (<2, > 2 μm), macrophytes (biomass, diversity), macrobenthos (richness, diversity), water (DO, Chl, Chl/phaeo, turbidity, SRP, TP, TN, NO <sub>2</sub> , NO <sub>3</sub> , NH <sub>4</sub> ), sediment (OM, TN, TP)
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<sup>a</sup>USEPA, 2008b.

<sup>b</sup>Vollenweider *et al.*, 1998

<sup>c</sup>Giordani *et al.*, 2009.

<sup>d</sup>Andersen and Laamanen, 2009

<sup>e</sup>Bricker *et al.*, 2003, 2007.

<sup>f</sup>Devlin *et al.*, 2011.

<sup>g</sup>OSPAR, 2002.

<sup>h</sup>Souchu *et al.*, 2000.

References:.

data. The WFD assessment results in ratings for high, good, moderate, poor, and bad for which thresholds between categories were developed during WFD inter-calibration exercises and reflect the location of the assessment (i.e., Basque coast in the [Revilla et al., 2009](#) study). The reference conditions for OSPAR COMPP are developed from reference sites and final ratings are determined as: (1) problem area if the measured Chl-*a* is greater than the reference condition + 50%; (2) potential problem area if it is between the reference concentration and + 50%, and (3) non-problem area if it is equal to or less than the reference site concentration.

The IFREMER method for lagoons uses mean annual or mean seasonal data compared to a fixed scale to determine the status for Chl-*a*, with final ratings being color-coded to match the WFD scaling from best (blue) to worst (red) ([Souchu et al., 2000](#)). It is of note that the thresholds and ranges used by IFREMER, determined from historical studies such as Organization for Economic Cooperation and Development (OECD; [Souchu et al., 2000](#)), are roughly consistent with the scales reported for TWQI/LWQI, EPA NCA, and ASSETS ([Table 3](#)). It is also important to note that although the reference conditions for EPA NCA and ASSETS are determined from national studies rather than on a case-by-case basis, each of these methods has a different scaling for systems that are sensitive (i.e., Florida Bay).

*Phytoplankton abundance and indicator species, duration and frequency of blooms.* Most of the assessment methods recognize that high Chl-*a* concentrations are only one of the potential impacts of nutrient enrichment. To provide a complete picture of eutrophic conditions, other characteristics should be included such as changes in community composition to include more nuisance and toxic species that result from changes in nutrient ratios, and increased duration and frequency of blooms which result from increases in nutrient loads. [Serre-Fredj et al. \(2021\)](#) showed that the excessive blooms of the harmful algal species *Lepidodinium chlorophorum*, and high toxin production, were favored by unbalanced ratios of N and P inputs. Similarly, [Rabalais et al. \(1996\)](#) showed that the dominance of *Nitzschia pungens* within the phytoplankton community of coastal Louisiana had increased greatly since the 1950s due to increasing N availability and decreasing Si:N ratios. The impact of nutrient concentrations and ratios on species dominance was also shown in by a study in the Bay of Seine where domoic acid concentrations and toxic *Pseudo-nitzschia* species coincided with silicate limitation and higher nitrogen concentrations ([Thorel et al., 2017](#)). It is clear that occurrences of nuisance and toxic algal blooms are linked to nutrient concentrations and ratios and could be used to provide additional power to assessments of water quality impairment.

All but three of the methods from [Table 3](#) include some measure of changes in community composition to potentially harmful or toxic species which usually includes changes in frequency and duration of blooms. The methods HEAT and OSPAR COMPP monitor for toxic bloom species looking at changes in specific groups (i.e., dinoflagellates, diatoms, and cyanobacteria). The ASSETS nuisance and toxic bloom index uses a combination of observations of nuisance and toxic blooms and the frequency and duration of the blooms to determine the status. The WFD approach, used by [Revilla et al. \(2009\)](#), and the IFREMER method use abundance of phytoplankton larger and smaller than 2  $\mu\text{m}$  as an indicator of the potential presence of toxic bloom organisms.

### **How well do these phytoplankton indices assess eutrophication?**

It is important to examine the behavior of the different phytoplankton indices. In cases where thresholds denoting the boundaries indicating small or large impacts are required, there is an approximate consistency among the named thresholds. As these measures have been determined independently, this suggests that there is equivalency among water bodies globally in the response to nutrient loads as well as a global understanding of undesirable levels of Chl-*a* ([Salas Herrero et al., 2019](#)). In the case of the EPA NCA and ASSETS, there is recognition that some systems, such as Florida Bay (a sensitive carbonate-based system), require thresholds and ranges that are lower but overall there is general agreement on the thresholds of Chl-*a* concentrations that indicate high-level impacts. It is also of note that the concentration ranges appear to be useful in both lagoons and estuaries ([Giordani et al., 2009](#)).

Most methods are related to the spatial scales of sampling to ensure results that are representative of the system. This is important, as typically one part of the system is more heavily impacted than others, and thus the methods must include some way to recognize the patchy spatial nature of these impacts. Most of the methods use an average of samples from different sites, whereas the EPA NCA looks at the ratio of stations above a threshold and can estimate the area of the region that is impaired given the statistical sampling design. The time frames of sampling vary from an annual to a summertime/growing season ([Table 3](#)). While extreme Chl-*a* concentrations are typically observed during the growing season that, in some cases, the restriction to an index period may under- or overestimate concentrations due to the variability in bloom timing as a result of variable climate and freshwater inflow.

The other most common phytoplankton indicator relates to changes in community reflected in the increase in nuisance and toxic species, including increases in bloom frequency and duration. In some cases, this is done on a species-by-species basis using indicator species as the measure of change, such as the method used by HEAT. This also includes the diatom to dinoflagellate ratio, given that the latter maybe more indicative of stressed or eutrophic conditions. The relative abundance of size categories is also used with the smaller forms indicative of possible toxic forms (e.g., WFD and IFREMER). This has been used for the assessment of the ecological status of phytoplankton on the Basque coast under the guidance of the WFD. In this case, it is notable that using different definitions of the groups and thresholds that are included in the size analysis resulted in different final assessments of conditions ([Revilla et al., 2009](#)). The different approaches also have different analytical requirements, that is, greater taxonomic expertise as well as different criteria for monitoring. For sustainability of a monitoring and assessment program, these differences must be taken into consideration.

A measure of the success of the phytoplankton indicator is whether it accurately reflects the extent and significance of nutrient-related impacts for the system where it is applied. Here we see that these methods are able to capture changes in biomass and species composition and, as such, are successful in noting nutrient-related/induced changes. In addition, it is recommended that

the phytoplankton indices used to assess eutrophication should be intercalibrated to ensure comparability, as has been done in the case of the different European indices within the WFD (Birk *et al.*, 2013; Poikane *et al.*, 2014; European Commission, 2018).

### Zooplankton

Zooplankton is not included in some legislation as a biological quality element to be monitored and assessed (e.g. in the European WFD), and historically has been used mainly to monitor seas in relation to climatic factors (Harris *et al.*, 2015). As such, the development of methods to assess the ecological status in estuarine systems has been very limited, but has been used in some locations such as Northern Spain (Intxausti *et al.*, 2012; Cajaraville *et al.*, 2016), Portugal (Falcao *et al.*, 2012), Belgium (Mialet *et al.*, 2011), Brazil (Veríssimo *et al.*, 2017), France (Chaalali *et al.*, 2013), and the USA (Carpenter *et al.*, 2006).

The estuarine zooplankton community shows similar features to those of the other components in having a few dominant species which in turn are governed by the physico-chemical conditions (Taylor, 1987, 1993). The communities can be classified according to those environmental variables such as the relationship between salinity and temperature as shown by Bary diagrams. This analysis also indicates the mixture of *Acartia* spp. in which the relative proportions of the congeneric species vary with environmental conditions. However, as there has been no requirement for adding zooplankton as an assessment component, their analysis has not been taken further with the development of quantitative indicators. Therefore, despite the increasing number of papers published in the last decade in this topic, zooplankton remains as an underrepresented indicator of estuarine health conditions.

The ubiquitous upper-estuarine, euryoecious Atlantic copepod *Eurytemora affinis*, which is a complex of sibling species, could be an excellent candidate to indicate the ecological status of estuarine mesozooplankton communities due to its ability to adapt its biological strategy to future climate warming (see Sukhikh *et al.*, 2013; Souissi *et al.*, 2016; Richirt *et al.*, 2019; Karlsson and Winder, 2020). However, it is also highly tolerant of naturally and anthropogenically variable conditions which may reflect the estuarine quality paradox (see above).

### Macroalgae

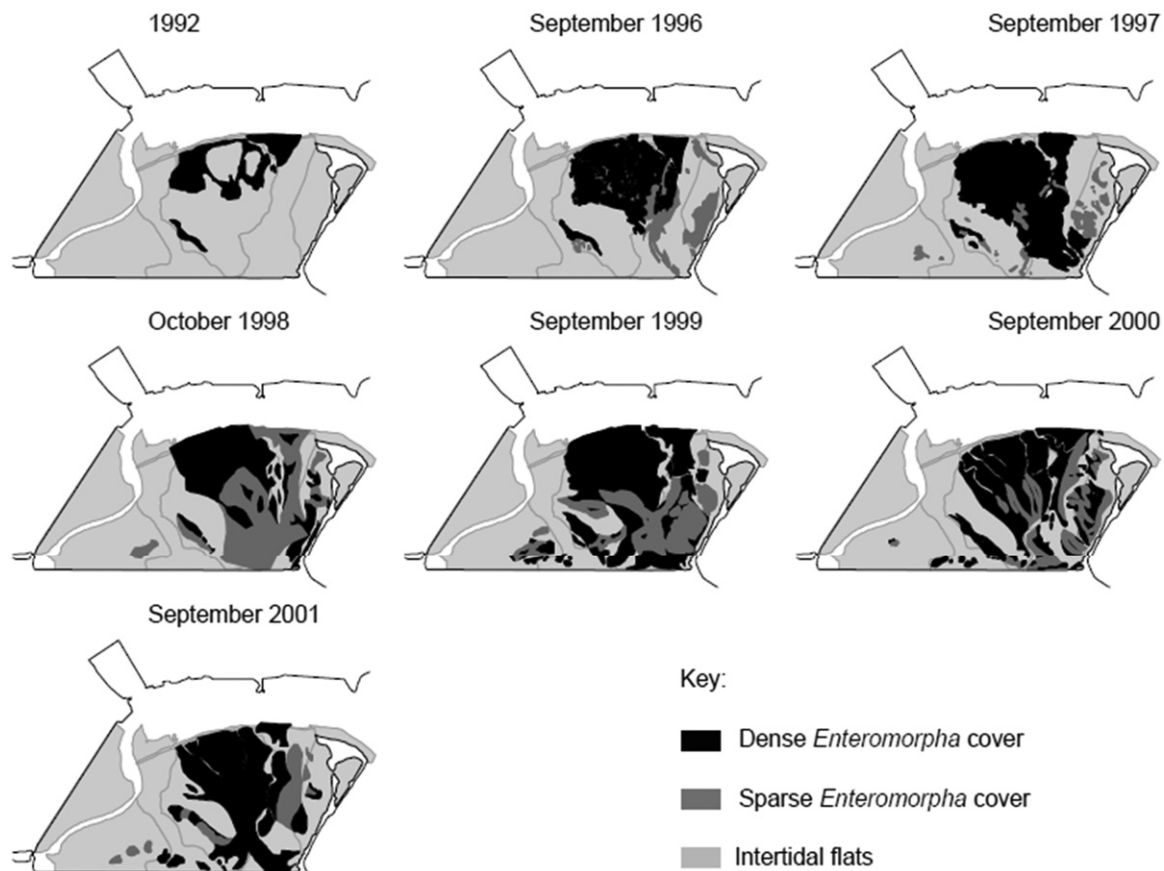
Prior to the last decade, the methods for assessing macroalgae were less-well developed compared to other biological elements. Macroalgae in estuarine and coastal marine waters have been used extensively as indicators of marine quality, and the development of new assessment methods increased after 2010. However, most of these methods are used in coastal areas, and especially on rocky shores, those for transitional systems are still limited (see a recent review in D'Archino and Piazzi, 2021). The use of macroalgae as indicators is recommended as, together with seagrasses, they are

1. important primary producers in estuaries;
2. a food source for waterfowl;
3. a habitat and nursery area for commercially and recreationally important fish species;
4. a protection against erosion, and
5. a buffering mechanism for excessive nutrient loadings (Gibson *et al.*, 2000).

As photosynthetic sessile organisms, they respond directly to abiotic and biotic environmental factors, thus representing sensitive bioindicators of natural and anthropogenic changes (Orfanidis *et al.*, 2001, 2003). In general, the different types of seaweed have differences in environmental tolerances, with opportunistic green algae being most tolerant of brackish conditions, brown algae being relatively hardy, and red algae being more delicate and suited to full salinity marine conditions (Wilkinson, *et al.*, 2007). However, due to this double response, they do not stand alone as an indicator of ecosystem condition, and additional parameters (e.g., water column nutrient concentrations and light penetration) are required to interpret macroalgae data (Gibson *et al.*, 2000; de Jonge and Elliott, 2002).

Despite this, methods based upon macroalgae in assessing environmental pollution are more developed in coastal areas than in estuarine habitats (Wilkinson and Rendall, 1985). Probably this is because of the complex natural gradients within estuaries, exemplified by the estuarine quality paradox. In addition, high concentrations of suspended matter, mainly in the Maximum Turbidity Zone, inhibit light penetration and macroalgae photosynthesis. Hence, Wilkinson *et al.* (1995, 2007) identified three algal zones (A, B, and C), within British estuaries. Zone A is the sheltered open coast at the mouth of the estuary and the lowermost part of the estuary, with 50–100 seaweed species; zone B contains the lower estuarine flora (fucoid-dominated), in the more saline part of the estuary, with richness reduced to 10–40 species; and zone C contains the upper estuarine flora, with mat-forming opportunists, and fucoids completely absent, with only 0–10 species.

Notably, as an example of monitoring macroalgae for management, the extent of intertidal or floating mats of green macroalgae, such as *Ulva* spp., is used. Such mats are a symptom of eutrophication and more notable for preventing wading birds feeding on infaunal invertebrates and for creating anoxic conditions under the mats (de Jonge and Elliott, 2002). As an example, the spatial extent of *Ulva* mats on Seal Sands in the Tees Estuary, England, has been used both as an indication of reduction of toxicants in the estuary and the interference with estuarine processes (Fig. 2). With an increasing water quality and the reduction of toxic persistent pollutants, the macroalgae capitalized on the nutrient levels in the estuary and the changing sediment and hydrodynamic conditions.



**Fig. 2** The coverage of *Enteromorpha* on Seal Sands, Tees Estuary, NE England from 1992 to 2001 (data provided by English Environment Agency).

In this section, methods including macroalgae together with other biological quality elements (i.e., phytoplankton and benthos) have not been considered. However, some examples of this approach are the TWQI (Giordani *et al.*, 2009), ASSETS (Bricker *et al.*, 2003, 2007), and the Chesapeake Bay health index (Williams *et al.*, 2009). Methods using macroalgae alone include different metrics or indicators in the quality assessment approach (Table 4). Hence, most of the methods include some measurement of richness (even as presence/absence) and abundance (generally as percentage of cover, and also as biomass). Several methods use the ecological or functional groups (see Orfanidis *et al.*, 2001) or the presence of indicator species (opportunistic or sensitive) as a way of detecting disturbances in the studied area. Very few methods use other metrics, such as the algae penetration into the estuary (Wilkinson *et al.*, 2007), the depth range (Selig *et al.*, 2007), or the Rhodophyceae/Chlorophyceae ratio (Sfriso *et al.*, 2007) (Table 4).

All of these methods have been developed since the year 2000, most of them being proposed within the European WFD and have been inter-calibrated among European countries (European Commission, 2018). As advocated by Borja and Dauer (2008), any index developed for quality assessment should be validated, including (1) testing of the index using an independent data set, different from the index development data set (calibration data set); (2) setting an a priori correct classification criteria; and/or (3) presentation of a strong *a posteriori* justification for use based upon best professional judgment. However, very few of the above-mentioned methods have been validated or used outside the countries where the method was developed. Hence, the ecological evaluation index (EEI) has been used in other countries of the Mediterranean (as an example, see Orlando-Bonaca *et al.*, 2008 in Slovenia); the opportunistic macroalgal blooms approach has been used in Portugal (Patrício *et al.*, 2007) and New Zealand (Plew *et al.*, 2020); and the macrophyte quality index (MaQI) has been validated in Venice Lagoon (Sfriso *et al.*, 2009) and Mar Menor Lagoon (García-Sánchez *et al.*, 2012). The scarce validation of some methods, due to the recent use in estuarine quality assessment, has been compensated in the last decade by the European inter-calibration exercises (European Commission, 2018).

All these methods link eutrophication pressure and macroalgae response, as responses to other anthropogenic pressures such as hydro-morphological changes within the estuary, harmful substances discharge, etc. (but see Melville and Pulkownik, 2006). Krause-Jensen *et al.* (2008) studied the relationships linking distribution and abundance of marine vegetation (both seagrasses and macroalgae) to eutrophication, by collating 73 relationships originating from 38 publications from the period 1982–2007 and covering a wide range of ecosystems (both coastal and estuarine). Of the 73 relationships, 38 link vegetation responses significantly to eutrophication pressure as expressed by nutrient concentration or water transparency, 18 link the responses to

**Table 4** Methods to evaluate the status of macroalgae in estuarine water bodies. Note: the proposal from Selig *et al.* (2007) is for Baltic coasts but has been used in habitats similar to estuarine areas.

