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Water quality for bivalve molluscs and consumer safety: Application of novel and adapted multimetric indices in a coastal lagoon system exposed to wastewater discharges

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A B S T R A C T

Water quality degradation associated with wastewater discharges compromises the production of marine living resources. Water quality indices (WQIs) are relevant tools for water quality management, but most applications are limited to the suitability of freshwater for drinking. In this study, a novel WQI was developed to assess the effects of urban wastewater treatment plant (WWTP) discharges on the water quality in Ria Formosa coastal lagoon, targeting the condition of bivalve molluscs and consumer food safety (WQIB). The application of WQIB was compared with an adapted version of the Canadian Council of Ministers of the Environment Water Quality Index, using similar parameters (CCME-WQI_B). WQIB and CCME-WQI_B were applied to four areas next to WWTPs, over a 2-year period. WQIB integrated seven sub-indices (salinity, unionized ammonia, dissolved oxygen, suspended solids, chlorophyll-a, *Escherichia coli* and toxigenic phytoplankton), using a weighted additive aggregation function. Water quality ranged from very poor to very good and generally improved with distance from the effluent discharge points, and during the cold period. Highest influence of WWTP discharges was detected in areas under weak hydrodynamics. In areas under strong hydrodynamics, poor water quality was caused by the advection of toxigenic phytoplankton from adjacent coastal waters during the warm period. Although correlated, the use of WQIB should be preferred over CCME-WQI_B due its greater sensitivity, use of weighted parameters and application at the sampling event scale. Our novel index extends the limited number of WQIs applied to marine systems and can be adapted to other systems and water use purposes.

1. Introduction

Coastal lagoons are among the most productive systems on Earth (Kennish and Paerl, 2010), providing a wide range of goods and ecological services that are highly valued by man, including the production of edible resources such as bivalve molluscs (Chapman, 2012; Newton et al., 2018; Nunes et al., 2021). However, the sustainability of coastal lagoon services is currently being challenged by water quality issues that require the implementation of appropriate ecosystem management strategies (e.g., Filgueira et al., 2016; Bonometto et al., 2022). In recent decades, the discharge of wastewater effluents into coastal ecosystems has generally impaired the water quality (Seiler et al., 2020), namely by promoting eutrophication problems and harmful algal blooms (HABs), reduction in dissolved oxygen levels, and the contamination with faecal material and associated pathogenic microbes (e.g., Cravo Uddin et al., 2022a, and references therein). These anthropogenically-induced environmental changes have been reported for coastal lagoons worldwide, including major bivalve mollusc production centers in southern Europe, such as the Thau (Derolez et al., 2020) and Ria Formosa lagoons (Cravo et al., 2015, 2022a). In these systems, water quality degradation can negatively affect the physiological condition of bivalves (Mendonça Uddin et al., 2022), and compromise their safety as food for human consumers, posing a relevant threat to public health. These issues globally limit production, sustainability (e.g., Gray, 2019; Cravo Uddin et al., 2022a) and other ecosystem services provided by shellfish production areas (review by Olivier et al., 2020).

The use of specific environmental policies and legislation (e.g.,

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Urban Wastewater Treatment Directive, Council Directive 91/271/EEC, 1991; Shellfish Waters Directive, Directive 2006/113/EC, 2006; Water Framework Directive, WFD, Directive 2000/60/EC, 2000), along with well-grounded monitoring strategies, enables the selection of effective ecosystem management actions, a key challenge for the protection of shellfish production areas and the safety of human consumers (Gray, 2019; Brown et al., 2020; Webber et al., 2021; Braga et al., 2023). The diversity and complexity of water quality monitoring data stimulated the development of water quality indices (WQIs), which synthesize multiple water quality parameters into a single value that can be easily perceived and effectively interpreted. These indices have been a popular tool for management purposes since the 1970s. Overall, the definition of any WQI involves four sequential stages: (1) selection of a set of parameters representative of the water quality; (2) development of sub-index functions and unitless values for each parameter; (3) assignment of the parameter weighting values; and (4) aggregation of weighted sub-index values (reviews by Gupta and Gupta, 2021; Uddin et al., 2021; Chidiac et al., 2023; Lukhabi et al., 2023; Mogane et al., 2023). Despite this generalized workflow, the index structure, number and type of water quality parameters, parameter metrics and transformations, and aggregation functions used to establish WQIs are diverse, resulting in different WQIs (e.g., Weighted Arithmetic Water Quality Index, US National Sanitation Foundation Water Quality Index, Canadian Council of Ministers of the Environment Water Quality Index, Irish Water Quality Index). This index diversity also reflects data availability, local water quality regulatory guidelines, and water body type and intended uses (reviews by Sivaranjani et al., 2015; Kachroud et al., 2019; Uddin et al., 2021, 2023; Mogane et al., 2023). Despite their widespread use, none of the WQIs has so far been universally accepted (e.g., Gupta and Gupta, 2021). In fact, many studies have applied multiple WQIs and approaches and analysed their relative advantages and disadvantages when applied to freshwater (Landwehr and Deininger, 1976; Said et al., 2004; Lumb et al., 2011; Akkoyunly and Akiner, 2012; Fataei et al., 2013; Behmanesh, 2014; Finotti et al., 2015; Darvishi et al., 2016; Hamlat et al., 2017; Kachroud et al., 2019; Zotou et al., 2019; Alexakis, 2020; Calmuc et al., 2020; Lencha et al., 2021; Ramírez-Morales et al., 2021; Gamvroula and Alexakis, 2022; Marcelina Uddin et al., 2022), transitional (Tiwari Uddin et al., 2022) and marine (Gupta et al., 2003; Woodward et al., 2020; Uddin Uddin et al., 2022; Nada et al., 2024) ecosystems. Overall, ca. 91 % of WQI applications have been used to assess water quality in freshwater systems (Uddin et al., 2021), mostly to examine the suitability of water quality for drinking purposes (Gupta and Gupta, 2021). Therefore, the development and application of WQIs in transitional and marine systems, namely for other beneficial uses of water, including their use to support bivalve production, is largely unexplored.

The Ria Formosa (RF) is a highly productive, coastal lagoon system, on the southern coast of Portugal that represents the most important bivalve mollusc production area, providing multiple ecosystem services with high socio-economic value (Newton et al., 2014, 2018, 2020; Cravo et al., 2022a). However, the RF water quality has been negatively impacted by the discharges from urban wastewater treatment plants, WWTPs (e.g., Martins et al., 2006; Cravo et al., 2015, 2022a, 2022b; Jacob et al., 2020; Newton et al., 2022; Rosa et al., 2022; Caetano et al., 2023). Most studies addressing water quality issues in the RF have used physico-chemical quality elements and chlorophyll-a (Newton et al., 2003; Loureiro et al., 2006; Goela et al., 2009; Brito et al., 2012; Cravo et al., 2015; Rodrigues et al., 2021; Newton et al., 2022; Rosa et al., 2022). However, most of these approaches have neglected critical water quality parameters (e.g., faecal contaminants, phycotoxins) that are relevant for appropriate protection and management of bivalve shellfish production areas. WQIs are potentially important tools for assessing the impacts of WWTP discharges on RF receiving waters and nearby shellfish production areas, thus contributing to the implementation of management actions, and evaluation of their effectiveness. In addition, there is a need to establish integrative WQIs, that can also be applied to different temporal scales, allowing the identification of critical episodes in terms of water quality and bivalve safety for consumers, as well as long-term trends in water quality classification.

In this context, the aim of this study was to develop a novel WQI to assess the impact of WWTP discharges, specifically targeting the physiological condition of bivalves and consumer food safety (WQIB). In addition, WQIB was compared with an adapted version of the conventional Canadian Council of Ministers of the Environment Water Quality Index (CCME-WQI), whose flexibility enabled the use of similar water quality parameters. The Trophic Index (TRIX) was also used to evaluate the impact of treated effluent disposal on the trophic status of the study areas. WQIs were applied to lagoon areas in the vicinity of the main WWTP discharge areas, exposed to variable environmental conditions and wastewater loading regimes. A set of water quality parameters directly affecting the physiological condition of bivalves and/or food safety for human consumers, including chemical and biological variables, were considered in both WQIs.

2. Material and methods

2.1. Ria Formosa coastal lagoon: Description of the study areas

The RF is a shallow coastal lagoon system, that extends ca. 55 km along the southern coast of Portugal (total wet area: 105 km^2) and is connected to the ocean by six inlets (Fig. 1). RF is an euryhaline mesotidal, well mixed system, with several main channels and a branched system of creeks (review by Barbosa, 2010). The tides are semidiurnal, with a mean tidal range around 2 m, and ca. 50–75 % of the lagoon water volume is daily exchanged with the ocean (Mudge et al., 2008). The Gilão River is the only permanent natural freshwater source affecting the lagoon, but with a low mean annual discharge rate (2.44 m^3s^{-1}; Lima et al., 2023), and the adjacent coastal ocean is affected by regular upwelling events that ultimately affect the outer lagoon areas (Barbosa, 2010; Cravo et al., 2014, 2020). Despite its semi-confined nature, the lack of significant salinity gradients and freshwater inputs is associated with the classification of the RF lagoon system as sheltered coastal waters, rather than transitional waters, in the context of the WFD (Newton et al., 2014). The RF coastal lagoon is a protected area, under national and international conventions (Newton et al., 2022), and is a breeding and nursery ground for many species of fish and molluscs, especially bivalves (Newton et al., 2003, 2020, 2022). The RF represents the main bivalve production area in Portugal, an activity that directly and indirectly involves about 10,000 people (Cravo et al., 2015). The three main municipalities in the RF drainage basin (Faro, Olhão and Tavira) have ca. 140,000 resident inhabitants (PORDATA, the Database of Contemporary Portugal; https://www.pordata.pt/#AnchorCensos), but this number increases markedly during the summer due to the influx of visitors.

Until recently, five main WWTPs located near the cities of Faro, Olhão and Tavira discharged their effluent into five RF lagoon areas (see Fig. 1): (1) Northwestern Faro (FNW); (2) Faro-Olhão (F—O); (3) Western Olhão (OW); (4) Eastern Olhão (OE); and (5) Almargem-Tavira (Alm-T). However, in late October 2018, two of the WWTPs underwent major alterations: the OW WWTP (with aerobic stabilization ponds) was decommissioned, and its influent was redirected to the upgraded F—O WWTP. Since then, the later WWTP has received the influents from both Faro and Olhão cities, which has increased the equivalent population served and the effluent discharge (from 10,000 to 16,000 $m^3\ d^{-1}$) by about 60 % (Jacob et al., 2020; Cravo et al., 2022a). Wastewater treatment at the FNW, OE, and Alm-T WWTPs includes primary physical treatment, followed by secondary biological treatment using activated sludge, and final ultraviolet disinfection. The upgraded F—O WWTP includes an advanced biological treatment, NEREDA®, designed to remove N and P nutrients (Khan et al., 2015), and the introduction of ultraviolet (UV) disinfection of effluent, after September 2019 (Jacob et al., 2020). The main characteristics of the effluents discharged by

Fig. 1. Map of the Ria Formosa (RF) coastal lagoon, and location of all the five study areas and sampling stations. The red stripped polygon, retrieved from the Portuguese Institute of the Sea and Atmosphere (https://www.ipma.pt/en/bivalves/docs/), represent the classified lagoon bivalve production area "Olhão 2". Study areas (coloured boxes): Northwestern Faro (FNW); Faro-Olhão (F—O); Western Olhão (OW); Eastern Olhão (OE); and Almargem-Tavira (Alm-T). For each area, blue arrows represent the location of the effluent discharge points from the main urban wastewater treatment plants (WWTP), white circles denote the main sampling stations used to derive the water quality indices (WQIB and CCME-WQI$_B$) and the trophic index (TRIX), and bi-coloured white-red circles represent stations used for TRIX only, and the white-delimited polygons represent bivalve mollusc harvesting grounds, provided by Agência Portuguesa do Ambiente. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

these five WWTPs, and the five receiving study areas, including hydrodynamic conditions, are summarized in Table S1 (Supplementary Material, Section E). All lagoon areas in the vicinity of the WWTP discharge points, except Alm-T, include shellfish harvesting grounds, which are more extensive in the OE study area (Fig. 1).

2.2. Sampling strategy

The impact of effluents derived from five WWTPs on the water quality of Ria Formosa was investigated for five lagoon areas, during a ca. 2-year period (September 2018–September 2020). Sampling was generally undertaken monthly, during low neap tides, the most critical tidal stage for lagoon water quality (Cravo et al., 2022a). However, sampling in the OW area was less frequent, especially for biological variables, due to the decommissioning of the WWTP.

For each study area, water samples were collected along longitudinal transects of the effluent dispersion, starting at 250 m from the WWTP discharge point (EDP) to a maximum distance of 2000 m (Fig. 1). As the impact of effluent discharges has been detected up to 750 m from the EDPs (Cabaço et al., 2008; Cravo et al., 2015), the first three stations sampled for each area were equally spaced, by 250 m, up to 750 m. Farthest stations from the EDPs were located in main lagoon channels and/or in the vicinity of bivalve mollusc production areas, and distance between intermediate stations generally increased due to weaker environmental gradients (Cabaço et al., 2008; Cravo et al., 2015). Thus, from four to 11 stations were sampled for each of the five study areas (total: 29 stations; Fig. 1). Basic water physico-chemical variables (water temperature, salinity, dissolved oxygen) were measured in situ, and surface water samples (ca. 20–30 cm) were collected for the analysis of dissolved inorganic macro-nutrients, suspended solids, *Escherichia coli*, chlorophyll-*a*, and phytoplankton abundance and composition. Water physico-chemical variables and chlorophyll-a were measured at all 29 stations, but the abundance of toxigenic phytoplankton and *E. coli* and the WQIs were only analysed for 16 stations (see white circles in Fig. 1).

2.3. Analysis of variables indicative of water quality

Water temperature, salinity, dissolved oxygen, and pH were measured in situ with a multiparametric probe YSI (EXO 2; see Cravo et al., 2020 for details on range and accuracy). Suspended solids and dissolved inorganic macro-nutrients (nitrate, nitrite, ammonium, phosphate, and silicate) were quantified using gravimetric and spectrophotometric methods, respectively (Grasshoff et al., 1999; see Cravo et al., 2022a for details). Unionized ammonia (NH_3) was calculated as a fraction of ionized ammonia (ammonium ion, NH_4^+) concentrations, based on water temperature and pH (Emerson et al., 1975). Abundance of *E. coli* in water samples was assessed using the Quanti-Tray method, with the defined-substrate assay Colilert system (see Cravo et al., 2022a for further details).

Chlorophyll-a concentration (Chl-a), used as a proxy for total phytoplankton biomass, was analysed spectrophotometrically (Lorenzen, 1967). Phytoplankton abundance and species composition, based only on strictly autotrophic and mixotrophic taxa, were evaluated using inverted microscopy, according to Utermöhl (1958). This strategy prevented the analysis of picophytoplankton (e.g., cyanobacteria and eukaryotes <2 μm) and morphologically inconspicuous nano-phytoplankton (2–20 μm). Phytoplankton were identified to the lowest taxonomic level possible (see Cravo et al., 2022a, and Lima et al., 2023, for details). In addition to our phytoplankton dataset, information on the abundance of potentially toxigenic phytoplankton taxa for the classified shellfish production area 'Olhão 2' (ca. 200 m east from station OE 250), analysed as part of the Portuguese shellfish safety monitoring program, was retrieved from the public database of the Portuguese Institute of Sea and Atmosphere (IPMA) (http://www.ipma.pt/pt/index.html/), for the study period (IPMA, 2018, 2019, 2020). This database, derived from approximately weekly sampling, included the abundance of the toxigenic taxa commonly reported in Portuguese coastal exposed and confined ecosystems. These taxa included *Pseudo-nitzschia* spp., *Dinophysis* spp., and *Gymnodinium catenatum*, which are responsible for the amnesic, diarrhetic and paralytic shellfish poisoning syndromes in humans (ASP, DSP, and PSP), respectively (for details on the use of the IPMA database, see Lima et al., 2022, 2023). Information from other classified RF lagoon shellfish production areas was not used due to the large distance between the IPMA sampling sites and our study areas.

2.4. Analysis of the trophic state of the study areas

The trophic index TRIX (Vollenweider et al., 1998) was used to characterize the general trophic status of the study areas and evaluate the influence of treated effluent disposal. TRIX combines pressure

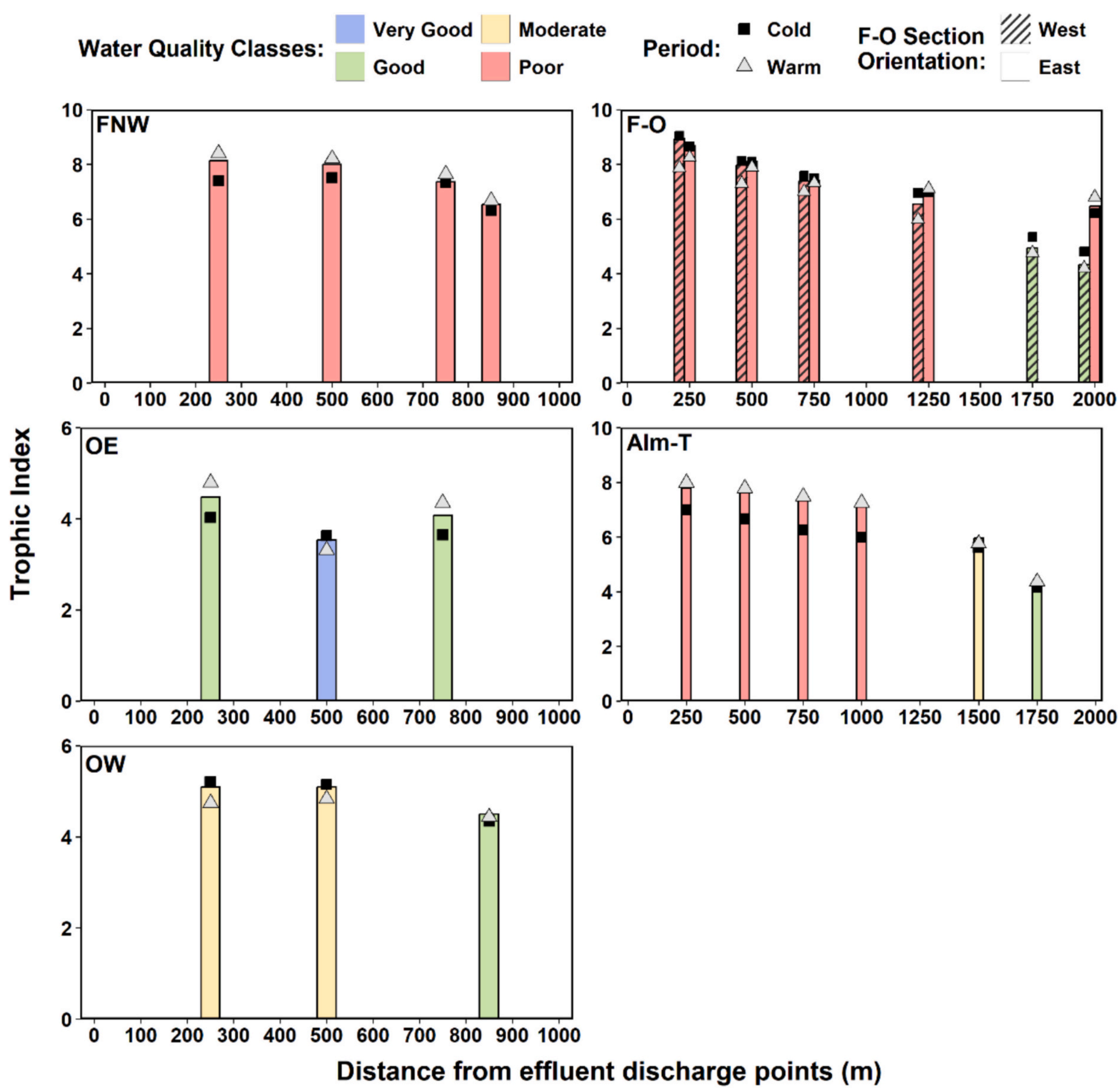

Fig. 2. Spatial variability of the mean values of the trophic index TRIX (bars) with distance (in meters) from the effluent discharge points in all the five study areas, between September 2018 and September 2020. The bar colours represent the trophic statuses and water quality classes associated with the mean TRIX values. Black squares and gray triangles represent mean values for the cold and warm period, respectively. Note differences in scales for both axes. See Fig. 1 for location of study areas and sampling stations.

variables (nutrients) and response variables (oxygen and Chl-a) related to eutrophication and was calculated according to Vollenweider et al. (1998; see Supplementary Material, Section A). TRIX index values were categorized into four trophic statuses, associated to four quality classes, using the following quality boundary values (Penna et al., 2004; Salas et al., 2008; Cabrita et al., 2015; Cervantes-Duarte et al., 2021): (a) Low trophic level/Oligotrophic (high quality): [0–4[; (b) Moderate trophic level/Mesotrophic (good quality): [4–5[; (c) High trophic level/Mesotrophic to Eutrophic (moderate quality): [5–6[; and (d) Very high trophic level/Eutrophic (poor quality): [6–10]. This index was applied to the five study areas (FNW, F—O, OW, OE and Alm-T) and to each of the 29 stations (Fig. 2) and, for each station, integrated over the entire 2-year study period, and over two specific periods (warm period: April–September; and cold period: October–March), as also applied for the WQIB and CCME-WQBI indices.

2.5. Development of a novel water quality index for bivalves and consumer safety (WQIB)

2.5.1. Water quality parameters, and parameter sub-index functions and values

Our novel index, specifically targeting the condition of bivalve molluscs and consumer safety (WQIB), integrates nine parameters: (a) key environmental determinants of water quality and physiological condition of bivalve molluscs, including suspended solids (e.g., Sobral and Widdows, 2000; Webber et al., 2021), Chl-a (e.g., Vaz et al., 2021), dissolved oxygen saturation (e.g., Sobral and Widdows, 1997; Sampaio et al., 2021; Scanes and Byrne, 2023), salinity (Navarro and Gonzalez, 1998; Carregosa et al., 2014; Pourmozaffar et al., 2020; Rato et al., 2022), and unionized ammonia (Batley and Batley and Simpson, 2009; Salmond and Wing, 2022); and (b) variables that mostly control shellfish safety for consumers (*E. coli*, and three common toxigenic phytoplankton taxa). Some of these water quality parameters (salinity, suspended solids, and *E. coli*) have been used as proxies for wastewater discharges (Cravo et al., 2022a; Mendonça et al., 2022) and have been included in legal frameworks for shellfish waters (Directive 2006/113/EC, 2006, no longer in force but transposed into national legislation, see Decree-Law (DL) 236/98; Diário da República, 1998). *E. coli* was selected since it is currently considered the preferred indicator for faecal contamination (Uddin et al., 2021). As generally recommended (Swamee and Tyagi, 2000; CCME, 2017; Ma et al., 2020), the water quality parameters used in the WQIB were not highly correlated (see sub-section 2.7), considering a threshold correlation coefficient (r_S) of $|r_S| > 0.70$ (Dormann et al., 2013; Lima et al., 2023).

The concentration or level of each WQIB parameter was transformed into unitless sub-index values by categorical scaling (i.e., linear segmented functions), considering integer numbers between 1 (best quality) and 4 (worst quality), as follows: 1 - very good; 2 – good; 3 – moderate; and 4 – poor. For each parameter, the threshold levels used to define the worst and best quality categories (Tables 1 and 2) were selected using non-arbitrary regulatory or recommended water quality standards and, in the case of Chl-a, from analysis of in situ observations from the RF lagoon (see below). Threshold parameter levels used to classify intermediate quality categories were further defined as quasi-proportional deviations from extreme threshold levels. Despite some degree of subjectivity associated with the later stage, this strategy for determining the sub-index values for each parameter avoided the normalization of observed parameter levels to sometimes subjective rating curves or functions (e.g., Giordani et al., 2009; Shah and Joshi, 2017). The definition of parameter thresholds based on species-specific tolerance and optimum levels (e.g., Vincenzi et al., 2006; Vaz et al., 2021) was also not considered due to their variability for different RF bivalve mollusc species (e.g., MMO, 2019), and the need to use the index parameters as indicators of the influence of WWTP discharges. Detailed information on the rationale behind the definition of water quality parameter sub-index functions and values, and supporting references, are provided as Supplementary Material (see Section B).

Table 1
Water quality parameters used in the water quality index for bivalve and consumer safety, WQIB (excluding toxigenic phytoplankton), threshold or range levels used to define each of the four quality categories, and associated sub-index values.

Parameters (units)	Sub-index values for each parameter quality category			
	1 (Very good)	2 (Good)	3 (Moderate)	4 (Poor)
Salinity (% variation in respect to reference)	< 5	[5–10[	[10–20[	≥20
Suspended Solids (% variation in respect to reference)	<10	[10–20[	[20–30[	≥30
Dissolved Oxygen (% saturation)	100–120	[80–100[, [120–140[	[60–80[, [140–160[	<60, ≥160
Unionized ammonia (NH_3-N L^{-1}, mg L^{-1})	<0.02	[0.02–0.1[	[0.1–0.2[	≥0.2
Escherichia coli (Most Probable Number/100 mL)	<100	[100–200[	[200–300[	≥300 (sensitive areas)
	<100	[100–500[	[500–2000[	≥2000 (non-sensitive areas)
Chlorophyll-a (μg L^{-1})	<2	[2–8[	[8–15[	≥15

Table 2
Potentially toxigenic phytoplankton taxa used in the water quality index for bivalve and consumer safety (WQIB), threshold or range levels used to define each of the four quality categories for each taxa, and associated sub-index values. Alert levels are underlined, and interdiction levels are shown in bold.

Phytoplankton taxa (units)	Sub-index values for each parameter quality category			
	1 (Very good; <alert level/2)	2 (Good; alert level/2 – alert level)	3 (Moderate; alert level – interdiction level)	4 (Poor; ≥ interdiction level)
Pseudo-nitzschia spp. (cells L^{-1})	<40,000	[40000–80,000[	[80000–200,000[	≥**200,000**
Dinophysis spp. (cells L^{-1})	<100	[100–200[	[200–500[	≥**500**
Gymnodinium catenatum (cells L^{-1})	<250	[250–500[	[500–1500[	≥**1500**

The abundances of key potentially toxigenic phytoplankton taxa, including *Pseudo-nitzschia* spp., *Dinophysis* spp. and *Gymnodinium catenatum*, were also used but aggregated into a single component of the WQIB index. In the case of the OE study area, the highest phytoplankton abundance between our estimates and the data from 'Olhão 2' classified shellfish production area, retrieved from the IPMA public database for the date closest to our sampling dates, was applied to all stations. For each toxigenic phytoplankton taxa, the threshold levels for the four water quality classes (Table 2) were based on the regulatory alert (trigger) and interdiction levels used by the IPMA regular monitoring program (https://www.ipma.pt/en/bivalves/docs/). The alert level, which imposes an increase in sampling frequency but allows bivalve harvesting, was used as the lower threshold for moderate water quality. The interdiction level, which imposes a closure of bivalve harvesting due to risks to human health regardless of bivalve phycotoxin content, was used as the threshold for the worst ('poor') water quality (Table 2). The value for this index component was the worst value of the three toxigenic taxa, as required by the "One Out - All Out" precautionary principle of the WFD (Directive 2000/60/EC, 2000).

2.5.2. Sub-index weighting factors and aggregation of weighted sub-index values

The final WQIB index value was estimated using a weighted additive function (e.g., Uddin et al., 2021) based on the values of each sub-index and the weight given to each sub-index, using Eqs. 1 and 2:

$$\mathrm{WQIB} = \sum_{i=1}^{n} \mathrm{SIi\ x\ Wi} \tag{1}$$

$$\sum_{i=1}^{n} Wi = 1 \tag{2}$$

where SI_i is the sub-index value for the *i*-th water quality parameter ($1 \leq SI_i \leq 4$, unitless integer), W_i is the weighting factor of the *i*-th water quality parameter, i.e., its importance with respect to the other parameters ($0 < W_i < 1$, unitless), and *n* is the number of aggregated sub-indices (seven).