Method/Approach	Country using method	Indicators/metrics	Reference
Macrophyte assessment	USA	Richness; cover (%); density, biomass	Gibson <i>et al.</i> , 2000
Macroalgae metrics	USA	Cover (%), biomass	Sutula <i>et al.</i> (2014)
Ecological Evaluation Index (EEI-c)	Greece	Mean abundance (%); two ecological (functional) groups	Orfanidis <i>et al.</i> , 2001, 2003; European Commission, 2018
Macroalgae assessment	Spain	Richness; presence of pollution indicator species; cover (%); ratio between green algae and the rest	Borja <i>et al.</i> , 2004a
Fucoid presence	United Kingdom	Presence/absence; algae penetration into the estuary	Wilkinson <i>et al.</i> , 2007
Opportunistic macroalgal blooms	United Kingdom; Ireland	Biomass; cover (%); opportunistic	Scanlan <i>et al.</i> , 2007
Total and opportunistic algal cover	Denmark	Macroalgal cover (%); opportunistic fraction	Krause-Jensen <i>et al.</i> , 2007
Depth distribution	Germany	Presence; depth range (m); cover (%)	Selig <i>et al.</i> , 2007
Macrophyte Quality Index (MaQI)	Italy	Presence/absence; Rhodophyceae/Chlorophyceae ratio	Sfriso <i>et al.</i> , 2007; European Commission, 2018
Opportunistic Macroalgae Metrics	Portugal	Opportunistic macroalgae cover (%) and areal extent	Patricio <i>et al.</i> (2007)
Macroalgal Bloom Assessment (TWOGA)	France	Blooms of species	European Commission, 2018
Opportunistic Green Macroalgal Abundance (OGA)	Ireland	Opportunistic Chlorophyceae species abundance	European Commission, 2018
Opportunistic Macroalgal Blooming Tool (OMBT)	United Kingdom	Biomass; cover (%); opportunistic	European Commission, 2018
Opportunistic Macroalgal Blooming Tool (OMBT)	New Zealand	Biomass; cover (%); opportunistic (adapted from UK)	Plew <i>et al.</i> , 2020
Ulva bioindicators	New Zealand	Ulva cover (%) and biomass	Barr <i>et al.</i> (2020)

combinations of eutrophication pressure and ecosystem characteristics, and nine link the responses to ecosystem characteristics alone. The compilation demonstrates that macroalgae generally respond quantitatively to changes in eutrophication pressure by growing deeper, being more abundant, and more widely distributed in clear waters with low nutrient concentration as compared with more turbid and nutrient-rich ecosystems.

## Angiosperms

Seagrasses are marine angiosperms that form meadows in near-shore and shallow brackish or marine waters, in temperate and tropical regions, and provide important ecosystem services such as carbon sequestration, coastal protection, and fish protection and nursery (Nordlund *et al.*, 2016; Whitfield *et al.*, 2022). Seagrasses suffer many human pressures and for decades have experienced a decline in the area occupied (Short *et al.*, 2006); however, in recent times, a trend reversal has been detected in many locations around the world, probably due to better management and restoration actions and the end of the disease impacting *Zostera marina* in northern Atlantic estuaries and coastal waters (de los Santos *et al.*, 2019; Dunic *et al.*, 2021).

As a function of complex biogeochemical processes (Harris, 1999; Hansen *et al.*, 2000; Eyre and Ferguson, 2002), seagrass meadows are extremely productive systems, exhibiting high biodiversity and supporting complex food webs, as well as constituting a habitat refuge for a number of organisms (Orth, 1992; Boström and Bonsdorff, 2000; Borum *et al.*, 2005). Changes in seagrass areas are often a symptom of major changes in environmental characteristics and therefore constitute an important indicator for assessing the state of the environment.

Seagrasses respond to natural variations in light availability in relation to water turbidity, nutrient and trace element availability (Duarte, 1995), grazing pressure, marine pests and pathogens (Giesen *et al.*, 1990), weather patterns, and episodic floods and cyclones. Due to their high minimum light requirements, the most widespread and pervasive cause of seagrass decline is a reduction in available light, which may be due to turbidity events during floods, harbor works which enhance suspended sediment loads, or elevated nutrient concentrations that cause phytoplankton proliferation (Boström *et al.*, 2002). In addition, phytoplankton and fast-growing macroalgae are better competitors for light than benthic plants, and their biomass can shade seagrasses during progressive eutrophication (Fourqurean and Zieman, 1991; Frost-Christensen and Sand Jensen, 1992; Walker and McComb, 1992; Dennison *et al.*, 1993; Terrados *et al.*, 1999). Similarly, seagrass species can suffer from the toxic effects of trace metal contamination (Prange and Dennison, 2000) or, in other cases, bioaccumulate it, which can have ramifications for grazers.

Seagrasses can also change in response to physical disturbances (e.g., ports and marinas, temperature increase, and fishing pressure represented by commercial and recreational harvesting of fish and shellfish) which may cause direct damage to plants. Finally, removal of forage or predator species can also have detrimental effects that cascade through seagrass associated food chains.

The causes for changes in seagrass areas may therefore be natural or anthropogenic, and it is often difficult to differentiate what changes are attributable to human activities. Nevertheless, marine angiosperms appear to be robust ecological indicators of estuarine and coastal waters and sediment quality, and because of their susceptibility to human disturbances were included as one of the biological quality elements included in the implementation of the European WFD.

To establish reference conditions for marine angiosperms is rather difficult as meadows are extremely variable in terms of spatial cover, density, and species composition. Moreover, these characteristics depend on the geographic location and the hydrodynamic regime, and therefore reference conditions must be defined using the typology of the habitat where the meadow occurs (Foden and Brazier, 2007). To accomplish these complex tasks, and as shown by the recommendations in the WFD for defining reference conditions, four methods can be used (Borja *et al.*, 2012b):

1. *Historical data.* When existing, these are normally relatively easy to obtain, although they may be heterogeneous and more often do not correspond to any metrics. Data quality may therefore be poor or even unknown. Of course, in many cases, such data do not exist.
2. *Expert judgment.* This is usually the way to integrate and interpret historical data, the main inconvenience being the expert subjectiveness (Dauvin *et al.*, 2010).
3. *Modeling.* This is region specific and requires large data sets for calibration and validation.
4. *Using a physical control area.* That is to choose an area similar in its physical characteristics, situation, latitude and aspect but without anthropogenic stressors,

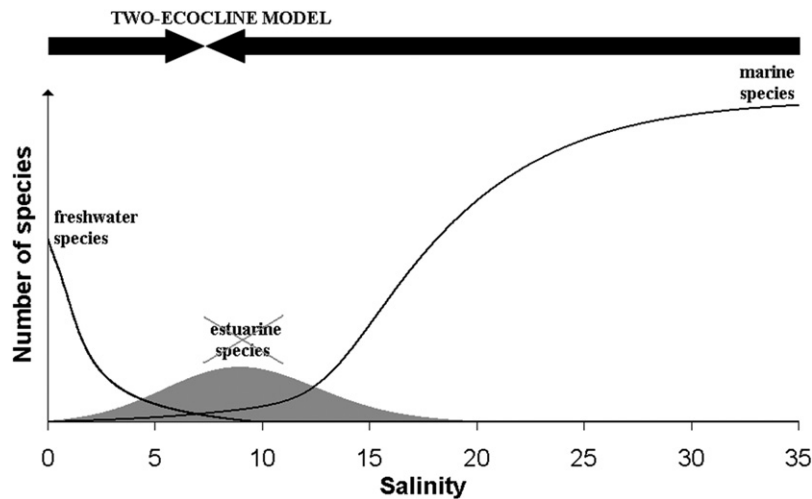
Although all these approaches have some limitations, their common idea is that a reference point cannot be a disturbed point (Gerritsen, 2005; Moreno *et al.*, 2001; Reynoldson *et al.*, 2001), and therefore determining such points must be done based on the prevalence of high ecological quality parameters. The metrics required are usually: (1) abundance, expressed as density in  $\text{g m}^{-2}$  of dry weight, possibly distinguishing between rooted and aerial parts of the plants; (2) cover, expressed as the area in  $\text{m}^2$  or ha occupied by the meadow, and (3) taxonomic composition.

Aerial photography, satellite imagery, and systematic towed video surveys can be used to map the extent of seagrass coverage in some coastal waterways. In addition, there have been significant advances in determining seagrass properties other than coverage from satellite imagery (e.g., species composition and biomass), although local ground confirmation of the taxonomy to the genus level is advised. Nevertheless, high levels of turbidity may constitute a constraint to the application of this methodology in tide-dominated coastal waters (e.g., deltas, estuaries, and tidal creeks; Larkum *et al.*, 2007).

Seagrasses have been used as quality indicators under accepted protocols. Hence, established in 2001, SeagrassNet is a monitoring program for seagrasses worldwide, which uses a standardized protocol for detecting change in seagrass habitat to capture both seagrass parameters and environmental variables (Short *et al.*, 2006). This program is designed to statistically detect change over a relatively short time frame (1–2 years) through quarterly monitoring of permanent plots. As of 2022, SeagrassNet operates in 33 countries at 126 sites; at each site, a permanent transect is established and a team of people from the area collects data, which are sent to the SeagrassNet database for analysis (Short *et al.*, 2006). Moreover, after the publication of the WFD, several methods have been published in Europe for angiosperm quality assessment. Some of them focus on coastal species (Romero *et al.*, 2007; Montefalcone, 2009), but many others have been implemented for transitional and low-salinity coastal waters (Krause-Jensen *et al.*, 2005; Best *et al.*, 2007b; Cabaco *et al.*, 2007; Foden and de Jong, 2007; Selig *et al.*, 2007; García *et al.*, 2009; García-Marín *et al.*, 2013; Irving *et al.*, 2013; Marbà *et al.*, 2013; Neto *et al.*, 2013) and most of them have already been intercalibrated (European Commission, 2018).

## Macroinvertebrates

Transitional systems between freshwater and marine systems are affected by natural and anthropogenic stressors. The main stressor is salinity, which organizes species distribution into three main groups: (1) in the upper part of the estuaries, freshwater species colonize this area affected mainly by the dynamic tidal range; (2) the lower part of the estuaries, a polyhaline zone where mainly marine species occur; and (3) between these two extremes, where estuarine associated species are adapted to life in variable intermediate salinity ranges. This principle was mainly expressed by the Remane diagram and modifications thereof (Remane, 1934; Whitfield *et al.*, 2012; Smyth and Elliott, 2016). In addition, estuaries have been regarded as a complex of ecotones, each of which gives a discontinuity in quality assessment and classification schemes (Basset *et al.*, 2013a). This follows from Attrill and Rundle (2002) who introduced the concept of ecocline (gradient of change) for estuarine ecosystems. They suggested that the estuary represents a two-ecocline model, with a first ecocline from sea to mid-estuary where there is a salinity decrease overlapping with an ecocline from river to mid-estuary where salinity increases. Hence, there is a double gradient of decreasing species from the river to the estuaries, and from the marine part to the estuaries. In the mesohaline stretch, but mainly in the oligohaline zones, only the marine-derived species live at the edge of their range along the sea-estuary ecocline and freshwater-derived species at the limit of the river-estuary ecocline. Such organisms were considered the ‘true estuarine organisms’ represented in the Remane diagram (Fig. 3), but if the estuary is seen as a two-ecocline system, this category of



**Fig. 3** Remane's diagram of hypothetical distribution of benthic invertebrate diversity along a marine-freshwater salinity gradient (Remane, 1934), redrawn following the two ecocline model (from Attrill and Rundle, 2002). The estuarine species are removed, the number of freshwater species is lower than that of marine species.

organisms disappears to be replaced by two gradients, running from river or sea into the mid-estuary (Fig. 3) (Attrill and Rundle, 2002). The oligohaline zone appears as the poorest, whereas the mesohaline shows intermediate values; the polyhaline zone shows generally high species richness supplied from the marine surrounding zone (Dauvin and Desroy, 2005; de Paz *et al.*, 2008b). The tidal freshwater zone appears impoverished in comparison to the tributary rivers or from the upper part of the estuary in the typical freshwater part of the system.

Biodiversity changes in relation to the size of the estuary, the largest being the richest and the smallest the poorest, but also in relation to the number of other natural biotope variety, that is, diverse type of substratum, tidal range, depth range from the intertidal zone to the deep subtidal zone in the channels, and hydrodynamism. The variety of biotopes creates, in turn, a variety of ecological conditions which overlap those of single salinity; typically, estuaries show a mosaic of different biotopes (Escaravage *et al.*, 2004; Dauvin and Ruellet, 2009). Moreover, dredging activities, dyke constructions and other harbor works play an important role in estuarine soft-bottom biotope evolution, and creation of hard bottom which can be colonized by algae and invertebrate as a new biotope (Dauvin and Ruellet, 2009).

Basset *et al.* (2013a) considered the estuary as an even greater complex of ecotones, i.e. created by discontinuities in physical, chemical and biological characteristics. This complexity of ecotones incorporates those from the sea to the estuary, the estuary to the freshwaters, the bed to the water column, the water column to the surface, the surface to the air, and the sides to the adjacent terrestrial system. Each of these discontinuities needs to be incorporated into any analysis of status.

There is a high level of spatial and temporal variability among macroinvertebrate community characteristics which must be accommodated in any environmental assessment. However, each measurement of any parameter has constraints, with inherent variability requiring many replicates to detect a given signal (Franco *et al.*, 2015), and the behavior of indicators needing to be rigorously tested (Quintino *et al.*, 2006; Basset *et al.*, 2013b). For example, the estimation of species richness depends on the number of observations and the level of taxonomic identification, precision and accuracy must be separated, determined and quantified in any assessment (Elliott *et al.*, 2022b). For example, among the oligochaetes, most researchers do not identify them to species level, but richness can reach more than 20 species in anthropogenically modified estuaries, e. g., the Seine (Dauvin and Ruellet, 2009). An inventory of all aquatic invertebrates in the eastern part of the Bay of Seine, and the region of freshwater influence, was performed to establish the biodiversity pattern (Ruellet and Dauvin, 2008). Such an inventory shows that the region is highly diverse: 1485 taxa of aquatic invertebrates were encountered, including 77 genera, five families, and one subclass (Ruellet and Dauvin, 2008). The compiled data show that the distribution of the invertebrate species in the Seine River Estuary follows a two-ecocline model, as proposed by Attrill and Rundle (2002).

In northern European waters, two main benthic communities occur in the soft bottom of the marine part of estuaries: (1) an *Abra alba* muddy fine sand community, which impoverished from the marine pole to the mesohaline zone, and (2) a *Macoma balthica* mud community, common on the tidal flat in the mesohaline and oligohaline part, and at subtidal depth (Dauvin and Desroy, 2005). Common species, such as the polychaete *Hediste diversicolor*, the bivalve *Macoma balthica*, the amphipod *Corophium* spp. and spionid polychaetes such as *Pygospio elegans* and *Streblospio* spp., and cirratulids such as *Aphelochaeta* spp., have a very large latitudinal distribution within the Northern Hemisphere and are dominant in this community. In turn, within southern European estuaries, there is a shift in the composition of the species, with those typical of colder water disappearing and those related to warm waters becoming more prevalent (Borja *et al.*, 2004a).

Invasive species is a common problem in estuaries worldwide, and European estuarine communities have also been colonized by numerous introduced species, some of which show proliferation, such as the bivalve *Dreissena* spp., *Corbicula* spp., the shrimp



*Palaemon macrodactylatus*, the bristle worm *Hypania invalida*, and in the upper estuary, *Crangonyx pseudogracilis* and *Dikerogammarus villosus*. In general, community vulnerability to invasions is ascribed to combinations of several factors such as the presence of vacant niches, habitat modification, and disturbances before and during invasions (Olenin *et al.*, 2011). Although the link between the biodiversity of communities and their vulnerability to invasions remains to be proved, invasion potential is known to increase if a community lacks certain species or species with certain biological traits, which ought to be present under normal conditions. A new hypothesis linking the various explanations of increased invasion potential is that of fluctuating resource availability such as an increased number of unused resources (Davis *et al.*, 2000).

The estuarine communities are also characterized by high contrast between zones with poor macroinvertebrate abundance and therefore biomass and production, and zones with high abundance, biomass and secondary production (Dauvin and Desroy, 2005; Elliott and Whitfield, 2011). Heip *et al.* (1995) underlined the positive relationship between pelagic primary production and macrobenthic biomass in estuaries. In general, benthic production represents a high source of food for fish and birds, and is usually the more productive zone in estuaries. The conservation and restoration of tidal flats, due to their high importance in terms of estuarine functionality, are therefore often underlined in estuarine management.

The implementation of the European WFD has provoked a large debate on the use of benthic bio-indicators and indices to determine the quality of the estuarine (transitional) and coastal waters in terms of the establishment of ecological quality status (Borja, 2005; Borja *et al.*, 2009c; Dauvin and Ruellet, 2009). Nevertheless, assessing estuarine quality by macroinvertebrates remains difficult due to the high variability of natural conditions in such ecosystems; moreover, estuaries are highly modified in many countries. There are numerous definitions of indicators but, essentially, they are quantitative or qualitative parameters, attributes or variables which characterize the environmental status and/or the pressures which may affect that status (Rossberg *et al.*, 2017). They are selected according to their ability to characterize the overall state of an ecosystem, thus simplifying an extremely diverse range of metrics to a small group of environmental integrative indicators (Aubry and Elliott, 2006). Moreover, indicators need to provide a valuable means of communication to stakeholders and policymakers (Aubry and Elliott, 2006). Furthermore, ecological indices are quantitative tools in simplifying, through discrete and rigorous methodologies, the attributes and weights of multiple indicators with the intention of providing broader indication of a resource, or the resource attributes, being assessed (Pinto *et al.*, 2009).