However, if the sub-index value of critical water quality parameters for bivalve condition and/or consumer safety was equal to 4 (worst quality, i.e., 'poor'), regardless of the values of other sub-index components, the final WQIB index value was also scored as 4 ('poor'), as prescribed by the WFD precautionary principle ('One Out - All Out'; Directive 2000/60/EC, 2000). This approach represents an adjustment of the "minimum operator function" (Mogane et al., 2023 and references therein). These critical parameters included concentration of dissolved oxygen and unionized ammonia, and abundance of *E. coli* (Table 1) and potentially toxigenic phytoplankton taxa (Table 2). The final WQIB index values were categorized into the following four water quality classes: (a) Very Good: WQIB = [1.0–1.5[; (b) Good: WQIB = [1.5–2.5[; (c) Moderate: WQIB = [2.5–3.5[; and (d) Poor: WQIB = [3.5–4.0].

A sensitivity analysis was run to determine how changes in parameter weights affected the WQIB values. The first scenario assumed similar weights (0.143) for each of the seven parameters (Scenario 1, unweighted function). Other scenarios tested (Scenarios 2–4) used variable weights for each parameter (from 0.05 to 0.30; see Table 3). These were defined using published information on their potential impact on bivalve condition and consumer safety, expert knowledge from the RF lagoon system, and considering the need to use the index parameters as indicators of the potential impacts of WWTP discharges on bivalve condition and consumer safety, including unquantified environmental pressures (e.g., pesticides, metals, microplastics, pharmaceutical contaminants, potentially associated with discharges). The rationale behind the four scenarios tested and the results are included as Supplementary Material (see Section C and Table S2, respectively).

Sensitivity analysis showed no significant differences in the WQIB values between the four different scenarios, either for the entire dataset (Kruskal Wallis test, $p = 0.843$; see Table 3) or for each of the 16 sampling stations (Kruskal Wallis test, $p > 0.05$; see Table S2). However, due to the variable impact of each water quality parameter on bivalve condition and consumer safety (see Supplementary Material, Sections B and C), we considered that the water quality targeting bivalve condition and consumer safety was best described by the individual parameter weights used in Scenario 4 (Table 3). In total, the summed weight of index parameters related to the condition of bivalves (oxygen saturation, unionized ammonia and salinity) and consumer safety (toxigenic phytoplankton and *E. coli*) was 0.55 and 0.35, respectively.

The WQIB value was estimated for four study areas (FNW, F—O, OE and Alm-T), and for each of the 16 stations and sampling dates, during the 2-year study period. If, for any reason, information on one or more index parameters was not available, the final WQIB value was not calculated. Thus, the less frequent sampling of biological parameters precluded the analysis of WQIB in the OW area. For comparison purposes with other WQIs, mean WQIB values were also calculated for the entire study period and for two specific time periods. These included the warm period: April – September, i.e., spring – summer), and the cold period cold period: October – March, i.e., autumn - winter).

2.6. Application of the adapted Canadian Council of Ministers of the Environment Water Quality Index (CCME-QWI) for bivalves and consumer safety

The CCME-WQI has been widely used to assess the quality of riverine and marine waters and protect aquatic life (e.g., CCME, 2001; Gupta and Gupta, 2021). This flexible index requires the use of at least four water quality parameters, and is calculated, objectively, according to specific quality guidelines and their violations (Tyagi et al., 2013; Uddin et al., 2021). Considering our study aims, a CCME-WQI index targeting the condition of bivalve molluscs and consumer safety was developed ($\mathrm{CCME\text{-}WQI_B}$), based on all water quality parameters used in our novel WQIB index, except suspended solids. Considering that each CCME-WQI parameter has a fixed weight and increases in suspended solids at shallower stations can result not only from WWTP discharges, but also from sampling and/or natural resuspension events, this parameter was not considered. The eight water quality parameters and guidelines used were as follows: (1) salinity >30 (typical value for marine waters); (2) dissolved oxygen close to saturation, 100 % ± 20 % (80 %–120 %, Decree Law 236/98); (3) Chl-a $\leq 5\ \mu g\ L^{-1}$ (typical values for the RF lagoon areas not impacted by effluent disposal; Barbosa, 2010; Cravo et al., 2022a); (4) unionized ammonia $\leq 0.05\ NH_3\text{-}N\ L^{-1}\ mg\ L^{-1}$ (see values referred for marine organisms by Haywood, 1983 and US EPA, 1989); (5) *E. coli* < 300 MPN/100 mL or < 2000 MPN/100 mL (see Table 1); (6–8) abundance of specific toxigenic phytoplankton taxa below regulatory alert levels imposed by IPMA (see Table 2). Conservative guidelines were selected for most index parameters (usually within good to very good WQIB levels, see Tables 1 and 2), as this is considered more accurate (Lumb et al., 2011; CCME, 2017).

The $\mathrm{CCME\text{-}WQI_B}$ index value was calculated according to CCME (2017), based on three measures of variance from the selected water quality guidelines (see Supplementary Material, Section D): (a) scope of exceedance; (b) frequency; and (c) amplitude. The $\mathrm{CCME\text{-}WQI_B}$ index values, which range from zero (worst quality) to 100 (best quality), were converted into four water quality classes using the following categorization scheme (CCME, 2017): (a) Excellent (i.e., Very good): $\mathrm{CCME\text{-}WQI_B}$ = 95–100 (virtual absence of threat or impairment); (b) Good: $\mathrm{CCME\text{-}WQI_B}$ = 80–94 (minor degree of threat or impairment); (c) Fair plus Marginal (i.e., Moderate): $\mathrm{CCME\text{-}WQI_B}$ = 45–79 (occasional to

Table 3
The four scenarios used to test the influence of parameter weighting factors (0–1) on the water quality index for bivalves and consumer safety (WQIB) in the Ria Formosa coastal lagoon, considering the entire dataset (period: September 2018 – October 2020), with mean WQIB values (± 1 standard error) for each scenario. Index parameters - O_2 sat.: oxygen saturation; *E. coli*: abundance of *Escherichia coli*; HABs: abundance of potentially toxigenic phytoplankton; NH_3: concentration of unionized ammonia; Chla: chlorophyll-a concentration; Sal: salinity; and SS: suspended solids.

Scenario	Parameter weighting factors							WQIB
	O_2 sat.	*E. coli*	HABs	NH_3	Chla	Sal.	SS	Mean ± SE
1	0.143	0.143	0.143	0.143	0.143	0.143	0.143	2.50 ± 1.25
2	0.30	0.20	0.20	0.10	0.075	0.075	0.05	2.47 ± 1.26
3	0.25	0.15	0.175	0.225	0.075	0.075	0.05	2.45 ± 1.27
4	0.25	0.15	0.20	0.20	0.05	0.10	0.05	2.46 ± 1.27

frequent threat or impairment); and (d) Poor: CCME-WQI_B = 0–44 (almost always threatened or impaired). This strategy represents an adaptation of the original five quality classes (CCME, 2001; 2017). Merging the CCME-WQI original marginal and fair classes into a moderate class allowed for a direct comparison with our WQIB values.

CCME-WQI_B index values were calculated for each of the 16 stations in the four study areas and integrated for the entire 2-year study period and for two specific periods, warm period (April – September) and cold period (October – March). Since at least four different samples within the same period are required to estimate the CCME-WQI (Tyagi et al., 2013), this index was not calculated for individual sampling events, as was applied for the WQIB. Our strategy for deriving CCME-WQI_B values (eight water quality parameters and 12 samples per sampling site and year) followed the recommendations of CCME (2017). These include a minimum of eight quality parameters and about 10 samples per sampling site and year, to ensure meaningful index results, that are representative of natural site variability (CCME, 2017).

2.7. Statistical analysis

Basic assumptions for parametric analyses, including data normality and homogeneity of variance, were tested using the Shapiro-Wilk and Levene tests, respectively, and nonparametric methods were used when required. All statistical analyses were considered at a significance level of $\alpha = 0.05$ and conducted using R version 4.0.3 (R Core Team, 2020). Differences in variables between study areas, considering all stations, were tested using the Kruskal Wallis test, a one-way analysis of variance by ranks, followed by Dunn's post-hoc test (Sokal and Rohlf, 1995). Assuming dependence along longitudinal transects, differences between stations for each study area and variable were assessed using the nonparametric Durbin test, a Friedman-type analysis of variance by ranks, for balanced incomplete block designs, followed by multiple pairwise comparisons using the corresponding post-hoc test (Durbin, 1951).

The strength of monotonic relationships between environmental variables, potential index parameters, and WQIs were assessed using the Spearman rank correlation coefficient, r_S (see correlation coefficients in Table S3). Variables highly correlated ($|r_S| > 0.70$; Dormann et al., 2013) were not used as index parameters. The influence of index parameter weighting factors on the WQIB values, evaluated for four different scenarios (Table 3), considering the entire dataset or each of the sampling station, was tested using the Kruskal Wallis test, followed by Dunn's post-hoc test (Sokal and Rohlf, 1995). This strategy was also used to test differences in the values of trophic index TRIX and the water quality indices (WQIB and CCME-WQI) between study areas, and differences in WQIB between sampling sites within each study area, and time periods (spring – summer, and autumn – winter).

3. Results

3.1. Environmental variability between and within the study areas

Basic statistical information on the water quality variables for the five lagoon areas and temporal variability for each variable and study area are included as Supplementary Material (see Table S4, and Figs. S1 and S2). Considering the variables conventionally used as indicators of wastewater discharges, FNW, F—O, and Alm-T study areas showed a higher influence of WWTP discharges, with lower salinities, and higher concentrations of suspended solids and *E. coli*, compared to OW and OE areas ($p < 0.01$; Table S4 and Fig. S1). Higher concentrations of ionized ammonia, ammonium, and Chl-a were also detected in the former study areas ($p < 0.01$), namely ammonium in FNW and F—O (Fig. S1), and Chl-a in F—O and Alm-T areas (Fig. S2). Potentially toxigenic phytoplankton generally showed higher abundances in OE, OW and Alm-T areas ($p < 0.01$), namely *Pseudo-nitzschia* spp. ($p < 0.01$; Table S4 and Fig. S2).

Spatial variability along the longitudinal transects of effluent dispersal was detected for most variables in all study areas ($p < 0.05$), except in the FNW (Chl-a) and OE (temperature, suspended solids, Chl-a and *Dinophysis* spp.; data not shown). In the FNW, F—O and Alm-T areas, lower salinities were recorded at the stations closest to the EDPs ($p < 0.01$), and values below 20 occurred episodically in F—O and Alm-T but frequently in FNW (Fig. S1). Salinities lower than 20 % of the reference conditions, the threshold for poor water quality (Table 1), reached sites up to 750 m from the EDPs and were detected more frequently (ca. 50 %) in FNW area. Concentrations of suspended solids were higher at the stations closest to the EDPs ($p < 0.01$), namely at FNW and F—O (Fig. S1). Values above 30 % of the reference conditions, used as a threshold for poor water quality (Table 1), were detected at stations up to 750 m from the EDPs, more frequently in FNW (33 %) and F—O (ca. 50 %) areas. Concentration of nitrate, ammonium, phosphate and silicate increased at stations closest to the EDPs in all study areas ($p < 0.01$; data not shown). Chl-a increased at these stations in the F—O, Alm-T ($p < 0.01$), and OW ($p < 0.05$) areas, and values above 15 μg L^{-1}, the threshold for poor water quality (Table 1), were not detected in OW and OE areas, rarely in FNW and Alm-T but regularly detected in F—O area up to 750 m from the EDPs; Table S4 and Fig. S2).

Unionized ammonia was higher than 0.2 mg L^{-1}, the lethal threshold concentration (Table 1), only in the FNW area, at stations closest to the EDP (Table S3). *E. coli* abundance was higher at the stations closest to the EDPs, for all study areas ($p < 0.01$), reaching mean abundances ca. 500-fold higher than at the furthest station in the F—O area (Table S4 and Fig. S1). In the FNW and F—O areas, *E. coli* threshold abundance for poor water quality (300 MPN/100 mL; Table 1) was frequently exceeded up to 750 m of the EDPs (Fig. S1). In OE and Alm-T, *E. coli* threshold abundance for poor water quality (2000 MPN/100 mL; Table 1) was surpassed only once (Fig. S1). Dissolved oxygen saturation levels indicative of poor water quality (<60 % or ≥ 160 %; Table 1) were regularly detected in the FNW, F—O and Alm-T areas (Fig. S1), at stations closest to the EDPs (Table S4). The abundance of toxigenic phytoplankton was homogeneous within the FNW, OW and OE areas, but higher in OE, namely in case of *Pseudo-nitzschia* spp. (Table S4 and Fig. S2). In OE, abundances above the thresholds for poor water quality were regularly detected, generally affecting all stations simultaneously. In the F—O and Alm-T areas, toxigenic phytoplankton increased significantly with distance from the EDPs ($p < 0.01$).

Temporal variability showed consistent intra-annual patterns for most water quality variables. Water temperature (data not shown), and salinity were generally higher during the late spring – summer period, in all study areas (Fig. S2). Concentrations of ammonium (Fig. S2) and other dissolved inorganic nutrients (data not shown) were usually higher during the autumn-winter period. By contrast, Chl-a showed maxima in spring (F—O area) or summer (FNW, OE, OW and Alm-T areas; Fig. S2). In the Alm-T area, extreme oxygen oversaturation levels (> 300 %) were episodically detected up to 750 m of the EDP, during a mixed bloom of macroalgae and phytoplankton (July 2019; ca. 140 μg Chl-a L^{-1}; Table S4, and Figs. S1 and S2). Potentially toxigenic phytoplankton were also more abundant during the summer period, for most study areas and taxa (Fig. S2). The abundances of *Pseudo-nitzschia* spp. and *Dinophysis* spp. in the classified lagoon shellfish production areas, retrieved from the IPMA public database, were also generally higher during summer (Fig. S3). Overall, summer appeared to be the most critical period for water quality considering chemical and phytoplankton variables. However, in the FNW, F—O and Alm-T areas, increases in the abundance of *E. coli* further promoted a relative water quality degradation during the cold period (Fig. S1). The decommissioning of the OW WWTP, in late October 2018, was followed by a marked reduction in ammonium, *E. coli* and Chl-a concentrations, and the upgrading of the F—O WWTP was followed by a 6-month period of elevated and highly variable concentrations for these variables, and a later improvement in water quality (Figs. S1 and Fig. S2).