A clear distinction between indices and indicators must be made. Any measure that allows the assessment and evaluation of the system status (descriptive indicators, environmental quality indicators, and performance indicators), as well as assessment of any management actions for conservation and preservation that occur in the ecosystem, is considered an indicator; indices are considered one possible measure of a system status (Dauvin, 2007; Dauvin and Ruellet, 2009).

The advantages of using macroinvertebrates to assess ecological quality are multiple: (1) these organisms are relatively sedentary, meaning that they cannot avoid deteriorating water/sediment quality conditions; (2) some have relatively long-life spans but the admixture of r- and k-strategists can give vital information; (3) they comprise diverse species that exhibit different tolerances to stress; (4) reflect the hydrodynamic and sedimentological conditions, and (5) they play an important role in cycling nutrients and materials between the underlying sediments and the overlying water column (Dauvin, 2007; Gray and Elliott, 2009). Still, Rakocinski and Zapfe (2005) have underlined several disadvantages of the existing benthic indices:

1. they represent a static expression of an ecological condition;
2. they are not explicitly linked to changes in ecological function;
3. they may not be specific with respect to different kinds of stressors;
4. they are subject to underlying taxonomic changes across estuarine gradients;
5. their use can be labor intensive, and
6. they are not applied consistently across biogeographic provinces.

Univariate indices were largely developed for marine or coastal ecosystems; however, new functional and multimetric indices are being developed for macroinvertebrates (see Díaz *et al.*, 2004; Blanchet *et al.*, 2008; Borja and Dauer, 2008; ICES, 2008; Pinto *et al.*, 2009; Borja *et al.*, 2015; Thrush *et al.*, 2021).

The benthic indicators and indices can be classified into four categories: (1) based on diversity, (2) based on ecological groups, (3) based on functional groups such as biological traits, and (4) indices synthesizing several other indicators. Most of the recently developed indices in the second category are based on dividing soft benthic species into previously defined ecological groups and then determining the respective proportion of the different groups in the benthic communities (Borja *et al.*, 2000). They provide information about the relative abundances of the sensitive species faced with increasing organic matter in the sediment and those of the species that are resistant or indifferent to such increases, or even favored by such conditions (e.g., the opportunistic species that proliferate when the sediment is rich in organic matter which often results in the sensitive species disappear). However, the main problem is that most of the indices, which aim to determine anthropogenic stress, relate to abundances of stress-tolerant species, which may also be tolerant of natural stressors such as in estuaries (see Section “The Estuarine Quality Paradox and Environmental Homeostasis”). Similarly, many indices described relate to anthropogenically organic-rich systems, whereas estuaries are naturally organic-rich systems.

Weisberg *et al.* (1997) developed a multimetric benthic index of biotic integrity (B-IBI) (for acronyms within this section, see Table 5) which was based on 17 candidate measures. These included primary and derived community indices (species richness,

abundance, diversity, etc.) as well as the percentage of abundance of different functional groups. By accommodating differences in salinity and substratum, comparing test and reference areas, and by using a rank-scoring system for the deviation by different metrics to reference conditions, Weisberg *et al.* (1997) were able to separate stressed benthic areas from reference conditions.

Other multicriteria methods have been developed in European waters, those based upon AMBI and BQI (see Borja *et al.*, 2009c) being the most successful. As an example, Quintino *et al.* (2006) analyzed data from the Portuguese coasts to produce univariate indices used for the WFD: abundance, species richness, biomass, Margalef index, Pielou evenness index, Shannon–Wiener index, Simpson’s index, AMBI and its reciprocal (1/AMBI), EQR (calculated according to the UK multi-metric approach), BQI, A/S, and B/A. They found that some of the indices gave an underrepresentation and others an overrepresentation of the ecological status. They cautioned that this was not merely of academic interest as misclassifying a good status area as being of moderate status could result in a large expense to make unnecessary remedial work (Gray and Elliott, 2009). It was particularly notable that many of the indices needed refining to cope with the naturally low diverse areas in estuaries and other transitional areas. Hence, when these indices were applied to highly stressed natural estuarine or lagoonal environments, the low species richness and dominance of a few tolerant species in these systems presented a challenge to the application of the biotic indices (Puente and Díaz, 2008).

Similarly, based on the B-IBI, a multimetric approach was developed for Arcachon Bay, an Atlantic lagoon, to correctly assess the benthic ecological status of an area that was physically perturbed by a deposit of dredged sediment (Lavesque *et al.*, 2009). This approach, called the macrobenthic index for sheltered systems (MISS), incorporated the natural variability of a set of variables describing the biological integrity of the reference conditions. These are classified into three groups: (1) community structure (abundance, biomass, number of species, Shannon’s diversity, and evenness); (2) trophic composition (grazer, selective deposit feeder, nonselective deposit feeder, suspension feeder, and carrion feeder), and (3) pollution indicators (AMBI, benthic opportunistic polychaetes/amphipods ratio (BOPA), W-statistic, and abundance of sensitive, and tolerant and opportunistic species) (Lavesque *et al.*, 2009). The MISS approach clearly proved that no single biotic index can correctly assess the ecological status of a given benthic invertebrate community.

**Table 5** shows an updated list of benthic indices found in the literature (mainly from Díaz *et al.*, 2004; Borja and Dauer, 2008; ICES, 2008; Pinto *et al.*, 2009; Borja *et al.*, 2009c; Ducrottoy *et al.*, 2011; Borja *et al.*, 2015; Dauvin, 2018). Hence, there is a plethora of univariate and multivariate indices and methods to assess the status or the integrity of estuaries using macroinvertebrates, sometimes associated with other environmental or biological variables. Most of them, as the indices of diversity, are long-established, whereas others have been published more recently and often used in a limited number of cases. AMBI and multivariate AMBI (M-AMBI), BQI (and its various adaptations), B-IBI, and infaunal trophic index (ITI), Polychaete/Amphipod ratios (BOPA, BO2A) are among the more universal methods used in several geographical zones, not only in European and American waters but also in other coastal and transitional zones around the world, that is, Mediterranean Sea, Indian Ocean, South- and North-American estuaries, Asia, etc. (see ICES, 2008, but also Borja *et al.*, 2015, 2019; Dauvin, 2018).

Some supplementary developments on macroinvertebrates include the species level as indicators. Ducrottoy *et al.* (2011) highlighted that indicator species can be considered to reflect the quality status of their habitat, whether they are present or absent from samples. There are many examples of such indicator species in the marine environment, particularly those living in sediments, that is, the polychaete *Capitella capitata* is often considered as a good indicator of organic enrichment. In this scope, dominant, keystone, sentinel, introduced, invasive species can be used as possible indicators. A specific group can be also used as the spionid polychaetes as environmental indicators in the Tampa Bay, Florida (Dix *et al.*, 2005).

Nevertheless, research can be continued to reexamine and adapt the different index thresholds for transitional waters (Borja *et al.*, 2009c). Over a decade ago, we stated that additional research was needed (Borja *et al.*, 2009e), including the need to take physical disturbances into account (e.g., dynamic forcing of the systems) and to favor multicriteria approaches, including the indices that are based on the structure and production of the communities, in the development of an operating report. Some studies have been published since then, covering those topics (e.g., Borja *et al.*, 2019; Brugnoli *et al.*, 2021). Moreover, testing indices with different human pressures have been undertaken in many cases and geographical areas around the world (Calabretta and Oviatt, 2008; Chainho *et al.*, 2008; de Paz *et al.*, 2008a; Josefson *et al.*, 2008; Thrush *et al.*, 2008; Borja *et al.*, 2009d, 2015, 2019; de-la-Ossa-Carretero *et al.*, 2009; Franco *et al.*, 2015; Dauvin *et al.*, 2017; Dauvin, 2018; Berthelsen *et al.*, 2018; Poikane *et al.*, 2020; Thrush *et al.*, 2021).

## Fishes

The survival and development of healthy estuarine fish communities require good environmental (physical, chemical, and biological) conditions (Marchand *et al.*, 2002; Whitfield *et al.*, 2022). Fishes therefore can provide a good indication of estuarine health or condition (Cabral *et al.*, 2022; Elliott *et al.*, 2022a,b,c; Franco, *et al.*, 2022). The use of fishes includes assessments at various levels of biological organization – the subcellular level, cellular level, organ level, individual level, population level, and community level (Table 6).

In general, measures restricted to lower levels of biological organization, such as molecular, biochemical, cellular, or physiological changes that occur at the subcellular, cellular, and organ levels are referred to as ‘biomarkers’, whereas changes that occur at higher levels of biological organization (individual, population, and community) are more usually referred to as ‘bioindicators’ (Lawrence and Hemingway, 2003; Lam and Gray, 2003; Franco *et al.*, 2022; Elliott *et al.*, 2022b). Biomarkers typically are used in the detection of pollution or contaminants; they respond rapidly to environmental conditions and as such are effective early warning systems of potential problems before they appear at higher levels of biological organization.

**Table 5** Indices for assessing environmental quality based on the structure of macroinvertebrates in transitional waters.**UNIVARIATE INDICES****Descriptors**

Number of species (species richness), Abundance (A), Biomass (B)

**Indices of diversity**

Shannon-Wiener Diversity Index ( $H'$ ) (Shannon and Weaver, 1949)  
 Simpson's indices of dominance, diversity and evenness (Simpson, 1949)  
 Brillouin indices of diversity and evenness (Brillouin, 1956)  
 Pielou evenness index ( $J'$ ) (Pielou, 1966)  
 Margalef's index (Margalef, 1968)  
 Hurlbert index (Hurlbert, 1971)  
 Hill's diversity numbers and evenness measures (Hill, 1973)  
 BPI: Benthic Pollution Index (Leppäkoski, 1975).  
 W-Statistic Index (Warwick and Clarke, 1994)  
 Taxonomic diversity index and Taxonomic distinctness (Warwick and Clarke, 1995)

**Graphical methods**

RFD: Rank-Frequency Diagram (Frontier, 1977)  
 K-dominance curves (Lamshead *et al.*, 1983)  
 ABC curves (Warwick and Clarke, 1994).

**Ecological groups**

Indice Annéidien de Pollution (Bellan, 1980)  
 Biotic Index (Hily, 1984)  
 MMI: Macrofauna monitoring index (Roberts *et al.*, 1998)  
 AMBI: AZTI's Marine Biotic Index (Borja *et al.*, 2000)  
 BENTIX (Simboura and Zenetos, 2002)  
 ISI: Indicator Species Index (Rygg, 2002)  
 IE2C: Indice Biotique et Indice d'Evaluation de l'Endofaune Côtière (Grall and Glémarec, 2003).  
 BOPA: Benthic Opportunistic Polychaetes / Amphipods ratio (Dauvin and Ruellet, 2007)  
 MEDOCC: Mediterranean Occidental Index (Pinedo and Jordana, 2007)  
 BIOTRESS: based on the abundance of 5 polychaetes (Ugland *et al.*, 2008)  
 BITS: Benthic Index based on Taxonomic Sufficiency (Mistri and Munari, 2008)  
 BO2A: Benthic Opportunistic Annelids / Amphipods ratio (Dauvin and Ruellet, 2009; Dauvin, 2018)  
 ZKI: Macrozoobenthos Community Index (Lauringson *et al.*, 2012)  
 NSI: Norwegian Sensitivity Index (Rygg and Norling, 2013)

**Size spectra**

ISD: Index of Size Distribution (Reizopoulou and Nicolaidou, 2007)  
 ISS: Index of Size Spectra Sensitivity (Bassett *et al.*, 2012)

**FUNCTIONAL INDICES**

ITI: Infaunal Trophic Index (Word, 1979)  
 EQI: Ecofunctional Quality Index (Fano *et al.*, 2003)  
 BTA: Biological Trait Analysis (Bremner *et al.*, 2006)

**MULTI-METRIC INDICES**

CoP: Pollution Coefficient (Satsmadjis, 1982)  
 BQI: Biological Quality Index (Jeffrey *et al.*, 1985)  
 Organism sediment index (Rhoads and Germano, 1986)  
 RTR: Infauna Ratio-to-Reference of sediment Quality Triad (Chapman *et al.*, 1987)  
 BIEC: Benthic Index of Estuarine Condition (Weisberg *et al.*, 1993)  
 B-IBI: Benthic Index of biotic integrity (Ranasinghe *et al.*, 1994)  
 BCI: Benthic condition Index (Engle *et al.*, 1994)  
 BHQ: Benthic Habitat Quality (Nilsson and Rosenberg, 1997)  
 VPBI: Virginia province benthic index (Paul *et al.*, 2001)  
 NQI: Norwegian Quality Index (Rygg, 2002)  
 IEI: Index of environmental integrity (Paul, 2003)  
 BQI: Benthic Quality Index (Rosenberg *et al.*, 2004)  
 IQI: Infaunal Quality Index (Prior *et al.*, 2004)  
 INES: fuzzy index of environmental integrity for transitional environments (Mistri *et al.*, 2005)  
 MarBIT: Marine Biotic Index Tool (Meyer *et al.*, 2006)  
 DKI: Danske Kvalitet Indeks (Borja *et al.*, 2007)  
 BEQI: Benthic Ecosystem Quality Index (Van Hoey *et al.*, 2007)  
 BBI: Brackish water Benthic Index (Perus *et al.*, 2007)  
 DAPHNE (Forni and Occhipinti-Ambrogi, 2007)

FINE: Fuzzy Index of Ecosystem Integrity (Munari and Mistri, 2008)  
 MISS (Macrobenthic Index for Semi-sheltered Systems) (Lavesque *et al.*, 2009)  
 BQI modified: Benthic Quality Index (Leonardsson *et al.*, 2009)  
 MIBIIN: Multimetric index of the Balearic Island based on invertebrate communities (Lucena-Moya and Pardo (2012)  
 TUBI: Turkish Benthic Index (Çinar *et al.*, 2015)  
 BEQI-2: Benthic Ecosystem Quality Index-2 (van Loon *et al.*, 2015)  
 BENFES: Benthic Families Ecological Status Index (Sánchez-Moyano *et al.*, 2017)

#### MULTIVARIATE APPROACHES, PACKAGES AND MODELS

PLI : Pollution Load Index (Jeffrey *et al.*, 1985)  
 CDI: Community Disturbance Index (Massart *et al.*, 1996)  
 BRI: Benthic Response Index (Smith *et al.*, 2001)  
 M-AMBI: Multivariate-AMBI (Borja *et al.*, 2004a; Muxika *et al.*, 2007)  
 PRC: Principal Response Curves (Pardal *et al.*, 2004)  
 TICOR: Typology and Reference Conditions for Portuguese and Coastal Waters (Bettencourt *et al.*, 2004)  
 Combination of indices: B-IBI and TICOR (Chainho *et al.*, 2008)  
 APBI: Acadian province benthic Index (Hale and Helshe, 2008)  
 BAT: Benthic assessment tool (Pinto *et al.*, 2009)

**Table 6** Levels of biological organization in fishes and examples of measures used at each level.

<i>Biological organization</i>	<i>Example</i>
Sub-cellular	Bioaccumulation of contaminants (e.g. metals, PAHs)
Cellular	Enzyme activity, stress proteins, DNA integrity
Organ	Liver histopathology
Individual	Condition, disease, physical deformities, parasite load
Population	Abundance, age/size structure, indicator species
Community	Species diversity, species abundance/biomass, guild composition

#### **Subcellular**

In most monitoring programs, an assessment of anthropogenic inputs of pollutants into estuaries usually includes chemical analyses of the water and sediments. However, the biological uptake (bioaccumulation) of these pollutants is probably of more interest than the absolute concentration in the water or sediments; this is particularly relevant if the species in question is used for human consumption (Marchand *et al.*, 2002; Cabral *et al.*, 2022). Chemical contaminants may enter fishes in several ways: either by direct uptake from the water (via the gills), consumption of contaminated sediment, or via the food chain by consumption of contaminated prey. Contaminants that cannot be excreted tend to accumulate in the animal tissues, especially those of the higher trophic levels, which therefore can be used to directly measure the occurrence and levels of various chemical compounds such as metals and organic pollutants (Souza *et al.*, 2022). Many marine juvenile fishes utilize estuaries as nursery areas and contamination by pollutants (metals, pesticides, etc.) may occur during this intensive feeding and growth period (Marchand *et al.*, 2002; Whitfield *et al.*, 2022). As many of these species are commercially exploited, their health, quality, and survival are of direct concern to man. The chemical analyses of pollutants in the tissues of fishes can provide an indication of actual or potential problems (Cabral *et al.*, 2022).

The accumulation of chemical pollutants and their bioaccumulation, however, may differ between organisms, the individual tissues of an organism, and between chemicals. A variety of internal and external factors can affect the biological uptake of pollutants, and these include physiological factors (variations in reproduction and nutrition), environmental factors (salinity, temperature, and pH of the water, the presence of other chemicals/metals in solution, and the geochemistry of the local area), and the chemical nature of binding of the pollutant (Elliott *et al.*, 2002; Marchand *et al.*, 2002; Cabral *et al.*, 2022). Chemical pollutants, particularly heavy metals, may also be present in the tissues of non-polluted organisms, and in order to assess the level of bioaccumulation of metals in organisms, baseline data are necessary on metal concentrations in individuals from clean areas (Marchand *et al.*, 2002).