3.2. Variability in the trophic status between and within the study areas

The mean values (± 1 standard error) of TRIX for the five study areas, considering the two-year study period and all stations within each area ($n = 29$; Fig. 2), ranged from 4.0 ± 0.2 (mesotrophic, good quality) to 7.5 ± 0.4 (eutrophic, poor quality) in OE and FNW, respectively. Differences in TRIX values were detected between the study areas, with higher values (i.e., lower water quality) in the FNW and F—O areas, and lower values (i.e., higher water quality) in OE area, where all stations are in a main lagoon channel ($p < 0.01$). Mean TRIX values for the entire study period generally decreased with distance from the EDPs in all five study areas, more markedly in the FNW and Alm-T areas (Fig. 2). Values were generally higher during the warm period (April–September) compared to the cold period (October–March) for most study areas, namely stations closer to the EDPs. However, in the F—O area, stations located along the western section showed a higher trophic status during the cold period (Fig. 2).

3.3. Application of a novel water quality index for bivalves and consumer safety (WQIB)

The mean values (± 1 standard error) of the WQIB for the four study areas considering the two-year study period and all stations within each area were, in descending order (i.e., ascending water quality), the following: (a) FNW: 3.15 ± 0.13 (moderate quality); (b) OE: 2.38 ± 0.17 (good quality); (c) F-O: 2.19 ± 0.11 (good quality); and (d) Alm-T: 2.13 ± 0.14 (good quality). WQIB values were detected between the study areas, with similar lower values in the Alm-T, F—O and OE areas, and higher values in the FNW area ($p < 0.01$). The relatively low mean values detected in the F—O area were partly due to the inclusion of two stations further away from the EDP, compared to other areas (see below). The mean WQIB value without these stations (2.53 ± 0.14; moderate quality) still provided the same spatial differences between areas, yet statistically less strong ($p < 0.05$).

The spatial variability in the mean WQIB values within study areas, was significant for all study areas except OE, yet weaker in Alm-T ($p < 0.05$) than in FNW and F—O areas ($p < 0.01$). Mean WQIB values progressively decreased along the longitudinal transect from the EDPs, indicating an improvement in water quality, also observed in TRIX, and differences were detected between the closest and the furthest stations in the FNW, F—O (more marked for the western section), and Alm-T areas (Fig. 3). In the FNW area, the percentage of samples classified as poor ranged from ca. 43 % to 86 % at FNW 850 and FNW 250 (station designation in meters from the EDP), respectively (Fig. S4). In the OE area, the proportion of samples classified as poor (38–43 %) and very good (38–57 %) was relatively similar, for all stations (Fig. S4). The percentage of samples classified as poor ranged from 20 % to 52 % in the F—O area and was <25 % in the Alm-T area, for all stations (Fig. S4).

The temporal variability of the WQIB values for each sampling event and station allowed the identification of episodic water quality problems

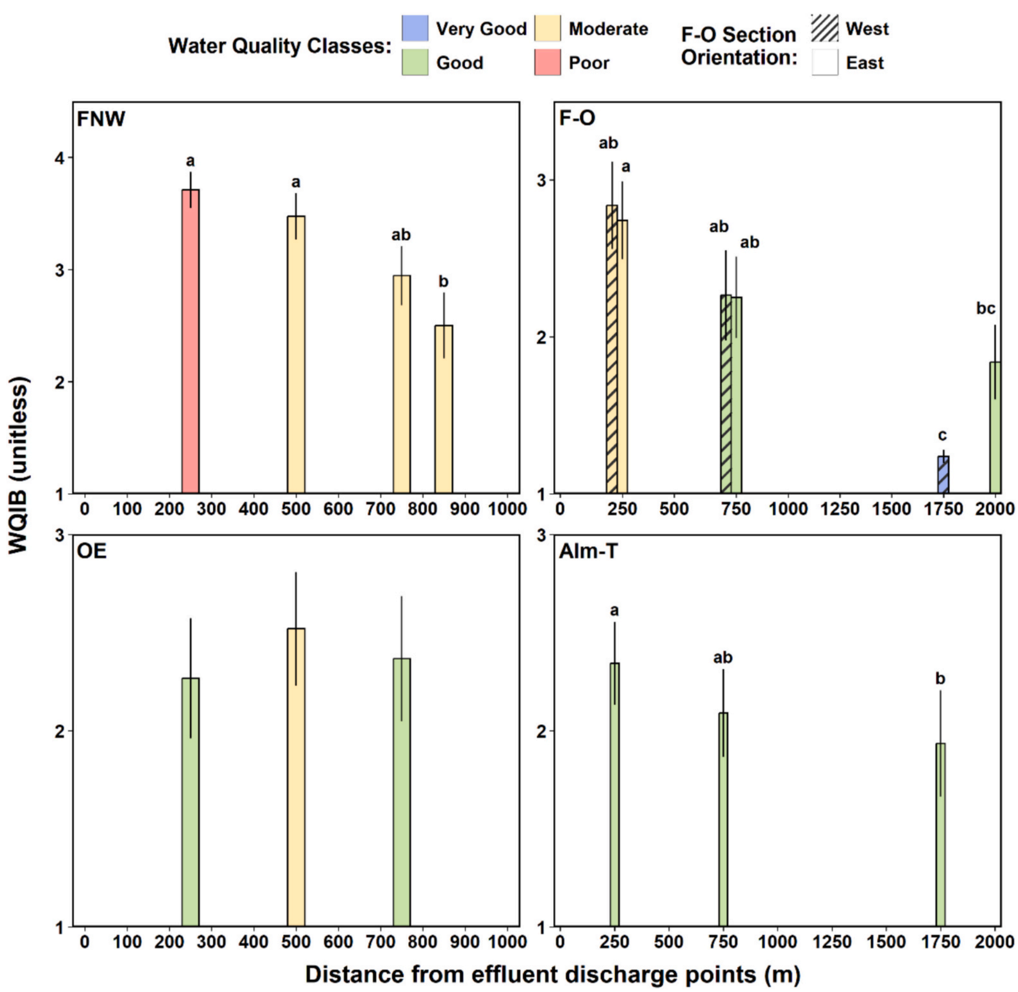

Fig. 3. Spatial variability of the mean values (± 1 standard error) of the Water Quality Index for Bivalve condition and consumer safety (WQIB) with distance (in meters) from the effluent discharge points in the four main study areas, between September 2018 and September 2020. The bar colours represent the water quality classes associated with the mean WQIB values. For each study area, different letters above the bars indicate significant differences between stations ($p < 0.05$). Note differences in scales for both axes. See Fig. 1 for location of study areas and sampling stations.

Table 4
Temporal variability of the value of the water quality index for bivalve condition and consumer safety (WQIB), calculated for each sampling event and station, for the four main study areas, for the period between September 2018 and September 2020). WQIB values were categorized into five water quality classes, represented by the following colours: Blue -; very good); Green - good); Yellow - moderate); and Red - poor). If the assigned classification was 'poor' (marked in red), the responsible index parameter(s) are indicated (abbreviations in white), and WQIB values are omitted. Index parameters: D – abundance of *Dinophysis* spp.; Ec – abundance of *Escherichia coli*; G – abundance of *Gymnodinium catenatum*; NH3 – concentration of unionized ammonia; O2 – saturation of dissolved oxygen; and P - abundance of *Pseudo-nitzschia* spp. White cells: WQIB values not available. Mean WQIB values for the cold period (October – March; dates labelled in gray), the warm period (April – September), and the entire study period are also provided (see lower table rows). For each period (cold or warm), asterisks indicate significantly higher values ($p < 0.05$) for each station. See Fig. 1 for location of study areas and sampling stations.

Date (Month/Year)	FNW				F-O						OE			Alm-T		
	250	500	750	850	1750W	750W	250W	250	750	2000	250	500	750	250	750	1750
09/2018	Ec	Ec, O2	Ec	Ec	1.0	1.4	1.6	Ec	O2, G	1.2	P	P	P	1.6	1.6	1.7
10/2018	Ec	Ec	1.4	1.2	1.4	Ec	Ec, O2	Ec, O2	Ec	Ec	1.4	2.0	1.8	2.2	1.8	P
11/2018	Ec, NH3			Ec	1.4		Ec, O2	Ec, O2		1.2	1.1	1.2	1.0	1.9		1.4
12/2018	1.9	1.8	1.8	1.7	1.2	Ec	Ec, O2	Ec, O2	Ec	1.5	1.4	1.2	1.0	Ec	1.7	1.0
02/2019	Ec	Ec	Ec, O2	Ec	1.6	Ec	Ec, O2	2.3	2	1.4	1.2	1.2	1.0	1.7	1.2	1.0
03/2019	NH3	NH3	O2	1.5	1.6	Ec	Ec	Ec, O2	Ec	1.6	1.0	1.2	1.0	1.9	1.4	1.2
04/2019	Ec	Ec	O2	O2	1.3	1.6	2	Ec	Ec	1.7	1.0	1.5	1.2	2.1	1.9	1.2
05/2019	1.9	Ec	1.7	1.2	1.2	1.5	2.1	Ec, O2	1.8	O2	1.2	1.2	P	1.9	O2	1.2
06/2019	2.1	Ec, O2	1.7	1.2	1.0	1.2	1.4	1.6	1.5	O2	P	P	P	O2	O2	G
07/2019	Ec	Ec	Ec	Ec	1.4	O2	O2	2.1	1.7	1.8	D	D	D	O2	O2	1.3
08/2019	Ec	Ec	O2	Ec	1.3	1.3	1.8	1.9	1.4	1.3	P	P	P	O2	1.9	1.3
09/2019	Ec, O2	O2	O2	O2	1.4	O2	O2	O2	O2	Ec	P	P	P	1.9	1.9	1.7
10/2019	Ec	Ec	Ec	Ec	1.0	1.4	1.2	2.1	1.8	1.2	P	P	P, G	1.9	1.4	
11/2019	Ec	1.7	1.6	1.1	1.0	1.0	1.1	1.8	1.3	1.2	1.1	1.7	1.0	1.7	1.2	1.0
01/2020	Ec	2.3	1.6	1.4	1.2	1.4	Ec	1.8	1.3	1.2	1.2	O2	1.0	1.3	1.1	1.0
03/2020	NH3	1.9	1.7	1.4	1.2	1.0	1.0	1.6	1.5	1.2	1.0	1.2	1.0	1.6	1.5	1.2
05/2020	Ec	Ec	Ec	Ec	1.3	1.0	Ec	Ec	1.6	1.0	1.1	1.4	1.0	2.0	2.1	1.0
06/2020	Ec	1.8	1.4	1.2	1.3	O2	O2	1.7	1.3	1.3	1.6	1.7	1.5	2.1	2.2	P
07/2020	Ec	Ec, O2	O2	1.7	1.0	1.4	O2, D	1.6	1.2	1.0	P	O2	P	O2	O2, P	P
08/2020	Ec	Ec	2.0	1.5	1.2	1.6	1.7	1.2	1.4	1.4	1.3	1.4	1.2	1.9	1.7	1.5
09/2020	Ec	Ec	Ec	1.4	1.0	1.5	1.7	1.9	1.2	1.4	P	P	P	1.5	1.2	P
Warm period	3.7	3.8*	3.2	2.7	1.2	2.0	2.7	2.7	2.1	2.0	2.9*	2.9	3.1*	2.6	2.5*	2.2*
Cold period	3.8	3.0	2.5	2.3	1.3	2.6	3.0	2.8	2.5	1.6	1.5	2.0	1.4	2.0	1.4	1.5
Entire period	3.7	3.5	2.9	2.5	1.2	2.3	2.8	2.7	2.3	1.8	2.3	2.5	2.4	2.3	2.1	1.9

(Table 4). In the FNW area, poor water quality (52 out of 82 samples, i.e., 63 %) was primarily due to unconformities associated with *E. coli* (ca. 44 %), oxygen saturation (ca. 10 %) and *E. coli* combined with oxygen saturation (ca. 4 %). NH_3 concentrations above 0.2 mg L^{-1} were detected only at FNW, in ca. 5 % of samples (Table 4). Poor water quality in the FNW area was more frequent in summer than in other seasons, but differences between cold and warm periods were detected only at FNW 500 ($p < 0.05$; Table 4). In the F—O area, poor water quality (37 out of 124 samples, i.e., 30 %) was mostly due to unconformities associated with *E. coli* (26 %) and oxygen saturation (ca. 17 %). Differences between cold and warm periods were not detected ($p > 0.05$), but a higher frequency of poor water quality was detected up to five months after the F—O WWTP upgrade, namely at stations up to 750 m from the EDP (Table 4). In the OE area, poor water quality (26 out of 63 samples, i.e., 41 %) was mainly associated with potentially harmful phytoplankton, namely *Pseudo-nitzschia* spp., alone (ca. 32 %) or combined with other toxigenic taxa (ca. 7 %). Mean WQIB values were higher in warm periods compared to cold periods at OE 250 ($p < 0.05$) and OE 750 ($p < 0.01$; Table 4). In Alm-T area, poor water quality (14 out of 61 samples, i.e., 23 %) was mostly due to unconformities related with oxygen saturation levels (ca. 13 %) and harmful phytoplankton (ca. 10 %), namely *Pseudo-nitzschia* spp. at the station furthest from the EDP. Mean WQIB values were also higher in warm periods compared to cold periods at Alm-T 750 ($p < 0.01$) and Alm-T 1750 ($p < 0.05$; Table 4).

3.4. Application of the adapted Canadian Council of Ministers of the Environment Water Quality Index for bivalves and consumer safety (CCME-WQI_B)

The mean values (± 1 standard error) of the CCME-WQI_B for the four study areas, considering the two-year study period and all stations within each area, were, in ascending order, the following: (a) FNW: 51.3 ± 8.4 (moderate quality); (b) F-O: 57.4 ± 8.3 (moderate quality); (c) Alm-T: 61.9 ± 5.9 (moderate quality); and (d) OE: 70.2 ± 2.0 (moderate quality). In contrast to the TRIX and WQIB, no significant differences in median CCME-WQI_B values were detected between the study areas ($p > 0.05$).