#### **Cellular**

Although the analysis of fish tissues can directly measure the bioaccumulation of various pollutants (or xenobiotics), exposure to such compounds may trigger certain biochemical responses which serve either to metabolize the chemical, or to store it, thereby preventing interference with essential biochemical reactions within the cell (Elliott *et al.*, 2002; Lawrence and Hemingway, 2003). Some chemicals bind proteins or enzymes that are concerned with their metabolism and biotransformation (Lam and Gray, 2003). The measurement of these enzymes or intermediates can signal the presence of certain chemicals or even toxic effects, especially where intermediate stages in detoxification can be carcinogenic (Elliott *et al.*, 2003). Many of these enzymes are also

specific to certain classes of compounds such as the induction of metallothionein synthesis by exposure to heavy metals (mercury, cadmium, lead, zinc, etc.). The mixed-function oxygenases (MFOs) are involved in the biotransformation and elimination of chlorinated and aromatic hydrocarbons such as polychlorinated biphenyls (PCBs), dioxins, and polycyclic aromatic hydrocarbons (PAHs) (Vasseur and Cossu-Leguille, 2003). Ethoxyresoufin O-deethylase (EROD) enzyme activity is widely used to detect exposure to PCBs and PAHs. The inhibition of acetylcholinesterase (AChE) enzyme activity also represents a specific marker of exposure to agricultural pesticides such as organophosphate and carbamate insecticides (Corsi *et al.*, 2003; Lam and Gray, 2003; Vasseur and Cossu-Leguille, 2003). Other cellular-level biomarkers may also include parts of cells confined to certain tissues, or nucleic acids or specific regions of protein within nerve synapses or cell membranes (Lam and Gray, 2003). Lipid peroxidation, a process resulting in the degradation of cell membranes, can be observed when antioxidant and detoxifying systems are deficient, whereas permanent changes in DNA structure are biomarkers of exposure to mutagens or carcinogens (Vasseur and Cossu-Leguille, 2003). The measurement of stress proteins can also be used to provide a general indication of overall stress within an organism (Lam and Gray, 2003).

Cellular biomarkers serve as early indicators of disturbance and act as an early warning of possible perturbations at the individual, population, and community levels, which may deteriorate over time. Although cellular-level biomarkers can serve as early warning systems for exposure to and/or toxicity of certain compounds, several biomarkers are also influenced by other factors such as hormones, growth factors, reproductive stage, and stress as well as other chemical compounds; they can also be tissue- and species-dependent (Vasseur and Cossu-Leguille, 2003). The influence of season, sex, age, reproductive stage, and environmental conditions therefore must be known for these tools to be effective for environmental monitoring (Corsi *et al.*, 2003; Vasseur and Cossu-Leguille, 2003; Sánchez and Porcher, 2009).

### **Organ/tissue**

The ability to detoxify pollutants is essential to fish; pollutants entering an organism may be either metabolized or stored in particular organs within the body and different organs behave differently to different contaminants (Souza *et al.*, 2018). In fish, the liver is the main storage organ and is also the site of detoxification (Elliott *et al.*, 2002). If the stress persists or if the detoxifying mechanisms fail, then this can result in cell damage and physiological changes (Corsi *et al.*, 2003; Vasseur and Cossu-Leguille, 2003). Fish liver structure or histopathology can provide a good indication of exposure to xenobiotics, and can also indicate the ability of an individual to resist future insults (Stentiford *et al.*, 2003). In addition to the liver, histopathological studies can include other organs such as the kidneys, gills, and ovaries, metabolically highly active sites but also organs which may store fat and therefore be sites attractive to lipophilic compounds (Souza *et al.*, 2018). A knowledge of the storage and sequestration mechanisms of contaminants in fishes then indicates the pathways of uptake, effect and excretion (Souza *et al.*, 2019).

Although organ structure can be used to indicate stress or exposure to xenobiotics, they are not often used as a diagnostic tool, and it is difficult to determine whether it was a particular pollutant or combination of contaminants that caused the alterations.

### **Individual**

At the whole-organism level, pollution or stress can result in either mortality or indirectly by causing changes in behavior such as impairing feeding and reproduction; these effects on individuals can reduce population growth, which, in turn, will result in effects at the community level (Elliott *et al.*, 2002; Lawrence and Hemingway, 2003). Measures of the health or condition of individual fish can include some measure of the weights of individual body organs or tissues (Elliott *et al.*, 2022b). A general condition index, which is a measure based on the relationship between weight and size (length), can provide a coarse measure of the food intake and nutritional health of an organism. The gonado-somatic index (GSI) compares the mass of the gonad with the total mass of the animal and assumes that an ovary increases in size with increasing development; the liver somatic index (LSI) relates liver weight to whole body weight and can also provide an indication of the health of an individual; if an organism is not feeding, then liver weight will decrease (Elliott *et al.*, 2022b; Corsi *et al.*, 2003). Any effect by contaminants or stress on the integrity of the gonads and/or liver will affect the GSI and LSI.

Morphological disorders, disease, and parasite infestation can also be used to assess individual fish health (Elliott *et al.*, 1988, 2022b). Morphological disorders can include blemishes, lesions, lymphocystis-type nodules, fin rot, eye deformity, mouth ulceration, and skeletal deformities (Hemingway and Elliott, 2002). Stressed fishes are more susceptible to disease and parasite infestation, which can affect the growth and body condition of that organism (Elliott *et al.*, 2002; Cabral *et al.*, 2022).

It should be noted that condition factors or indices often vary between life stages within a population and also with feeding status; emaciation created by spawning, poor food conditions, or overwintering will be reflected by these indices as well as environmental stress (Elliott *et al.*, 2022b).

### **Population**

Population parameters are restricted to a single species and include measures of abundance, biomass, length and year classes, and distribution patterns. Estimates of abundance will indicate the size of the populations of the species, and temporal variations will show seasonal and annual cyclical patterns (Elliott *et al.*, 2022b; Franco *et al.*, 2022; Blaber *et al.*, 2022). The accuracy of measures of abundance, however, depends on the sampling methods used and assumes that these samples are representative of the whole population. Cohort analysis is based on catch data obtained from different age or size groups of the population. Most populations exhibit polymodal size distributions and an analysis of size-frequency information can be used to determine recruitment success, growth, and population changes (Elliott *et al.*, 2022b; Franco *et al.*, 2022).

Indicator species include sensitive taxa that have narrow water-quality and habitat requirements. Monitoring these populations is a useful indicator of environmental quality as they are often the most sensitive to environmental change and will be the first to disappear when conditions deteriorate. As the sensitive fauna is eliminated, they are often replaced by more tolerant species, which may thrive and become more abundant (Elliott *et al.*, 2022a,b,c). Indicator species may also include rare or threatened species, which are of conservation value. Because rare species are fragile, they may become endangered or even locally extinct with increasing anthropogenic stress (Costello *et al.*, 2002; Cowley *et al.*, 2022). However, it should be noted, that the status of some fish species might vary geographically. For instance, a particular species may be abundant in one region, but threatened in another; this is because some rare fishes are at the limits of their geographic distribution (Seegert, 2000; Costello *et al.*, 2002; Cowley *et al.*, 2022). It should be noted that changes in the distribution range and abundance of certain species could also be a result of global factors such as climate change rather than local conditions (Gillanders *et al.*, 2022). In particular, climate change induced features of the poleward expansion of species, changes to physiological processes and even the enhanced introduction of non-indigenous species will all influence any population and community indices of fishes (Elliott, *et al.*, 2015). Hence, the occurrence, distribution, and abundance of populations of exotic or introduced species also represent a potential threat to naturally occurring taxa through competitive exclusion and predation (Marchand *et al.*, 2002; Moyle and Stompe, 2022).

### Community

Any indices used to describe fish community change are often the same as those used for the macrobenthos (see above). Fish communities can be described according to the number of species present (richness) and the distribution of individuals or biomass among those species (Elliott *et al.*, 2022b; Cabral *et al.*, 2022). These variables can be used to derive other measures such as diversity indices; the Shannon–Wiener index ( $H'$ ), for example, gives a measure of species richness and evenness within a community. Another means of interpreting fish community structure uses the concept of functional groups or guilds; these can be based on the ecological preferences of a species, their use of the estuary or its associated catchment and marine area, their place in the water column or on the bed, their reproductive strategies, or their feeding modes (Elliott *et al.*, 2007; Potter *et al.*, 2015; Cabral *et al.*, 2022). However, some authors (Selleslagh *et al.*, 2009) suggest that the guild approach may not be useful to provide valuable information on the ecological status of small estuaries.

Waugh *et al.* (2019) used a large estuarine dataset to test paradigms relating to richness of fish species in estuaries in response to the types of estuaries and their latitudinal position. This also showed the importance of rigorously testing the basis behind fish-based indicators, especially for policy instruments such as the European WFD. More recently, Harrison and Whitfield (2021) developed a numerical fish estuary-association scoring system which allowed the functional composition of estuarine fish communities to be statistically analysed and compared; the potential use of this system as a monitoring and assessment tool was also demonstrated.

As fish communities respond to a variety of environmental factors (physical, chemical, and biological) they provide an integrated measure of estuarine conditions or health (Cabral *et al.*, 2022; Elliott *et al.*, 2022a). A fish community has the ability, to a limited extent, to absorb change within the various levels of biological organization; it is able to compensate for short-term localized stress such as disease or low dose and/or infrequent pollution events. However, if this stress is prolonged or too intense, the biological community will change from a relatively diverse and complex community to one that is relatively simple and species poor (Whitfield and Elliott, 2002; Whitfield *et al.*, 2022). Trends in fish community attributes can be described and compared with data from other systems or with some reference condition and these results be used to assess the overall condition of the ecosystem (Elliott *et al.*, 2002, 2022b; Pérez-Domínguez *et al.*, 2012).

Community attributes such as species richness, abundance, and diversity indices, however, are heavily influenced by sampling effort. Furthermore, different combinations of species and abundance can yield the same diversity ( $H'$ ) value. It is also important to be able to distinguish between natural variations and anthropogenic impacts when assessing fish community structure and function (Martinho *et al.*, 2008) and also to determine the sensitive and responsive nature of the indices and particularly their use in legislative instruments such as the EU WFD (Alvarez *et al.*, 2013). Although fish communities can provide a good integrated measure of ecosystem condition, their response to disturbance or impact can only be diagnosed only after degradation or impact has occurred (Vasseur and Cossu-Leguille, 2003). Furthermore, because fish communities integrate environmental conditions both inside the estuary but also in the catchment and/or marine areas in the case of some species, it is often difficult to assign causes responsible for degradation.

Given that the assessment of the estuarine fish community is highly dependent on the type of habitat which exists, has been lost or can be created then habitat condition and extent indices are often more valuable and may be regarded as a surrogate for the health of the fish community (Amorim *et al.*, 2016, 2017). Hence, while the assessment requires the need for restocking fish populations (Type B ecoengineering), it primarily requires creating the appropriate habitat and allowing the community to develop (the Type A ecoengineering) (Elliott *et al.*, 2016; Wolanski and Elliott, 2016). Hence indicators of estuarine fish community require to be used together with indicators of habitat quality and extent.

### Biomarkers and bioindicators in environmental monitoring and assessment

Most estuarine and coastal monitoring programs have the objective of measuring the quality of the environment and fishes are valuable for such bio-monitoring programs and estuarine management schemes (Elliott *et al.*, 2022c). With any survey of fishes in estuaries, information may be required at any one or several levels of biological organization; for example, information may be required at the cellular, individual, population, community, or ecosystem level. With a progression through each level, the speed of response to environmental change decreases and the inherent variability in the ecosystem increases; for example, an individual fish will respond rapidly to a change in water quality, whereas the community will take longer to show changes (Elliott *et al.*, 2002, 2022c; Cabral *et al.*, 2022). Biomarkers can also

complement chemical and bioindicator assessments in that they can provide an early warning signal of contamination and help establish relationships among chemical quality, fish health, and ecological status (Sánchez and Porcher, 2009; Souza *et al.*, 2018, 2019). Ideally, biomonitoring programs should include measures of stress at the subcellular, cellular, and organ levels (biomarkers), as well as at the individual, population, and community levels (bioindicators). Despite having a high resonance with the public and policy-makers, due to perceptions of the health of fish and their habitat, such approaches, however, are difficult to apply in large monitoring networks due to practical and economic constraints.

Fish biomarkers have been used in some monitoring programs to assess the environmental condition of coastal and estuarine waters. In the USA, a fish contaminants index, which examines the bioaccumulation of contaminants (e.g., metals, dichlorodiphenyltrichloroethane (DDT), dieldrin, lindane, PAH, and PCB) in fish tissues, has been developed as part of a national estuary monitoring program (USEPA, 2001a). Part of a national monitoring program for coastal and estuarine waters within the UK includes a range of fish biomarkers (e.g., bioaccumulation, metallothionein, vitellogenin, EROD activity, bile metabolites, DNA adducts, and liver pathology) (DEFRA, 2005).

Fish bioindicators have also been used to monitor and assess estuaries. One approach has been to include some attributes (or metrics) into a single, integrated measure. Such a multimetric approach has been developed and applied in the United States (Deegan *et al.*, 1997; Hughes *et al.*, 2002), South Africa (Harrison and Whitfield, 2004, 2006), Spain (Borja *et al.*, 2004b; Uriarte and Borja, 2009), Belgium (Breine *et al.*, 2007), and the United Kingdom (Coates *et al.*, 2007). A recent complete review of fish-based indices includes 27 methods covering North and South America, Europe, Africa and Australia (Cabral *et al.*, 2022) shows the value of multimetric approaches with metrics at various levels of biological organization from individual (e.g., number of diseased fishes), population (e.g., indicator species), to community (e.g., species richness, dominance, resident species, and piscivorous species) measures (Pérez-Domínguez *et al.*, 2012). Most of them have been evaluated to determine the efficacy in assessing ecological status (Henriques *et al.*, 2008; Martinho *et al.*, 2008), its response to human pressures (Uriarte and Borja, 2009; Pasquaud *et al.*, 2013; Teichert *et al.*, 2016), and have been intercalibrated (Lepage *et al.*, 2016; European Commission, 2018).

Key to any biomonitoring program, whether based on biomarkers, bioindicators, or both, is the ability to define the normal (natural) situation, measure any departure from this situation, assess whether any departure is significant, and explain the cause and effect (Hemingway and Elliott, 2002; Elliott *et al.*, 2022 a,b,c).

### Monitoring and Assessment Using Molecular Methods

All the monitoring and assessment methods presented in previous sections are based on the traditional morphological identification of the species. However, with the successive recent economic crises, some governments and agencies are seeking ways to restructure monitoring networks, by making them more cost-effective and hence reducing costs (Borja and Elliott, 2013, 2021). As laboratory work involving laborious taxonomy is usually expensive and slow in delivering results, managers are considering as a cheaper and faster possibility the use of molecular tools to identify the species and assess the status of marine biological elements (Bourlat *et al.*, 2013; Hering *et al.*, 2018). These tools include DNA and environmental DNA (eDNA) metabarcoding of different biological groups, from bacteria to mammals, and the creation of genomic-based biotic indices, which can be comparable to those based on morphological identification (Pawlowski *et al.*, 2018).

In some cases, these indices have been demonstrated to be as effective as the morphological ones, e.g., in the case of genomic AMBI (gAMBI), its performance yields similar results for macroinvertebrates, not only with the presence-absence of the species (Aylagas *et al.*, 2014), but also as abundance-biomass, using the number of reads (Aylagas *et al.*, 2018). In other elements, such as fish, good performance has also been demonstrated (Stoeckle *et al.*, 2017; Ahn *et al.*, 2020). However, many problems still remain, linked to the absence of suitable DNA barcode reference libraries for the species in estuaries (Weigand *et al.*, 2019), standards and guidelines to ensure replicability of the results (Rimet *et al.*, 2021), sustained collaboration between molecular ecologists and stakeholders to accelerate the adoption of molecular-based approaches for marine monitoring and assessment (Aylagas *et al.*, 2020), as well as suitable genomic-based biotic indices, fully comparable with those morphological currently in use, to ensure that the long-term series of ecological status available can be still be used by managers in taking decisions (Pawlowski *et al.*, 2018). Such eDNA techniques may be of value with relatively sedentary groups, in which a sample of water or sediment may indicate the species complement in the area. However, their use with more mobile species such as fishes and with non-indigenous species may pose more questions than answers: for example, while the eDNA could detect fish species anywhere in a catchment or the hydrodynamic receiving area of an estuary, this is unlikely to be focused on a small precise area, even if the genome database is sufficiently large to contain all possible species.

## Integrating Multiple Compartments of the Ecosystem in Assessing Ecological Quality

### North America

There are many large spatial scale assessments of aquatic environmental condition in several countries, especially those with a long history of environmental monitoring and assessment such as the United States of America (Table 7), Europe, Australia and South Africa (Elliott *et al.*, 2022c) but many are designed to address single types of environmental stress. For instance, in the USA, there are national assessments of contaminant accumulation (National Status and Trends (NS&T), Mussel Watch, Kimbrough *et al.*, 2008; Maruya *et al.*, 2014), bacterial concentrations on beaches (Dorfman and Stoner, 2007), nutrient effects in estuaries (Bricker *et al.*, 2007, 2008), wetlands condition and assessment of stressors to estuarine fish habitats (Greene *et al.*, 2015). There are also

**Table 7** North American methods in assessing ecological quality using multiple compartments of the ecosystem.

<i>Method/Report Name</i>	<i>Organization</i>	<i>Stressor</i>	<i>Ecosystem and/or Organism</i>	<i>Human Use/ Human Health</i>	<i>References</i>
Single stressor/ecosystem/organism methods					
ASSETS/NEEA (Assessment of Estuarine Trophic Status / National Estuarine Eutrophication Assessment)	NOAA NOS	nutrients	estuaries, coastal waters	no	Bricker <i>et al.</i> , 1999, (2003,2007) <a href="http://www.eutro.org">www.eutro.org</a>
NS&T (National Status and Trends Program) Mussel Watch	NOAA NOS	inorganic and organic contaminants	estuaries, coastal waters, mussels	no	Kimbrough <i>et al.</i> , 2008
IBIs	USACE, IBIs	contaminants	streams, coastal waters, benthic invertebrates	no	Karr, 1981,1991
Annual Beach Report Card	Heal the Bay	bacteria	California beaches, coastal waters	use, health	<a href="http://www.healthebay.org/brc/annual/2006/counties/la/analysis.asp">www.healthebay.org/brc/annual/2006/counties/la/analysis.asp</a> , <a href="http://www.healthebay.org/brc/annual/2006/execsumm.asp">www.healthebay.org/brc/annual/2006/execsumm.asp</a>
Testing the Waters: A Guide to Water Quality at Vacation Beaches	NRDC	bacteria	National Beaches	use, health	<a href="http://www.nrdc.org/water/oceans/tw/titinx.asp">www.nrdc.org/water/oceans/tw/titinx.asp</a> , <a href="http://www.nrdc.org/water/oceans/tw/tw2008.pdf">www.nrdc.org/water/oceans/tw/tw2008.pdf</a>
Our Living Oceans: Report on the Status of U.S. Living Marine Resources	NOAA NMFS	fishing pressure	nearshore fish species and marine mammals and sea turtles	use, health	<a href="http://spo.nwr.noaa.gov/national.pdf">http://spo.nwr.noaa.gov/national.pdf</a>
Status of US Fisheries Recovery Program for Threatened and Endangered Species	NOAA NMFS NOAA NMFS	fishing pressure fishing pressure	US fish stock endangered species	use use	NMFS, 2008 NMFS, 2006
SQT (Sediment Quality Triad)	USEPA, NOAA NOS, Environment Canada	inorganic and organic contaminants	sediment chemistry and sediment toxicity, benthic community composition	no	Chapman, 1986; Chapman <i>et al.</i> , 1987
EMAN (Ecological Monitoring and Assessment Network)	Environment Canada, citizen monitoring	Difficult to tell	also difficult to tell	???	<a href="http://www.eman-rese.ca">www.eman-rese.ca</a>
The state of coral reef ecosystems of the United States and Pacific freely associated states: 2008	NOAA NOS	climate change and coral bleaching, coral disease, tropical storms, coastal development/runoff, tourism/recreation, commercial fishing, subsistence and recreational fishing, vessel damage, marine debris, aquatic invasive species	coral, reef fish populations, macroinvertebrates	use	Waddell and Clarke, 2008
Integrative Methods					
National Coastal Conditions Reports	EPA (lead) with NOAA, FWS, USGS	nutrients, contaminants	estuaries, coastal waters, wetlands, fish, some socio-economics	uses, health (i.e. consumption advisories, beach closures)	USEPA, 2001b;USEPA, 2005;USEPA, 2008b; USEPA, 2012
The State of the Nation's Ecosystems: Measuring the lands, waters, and living resources of the United States	The H. John Heinz III Center for Science, Economics and the Environment	nutrients, carbon, oxygen, chemical contaminants, physical conditions (i.e. temperature)	coasts and oceans, farmlands, forests, freshwaters, grass and shrub lands, plants and animals, socio-economics. Leaves to others the analysis of pressures and the effects of actions taken to reduce stress	uses, health (i.e. beach closures)	Heinz, 2008



EPA Report on Environment	EPA	inorganic and organic contaminants, nutrients, oxygen, climate change	air, water (fresh and sea), wetlands, land, human exposure and health, ecological condition, fish	health	USEPA, 2008a
State of the Maryland Coastal Bays	Maryland DNR	nutrients, sediment toxicity	stream and lagoon, water quality, sediment quality, sea grass, wetlands, benthic community, fish and shellfish	uses	Wazniak <i>et al.</i> , 2004
A National Assessment of Stressors to Estuarine Fish Habitats in the Contiguous	NOAA NOS and NMFS	Land cover/land use, alteration of river Flow, pollution sources, eutrophication	Estuaries, coastal waters	Human use	Greene <i>et al.</i> , 2015.

biotic assessments of fishery condition (NMFS, 2008), endangered species (NMFS, 2006), and coral reefs (Waddell and Clarke, 2008). Several of these then result in summary report cards which show both the need for management and the efficacy of management measures (Elliott *et al.*, 2022c).

In the US, there are only three programs that integrate across the types of stressors for marine environments at the national level. One of these is the National Coastal Condition Report (NCCR), which includes many components of the coastal ecosystem and also considers at a lesser level the connections to human uses and human health. The NCCR is led by the US EPA with collaboration from the National Oceanic and Atmospheric Administration (NOAA), the US Fish and Wildlife Service (FWS), and the US Geological Survey (USGS). The report also includes case-study contributions from states and indigenous tribes. It is a comprehensive report on the condition of the nation's estuarine regions, coastal wetlands, seagrass meadows, coral reefs, mangrove and kelp forests, upwelling areas, and coastal fisheries that together present a broad baseline picture of conditions within the coastal ecosystem. The assessment combines five primary indicators: water quality (nutrient related), sediment quality (inorganic and organic pollutants), benthic (benthic population and communities), coastal habitat (wetland loss rate), and fish tissue contaminants (fish and shellfish tissue contaminant concentration) into a rating for the overall condition of the coastal ecosystem. Coastal monitoring data from programs such as EPA's National Coastal Assessment Program, NOAA's NS&T Program, FWS's National Wetland Inventory, and data from the Great Lakes National Program Office (GLNPO) are used to develop these indices of condition.

The NCCR primary indices focus on ecological conditions, showing that overall conditions are rated fair and have improved slightly since the initial NCCRI in 2001, the most recent being that from 2012 (USEPA, 2012), although more recent results are available (see "Relevant Websites" section). The worst ecological conditions were recorded in the Northeast, Gulf of Mexico, Great Lakes, and Puerto Rico regions, and the best in South Central Alaska and Hawaiian regions as they do not report by individual system. The report also includes data on human use and human health aspects of the coastal ecosystem. For instance, it includes information on fish stocks and catches, fish consumption advisories, and beach advisory statistics. The report includes data collected through EPA Clean Water Act Section 305(b) Program, the National Listing of Fish and Wildlife Advisories Program, and the Beaches Environmental Assessment, Closure and Health Program databases, as well as NOAA's National Marine Fisheries Service statistics on fish populations and catches. Although these data sets are not as robust as the data sets supporting the ecological indicators, they highlight the extent of human use of the coastal ecosystem as well as human health risks associated with polluted beaches and contaminated fish, thus providing a more complete/integrated picture of the ecosystem condition. As an example, the results show that fish consumption advisory statements have been issued for an estimated 77% of the US coastal waters for a total of 23 individual chemical contaminants, although four primary contaminants (PCBs; mercury; DDT and its degradation products; and dioxins and furans) were responsible for 92% of all fish consumption advisory notices in 2003.

The Heinz Center's State of the Nation's Ecosystems (Heinz, 2008) and the EPA's Report on the Environment (USEPA, 2008a) also provide a broad view of conditions nationally across many ecosystems, but both of these reports are broader in scope than the NCCR, including both terrestrial and coastal ecosystems. The Heinz report provides results for coasts and oceans, farmlands, forests, freshwaters, grasslands and shrublands, and urban and suburban areas, whereas the EPA report includes evaluation of air, water, land, human exposure and health, and ecological condition. Like the NCCR, these reports are multi-agency collaborations.

The Heinz report also provides condition indicators for each of the major ecosystem types and 10 core national indicators that provide a broad perspective on national trends and conditions. A list of 108 indicators describes 10 major ecosystem characteristics within five groups: physical dimensions, chemical and physical conditions, biological components, and human uses. Of the 108 indicators, 63 % have some or all data and can be reported nationally, whereas 37 % cannot be reported nationally due to inadequate data or needed further development of the indicator. Although the report does not include an in-depth economic analysis, it includes highlights of the relative economic significance of resources for human uses as well as events such as blooms of toxic algae that can cause economic losses. However, this report does not include a combined overall evaluation for condition of any one ecosystem or combined multi-ecosystem assessment. Data sources are national in scope, derived mostly from federal agency reports and databases. Results do not address individual water bodies but rather larger regions.

EPA's Report on the Environment (see "Relevant Websites" section) uses a set of indicators to answer 23 questions about stressors to air, water, and land, their effects on human health and the environment, and the condition of the environment. These questions are related to EPA's five strategic goals: clean air, clean and safe waters, healthy land, healthy communities and ecosystems, and stewardship and compliance, and also focus on protection of human health and the environment. The answers are provided by 86 indicators of environmental and human health conditions, but there are no integrated assessments provided for any of the ecosystems.

These reports are all limited by a lack of national sampling programs that provide comprehensive supporting data sets; however, there are some regional or water-body-specific reports which are supported by dedicated monitoring efforts. One such example is the State of the Maryland Coastal Bays Assessment (Wazniak *et al.*, 2004). The report contains 13 indicators of water quality, living resources, and habitat to evaluate the overall health of the coastal bays and to track changes over time. The component indicators are combined to give an overall assessment of the ecosystem which integrates across ecosystem components and stressors, and includes impacts to human uses and human health. The report also goes beyond that of the national reports in relating the outcomes to management objectives for ecosystem components (e.g., reduce and control invasive/exotic species, increase seagrass abundance, and reduce nitrogen loading to streams), which provides insight and information to guide a management framework.

Many of the regional and water-body-specific assessments in the United States (e.g., State of the MD Coastal Bays, Wazniak *et al.*, 2004; State of Santa Monica Bay; Santa Monica Bay Restoration Commission, 2004; State of Barnegat Bay; Barnegat Bay Estuary Program, 2005; or Southwest Florida tidal creeks (Wessel *et al.*, 2022)) have been associated with the various national estuary programs, which were created by US EPA to provide integrated management units. Although the national reports can provide the larger perspective, management is typically done on the local scale. The National Estuary Program water body assessments and recommendations for management are good examples of the use of integrated assessments at the local scale.

## Europe

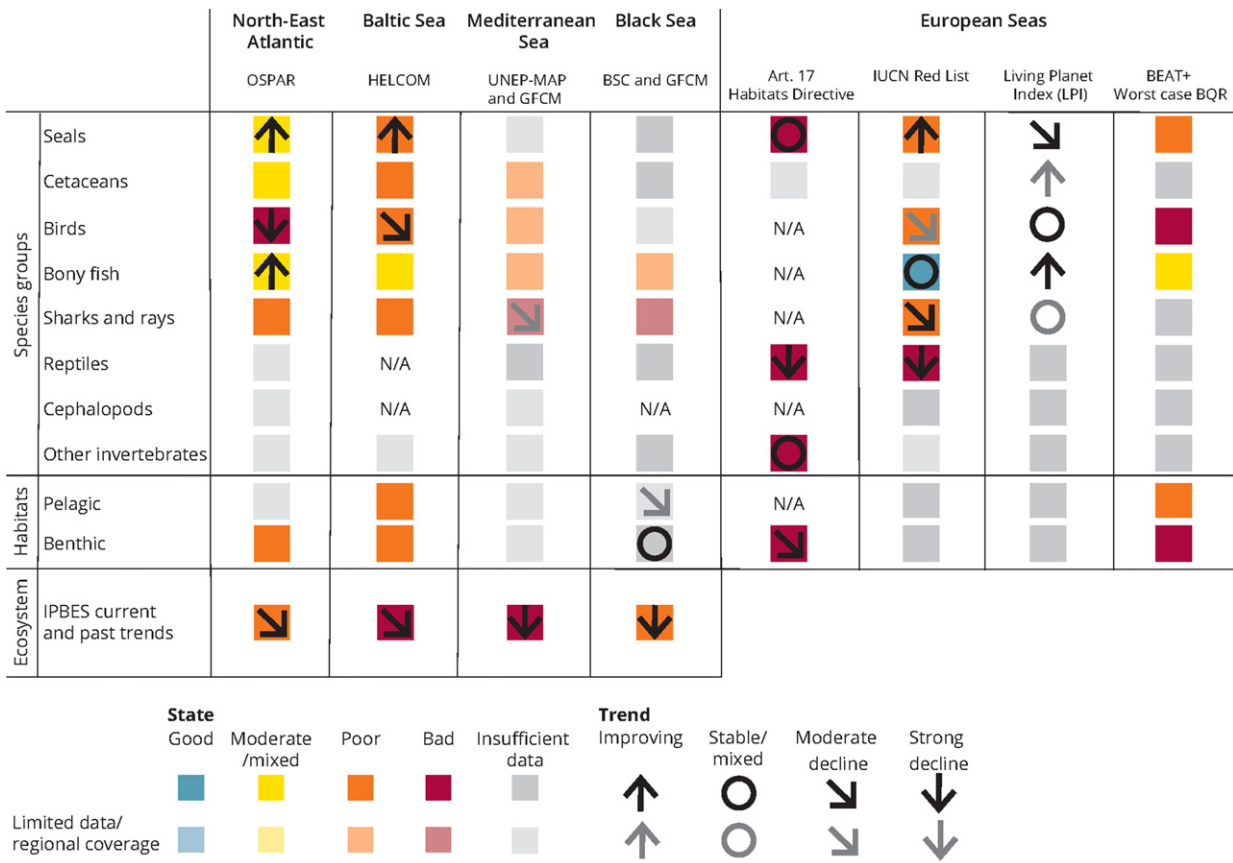
Increasing pressures and impacts within European coastal and transitional waters (e.g. estuaries and lagoons) led to the approval of a series of laws which focus on water management, the WFD being the most important (see details in Borja, 2005; Borja *et al.*, 2008a, 2010, 2013a). This Directive emphasizes the increasing need to protect European coastal, estuarine and lagoonal ecosystems and to move toward marine integrative management. The main objective of the WFD is to achieve a good ecological status for all continental, transitional and coastal European water bodies by 2027, and covers the catchment, estuary to the 'bay-closing line', and the coastal area out to 1 nm (Boyes *et al.*, 2016).

To achieve such an objective, the WFD requires the development of tools and methodologies to assess the status of several elements of the ecosystem, including physico-chemical and biological elements (phytoplankton, macroinvertebrates, macroalgae, phanerogams, and fishes only for transitional waters), as shown above (Birk *et al.*, 2012). From the 423 methods to assess the status in superficial waters, only 66 are specific for transitional waters, being multi-pressure indices and addressing mostly eutrophication (49%) and hydro-morphology (35 %) (Poikane *et al.*, 2020). However, the WFD, instead of using all these elements in assessing environmental quality in an integrative way, uses a simple approach known as the 'one out, all out' (OOAO) principle (Borja and Rodríguez, 2010). This principle takes the final quality of a water body from the worst-rated element, which may be a useful starting point but should be avoided due to the problems that arise in the final classification (Borja *et al.*, 2004b; Moss, 2008; Tueros *et al.*, 2009). Although there are several ways of weighting a combination of metrics, it is argued that this OOAO is a precautionary approach in that the status class is based on the worst-rated biological component (Borja *et al.*, 2013b).

The European Environment Agency (EEA), with the support of the European Topic Center on Inland, Coastal and Marine Waters, has reported in recent years about the state of the European environment (EEA, 2019), the multiple pressures from human activities and their combined effects in European seas (Korpinen *et al.*, 2019), the status of the marine and estuarine waters in the four regional seas (Reker *et al.*, 2019), and the status of marine biodiversity (Vaughan *et al.*, 2019). These reports include information coming from all member states, responding to legislation requirements such as the WFD, the Marine Strategy Framework Directive (European Commission, 2008) or the Biodiversity Strategy (European Commission, 2020).

The results from these reports show that, for 2011–2016, almost the entire European marine waters supported many human activities producing multiple pressures, including introduction of hazardous substances, sediment dredging and extraction, desalination brine disposals, fishing exploitation, climate change, underwater noise, introduction of invasive species, marine litter and nutrient enrichment (Korpinen *et al.*, 2019). The effects of climate change are the most widespread, including increases in water temperature and acidification (Korpinen *et al.*, 2019). The combined effects of the multiple pressures on marine ecosystems are found in most of marine areas, but especially in the North Sea, Southern Baltic Sea, Adriatic and Western Mediterranean (Korpinen *et al.*, 2019). Despite this panorama, the report states that some areas are starting to recover from degradation as a result of management measures being implemented (Korpinen *et al.*, 2019).

Given these pressures, it is not unexpected to find that the state and trends in marine biodiversity indicate a high proportion of species and habitats are in an unfavorable status (Fig. 4). Although there is a large variation in data availability across species and regional seas, the data indicate that the objectives of halting the loss of marine biodiversity loss by 2030 (European Commission, 2020) require a major effort to reduce pressures and restore habitats and species. Despite this, where regional cooperation has been established and implemented consistently, negative trends are beginning to be reversed (e.g., with regard to nutrients, pollutants and invasive species) (Reker *et al.*, 2019), and some biological elements are recovering (e.g., seagrasses) (de los Santos *et al.*, 2019).



N/A = Not applicable

**Fig. 4** Notes: NE: north-east; BSC: Black Sea Commission; GFCM: General Fisheries Council of the Mediterranean; BQR: Biological Quality Ratio. For the sources therein, consult [Vaughan et al. \(2019\)](#) Overall summary of the state and trends in European marine biodiversity, after the Regional Seas Conventions (RSCs) and European assessments. (Source courtesy: [Vaughan et al., 2019](#), updated in 2022, from the European Environment Agency (EEA), from Overall biodiversity condition and trends in Europe’s Seas — European Environment Agency (europa.eu).