Mean CCME-WQI_B values generally increased with distance from the EDPs, indicating an improvement in water quality more pronounced in the FNW and F—O areas, namely for the western F—O section (Fig. 4). Regarding temporal variability, mean CCME-WQI_B values were generally lower during the warm period compared to the cold period for most study areas and stations, except in F—O. In the latter area, stations located near or up to 750 m west from the EDP showed a worse water quality during the cold period (Fig. 4).

3.5. Comparative analysis of novel and adapted water quality indices

The mean classifications of the water quality assigned by the WQIB and the CME-WQI were significantly correlated, considering both the entire study period ($r_S = -0.67$, $n = 16$, $p < 0.01$) and the cold ($r_S = -0.83$, $n = 16$, $p < 0.00001$) and warm periods ($r_S = -0.59$, $n = 16$, $p < 0.05$), separately. The application of the trophic index TRIX, the novel

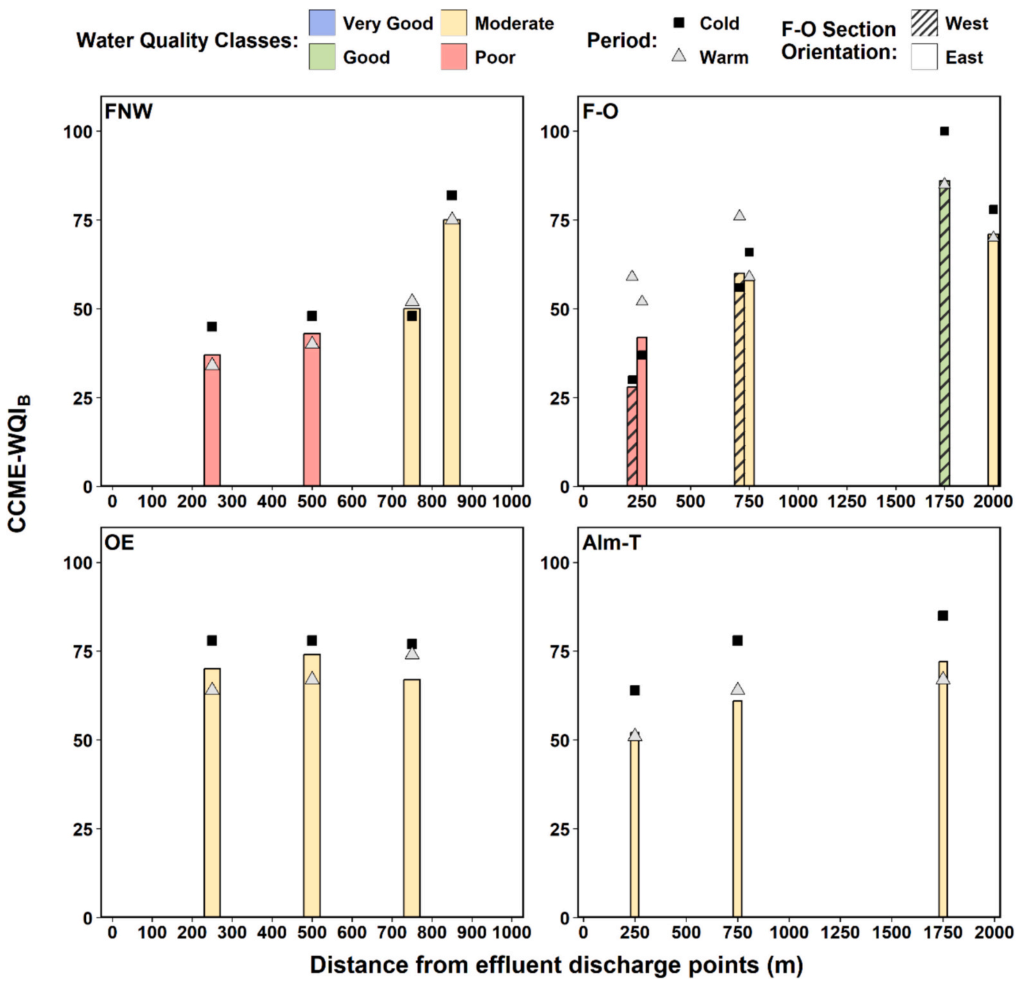

Fig. 4. Spatial variability in the mean values (bars) of the Canadian Council of Ministers of the Environment Water Quality Index for bivalves and consumer safety (CCME-WQI$_B$) with distance (in meters) from the effluent discharge points in the four main study areas, between September 2018 and September 2020. The bar colours represent the water quality classes associated with the mean CCME-WQI$_B$ values. Black squares and gray triangles represent the mean index values for the cold s and warm periods, respectively. Note differences in the x-axis scales. See Fig. 1 for location of study areas and sampling stations.

WQIB and the adapted CCME-WQI$_B$ revealed common trends in terms of the spatial variability, including a general increase in water quality along the longitudinal transects from the EDPs. Nonetheless, the mean classification assigned by the WQIB was higher (12 out of 16 stations) or similar (4 out of 16 stations) to that of the CCME-WQI (Fig. 5). TRIX provided the worst classification for all study sites except the more hydrodynamically exposed sites, including the OE area and Alm-T 1750 (Fig. 5), where good or very good classifications were detected.

4. Discussion

This study developed a novel water quality index specifically targeting the condition of bivalve molluscs and consumer food safety (WQIB) in a coastal lagoon system exposed to effluents from WWTPs. This is, to the best of our knowledge, the first WQI designed for this specific purpose, and also extends the limited number of WQIs designed and/or applied to transitional and marine systems. The performance of WQIB was compared with the adapted CCME-WQI$_B$, a flexible index widely used for water quality assessment (Gupta and Gupta, 2021; Uddin et al., 2021). Water quality at the study sites ranged from very poor to very good, depending on the location, time after the implementation of the WWTP upgrade (F—O area), period of the year, and the specific index applied.

4.1. Variability of the water quality to bivalve mollusc condition and consumer safety in the Ria Formosa coastal lagoon exposed to wastewater discharges

The environmental conditions and trophic statuses detected next to the EDPs are consistent with previous studies in the RF lagoon in areas adjacent to WWTP discharges (Cravo et al., 2015; Rosa et al., 2022), some of which used part of our dataset (Jacob et al., 2020; Cravo et al., 2022a; Caetano et al., 2023; Lima et al., 2023). Due to strong tidal flushing, the conditions at the farthest stations from the EDPs, close to main lagoons channels, were similar to those typically reported for the RF lagoon (Falcão and Vale, 2003; Newton and Mudge, 2005; Saraiva et al., 2007; Barbosa, 2010; Cravo et al., 2014, 2015, 2019; Rosa et al., 2019, 2022; Domingues, 2022; Newton et al., 2022). However, previous studies using single variables to assess water quality in the RF lagoon have showed contrasting classifications ranging from high/good based on Chl-a (Newton et al., 2003; Goela et al., 2009; Loureiro et al., 2006; Brito et al., 2012) to poor/bad status based on nutrient concentrations (Newton et al., 2003; Brito et al., 2012). Recent assessments of water quality in the RF lagoon have also yielded variable classifications, ranging from mostly "moderate", likely the result of inappropriate boundary settings based strictly on percentiles (Newton et al., 2022), to high/good status (Rosa et al., 2022).

All multimetric indicators (WQIB, CCME-WQI$_B$ and TRIX) consistently showed an improvement in water quality with distance from the

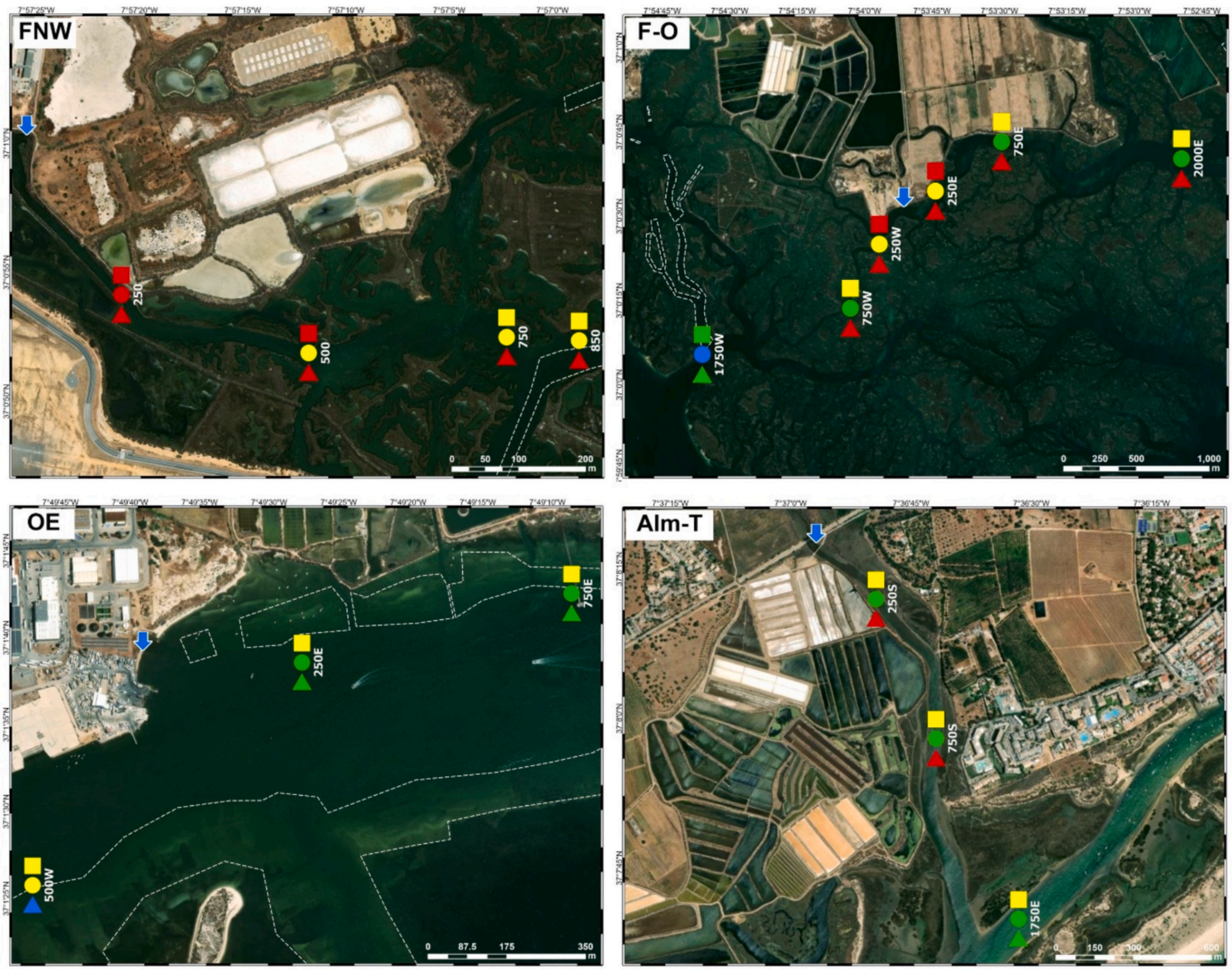

Fig. 5. Spatial variability of the trophic status and associated water quality (TRIX, triangles), the Water Quality Index for Bivalve condition and consumer safety (WQIB, circles), and the Canadian Council of Ministers of the Environment Water Quality Index for bivalves and consumer safety (CCME-WQI$_B$, squares) with distance from the effluent discharge points in the four main study areas, between September 2018 and September 2020. The notation used for each station represents the distance (in meters), from the effluent discharge points, represented as blue arrows. For each station, the position of circles (WQIB) represents the exact location of each station, and other symbols are shifted to allow their visualization. The symbol colours represent the water quality classes associated with the mean values of TRIX, WQIB, and CCME-WQI$_B$ (very good - blue; good - green; moderate - yellow; and poor - red). The white-delimited polygons represent bivalve mollusc harvesting grounds, provided by Agência Portuguesa do Ambiente. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

EDPs in all areas except the OE area, subjected to stronger hydrodynamic forcing. Considering only stations up to 750–850 m from the EDPs, the highest influence of WWTP discharges was detected in areas subject to variable effluent (N and P) loads but weak hydrodynamic conditions (see Table S1), the FNW (minimum loads) and the F—O areas (maximum loads). Indeed, as previously reported, the intensity and spatial extent of the wastewater footprint in the RF lagoon are strongly modulated by local hydrodynamic conditions (Cravo et al., 2015, 2022a; Jacob et al., 2020; Caetano et al., 2023; Lima et al., 2023), as also demonstrated by a dedicated hydrodynamic modelling study (Fabião et al., 2016). Overall, the strong tidal mixing and lagoon shallowness have generally limited the anthropogenic eutrophication problems to confined RF sites (Cravo et al., 2015; Domingues et al., 2017; Rosa et al., 2019, 2022; Newton et al., 2022) and, in case of WWTP, down to 500 m to 750 m from EDPs (Cravo et al., 2015, 2022a).

The water quality unconformities more frequently detected in the FNW, F—O and Alm-T areas, related to dissolved oxygen and/or *E. coli*, are commonly considered to be diagnostic of effluent discharge (Cravo et al., 2022a). Despite the increase in water quality with distance from the EDP for these study areas, at FNW 850, next to bivalve mollusc harvesting areas, water quality was frequently classified as poor. Indeed, faecal contamination of bivalves has been reported for areas next to WWTP discharges (Almeida and Soares, 2012; Botelho et al., 2015). In the F—O area, a more pronounced water quality improvement along the western channel section, implicit in all multimetric indicators, reflected the preferential circulation patterns toward the eastern channel section (Cravo et al., 2015; Fabião et al., 2016). However, at Alm-T 1750 and in the OE area, located in wider and deeper channels next to main lagoon inlets (see Fig. 1), high tidal exchange and rapid advection and dilution reduced the water quality problems associated with the WWTPs, but maximized the issues linked to the import of marine toxigenic phytoplankton. Indeed, both ASP (*Pseudo-nitzschia* spp.) and DSP (*Dinophysis* spp.) producers emerged as outer RF lagoon specialists (Lima et al., 2023). This pattern highlights the importance of coastal-lagoon connectivity and broader-scale coastal processes, not just WWTP discharges, as potential sources of water quality concerns, namely for bivalve mollusc consumers. This also reinforces the need to include phytoplankton metrics in WQIs, and bivalves and ecosystem management.