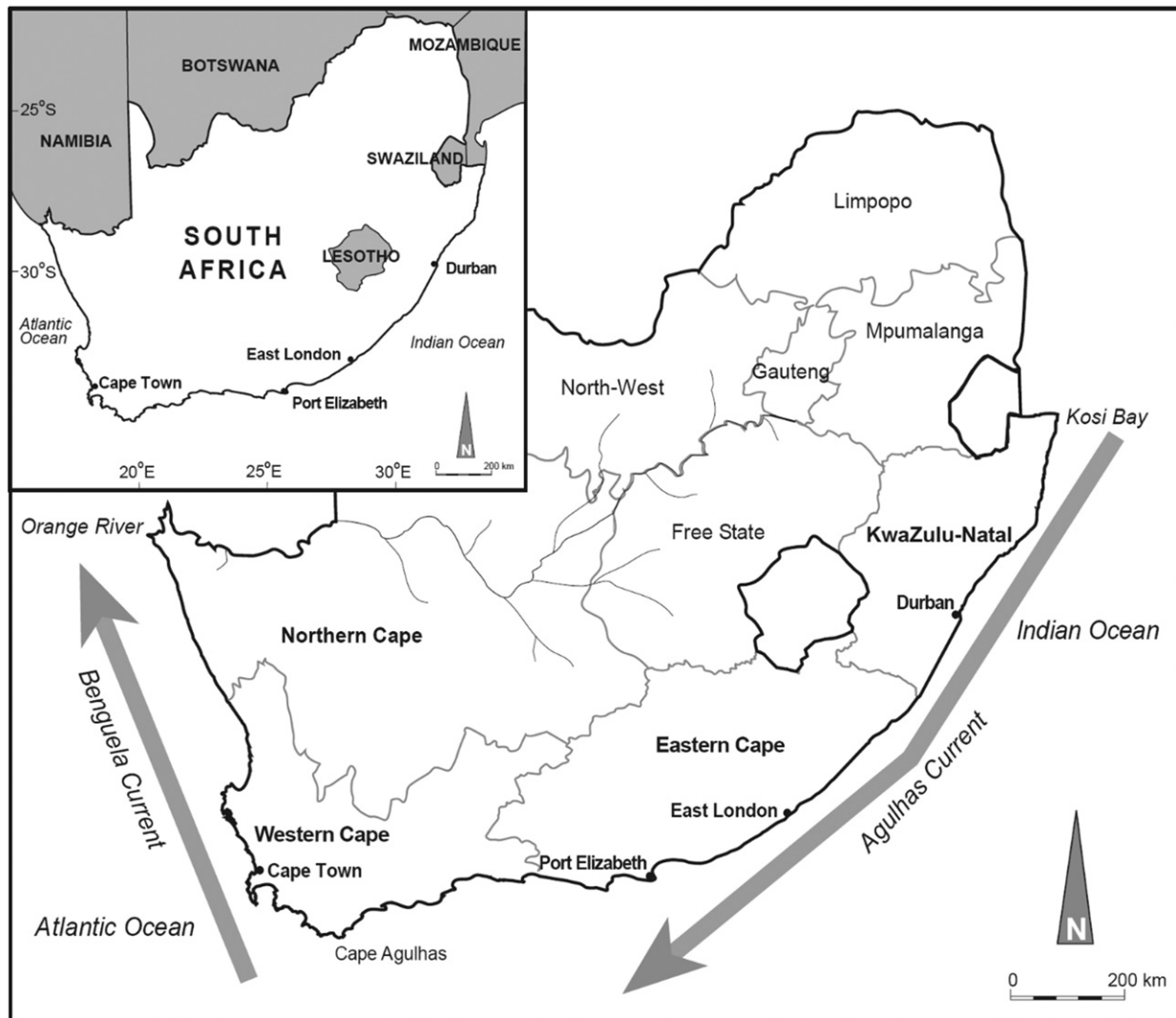
### South Africa

The South African coastline extends approximately 3000 km from the Orange (Gariep) River (28°38' S; 16°28' E) on the west coast to Kosi Bay (26°54' S; 32°53' E) on the east coast ([Fig. 5](#)). The west coast of South Africa is bordered by the Atlantic Ocean and is influenced by the north flowing Benguela Current of upwelled inshore waters. The east coast borders the Indian Ocean and is influenced by the south-flowing Agulhas Current; being tropical in origin, the waters of the Agulhas Current are relatively warm but as it flows south it tends to cool. The estuaries of South Africa cover three biogeographic regions, a cool-temperate west coast, a warm-temperate south coast, and a subtropical east coast ([Harrison, 2002](#)). Some 300 coastal outlets have been identified along the coast of South Africa and these include relatively large, permanently open estuaries, small estuaries that are often closed to the sea by the formation of sand barriers at the mouth, very small coastal streams, and even dry riverbeds that only occasionally contain water ([Whitfield, 2000](#)).

Regional assessments of South African estuaries have been concerned with either establishing ecological importance or assessing ecological health. Ecological importance is an expression of the contribution of an estuary to the maintenance of ecological diversity and the provision of goods and services at regional and national scales. Measures of health are used to describe an estuary’s condition and how well a particular system is fulfilling its ecological function relative to undisturbed or natural conditions.

### Estuarine importance

The importance of an estuary can be measured in terms of zoological, botanical, physico-chemical and socioeconomic factors such as the presence of rare or endangered species, well-developed and diverse plant communities, unique hydrological features, and important recreational or amenity value. All these factors contribute to the overall importance of an estuary.



**Fig. 5** Map of South Africa.

Several measures of estuarine importance have been developed and applied to South African estuaries. *Coetzee et al. (1996)* developed a botanical importance rating system, which incorporates factors such as plant community area cover, plant community condition (degree of impact), plant community importance within the estuary (dependence), and plant community richness. The botanical importance rating system has been applied to estuaries in the Western and Eastern Cape systems (*Coetzee et al., 1997; Collopy et al., 2000*). *Maree et al. (2003)* developed a fish importance rating (FIR) system to prioritize South African estuaries in terms of their importance to estuarine-associated fishes. The FIR is based on a scoring system of seven criteria that are considered to reflect the importance of estuaries to estuarine-associated fishes. The seven criteria were divided into two components: species importance (biological elements) and estuarine importance (physical/habitat elements). The FIR has been applied to estuaries spanning the entire South African coast.

*Turpie (1995)* used estuarine water birds for prioritizing South African estuaries for conservation. South African estuaries were ranked according to single- and multiple-criteria indices, which included measures of diversity, abundance, rarity, and conservation status. The value of certain estuaries for overwintering Palearctic migrant waders was also emphasized.

*Turpie et al. (2002)* also assessed the conservation priority of South African estuaries using some attributes, which included estuarine size, rarity of estuarine type, habitat diversity, and biodiversity in terms of plants, invertebrates, fish, and birds.

### Estuarine health

The community degradation index (CDI) represents the earliest attempt to assess the condition of South African estuaries. The CDI was originally developed by *Ramm (1988)* and was adapted and applied to South African estuaries along the eastern KwaZulu-Natal coast (*Ramm, 1990*).

The estuarine health index (EHI), which was also applied to east coast KwaZulu-Natal estuaries, is a multidisciplinary index that includes physical (geomorphology), biological (fishes), water-quality, and esthetic parameters, and condenses these into a single, composite measure of overall estuarine health (Cooper *et al.*, 1994).

The EHI was further developed and applied to the entire South African coast as part of a national state of the environment report for the Department of Environment and Tourism (Harrison *et al.*, 2000). This assessment included all the major components of the EHI (geomorphology, ichthyofauna, water quality, and esthetics) but with some modifications. Some 250 estuaries spanning the entire South African coast were assessed using this approach.

Harrison and Whitfield (2004) further developed an estuarine fish community index (EFCI), which is a multi-metric approach that included measures species of richness and composition, abundance, estuarine dependence, and trophic composition. Using data collected during the state of the environment survey, the EFCI has been applied to 190 South African estuaries (Harrison and Whitfield, 2006).

Richardson (2008) and Richardson *et al.* (2010, 2011) successfully combined the use of fish biomarkers and bioindicators to provide a biomonitoring tool that can be applied to fish assemblages in South African estuaries.

### **Resource-directed measures**

The National Water Act (Act 36 of 1998) in South Africa requires that the national water resources be protected, used, developed, conserved, managed, and controlled in an equitable, efficient, and sustainable manner. This Act requires the implementation of resource-directed measures (RDM), which involves the determination of the water quality and quantity required to meet basic human needs and for the protection of aquatic ecosystems (Adams *et al.*, 2002). Provision is made for a water reserve to be established prior to the authorization, through licensing, of water use (e.g., for agriculture, domestic and industrial uses). The ecological reserve is the quality and quantity of water required to maintain a desired level of ecosystem structure and function and this is defined by assigning each estuary to an ecological management class. The determination of the ecological management class is based on a combination of measures of estuarine health and estuarine importance (DWAF, 1999).

Estuarine health in this process is determined in terms of both abiotic (e.g., hydrology, water quality, physical habitat, and human disturbance) and biotic (e.g. microalgae, macrophytes, invertebrates, fish, and birds) components. Estuary importance is established following the procedure developed by Turpie *et al.* (2002).

The National Water Act represents a major shift in emphasis from water resource development to resource management; the natural environment is no longer regarded as a competitive user of water but rather the base from which the resource is obtained, and which must therefore be protected and managed (Adams *et al.*, 2002).

### **Australia**

When the combined state, territory, and federal governments of Australia adopted a national strategy for ecological sustainable development (ESD) in 1992 (COAG, 1992), one of the key objectives was to develop a system of State of the Environment (SoE) reporting to monitor the condition (or health) of the environment. 'Estuaries and the sea' was a major theme for the early Australian SoE reports (DEST, 1994; Zann, 1994) and these reports soon identified significant gaps in information and data concerning suitable indicators on which to base spatial and temporal comparisons of estuary condition (SEAC, 1996). As a result, Ward *et al.* (1998) reviewed 61 possible indicators for Australian estuaries and the seas, making recommendations and identifying possible data sources. Despite this early work, and a host of related scientific studies (Fairweather, 1997; Deeley and Paling, 1999; Harris and Silveira, 1999; Kennedy and Jacoby, 1999; Edgar and Barrett, 2000; Ward, 2000; Melville and Pulkownik, 2006; Hirst and Kilpatrick, 2007; Scanes *et al.*, 2007; Birch and Olmos, 2008; Wolanski, 2013), a clear set of national guidelines for monitoring Australian estuaries and marine waters have not been developed.

A major obstacle in developing a consistent approach to monitoring of natural resources in Australia has been the complex and often confusing process of coastal zone management, which is well documented (e.g., Zann, 2000). Other issues include the size of the Australian continent, with over 1000 estuaries; the large climatic variations across the nation; the huge diversity of estuaries between major regions, and the institutional and jurisdictional arrangements that often provide little clarity of key responsibilities. This has made the adoption of a single set of indicators particularly onerous and perhaps irrelevant. As a result, when a major assessment of Australia's catchments, rivers, and estuaries was carried out in 2002 (NLWRA, 2002), it relied heavily on a qualitative assessment of a set of general health criteria by an expert group (Table 8). The audit covered 972 waterbodies and concluded that the half (482) were near pristine (Table 9), although estuary condition varied greatly between the populated and unpopulated Australian states (NLWRA, 2002).

Although no Australia-wide standards exist for classifying ecological quality and integrity of estuaries, a long list of possible health indicators has been developed and most states and territories have monitoring programs based on "site relevant" sampling criteria (Table 10, see also Hallett *et al.*, 2016a). In addition, SoE reporting, including reporting on the condition of estuaries, has been carried out by national, state and local governments, in most cases for over a decade, and usually provides summaries of major environmental changes in many key estuarine environments (Kirkham, 1997; Hallett *et al.*, 2016a,b). The primary responsibility for monitoring estuary quality and integrity in Australia lies with the state and territory (=provincial) governments. For example, in New South Wales (NSW), the task of monitoring estuary health has recently been divided between the state government and a regional framework of Catchment Management Authorities (CMAs).

**Table 8** Criteria used in the initial assessment of Australian estuary condition (reprinted from NLWRA, 2002).

	<i>Near-pristine condition</i>	<i>Largely unmodified condition</i>	<i>Modified condition</i>	<i>Extensively modified condition</i>
	<i>Catchment natural cover &gt; 90%</i>	<i>Catchment natural cover ~ 65–90%</i>	<i>Catchment natural cover &lt; 65%</i>	<i>Catchment natural cover &lt; 35%</i>
Land use	Limited roads and disturbance to natural conditions and processes	No known gross impacts from land use e.g. sediments to waterways and estuary	Documented impacts from land use (e.g., sediments and nutrients to waterways)	Documented impacts from land use throughout waterways and into estuary
Catchment	hydrology	No dams or impoundments, virtually nil abstraction	No dams or significant impoundments, some abstraction	Dams and impoundments, significant abstraction modifying natural flows
Dams and	impoundments, significant abstraction modifying natural flows			
Tidal regime	No impediments to tidal flow, changes from natural morphology (e.g., training walls, barrages, bridges and causeways)	No significant impediments to tidal flow or changes from natural morphology	Impediments to tidal flow and/or changes from natural morphology (e.g., training walls, causeways, artificial opening of entrance)	Major changes to tidal flow and/or major changes from natural morphology
Floodplain	Wetlands intact in vegetation and hydrology, no alterations to flood pattern	Wetlands mostly intact in vegetation and hydrology, no alterations to flood pattern	Wetlands mostly cleared in vegetation and/or changes in hydrology (e.g., drains, tidal barrages, levees)	Wetlands mostly cleared in vegetation and/or changes in hydrology (e.g., major losses in fresh to brackish wetlands)
Estuary use	Extractive activities limited to Indigenous or limited and sustainable commercial and recreational fishing, no aquaculture	Extractive activities limited to sustainable commercial and recreational fishing, minor aquaculture	Extractive activities include dredging, extensive aquaculture, habitat modifying fishing methods (e.g., prawn trawling)	Extractive activities include dredging, extensive aquaculture, habitat modifying fishing methods (e.g., prawn trawling)
Pests and weeds	Minimal impact on estuary from catchment weeds and limited pests and weeds within estuary	Minimal impact on estuary from catchment weeds and limited pests and weeds within estuary	Significant impact on estuary from catchment weeds and impact on estuary ecology from pests and weeds within estuary	Significant impact on estuary from catchment weeds and impact on estuary ecology from pests and weeds within estuary
Estuarine ecology	Ecological systems and processes intact (e.g., benthic flora and fauna)	Ecological systems and processes mostly intact (e.g. some changes to benthic flora and fauna)	Ecological systems and processes modified (e.g., loss of benthic flora and fauna)	Ecological systems and processes degraded (e.g., major changes to habitats or species assemblages)

**Table 9** Condition of Australia's estuaries by process type (reprinted from NLWRA, 2002).

<i>Class</i>	<i>Subclass</i>	<i>Near-pristine</i>	<i>Largely unmodified</i>	<i>Modified</i>	<i>Extensively modified</i>	<i>Total</i>
Wave	Estuary	28	41	62	25	156
	Strandplain	36	13	10	1	60
	Other	40	30	22	17	109
Tide	Estuary	57	25	9	4	95
	Tidal flat/creek	210	43	16	15	284
	Other	40	17	23	9	89
River	Wave-dominated delta	28	24	30	12	94
	Tide-dominated delta	36	16	11	9	72
Notclassified		9	1	3	0	13
Total		484	210	186	92	972

A list of indicators has been recommended for monitoring estuarine health at the state level, derived from the national guidelines (Table 10). Each NSW CMA has developed its own set of indicators depending on local priorities and resources (Table 9) and will report on the quality of each estuary in a report-card format. This new framework builds on a previous system involving environmental reporting at local and state government authorities (e.g., NSWDECC, 2006) and is in an early stage of development. A major issue to be resolved relates to the standardization of methodologies. Although guidelines exist for many of the physico-chemical measures used in these health assessments, such as water analyses for nutrients, pollutants, and turbidity (e.g., ANZECC

**Table 10** Examples of estuarine quality and integrity ‘indicators’ proposed at the different jurisdictional levels within Australia (adapted from Fraser (2008))

<i>National estuary “indicators”</i>	<i>Examples of state estuary “indicators”</i>	<i>Examples of regional estuary “indicators”</i>
<p><b>Recommended Australian Estuarine Health Indicators</b></p> <ul style="list-style-type: none"> <li>● Algal blooms</li> <li>● Animal disease/lesions</li> <li>● Animal kills</li> <li>● Animal or plant species abundance</li> <li>● Animals killed or injured by litter (entanglement, starvation, suffocation)</li> <li>● Benthic microalgae biomass (in intertidal sand/mudflat communities)</li> <li>● Biomass, or number per unit area, of epiphytes (in seagrass or mangrove communities)</li> <li>● Biomass, or number per unit area, of macroalgae (in rocky shore, rocky reef or coral reef communities)</li> <li>● Chlorophyll a</li> <li>● Coral bleaching</li> <li>● Death of marine mammals, endangered sharks and reptiles caused by boat strike, shark nets or drum lines</li> <li>● Dissolved Oxygen (DO)</li> <li>● Estuary mouth opening/closing</li> <li>● Extent/distribution of key habitat types</li> <li>● Extent/distribution of subtidal macroalgae</li> <li>● Occurrence of imposex</li> <li>● Pest species (number, density, distribution)</li> <li>● pH</li> <li>● Presence/extent of litter</li> <li>● Salinity</li> <li>● Seagrass: depth range</li> <li>● Sedimentation/erosion rates</li> <li>● Targeted pathogen counts</li> <li>● Total nutrients in the sediment with dissolved nutrients in the sediment</li> <li>● Total nutrients in the water column with dissolved nutrients in the water column</li> <li>● Toxicants in biota</li> <li>● Toxicants in the sediment</li> <li>● Turbidity/water clarity</li> <li>● Water-current patterns</li> <li>● Water soluble toxicants in the water column</li> <li>● Water temperature</li> </ul>	<p><b>NSW Estuarine Health Indicators</b></p> <ul style="list-style-type: none"> <li>● Extent of mangroves, saltmarsh, seagrass and macrophytes</li> <li>● Freshwater inflow</li> <li>● Fish assemblages</li> <li>● Stress biomarkers</li> <li>● Pelagic chlorophyll a</li> <li>● Estuaries Baseline Data Collection Program</li> <li>● Hydrography survey</li> <li>● CMA regional monitoring</li> </ul> <p><b>QLD Estuarine Health Indicators</b></p> <ul style="list-style-type: none"> <li>● Extent and diversity of estuarine habitats</li> <li>● Estimated wild fish stocks</li> <li>● Algal blooms in estuarine and marine environments</li> <li>● Exceedences of marine and estuarine water quality guidelines</li> <li>● Number of hotspot areas causing acidified waterways</li> <li>● Sea level rise</li> <li>● Introduced species</li> </ul> <p><b>TAS Estuarine, Coastal and Marine Indicators (draft)</b></p> <ul style="list-style-type: none"> <li>● Physical-chemical condition (water quality parameters incl. toxicants)</li> <li>● Biological condition (e.g., species distributions and abundance; algal blooms; chlorophyll-a; pest species, mass mortality events; litter, etc.)</li> <li>● Habitat extent (extent/distribution of key habitat types, subtidal, inter/supratidal)</li> </ul>	<p><b>NSW Northern Rivers CMA</b></p> <ul style="list-style-type: none"> <li>● Water quality suite: pH, DO, salinity, conductivity, turbidity, temperature</li> <li>● Secchi disc</li> <li>● Benthic light (light loggers)</li> <li>● Total Nitrogen</li> <li>● Total Phosphorous</li> <li>● <math>\delta^{15}N</math> sewage plume mapping using oyster meat or aquatic plants</li> <li>● Chlorophyll a</li> <li>● Habitat assessment (health and presence of riparian, intertidal and subtidal habitats)</li> <li>● Seagrass depth range</li> <li>● Waterwatch and/or Bugwatch</li> </ul> <p><b>NSW Hunter Central Rivers CMA</b></p> <ul style="list-style-type: none"> <li>● Chlorophyll a</li> <li>● Seagrass and macro-algae extent</li> <li>● Seagrass depth</li> <li>● Modeled catchment nutrient loads</li> <li>● Water quality suite: Secchi disc, temperature, salinity, dissolved oxygen, pH</li> <li>● Saltmarsh and mangrove extent</li> <li>● Seagrass condition, including epiphytic growth.</li> <li>● Phytoplankton composition</li> <li>● Macro-algal blooms</li> <li>● Growth rates of sediment fans</li> <li>● Extent of mudflats</li> <li>● Shell fishery closures</li> <li>● Fecal coliforms and/or enterococci</li> </ul> <p><b>Southern Rivers CMA</b></p> <ul style="list-style-type: none"> <li>● Seagrass, mangrove, saltmarsh habitat extent</li> <li>● Seagrass Depth Limits</li> <li>● Water clarity/turbidity</li> <li>● Water quality suite (temperature, salinity, conductivity, pH)</li> <li>● Secchi depth</li> <li>● Chlorophyll a</li> <li>● Catchment nutrient and sediment loads</li> <li>● Fecal coliforms</li> <li>● Shellfish closures</li> <li>● Fish kills</li> <li>● Invasive species</li> </ul>

and ARMCANZ, 2000), ecological measures are often not standardized and a range of practices have been selected. For example, the extent of estuarine wetland communities (e.g., seagrasses) is an ecological indicator adopted by most regional, state, and national authorities (Table 10); however, no nationwide standard methods of mapping (e.g., scale or resolution) have been developed or adopted, a situation that may lead to inconsistencies in the future.

Despite this slow progress in developing a consistent approach across jurisdictions, a particularly successful and well-planned estuary monitoring program, the Southeast Queensland Healthy Waterways Program (Abal *et al.*, 2000, 2006), has been established in the Moreton Bay region through a cooperative approach between the national, state and local governments. A range of environmental and ecological quality parameters in approximately 18 major estuaries in SE Queensland are assessed, with the main ecological parameters relating to seagrasses (depth range and distribution), coral cover, and riparian condition. Detailed methods for this monitoring program are available in annual technical reports (e.g., EHMP, 2008). The parameters are used to develop a biological health rating (BHR). In addition, a suite of water-quality parameters is used to provide an EHI. A single BHR and a single EHI value are generated based on the number of sites within in each estuary that comply with established standards. These values are reviewed by an expert panel and combined into a report-card format for each estuary, providing a condition indicator for each estuary.

With an enormous diversity of estuary types (e.g., Roy *et al.*, 2001), a wide range of possible impacts and a scarcity of detailed site-specific data, an approach adopted in some jurisdictions has been to identify priorities for research and management, through an Ecological Risk Assessment (ERA) process (Astles *et al.*, 2009; Astles, 2015). For example, in New South Wales, an ecological threat and risk assessment has been carried out across the coastal waters, including estuaries (MEMA, 2015; BMT WBM, 2017). This resulted in a comprehensive summary of the relevant scientific studies, particularly related to stressors, ecological quality and assessment of environmental risks to estuaries and coastal waters (NSW DPI, 2017). One of the key stressors identified in SE Australia was climate change, in particular impacts on water temperature and acidity (e.g., Scanes *et al.*, 2020).

In summary, despite a long history of environmental reporting and some excellent local and regional examples of successful programs, a general framework for quantitatively classifying the ecological quality and integrity of estuaries across Australia is not well developed, and unlikely to emerge. Instead, due to the size of the continent, diversity of estuary types and variations in climate and geography, regional approaches to the quantification of ecological quality and integrity are likely to be more meaningful, with national responsibility mostly related to qualitative assessments and general guidelines.

## China

China has four regional seas and 11 coastal provinces. The marine studies started especially after the 1950 s, and have grown in recent decades (Liu, 2013). Due to its impressive economic development, most of the research undertaken was addressing the carrying capacity of the estuarine and coastal systems, as well as to spatial planning (Yue *et al.*, 2020). However, this growth has resulted in resources overexploitation, sea pollution, and degradation of some ecosystems (Sun, 2013). Environmental degradation, linked to pollution and land claim, has been one of the major threats to biodiversity in Chinese estuaries (Liu, 2013). Hence, since 2004, the “China Species Red List” has documented an increasing number of species endangered by the impact of human activities, including 26 scleractinian corals, 23 molluscs, 56 decapods, 53 holothuroids and 19 fish (Liu, 2013).

Despite this situation, the assessment of estuaries and coasts in China has been mainly focused on benthic biotic indices application in small areas or regional seas (e.g. Cai *et al.*, 2014; Qiu *et al.*, 2018; Liu *et al.*, 2019; Li *et al.*, 2021). However, partially driven by the World Ocean Assessment (United Nations, 2021a,b), recently some rapid methods to assess the status in data-poor regions have been implemented in the South China Sea (Feary *et al.*, 2014). Hence, a Marine Health assessment Index (MHI), developed by Yang *et al.* (2021), offers a comprehensive view of the health status in Chinese seas and regions. The South China Sea is considered to be in good status (MHI scores 0.72 over 1), while the other three seas are in moderate status (MHI 0.60 in the Yellow Sea, 0.59 in the Bohai Sea, and 0.56 in the East China Sea) (Yang *et al.*, 2021). It is interesting to note that most seas and provincial coasts decreased in quality from 2003 to 2018, in tandem with the increasing human developmental pressures in China (Yang *et al.*, 2021).

## International Methodologies and Comparison Across Geographies

The goal of methods developed to evaluate ecological condition is to reduce or summarize environmental indicators to a number that will provide adequate assessment to form the basis for management decisions. The more integrated methods allow for assessment at the ecosystem level, rather than only an ecosystem component (Borja *et al.*, 2016). In several countries with large indigenous communities, such as Australia, New Zealand and Canada, the wishes of those communities are paramount and emphasis is put on “custodianship” rather than “ownership” of water bodies (Elliott *et al.*, 2022c). Ideally, an assessment of ecological status will provide results showing the level of ecological impairment and the dominant source(s) and level of contaminant that has caused observed impairment so that management measures can be targeted for maximum effectiveness. Continued monitoring and application of the assessment method allow for tracking of management success through time (Borja and Elliott, 2021). To ensure their value in developing successful management measures, assessment methods must fulfill several requirements. One important aspect is to include contaminant sources and loads along with biological and physico-chemical indicators of adverse change, i.e. pollution per se. In this way, the level of contaminant load can be associated with the level of impairment (i.e., pollution) and, from this relationship, successful management criteria can be developed. Both biological and physico-chemical components should be used to provide an accurate evaluation of conditions. Using nutrients as an example, although there may be no problems with dissolved oxygen, which would indicate no significant nutrient-related problems, there may be losses of seagrasses and excessive algal blooms (micro- and macro-algae) which are indicators of the early stages of nutrient enrichment and system eutrophication (de Jonge and Elliott, 2002).



**Table 11** Method of eutrophication assessment, the biological and physico-chemical indicators that are used by the method, whether the method integrates nutrient load with ecological condition assessment and whether the method formulation results in one integrated rating. Key. Chl: chlorophyll, DO: dissolved oxygen, DIN: dissolved inorganic nitrogen, TP: total phosphorous, DIP: dissolved inorganic phosphorous, TN: total nitrogen, HAB: harmful algal blooms.

Method Name	Biological indicators	Physico-chemical indicators	Nutrient Load related to impairments	Integrated rating
TRIX <sup>a</sup>	Chl	DO, DIN, TP	no	yes
EPA NCA <sup>b</sup> Water Quality Index	Chl	Water clarity, DO, DIN, DIP	no	yes
ASSETS <sup>c</sup>	Chl, macroalgae, seagrass, HAB	DO	yes	yes
LWQI/TWQI <sup>d</sup>	Chl, macroalgae, seagrass	DO, DIN, DIP	no	yes
OSPAR COMPP <sup>e</sup>	Chl, macroalgae, microphytobenthos, seagrass, HAB	DO, TP, TN, DIN, DIP	no	yes
WFD <sup>f</sup>	Chl, macroalgae, microphytobenthos, seagrass, HAB	DO, TP, TN, DIN, DIP	no	yes
HEAT <sup>g</sup>	Chl, seagrass, benthic invertebrates, HAB	DIN, DIP, TN, TP, DO	no	yes
IFREMER <sup>h</sup>	Chl, seagrass, macrobenthos, HAB	DO, water clarity, SRP, TP, TN, DIN, sediment organic matter, sediment TN, TP	no	yes
AMBI <sup>i</sup>	Soft bottom macrobenthic community (5 classes)		no	yes
BENTIX <sup>j</sup>	Soft bottom macrobenthic community (3 classes)		no	yes
ISD <sup>k</sup> (lagoons)	Benthic community biomass size classes		no	yes
B-IBI <sup>l</sup>	Benthic community species diversity, productivity, indicator spp, trophic composition		no	yes
NEAT <sup>m</sup>	Chl, phytoplankton, HAB, macroalgae, seagrass, macrobenthos, fish	DO, TP, TN, DIN, sediment organic matter	no	yes

<sup>a</sup>Vollenweider *et al.*, 1998;

<sup>b</sup>USEPA, 2008a,b;

<sup>c</sup>Bricker *et al.*, 2003, 2007;

<sup>d</sup>Giordani *et al.*, 2009;

<sup>e</sup>OSPAR, 2002;

<sup>f</sup>Devlin *et al.*, 2011;

<sup>g</sup>Andersen and Laamanen, 2009;

<sup>h</sup>Souchu *et al.*, 2000,

<sup>i</sup>Borja *et al.*, 2000, 2007;

<sup>j</sup>Simboura and Zenetos, 2002;

<sup>k</sup>Reizopoulou and Nicolaidou, 2007;

<sup>l</sup>Weisberg *et al.*, 1997;

<sup>m</sup>Borja *et al.*, 2016.

As ecological degradation is a global problem, many methods have been developed worldwide to try to evaluate ecological status (i.e., see section “Classifying Biological Quality Elements”). Here, methods developed for eutrophication are used as examples in the discussion of integrated methods. Only screening models are considered as these are most useful for resource managers (Table 11). Dynamic models are not considered, despite their potential to help in understanding details of nutrient-related problems, because typically they are very complex with rigorous data requirements that are not necessarily needed for the screening process. The eutrophication assessment methods described here highlight a commonality among ecological assessment methods which typically focus on a single stressor/pollutant, e.g., nutrients. In addition, factors influencing the impact of nutrients on the water body (e.g., tidal flushing, freshwater inflow, stratification status, climate, etc.) are often included in the assessment tool. A true integrated assessment method would include additional stressors (e.g., as in the case of Nested Environmental status Assessment Tool (NEAT), Borja *et al.*, 2016). However, single stressors are typically the focus because of the complexity, with respect to study design and resource allocation, related to identifying and examining synergistic impacts of multiple stressors and addressing multiple stressors through management.

In particular, recent emphasis has been on determining the ‘footprints’ of activities, pressures and effects on both the natural and social systems (Elliott *et al.*, 2020b) and then the management response footprints (Cormier *et al.*, submitted). Hence there is the need for measures showing the spatial and temporal extent of these footprints, such that effective management can be agreed, and its achievement measured. While indicators of pressures (the mechanisms of effect) and human and natural effects are important, management more often focuses on the extent and duration of the activity.

**Table 12** Comparison of three eutrophication assessment methods results from application to Barnegat Bay, New Jersey, US. The color coding is consistent with the EU WFD color coding for ecological condition from best to worst (High – blue, Good – green, Moderate – yellow, Poor – orange, Bad – red).

ELEMENT	ASSETS <sup>a</sup>	EPA NCA WQI <sup>b</sup>	OSPAR COMPP <sup>c</sup>
Nutrient Load	High (OHI = 90% from watershed)	Not Used	Problem <sup>d</sup>
DIN Concentration	Not Used	Fair (0.17 mg/l)	No Problem (12.5 uM)
DIP Concentration	Not Used	Fair (0.031 mg/l)	No Problem (0.25 uM)
N:P ratio	Not Used	Not Used	No Problem (16.5)
Chlorophyll a	High (90 <sup>th</sup> % = 9.67 ug/l)	Good (4.74 ug/l)	No Problem (3.64 ug/l)
Macroalgae	Problem	Not Used	Problem
Submerged Aquatic Vegetation	Problem	Not Used	Problem
Dissolved Oxygen	No Problem (10 <sup>th</sup> % = 5.8 mg/l)	Good (6.29 mg/l)	No Problem (min. = 3.51 mg/l)
HABs	Problem	Not Used	Problem
Water Clarity	Not Used	Poor (0.56 WCI)	Not Used
Overall Waterbody Classification	BAD	FAIR	PROBLEM AREA

<sup>a</sup>Chl-a values are 90th percentile, DO values are 10th percentile based on NJDEP 2002–2003 data, other indicator assessments based on Kennish 2001a,b; Hunchak-Kariouk and Nicholson, 2001; Olsen and Mahoney, 2001 and Seitzinger *et al.*, 2001.

<sup>b</sup>Values are averages for samples taken in Aug and Sept of 2002–2003. This includes samples from 43 NJDEP stations (all 1600, 1700 and 1800 series plus 1506A and R08 – R20) 76 samples for Chl-a and DO and 104 for N and P during August and September of 2002–2003. Secchi depth = 0.835 m giving a value of 0.56 for the WCI based on the conversions Secchi depth \* Kd = 1.45 and Light at depth/Incident light = exp(-Kd) from Batiuk *et al.*, 2000.

<sup>c</sup>Data are from NJDEP oceanic station data 1989–2003. Reference values are winter means (Dec. 21 – April 21) for DIP (0.46 uM) and NO3 (9.15 uM), annual means for DO, and means for growing season for Chl-a (Feb 1 – July 31). Values in table, for stations detailed in 2 above, are winter means for DIP, NO3, annual mean for DO and growing season for Chl-a.

<sup>d</sup>No reference value was available for nutrient inputs and the rating of Problem for this component was determined from the OHI value calculated in the NEEA/ASSESTS method.

Another commonality is the emphasis on the status of ecological condition (even the biotic degradation, i.e., pollution per se) without a cause-and-effect linkage to contaminant source and load (Table 11). In these cases, although the ecological status is determined, the relationship of contaminant source/load and pollution impairment level cannot be determined, and thus the analysis is of limited usefulness toward development of management measures to address pollutant issues.

Although all methods have been developed with the intent of accurately evaluating eutrophic conditions, several important questions arise with the recognition that the methods have different formulations to determine the level of impairment (Table 11). Will they all give the same result if applied to the same water body? If not, does one or another give a more accurate determination of the extent of nutrient-related conditions? As indicated above, the main aim is to detect change from what is expected in an area or what change will occur if a human activity occurs or is permitted. Again, as indicated above, there are four ways of determining reference conditions: finding a physical and unaffected control area, hindcasting, numerical and predictive modeling and, if all else fails, expert judgement. Each of these methods has drawbacks and because reference conditions are determined differently, does this mean that thresholds for undesirable conditions differ with place? If so, can valid comparisons be made among results derived from different methods? What are the implications to management and how should the selection of a method be made?