At the level of temporal variability, the poorer water quality generally identified by both WQIs during the warm period has also been reported, and linked to extreme oxygen saturation levels, and high Chl-a and *E. coli* concentrations (Cravo et al., 2015, 2022a; Caetano et al., 2023; Lima et al., 2023). In the OE area and outer Alm-T station, however, increases in HAB taxa during the warm period, also referred for the RF lagoon and adjacent coastal waters (Lima et al., 2022, 2023), were responsible for the poor water quality. By contrast, in the F—O area, the poorer water quality during the first cold period (October 2018–March 2019; high ammonium, suspended solids and *E. coli*) probably reflected the increase in effluent discharge rate (60 %), and the period of stabilization of the new biological treatment (ca. 5-month). Interestingly, during the second cold period and, under similar rainfall patterns (Lima et al., 2023), higher water quality supported the positive influence of the WWTP treatment upgrade, as referred by Jacob et al. (2020).

4.2. Comparative analysis of novel and adapted water quality indices

Despite the common patterns of spatial variability between TRIX and the two WQIs, TRIX provided the worst classification for all study sites except the more hydrodynamically exposed sites (OE area and Alm-T 1750). TRIX is not directly comparable to WQIB or CCME-WQI_B due to differences in objectives and parameters used. Despite the widespread use of TRIX as an indicator of trophic status and a proxy for water quality (e.g., Pérez-Ruzafa et al., 2019; Cereja et al., 2022; Rosa et al., 2022; Reyes-Velarde et al., 2023), the integration of variables that are not fully independent (chlorophyll *a*, dissolved oxygen, and nutrients), providing redundant information, and the use of classification thresholds developed for Mediterranean coastal waters, typically more oligotrophic, make its application in transitional waters challenging (e.g., Pettine et al., 2007; Salas et al., 2008; Giordani et al., 2009).

The motivation behind the WQBI and the CCME-WQI_B and the use of quasi-identical water quality parameters make their comparison appropriate. Indeed, these indices were significantly correlated, but a higher water quality was generally assigned by the WQIB. These differences, which never exceeded more than one quality class (Fig. 5), were anticipated given the various approaches used. Indeed, considerable differences solely related to the aggregation functions have been reported for WQIs applied to freshwater (Landwehr and Deininger, 1976; Lumb et al., 2011) and marine systems (Gupta et al., 2003; Nguyen et al., 2013; Nguyen and Nguyen and Sevando, 2019; Uddin et al., 2022). Also, differences in parameter guidelines (de Rosemond et al., 2009; Nada et al., 2024; Papaevangelou et al., 2024) or the number of quality parameters (Lumb et al., 2011; Akkoyunly and Akiner, 2012; Gamvroula and Alexakis, 2022; Marcelina et al., 2022) could also partly explain the differences detected between the two WQIs used in our study. In contrast to the WQIB, CCME-WQI_B involved a complex unweighted harmonic aggregation function, based on the deviation from specific parameter guidelines, and was integrated over time. In addition, the scope of exceedance (see Section 2.6) has a strong influence on the index results, independently on the exceedance amplitude, affecting all parameters equally (Nguyen and Nguyen and Sevando, 2019; Calmuc et al., 2020; Uddin et al., 2021, 2022), even those considered less relevant in our weighted WQIB.

A poorer water quality derived from the CCME-WQI compared to other WQIs has also been reported in freshwater (Lumb et al., 2011; Akkoyunly and Akiner, 2012; Finotti et al., 2015; Hamlat et al., 2017; Zotou et al., 2019; Alexakis, 2020; Lencha et al., 2021; Ramírez-Morales et al., 2021; Marcelina et al., 2022), transitional and coastal systems (Nada et al., 2024), despite exceptions (Gamvroula and Alexakis, 2022; Tiwari et al., 2022; Uddin et al., 2022). The CCME-WQI is generally considered a more 'stringent' (Lumb et al., 2011) or 'strict' (Alexakis, 2020) index, recommended as conservative enough to avoid overestimation of the water quality (Zotou et al., 2019). Also, the ability of this index to combine user-defined variables and its adaptability to different parameter guideline requirements and water uses are often referred as key advantages (see review by Chidiac et al., 2023). However, the CCME-WQI is also considered to be less sensitive to spatio-temporal changes in water quality (Zotou et al., 2019; Uddin et al., 2022; but see Gamvroula and Alexakis, 2022) and limited in assessing water quality variability downstream of a point source discharge (de Rosemond et al., 2009). In fact, the variability and sensitivity of the CCME-WQI_B were reduced compared to the WQIB, especially over the more hydrodynamic study areas, OE and Alm-T. Thus, given our specific aims and target, and despite the subjectivity inherent in the definition of the parameter weighting factors, the use of the weighted WQIB index should be favoured over the CCMW-WQIB (see next subsection).

4.3. The novel water quality index for bivalve condition and consumer safety (WQIB): Critical analyses and future uses

Despite their extensive uses and advantages, WQIs are affected by several problems (eclipsing, ambiguity, rigidity, sampling and expert biasing), sometimes aggravated by limited sampling strategies and data availability (Akkoyunly and Akiner, 2012; Gupta and Gupta, 2021; Uddin et al., 2021, 2022, 2023; Lukhabi et al., 2023; Mogane et al., 2023), that create a general bias toward physical and chemical parameters over biological metrics (Swamee and Tyagi, 2000). Our novel WQIB integrated four chemical and three biological sub-indices, using a weighted additive arithmetic aggregation function. This function is prone to eclipsing effects, that occur when some water quality parameters exceed critical levels, but the overall index does not reflect this, thus underestimating quality problems (e.g., Swamee and Tyagi, 2000; Uddin et al., 2022, 2023). However, eclipsing was minimized in our WQIB by: (a) the limited number of parameters, compared to indices with many parameters of reduced importance (Swamee and Tyagi, 2000; CCME, 2017); and (b) the use of critical influencing parameters (dissolved oxygen, unionized ammonia, *E. coli* and HAB taxa) which, if rated "very poor", forced the index classification to "very poor" as well, an adjustment of the "minimum operator function" (Mogane et al., 2023). In contrast to eclipsing, ambiguity is a situation when the WQI indicates a worse water quality than expected from the sub-index values, and it is usually minimized by weighted aggregation functions (Gupta et al., 2023). It is worth noting, however, that ambiguity poses less risk than eclipsing when considering a precautionary approach to water and ecosystem management.

Sampling bias, another problem of WQIs, was minimized by monthly sampling of RF lagoon areas under a wide range of environmental drivers, pressures and trophic conditions (oligotrophic to eutrophic), over a 2-year period. The spatio-temporal variability of WQIB demonstrated its sensitivity under these variable conditions, while its integration over the entire study period minimized the influence of extreme events (CCME, 2017). Other sources of uncertainty of WQIs are related with expert bias. For the WQIB, the selection of parameters and relative weighting factors were strongly based on knowledge of their significance for bivalve mollusc condition and consumer safety, and the use of non-arbitrary regulatory guideline values to define the sub-index values (see sub-section 2.5). All our model parameters, except toxigenic phytoplankton, have been widely used in WQIs (reviews by Kachroud et al., 2019; Gupta and Gupta, 2021; Uddin et al., 2021; Lukhabi et al., 2023; Mogane et al., 2023), and some (dissolved oxygen, salinity, suspended solids, Chl-a) were also included in habitat suitability models for bivalve mollusc aquaculture (Vincenzi et al., 2006; Silva et al., 2011; Picado et al., 2020; Vaz et al., 2021; Webber et al., 2021). Also, the use of higher weighting factors for dissolved oxygen, and lower factors for suspended solids and Chl-a in the WQIB is a common practice in most WQIs (e.g., Akkoyunly and Akiner, 2012; Nguyen et al., 2013; Hamlat et al., 2017; Uddin et al., 2021, 2023; Marcelina et al., 2022; Mogane et al., 2023).

However, some variables commonly used as water quality criteria (e.g., dissolved inorganic nutrients; Uddin et al., 2021; Mogane et al., 2023) and/or descriptors of habitat suitability for bivalves (e.g., water temperature, current velocity; Vincenzi et al., 2006; Silva et al., 2011; Picado et al., 2020; Vaz et al., 2021) were not used. Water temperature was not used because it is not affected by WWTP discharges in the RF (Jacob et al., 2020; Cravo et al., 2022a) and was also not considered in some WQIs applied to transitional and coastal systems due to high spatio-temporal variability (e.g., Nguyen et al., 2013). Inorganic nutrients per se are not indicative of water quality for bivalves in shallow ecosystems (Giordani et al., 2009), and the direct and indirect negative effects of high nutrient levels were already reflected in Chl-a and dissolved oxygen (e.g., Nguyen et al., 2013; Devlin et al., 2023). Some parameters used in WQIs (e.g., biological oxygen demand - BOD, oil and grease, heavy metals; reviews by Kachroud et al., 2019; Gupta and Gupta, 2021; Uddin et al., 2021; Lukhabi et al., 2023; Mogane et al., 2023) and habitat suitability for bivalves (e.g., current velocity; e.g., Vaz et al., 2021) were not considered mainly due to lack of contemporaneous data available for the RF lagoon. Yet, the negative effects of high BOD levels were, to a certain extent, already reflected in oxygen saturation.

A more comprehensive water quality assessment would then require a higher number of physical variables, including hydrodynamics, chemical and biological variables, and sustained aquatic ecosystem monitoring efforts. Future developments of the novel WQIB should also include water contaminants potentially toxic to bivalve molluscs and/or human consumers (e.g., heavy metals, persistent organic pollutants, agri-chemicals and pharmaceuticals; Webber et al., 2021; Cravo et al., 2022b; Lukhabi et al., 2023). An increase in the number of water quality classes and a more objective selection of parameters and weighting factors, based on multivariate analysis and artificial intelligence (e.g., Gupta and Gupta, 2021; Uddin et al., 2022, 2023), could also improve the robustness of water quality classification. Ideally, quantitative metrics of bivalve physiological condition (e.g., growth rate, condition index, survival, tissue contaminants, biomarkers) and their effective responses and tolerance to specific environmental changes should be used to identify the best set of water quality parameters and their weighting factors.

The scope, flexibility, and sensitivity of our novel WQIB and its application to individual sampling events, enabling the analysis of short-term variability, represents a critical advantage for the viability of a sustainable mariculture product, needed to support a growing human population (e.g., Gray, 2019; Brown et al., 2020; Webber et al., 2021) and a rising wastewater production (Qadir et al., 2020). In addition, the rationale behind the WQIB could be applied to other aquatic organisms (e.g., fish, crustaceans), ecosystems (e.g., coastal lagoons, estuaries, exposed coastal areas), variable natural (e.g., discharges from submarine vents) and anthropogenic stressors (e.g., discharges from chemical industries, desalination plants, aquaculture facilities, nuclear power plant's cooling systems), and purposes (e.g., resource exploitation, conservation, restoration). This would require the identification of appropriate water quality parameters, indicative of specific stressors, the prioritization of the water quality parameters, variable depending on the sensitivity of target organisms and environmental setting and stressors, and the adjustment of their specific local/regional guideline values, overall increasing the flexibility and value of our novel index.

5. Conclusions

Our study developed a novel water quality index specifically targeting the condition of bivalve molluscs and consumer food safety (WQIB), the first developed for this specific purpose, thus extending the limited number of WQIs applied to transitional and marine systems.

This index was applied to the RF coastal lagoon, in areas exposed to variable WWTP effluent loading regimes, trophic status (oligotrophic to eutrophic) and hydrodynamic conditions, over a ca. 2-year period. The water quality assigned by the novel WQIB and the adapted CCME-WQI$_B$ ranged from very poor to very good, depending on the specific index applied, study area and station, time after the implementation of the WWTP upgrade (F—O area), and period of the year. Poorer water quality was caused by effluent discharges up to 750 m from the EDPs, and by the advection of toxigenic phytoplankton from adjacent coastal waters, at stations near main lagoon inlets, more frequently in the warm period. Thus, our study highlighted the importance of wastewater treatment strategies, natural temporal variability patterns, local hydrodynamics, and broader scale coastal processes on the water quality for bivalve mollusc condition and consumer safety.

Water quality assigned by the WQIB and the CCME-WQI$_B$ were significantly correlated, but the CCME-WQI$_B$ generally indicated lower quality. Given the higher sensitivity of the WQIB, its application at the sampling event scale and its flexibility, this index is a more valuable tool for adaptive ecosystem management and can be tailored to other aquatic ecosystems and water use purposes.

CRediT authorship contribution statement

Alexandra Cravo: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Ana B. Barbosa:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Maria João Lima:** Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Cristina Ferreira:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Cátia Correia:** Investigation, Formal analysis, Data curation. **André Matos:** Investigation, Formal analysis. **José Jacob:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis. **Sandra Caetano:** Supervision, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2025.117814.

Data availability

Data will be made available on request.

References

Akkoyunly, A., Akiner, M., 2012. Pollution evaluation in streams using water quality indices: A case study from Turkey's Sapanca Lake Basin. Ecol. Indic. 18, 501–511. https://doi.org/10.1016/j.ecolind.2011.12.018.

Alexakis, D.E., 2020. Meta-evaluation of water quality indices. Application into groundwater resources. Water 12 (7), 1890. https://doi.org/10.3390/w12071890.

Almeida, C., Soares, F., 2012. Microbiological monitoring of bivalves from the ria Formosa lagoon (south coast of Portugal): a 20 years of sanitary survey. Mar. Pollut. Bull. 64, 252–262. https://doi.org/10.1016/j.marpolbul.2011.11.025.