Some of these questions can be answered by comparing multiple model application to the same system. This comparison was made for the ASSETS, EPA NCA and OSPAR COMPP methods using Barnegat Bay, New Jersey, USA as the test systems (Bricker *et al.*, 2005; Table 12). Another comparison was made of the ASSETS, TRIX, EPA NCA, OSPAR COMPP and WFD methods using the UK Thames and Medway estuaries as test sites (Devlin *et al.*, 2011; Table 13). Comparisons applied to a shallow microtidal lagoon system (US Barnegat Bay, average depth 1.5 m, tidal range 0.24 m) and a deeper macrotidal estuarine system (Medway Estuary, average depth 10 m, tidal range 5 m) highlighted some of the differences among the methods. For example, in the application to Barnegat Bay, the two methods that included submerged aquatic vegetation, macroalgae, and harmful algal blooms (HABs), both resulted in a rating of problem or bad; whereas the EPA NCA, for which only water column indicators were used, showed the system to be in fair or moderate condition.

In these case studies, there is agreement among methods for most indicators; the exception is Chl-a, for which ASSETS gives a rating of high (worst) and the others as good and no problem. The difference is that ASSETS uses the 90th percentile of annual data, whereas the OSPAR COMPP and EPA NCA use growing season/summertime values. In this system, Chl-a concentrations may

**Table 13** Comparison of results of application of 5 eutrophication assessment methods to Medway Estuary, UK, for eutrophic condition only. The color coding is consistent with the EU WFD color coding for ecological condition from best to worst (High – blue, Good – green, Moderate – yellow, Poor – orange, Bad – red).

ELEMENT	WFD	ASSETS	EPA NCA WQI	OSPAR COMPP	TRIX
DIN	Moderate	Not Used	Poor	+	*
DIP	Not Used?	Not Used	Poor	+	*
Chlorophyll a	Good	High	Good	+	*
Dissolved oxygen	Good	No Problem	Good	-	*
Water Clarity	Poor	Not used	Poor	Not Used	Not Used
HAB/Algal Toxin	Not Used	No Problem	Not Used	Not Used	Not Used
PP Indicator spp	High	Not Used	Not Used	-	Not Used
Macroalgae	Moderate	Moderate	Not Used	+	Not Used
Submerged Aquatic Vegetation	Not Applicable***	Not Applicable***	Not Used	Not Used	Not Used
Zoobenthos/fishkills	Not Used	Not Used	Not Used	-	Not Used
Waterbody Eutrophic Condition	MODERATE	MODERATE	POOR	POTENTIAL PROBLEM AREA	MESOTROPHIC

\*SPM used as modifier in the nutrient assessment, \*\*No individual score for TRIX, 4 parameters combined/integrated to derive rating, \*\*\*No submerged aquatic vegetation is observed in this system.

reach a maximum beyond the limit of the index period used by the other two methods; thus, the Chl-*a* indicator is not accurately captured by using limited temporal data. This can be especially problematic in cases when the sampling is done over only one or two days per year during the index period as is done in the EPA NCA program (USEPA, 2001a). This may also be a problem in cases where only water column indicators are used; for example, using macroalgae as an indicator in lagoons together with Chl-*a* may be particularly important as it may be the macroalgal component that grows rather than the phytoplankton in this type of water body (Nobre *et al.*, 2005).

The comparison of results among the five methods applied to the Medway Estuary also shows discrepancies in the ratings for Chl-*a* with only ASSETS giving a high (worst) rating which is also related to the time frames of sampling – growing season/index period versus annual data. In this case, in the deeper water system, the use of submerged aquatic vegetation is not as important as there is none observed, but the macroalgae component is present. It is important to note that despite some discrepancies, in both comparisons (Tables 12 and 13), the integrated outcome for the water body is fairly consistent, with the Barnegat Bay ratings fair to bad and Medway ratings moderate to poor and bad. In the MSFD (European Commission, 2008), there is recognition that the five quality classes of the WFD are largely un-implementable with respect to meaningful type-specific thresholds (Borja *et al.*, 2010). The focus has been narrowed to a more practical approach that includes two classes of environmental status (i.e., good or otherwise) rather than the five ecological status classes of the WFD. In this case, both systems (Barnegat Bay and Medway Estuary) would be subject to the monitoring, assessment and management requirements that systems below ecological good status are required to undergo by WFD legislation.

Although these eutrophication assessment methods use an integrated approach by combining biological and physico-chemical indicators, there are other integrative methods that deserve mention. In the case of the methods that use soft-bottom benthic community analysis (i.e., AMBI, BENTIX, BOPA, BO2A, B-IBI, ISD and NEAT; Table 11), the data provide a result that is integrative but not in the same way as the other methods. The result is a reflection of integrated conditions with ratings ranging from pristine/unpolluted to extremely polluted (see Borja *et al.*, 2000; Simbora and Zenetos, 2002; Reizopoulou and Nicolaidou, 2007; Weisberg *et al.*, 1997; or Zaldivar *et al.*, 2008 for comparative discussion). In this case, application of the methods provides an integrated result; however, it is not possible to identify the primary stressor(s) that are causing the impairment and thus it has the opposite problem of the single stressor focus of the other methods (see also Poikane *et al.*, 2020). Although it has been shown that AMBI is reflective of dissolved oxygen conditions (Borja *et al.*, 2006, 2009d, 2019) and thus reflects one of the eutrophication indicators, it would be an interesting exercise to apply the AMBI and other benthic analysis methods to the Barnegat Bay and Medway Estuary and see if the results would be the same.

## Discussion

Monitoring programs worldwide seek integrative methodologies for assessing estuarine environmental or ecological status. However, following Díaz *et al.* (2004), rather than developing such integrative methods, we are assisting a repetitive development of new indices for particular biological elements (e.g., phytoplankton and macroinvertebrates), which appear to be endemic, self-

propagating, and rarely justified. This recent increase in the number of aquatic habitat quality indices suggests that there is little acceptance of any specific metric by environmental managers or scientists, although in the case of Europe they have accepted hundreds of them (European Commission, 2018; Poikane *et al.*, 2020).

The growth in the number of these indices has been fueled by the management desire for a reductionist approach to the assessment of habitat quality (Díaz *et al.*, 2004). In essence, the final outcome is the integration of multivariate data into a single site-specific numeric value that can be interpreted by a non-specialist within a good- versus bad-gradient, often to meet a minimum legislative requirement (i.e., the CWA or the WFD). However, it is generally agreed that the ecological assessment methodologies should respond to the drivers–activity–pressure–state–impacts–human welfare–response–management measures (DAPSI(W)R(M)) paradigm (Elliott *et al.*, 2017). This requires (1) assessment of ecological integrity; (2) evaluation of whether significant ecological degradation has occurred (in relation to anthropogenic disturbance); (3) identification of the spatial extent and location of ecological degradation (in relation to recent historical changes and/or reference conditions); (4) determination of causes of unacceptable degradation in order to guide management actions, and (5) indicators of the efficacy and cost-effectiveness of management measures (expanded from Borja and Dauer, 2008).

Most of the indices presented in this chapter that were developed for a particular biological element (e.g., soft bottom habitats) have similar merits: (1) multiple attributes (i.e., richness, diversity, opportunistic/sensitive species, etc.) are combined into a single measure designed to maximize the ability to distinguish between degraded versus non-degraded condition; (2) they are developed with an appropriate methodology that accounts for biological variability that is associated with natural estuarine controlling factors such as latitude, salinity, and sediment particle size, and (3) they allow the comparison of values that reflect the degree to which component measures of key biological attributes at one location deviate from corresponding optimum/trigger/threshold values expected under undisturbed or reference conditions (Díaz *et al.*, 2004). However, there is an urgent need to test the response of this plethora of indices to individual and multiple human pressures, within different geographies, as it has been done for some of them, e.g., Chainho *et al.* (2008); Henriques *et al.* (2008); Martinho *et al.* (2008); Borja *et al.* (2009d, 2015, 2019), Uriarte and Borja (2009); Teichert *et al.* (2016); Dauvin *et al.* (2017), or Berthelsen *et al.* (2018).

Perhaps the greatest challenge in estuarine and coastal status assessment is not the impact of one activity at one place and one time, that is usually straightforward as shown by a classical Environmental Impact Assessment (Gray and Elliott, 2009), but rather the effects of many activities at many places and over long times. The latter results in cumulative impacts and with the need to determine, assess and management those complex processes (see Lonsdale *et al.*, 2020). Although some of the reports described above (e.g., US Heinz Center Report, Report on the Environment, and Australian Ecosystem Health Monitoring Program) improve upon assessments limited to individual stressors, there is the pressing need for further improvements in how integrated assessments are conducted (see Borja *et al.*, 2009a, 2016). The biggest shortcoming is the lack of integration of the indices for different biological elements into an overall evaluation of ecosystem health.

While there are well-defined principles and approaches to determining environmental health (Tett *et al.*, 2013), there is the need to quantify such changes and create holistic and integrative approaches. The difficulty is mostly the lack of an agreed-upon methodology, rather than a lack of intent (Borja *et al.*, 2016). As an example, just for eutrophication assessment, ASSETS uses matrices to combine characteristics of the pressure–state–response components and uses a matrix to combine the results of the three components into a single rating. The OSPAR COMPP and WFD assessment use a ‘one-out all-out’ process to determine the overall status of conditions but do not include the pressure or response components.

Additionally, the integration into an overall evaluation should include human use and socioeconomic concerns so that the costs of environmental degradation can be highlighted. For instance, ASSETS considers physico-chemical and biological components and the interconnectedness between the watershed and coastal waters (i.e., land-based nutrient sources, e.g., Whitall *et al.*, 2007) and recognizes the economic impact of nutrient-related damage (e.g., Bricker *et al.*, 2006; Lipton, 2007), but ASSETS is still a single-issue focus (nutrients). Although present assessment methods are limited in the guidance they can provide to managers, development of multi-stressor assessment methods, albeit needed, will be complex and most effective if developed at the local level (Poikane *et al.*, 2020). However, a major issue is determining methods that are accurate and acceptable on a large-scale basis that can then be applied at a smaller scale and intercalibrated (Borja *et al.*, 2007; European Commission, 2018). The value of integrative assessment methods and Environmental Integrative Indicators (Aubry and Elliott, 2006) linked to an overall and GIS-based Estuarine Planning Support System (Lonsdale *et al.*, 2018, 2020) should lead to the required holistic assessment for policy management.

A second concern is that most of the integrated assessments are based on biological community endpoints, i.e. a change in community structure; however, in most cases, the biological indices used to conduct these assessments have been developed regionally and differ substantially in their formulation (Díaz *et al.*, 2004; Borja and Dauer, 2008). Many relate to the structure of the ecosystem (how many species, what diversity number, what area covered, etc.) rather than the functioning of the system, i.e. the rate processes in the ecological well-being. Regional development makes sense, as species composition and reference expectations for community parameters change naturally with ecoregion and habitat, but there is little assurance that regional indices are all calibrated to the same scale. The European efforts at intercalibration to ensure comparability and consistency in indicator use (Poikane *et al.*, 2014; European Commission, 2018), as well as the comparison of methodologies at large scale in USA (Gillett *et al.*, 2015), provide a basis for accurate and comparable assessments. Although biological assessments provide many advantages as they integrate the effects of multiple stressors over time, common scaling of the indices is essential for national assessments intended to accurately compare condition across regions (Borja *et al.*, 2009e; Teixeira *et al.*, 2010; Pelletier *et al.*, 2018).

Another concern with the use of biological indicators is the difficulty in relating observed effects back to a particular stressor that is causing impairment, i.e. the cause-consequence chain (see [Borja et al., 2009d](#)). While the integrative response that biological indicators offer is valuable, the actions taken by managers are typically directed to individual stressors and much management is directed mainly towards sanctioning individual activities. Distinguishing whether biological impairment results from habitat perturbation, invasive species, or pollutant stress, as well as which pollutant among many candidates, is essential to directing appropriate corrective actions (see [Teichert et al., 2016](#)). Some of them pose a technical challenge, given that the methods for developing stressor attribution are still in development and are more advanced for stream environments than for estuaries or lagoons.

An important issue is structuring assessment reports so that both stressors and response indicators are included and linked, such that management recommendations can be made, e.g., [Dauer et al. \(2000\)](#). Heavy metal contamination levels are generally higher in estuaries than in the open sea, with a wide variability of benthic responses to contamination in estuaries, probably due to the high spatio-temporal heterogeneity of these systems ([Dauvin, 2008](#)). Similarly, determining the effects at various levels of biological organization and due to trace contaminants is difficult to assess, given the inherent ability of estuarine ecosystems to absorb stress ([García-Alonso et al., 2011](#)). That ability has been termed environmental homeostasis and is arguable greater in variable systems such as estuaries ([Elliott and Quintino, 2007, 2019](#)).

Beyond these technical challenges, most integrated assessment reports do not have a well-defined audience and are not well linked to management activities. This is particularly problematic at the national level, e.g. the US Congress is often listed as the target audience. Although there have been legislative requests for such reports, the US Congress has not adopted them and used them as a focal point for hearings or triggers for large-scale directional changes. One of the difficulties is that management is best done at the local level and most of the national reports do not provide results by water body but rather by region. Hence, assessments or individual components and/or individual places and times are then combined to give a larger area/longer time quality assessment. Additionally, it is a challenge to identify the causative influences for environmental degradation as there is often a synergistic effect or possibly even an antagonistic effect. Moreover, monitoring, assessment, and management of a single stressor is very resource intensive, and thus typically it is the priority stressor or issue that receives the most scientific study and management attention, whereas other stressors that are not as easily linked to impacts, or might be more difficult to manage, are not given priority treatment when restoring ([Teichert et al., 2016](#)).

Although challenging at the national level, integrative reports more effectively stimulate management actions at the local level, for example, many of the US National Estuary Programs (e.g., for Chesapeake Bay, Puget Sound, etc.) use integrative reports and report cards to focus their management priorities ([Elliott et al., 2022c](#)). The national reports can be used to highlight a priority stressor of concern and gain momentum to manage that one stressor at the local level. It is also easier to develop management plans at the local level given that scientific study and report development is more participatory among groups with the same management goals, and there is potentially less disconnect between the scientists and the users of the report. Furthermore, the US NOAA approach for eutrophication status shows the value of using expert knowledge for each estuary to give 'soft intelligence' and then combining this is a rigorous framework to give 'hard data' ([Bricker et al., 1999, 2007, 2008](#)).

One challenge in making the management linkage is that the present reports focus on historically important stressors and do not provide much information on emerging issues (e.g., micro- and nano-plastics, pharmaceuticals, etc.) ([Borja and Elliott, 2021](#); [Elliott and Whitfield, 2022](#)). This is a natural outcome of emerging issues being too new to have yet been incorporated into large-scale monitoring programs and therefore the data sources to make them a focal point of a national assessment are lacking, but they are growing rapidly in recent years ([Borja and Elliott, 2021](#)). However, the result is that the reports focus on legacy issues for which management actions have already been undertaken, rather than on issues which managers are contemplating action. Theoretically emerging issues such as climate change or emerging contaminants are integrated into the biological responses that are key to these reports; however, relating the biological responses back to the stressors on which managers are considering action would substantially enhance the value of the reports.

Finally, the goal of environmental managers is to provide the public with understandable maps integrating transitional water condition information from the different elements, presenting condition (quality) categories using simple colors. Reducing complex information from multiple ecosystem elements to a single color is a substantial challenge to coastal scientists. Similarly, the process in going from detailed data to detailed reports to summary reports and report cards in which the qualifying remarks, degree of confidence and explanations have been lost, is anathema to many scientists. This often leads a geographical area which may have been marginal in quality being categorically placed in one quality band or another. Hence, when using indicators and indices, investigators should be as pragmatic as possible in making them related to policy and management; this constitutes sustainable solutions which have been defined as fulfilling the so-called 10-tenets: that the management actions should be environmentally sustainable, economically viable, technologically feasible, socially desirable/tolerable, legally permissible, administratively achievable, politically expedient, ethically defensible/morally correct, culturally inclusive and effectively communicable ([Elliott et al., 2020a](#)).

The means of integrating quality assessments for many components, areas and times will remain challenging. Assessing estuarine and marine quality will require the integration of different disciplines (chemists, engineers, biologists, ecologists, physicists, managers, communication specialists, etc.), to reach an agreement on the final assignment of ecological status ([Borja et al., 2009e, 2016](#)). Hence, emphasis needs to be directed at understanding the complexities of estuarine system functioning rather than simplifying, scaling down and deconstructing the system into smaller components ([Díaz et al., 2004](#)). Indeed, the process of deconstructing an ecosystem for study and then reconstructing it to give a holistic and weighted assessment is by far the greatest challenge in areas where there are many activities, pressures and effects.

## Conclusions

Despite the large number of traditional methods to assess the ecological status of single ecosystem components, its number is increasing every year, although very few have been demonstrated to be useful in multiple biogeographical areas and responding to multiple pressures. The possibilities facilitated by new methodologies, such as the molecular ones, could increase this number, but also represent an opportunity for some convergence and comparability across geographies. In addition to methods assessing the status of single ecosystem components, methods including multiple ecosystem components, both physico-chemical and biological, could be more useful to assess the status under an ecosystem approach. This will allow for the implementation of better management measures, to address increasing multiple pressures, producing different impacts in the environment and their ecosystem services.

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