Barbosa, A.B., 2010. Seasonal and interannual variability of planktonic microbes in a Mesotidal coastal lagoon (ria Formosa, SE Portugal), impact of climatic changes and local human influences. In: Paerl, H.W. (Ed.), Kennish, M.J. Coastal Lagoons, Critical Habitats of Environmental Change, pp. 336–366. https://doi.org/10.1201/EBK1420088304.

Batley, G.E., Simpson, S.L., 2009. Development of guidelines for ammonia in estuarine and marine water systems. Mar. Pollut. Bull. 58, 1472–1476. https://doi.org/10.1016/j.marpolbul.2009.06.005.

Behmanesh, A., 2014. Quality assessment of Babolroud river water based on qualitative index physicochemical characteristics and water's heavy metals. Adv. Environ. Biol. 8, 545–552.

Bonometto, A., Ponis, E., Cacciatore, F., Riccardi, E., Pigozzi, S., Parati, P., Novello, M., Ungaro, N., Acquavita, A., Manconi, P., Sfriso, A., Giordani, G., Brusà, R.B., 2022. A new multi-index method for the eutrophication assessment in transitional waters: large-scale implementation in Italian lagoons. Environments 9, 41. https://doi.org/10.3390/environments9040041.

Botelho, M.J., Soares, F., Matias, D., Vale, C., 2015. Nutrients and clam contamination by *Escherichia coli* in a meso-tidal coastal lagoon: seasonal variation in counter cycle to external sources. Mar. Pollut. Bull. 96, 188–196. https://doi.org/10.1016/j.marpolbul.2015.05.030.

Braga, A.C., Rodrigues, S.M., Lourenço, H.M., Costa, P.R., Pedro, S., 2023. Bivalve shellfish safety in Portugal: variability of Faecal levels, metal contaminants and marine biotoxins during the last decade (2011−2020). Toxins 15, 91. https://doi.org/10.3390/toxins15020091.

Brito, A.C., Quental, T., Coutinho, T.P., Branco, M.A.C., Falcão, M., Newton, A., Icely, J., Moita, T., 2012. Phytoplankton dynamics in southern Portuguese coastal lagoons during a discontinuous period of 40 years: an overview. Estuar. Coast. Shelf Sci. 110, 147–156. https://doi.org/10.1016/j.ecss.2012.04.014.

Brown, A.R., Webber, J., Zonneveld, S., Carless, D., Jackson, B., Artioli, Y., Miller, P.I., Holmyard, J., Baker-Austin, C., Kershaw, S., Bateman, B.J., Tyler, C.R., 2020. Stakeholder perspectives on the importance of water quality and other constraints for sustainable mariculture. Environ. Sci. Pol. 114, 506–518. https://doi.org/10.1016/j.envsci.2020.09.018.

Cabaço, S., Machás, R., Vieira, V., Santos, R., 2008. Impacts of urban wastewater discharge on seagrass meadows (Zostera noltii). Estuar. Coast. Shelf Sci. 78, 1–13. https://doi.org/10.1016/j.ecss.2007.11.005.

Cabrita, M.A., Silva, A., Oliveira, P.O., Angélico, M.A., Nogueira, M., 2015. Assessing eutrophication in the Portuguese continental exclusive economic zone within the European marine strategy framework directive. Ecol. Indic. 58, 286–299. https://doi.org/10.1016/j.ecolind.2015.05.044.

Caetano, S., Correia, C., Vidal, A.F., Matos, A., Ferreira, C., Cravo, A., 2023. Fate of microbial contamination in a south European coastal lagoon (ria Formosa) under the influence of treated effluents dispersal. J. Appl. Microbiol. 134(8), lxad166. https://doi.org/10.1093/jambio/lxad166.

Calmuc, M., Calmuc, V., Arseni, M., Topa, C., Timofti, M., Georgescu, L.P., Iticescu, C., 2020. A comparative approach to a series of Physico-chemical quality indices used in assessing water quality in the lower Danube. Water 12 (11), 3239. https://doi.org/10.3390/w12113239.

Carregosa, V., Velez, C., Soares, A.M.V.M., Figueira, E., Freitas, R., 2014. Physiological and biochemical responses of three Veneridae clams exposed to salinity changes. Comp. Biochem. Physiol. Part B 177–178, 1–9. https://doi.org/10.1016/j.cbpb.2014.08.001.

CCME (Canadian Council of Ministers of the Environment), 2001. Canadian Environmental Quality Guidelines. CCME Water Quality Index 1.0 User's Manual, (5p).

CCME (Canadian Environmental Quality Guidelines Canadian Council of Ministers of the Environment), 2017 - CCME Water Quality Index User's Manual 2017 Update, (23p).

Cereja, R., Brotas, V., Nunes, S., Rodrigues, M., Cruz, J.P.C., Brito, A.C., 2022. Tidal influence on water quality indicators in a temperate mesotidal estuary (Tagus estuary, Portugal). Ecol. Indic. 136, 108715. https://doi.org/10.1016/j.ecolind.2022.108715.

Cervantes-Duarte, R., Jimenez-Quiroz, M.dC., Funes-Rodriguez, R., Hernandez-Trujillo, S., Gonzalez-Armas, R., Anaya-Godinez, E., 2021. Interannual variability in the trophic status and water quality of Bahía Magdalena, Mexico, during the 2015–2018 period: TRIX. Reg. Stud. Mar. Sci. 42, 101638. https://doi.org/10.1016/j.rsma.2021.101638.

Chapman, P.M., 2012. Management of coastal lagoons under climate change. Estuar. Coast. Shelf Sci. 110, 32–35. https://doi.org/10.1016/j.ecss.2012.01.010.

Chidiac, S., El Najjar, P., Ouaini, N., El Rayess, Y., El Azzi, D., 2023. A comprehensive review of water quality indices (WQIs): history, models, attempts and perspectives. Rev. Environ. Sci. Biotechnol. 22, 349–395. https://doi.org/10.1007/s11157-023-09650-7.

Council Directive 91/271/EEC of 21 May 1991 concerning urban waste-water treatment. OJ L 135, 30/05/1991, p. 40–52.

Cravo, A., Barbosa, A.B., Correia, C., Matos, A., Caetano, S., Lima, M.J., Jacob, J., 2022a. Unravelling the effects of treated wastewater discharges on the water quality in a coastal lagoon system (ria Formosa, South Portugal): relevance of hydrodynamic conditions. Mar. Pollut. Bull. 174, 113296. https://doi.org/10.1016/j.marpolbul.2021.113296.

Cravo, A., Cardeira, S., Pereira, C., Rosa, M., Alcântara, P., Madureira, M., Rita, F., Correia, C., Rosa, A., Jacob, J., 2019. Nutrients and chlorophyll-a exchanges through an inlet of the ria Formosa lagoon, SW Iberia during the productive season – unravelling the role of the driving forces. J. Sea Res. 144, 133–141. https://doi.org/10.1016/j.seares.2018.12.001.

Cravo, A., Cardeira, S., Pereira, C., Rosa, M., Alcântara, P., Madureira, M., Rita, F., Luis, J., Jacob, J., 2014. Exchanges of nutrients and chlorophyll a through two inlets of ria Formosa, south of Portugal, during coastal upwelling events. J. Sea Res. 93, 63–74. https://doi.org/10.1016/j.seares.2014.04.004.

Cravo, A., Fernandes, D., Damião, T., Pereira, C., Reis, M.P., 2015. Determining the footprint of sewage discharges in a coastal lagoon in South-Western Europe. Mar. Pollut. Bull. 96, 197–209. https://doi.org/10.1016/j.marpolbul.2015.05.029.

Cravo, A., Rosa, A., Jacob, J., Correia, C., 2020. Dissolved oxygen dynamics in ria Formosa lagoon (South Portugal) - a real time monitoring station observatory. Mar. Chem. 223, 1–14. https://doi.org/10.1016/j.marchem.2020.103806.

Cravo, A., Silva, S., Rodrigues, J., Cardoso, V.V., Benoliel, M.J., Correia, C., Coelho, M.R., Rosa, M.J., Almeida, C.M.M., 2022b. Understanding the bioaccumulation of pharmaceutical active compounds by clams Ruditapes decussatus exposed to a UWWTP discharge. Environ. Res. 208, 112631. https://doi.org/10.1016/j.envres.2021.112632.

Darvishi, G., Ramezani, M., Kootenaei, F.G., Lotfi, E., Asgharnia, H., 2016. Comparative investigation of river water quality by OWQI, NSFWQI and Wilcox indexes (case study: the Talar River – IRAN). Arch. Environ. Prot. 42 (1), 41–48. https://doi.org/10.1515/aep-2016-0005.

de Rosemond, S., Duro, D.C., Dubé, M., 2009. Comparative analysis of regional water quality in Canada using the water quality index. Environ. Monit. Assess. 156, 223–240. https://doi.org/10.1007/s10661-008-0480-6.

Derolez, V., Malet, N., Fiandrino, A., Lagarde, F., Richard, M., Ouisse, V., Bec, B., Aliaume, C., 2020. Fifty years of ecological changes: regime shifts and drivers in a coastal Mediterranean lagoon during oligotrophication. Sci. Total Environ. 732, 139292. https://doi.org/10.1016/j.scitotenv.2020.139292.

Devlin, M.J., Prins, T.C., Enserink, L., Leujak, W., Heyden, B., Axe, P.G., Ruiter, H., Blauw, A., Bresnan, E., Collingridge, K., Devreker, D., Fernand, L., Gómez Jakobsen, F.J., Graves, C., Lefebvre, A., Lenhart, H., Markager, S., Nogueira, M., O'Donnell, G., Partner, H., Skarbøvik, E., Skogen, M.D., Sonesten, L., Van Leeuwen, S.M., Wilkes, R., Dening, E., Iglesias-Campos, A., 2023. A first ecological coherent assessment of eutrophication across the north-East Atlantic waters (2015–2020). Frontiers Ocean Sustainability 1, 1253923. https://doi.org/10.3389/focsu.2023.1253923.

Diário da República, 1998. Decree Law (Decreto-Lei) 236/98. Estabelece normas, critérios e objectivos de qualidade com a finalidade de proteger o meio aquático e melhorar a qualidade das águas em função dos seus principais usos. I SÉRIE-A, 176, 1 de Agosto 1998, 3676–3721.

Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. OJ L 327, 22/12/2000, p. 1–73.

Directive 2006/113/EC of the European Parliament and of the Council of 12 December 2006 on the quality required of shellfish waters (codified version) OJ L 376, 27/12/2006, p. 14–20.

Domingues, R.B., 2022. Seasonal and spatial variability of phytoplankton primary production in a shallow temperate coastal lagoon (ria Formosa, Portugal). Plants 11, 3511. https://doi.org/10.3390/plants11243511.

Domingues, R.B., Guerra, C.C., Barbosa, A.B., Galvão, H.M., 2017. Will nutrient and light limitation prevent eutrophication in an anthropogenically-impacted coastal lagoon? Cont. Shelf Res. 141, 11–25. https://doi.org/10.1016/j.csr.2017.05.003.

Dormann, C.F., Elith, J., Bacher, S., Carré, G.C.G., García Márquez, J.R., Gruber, B., Lafourcade, Leitão, P.J., Münkemüller, T., McClean, C.J., Osborne, P.E. Reneking, B. Schröder, B., Skidmore, A.K., Zurell, D., Lautenbach, S. 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography (Cop.) 36, 27–46. doi:https://doi.org/10.1111/j.1600-0587.2012.07348.x.

Durbin, J., 1951. Incomplete blocks in ranking experiments. Br. J. Psychol. Stat. Sect. 4, 85–90.

Emerson, K., Russo, R.C., Lund, R.E., Thurston, R.V., 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. J. Fish. Res. Board Can. 32, 2379–2383.

Fabião, J.P.F., Rodrigues, M.F.G., Fortunato, A.B., Jacob, J., Cravo, A., 2016. Water exchanges between a multi-inlet lagoon and the ocean: the role of forcing mechanisms. Ocean Dyn. 66, 173–194. https://doi.org/10.1007/s10236-015-0918-7.

Falcão, M., Vale, C., 2003. Nutrient dynamics in a coastal lagoon (ria Formosa, Portugal): the importance of lagoon-sea water exchanges on the biological productivity. Ciencias Mar. 29, 425–433. https://doi.org/10.7773/cm.v29i4.173.

Fataei, E., Seyyedsharifi, S.A., Seiiedsafaviyan, S.T., Nasrollahzadeh, S., 2013. Water quality assessment based on WQI and CWQI indexes in Balikhlou River. Iran J. Basic. Appl. Sci. Res. 3 (3), 263–269.

Filgueira, R., Guyondet, Th., Comeau, L.A., Tremblay, R., 2016. Bivalve aquaculture-environment interactions in the context of climate change. Glo. Change Biol. 22 (12), 3901–3913. https://doi.org/10.1111/gcb.13346.

Finotti, A.R., Finkler, R., Susin, N., Schneider, V.E., 2015. Use of water quality index as a tool for urban water resources management. Int. J. Sus. Dev. Plann. 10 (6), 781–794. https://doi.org/10.2495/SDP-V10-N6-781-794.

Gamvroula, D.E., Alexakis, D.E., 2022. Evaluating the performance of water quality indices: application in surface water of Lake union. Washington State-USA. Hydrology 9, 116. https://doi.org/10.3390/hydrology9070116.

Giordani, G., Zaldívar, J.M., Viaroli, P., 2009. Simple tools for assessing water quality and trophic status in transitional water ecosystems. Ecol. Indic. 9, 982–991. https://doi.org/10.1016/j.ecolind.2008.11.007.

Goela, P.C., Newton, A., Cristina, S., Fragoso, B., 2009. Water framework directive implementation: intercalibration exercise for biological quality elements - a case study for the south coast of Portugal. J. Coast. Res. 2, 1214–1218.

Grasshoff, K., Kremling, K., Erkhardt, M., 1999. Methods of Seawater Analysis, 3[th] ed. Wiley, New York. https://doi.org/10.1002/9783527613984.

Gray, L., 2019. Developing Criteria and Methodology for Determining Aquaculture Zones under Marine Spatial Planning in the EU. Aquaculture Advisory Council, Brussels.

Gupta, A.K., Gupta, S.K., Patil, R.S., 2003. A comparison of water quality indices for coastal water. J. Envir. Sci. Health. Part A 38 (11), 2711–2725. https://doi.org/10.1081/ESE-120024458.

Gupta, S., Gupta, K., 2021. A critical review on water quality index tool: genesis, evolution and future directions. Ecol. Inform. 63, 101299. https://doi.org/10.1016/j.ecoinf.2021.101299.

Hamlat, A., Guidoum, A., Koulala, I., 2017. Status and trends of water quality in the Tafna catchment: a comparative study using water quality indices. J. Wat. Reuse and Desal. 7 (2), 228–245. https://doi.org/10.2166/wrd.2016.155.

Haywood, G. P. 1983. Ammonia toxicity in teleost fishes: A review. Can. Tech. Rep. Fish. Aquat. Sci. 1177: iv + 35 p.

IPMA, 2018. Results of Harmful Phytoplankton (HAB Bulletins: October – December 2018), National Monitoring System of Bivalve Mollusks (Sistema Nacional de Monitorização de Moluscos Bivalves, Resultados das Determinações de Fitoplâncton Nocivo). Instituto Português Do Mar e da Atmosfera. accessed. 31/06/2022. http://www.ipma.pt/en/bivalves/fito/.

IPMA, 2019. Results of Harmful Phytoplankton (HAB Bulletins: January – December 2019), National Monitoring System of Bivalve Mollusks (Sistema Nacional de Monitorização de Moluscos Bivalves, Resultados das Determinações de Fitoplâncton Nocivo). Instituto Português Do Mar e da Atmosfera. accessed. 31/06/2022. http://www.ipma.pt/en/bivalves/fito/.

IPMA, 2020. Results of Harmful Phytoplankton (HAB Bulletins: January – September 2020), National Monitoring System of Bivalve Mollusks (Sistema Nacional de Monitorização de Moluscos Bivalves, Resultados das Determinações de Fitoplâncton Nocivo). Instituto Português Do Mar e da Atmosfera. accessed. 31/06/2022. http://www.ipma.pt/en/bivalves/fito/.

Jacob, J., Correia, C., Torres, A.F., Xufre, G., Matos, A., Ferreira, C., Reis, M.P., Caetano, S., Freitas, C.S., Barbosa, A.B., Cravo, A., 2020. Impacts of decommissioning and upgrading urban wastewater treatment plants on the water quality in a shellfish farming coastal lagoon (ria Formosa, South Portugal). J. Coast. Res. 95, 45–50. https://doi.org/10.2112/SI95-009.1.

Kachroud, M., Trolard, F., Kefi, M., Jebari, S., Bourrié, G., 2019. Water quality indices: challenges and application limits in the literature. Water 11 (2), 361. https://doi.org/10.3390/w11020361.

Kennish, M.J., Paerl, H.W., 2010. Coastal Lagoons: Critical Habitats of Environmental Change. Critical Habitats of Environmental Change. CRC Press. Boca Raton, Coastal Lagoons. https://doi.org/10.1201/EBK1420088304.

Khan, A.A., Ahmad, M., Giesen, A., 2015. NEREDA®: an emerging technology for sewage treatment. Water Pract. Technol. 10 (4). https://doi.org/10.2166/wpt.2015.098.

Landwehr, M., Deininger, R.A., 1976. A comparison of several water quality indexes. Wat. Pol. Cont. Fed. 48 (5), 954–958.

Lencha, S.M., Tränckner, J., Dananto, M., 2021. Assessing the water quality of Lake Hawassa Ethiopia—trophic state and suitability for anthropogenic uses—applying common water quality indices. J. Environ. Res. Public Health. 18, 8904. https://doi.org/10.3390/ijerph18178904.

Lima, M.J., Barbosa, A.B., Correia, C., Matos, A., Cravo, A., 2023. Patterns and predictors of phytoplankton assemblage structure in a coastal lagoon: species-specific analysis needed to disentangle anthropogenic pressures from ocean processes. Water 15 (24), 4238. https://doi.org/10.3390/w15244238.

Lima, M.J., Relvas, P., Barbosa, A.B., 2022. Variability patterns and phenology of harmful phytoplankton blooms off southern Portugal: looking for region-specific environmental drivers and predictors. Harmful Algae 116, 102254. https://doi.org/10.1016/j.hal.2022.102254.

Lorenzen, C., 1967. Determination of chlorophyll and pheopigments: spectrophotometric equations. Limnol. Oceanogr. 12, 343–346.

Loureiro, S., Newton, A., Icely, J., 2006. Boundary conditions for the European water framework directive in the ria Formosa lagoon, Portugal (physico-chemical and phytoplankton quality elements). Estuar. Coast. Shelf Sci. 67, 382–398.

Lukhabi, D.K., Mensah, P.K., Asare, N.K., Pulumuka-Kamanga, T., Ouma, K.O., 2023. Adapted water quality indices: limitations and potential for water quality monitoring in Africa. Water 15, 1736. https://doi.org/10.3390/w15091736.

Lumb, A., Sharma, T.C., Bibeault, J.-F., Klawunn, P., 2011. A comparative study of USA and Canadian water quality index models. Water Qual Expo Health 3, 203–216. https://doi.org/10.1007/s12403-011-0056-5.

Ma, Z., Lia, H., Ye, Z., Wen, J., Hu, Y., Liu, Y., 2020. Application of modified water quality index (WQI) in the assessment of coastal water quality in main aquaculture areas of Dalian. China. Mar. Pollut. Bull. 157, 111285. https://doi.org/10.1016/j.marpolbul.2020.111285.

Marcelina, M., Wibowo, F., Mushfiroh, A., 2022. Water quality index assessment methods for surface water: A case study of the Citarum River in Indonesia. Heliyon 8, e09848. https://doi.org/10.1016/j.heliyon.2022.e09848.

Martins, F., Reis, M.P., Neves, R., Cravo, A., Brito, A., Venâncio, A., 2006. Molluscan shellfish bacterial contamination in ria Formosa coastal lagoon: A modelling approach. J. Coast. Res. 39, 1551–1555.

Mendonça, J., Navoni, J., Medeiros, G., Mina, I., 2022. Ecotoxicological assessment of estuarine surface waters receiving treated and untreated sanitary wastewater. Environ. Monit. Assess. 194, 908. https://doi.org/10.1007/s10661-022-10636-1.

MMO (Marine Management Organization), 2019. Identification of Areas of Aquaculture Potential in English Waters. A report produced for the Marine Management Organisation by Centre for Environment Fisheries and Aquaculture Science, MMO Project No: 1184, May 2019, 107 pp.

Mogane, L.K., Masebe, T., Msagati, T.A.M., Ncube, E., 2023. A comprehensive review of water quality indices for lotic and lentic ecosystems. Environ. Monit. Assess. 195, 926. https://doi.org/10.1007/s10661-023-11512-2.

Mudge, S.M., Icely, J.D., Newton, A., 2008. Residence times in a hypersaline lagoon: using salinity as a tracer. Estuar. Coast. Shelf Sci. 77 (2), 278–284. https://doi.org/10.1016/j.ecss.2007.09.032.

Nada, A., Ibrahim, M.G., Elshemy, M., Fujii, M., Sharaan, M., 2024. Integrated water quality and performance assessment of seawater desalination plants along two coasts in Egypt. Desalination 586, 17844. https://doi.org/10.1016/j.desal.2024.117844.

Navarro, J.M., Gonzalez, C.M., 1998. Physiological responses of the Chilean scallop *Argopecten purpuratus* to decreasing salinities. Aquaculture 167, 315–327. https://doi.org/10.1016/S0044-8486(98)00310-X.

Newton, A., Brito, A.C., Icely, J.D., Derolez, V., Clara, I., Angus, S., Schernewski, G., Inácio, M., Lillebø, A.I., Sousa, A.I., Béjaoui, B., Solidoro, C., Tosic, M., Cañedo-Argüelles, M., Yamamuro, M., Reizopoulou, S., Tseng, H.-S., Canu, D., Roselli, L., Maanan, M., Cristina, S., Ruiz-Fernández, A.C., de Lima, R.F., Kjerfve, B., Rubio-Cisneros, N., Pérez-Ruzafa, A., Marcos, C., Pastres, R., Pranovi, F., Snoussi, M., Turpie, J., Tuchkovenko, Y., Dyack, B., Brookes, J., Povilanskas, R., Khokhlov, V., 2018. Assessing, quantifying and valuing the ecosystem services of coastal lagoons. J. Nat. Conserv. 44, 50–65. https://doi.org/10.1016/J.JNC.2018.02.009.

Newton, A., Cañedo-Argüelles, M., March, D., Goela, P., Cristina, S., Zacarias, M., Icely, J., 2022. Assessing the effectiveness of management measures in the ria Formosa coastal lagoon. Portugal. Front. Ecol. Evol. 10, 508218. https://doi.org/10.3389/fevo.2022.508218.

Newton, A., Icely, J., Cristina, S., Brito, A., Cardoso, A.C., Colijn, F., Riva, S.D., Gertz, F., Hansen, J.W., Holmer, M., Ivanova, K., Leppäkoski, E., Canu, D.M., Mocenni, C., Mudge, S., Murray, N., Pejrup, M., Razinkovas, A., Reizopoulou, S., Pérez-Ruzafa, A., Schernewski, G., Schubert, H., Carr, L., Solidoro, C., Viaroli, P., Zaldívar, J.M., 2014. An overview of ecological status, vulnerability and future perspectives of European large shallow, semi-enclosed coastal systems, lagoons and transitional waters. Estuar. Coast. Shelf Sci. 140, 95–122. https://doi.org/10.1016/j.ecss.2013.05.023.

Newton, A., Icely, J., Cristina, S., Perillo, G.M.E., Turner, R.E., Ashan, D., Cragg, S., Luo, Y., Tu, C., Li, Y., Zhang, H., Ramesh, R., Forbes, D.L., Solidoro, C., Béjaoui, B., Gao, S., Pastres, R., Kelsey, H., Taillie, D., Nhan, N., Brito, A.C., de Lima, R., Kuenzer, C., 2020. Anthropogenic, direct pressures on coastal wetlands. Front. Ecol. Evol. 8, 1–29. https://doi.org/10.3389/fevo.2020.00144.

Newton, A., Icely, J.D., Falcão, M., Nobre, A., Nunes, J.P., Ferreira, J.G., Vale, C., 2003. Evaluation of eutrophication in the ria Formosa coastal lagoon. Portugal. Cont. Shelf Res. 23, 1945–1961.

Newton, A., Mudge, S.M., 2005. Lagoon-sea exchanges, nutrient dynamics and water quality management of the ria Formosa (Portugal). Estuar. Coast. Shelf Sci. 62, 405–414. https://doi.org/10.1016/j.ecss.2004.09.005.

Nguyen, N.T.T., Loan, D.K., Hoi, N.C., 2013. Development of water quality index for coastal zone and application in the Ha Long Bay. VNU Jour. Earth Environ. Sci. 29 (4), 43–52.

Nguyen, N.T.T., Sevando, M., 2019. Assessing coastal water quality through an overall index. Pol. J. Environ. Stud. 28 (4), 2321–2330. https://doi.org/10.15244/pjoes/90836.

Nunes, A., Larson, M., Fragoso, C.R., Hanson, H., 2021. Modeling the salinity dynamics of a choked coastal lagoon and its impact on the Sururu mussel (*Mytella falcata*) population. Reg. Stud. Mar. Sci. 45, 101807. https://doi.org/10.1016/j.rsma.2021.101807.

Olivier, A., Jones, L., Le Vay, L., Christie, M., Wilson, J., Malham, S., 2020. A global review of the ecosystem services provided by bivalve aquaculture. Rev. Aquac. 12, 3–25. https://doi.org/10.1111/raq.12301.

Papaevangelou, V., Bakalakou, K.A., Ntislidou, C., Latinopoulos, D., Kokkos, N., Zachopoulos, K., Zoidou, M., Makri, A., Azis, K., Ioannidou, N., Sylaios, G., Melidis, P., Ntougias, S., Kagalou, I., Akratos, C.S., 2024. The spring to coast approach in small-scale catchments and adjacent coastal zone. Water 16, 259. https://doi.org/10.3390/w16020259.

Penna, N., Capellacci, S., Ricci, F., 2004. The influence of the Po River discharge on phytoplankton bloom dynamics along the coastline of Pesaro (Italy) in the Adriatic Sea. Mar. Pollut. Bull. 48, 321–326.

Pérez-Ruzafa, A., Campillo, S., Fernández-Palacios, J.M., García-Lacunza, A., García-Oliva, M., Ibañez, H., Navarro-Martínez, P.C., Pérez-Marcos, M., Pérez-Ruzafa, I.M., Quispe-Becerra, J.I., Sala-Mirete, A., Sánchez, O., Marcos, C., 2019. Long-term

dynamic in nutrients, chlorophyll a, and water quality parameters in a coastal lagoon during a process of eutrophication for decades, a sudden break and a relatively rapid recovery. Front. Mar. Sci. 6, 1–23. https://doi.org/10.3389/fmars.2019.00026.
Pettine, M., Casentini, B., Faza, S., Giovanardi, F., Pagnotta, R., 2007. A Revisitation of TRIX for trophic status assessment in the light of the European water framework directive: application to Italian coastal waters. Mar. Pollut. Bull. 54, 1413–1426. https://doi.org/10.1016/j.marpolbul.2007.05.013.
Picado, A., Oliveira, V., Pereira, H., Sousa, M.C., Costa, L., Almeida, A., Dias, J.M., 2020. Assessing the potential of Minho and Lima estuaries for aquaculture. J. Coast. Res. SI 95, 148–152.
Pourmozaffar, S., Jahromi, S.T., Rameshi, H., Sadeghi, A., Bagheri, T., Behzadi, S., Gozari, M., Zahedi, M.R., Lazarjani, S.A., 2020. The role of salinity in physiological responses of bivalves. Rev. Aquac. 12, 1548–1566. https://doi.org/10.1111/raq.12397.
Qadir, M., Drechsel, P., Cisneros, B.J., Kim, Y., Pramanik, A., Mehta, P., Olaniyan, O., 2020. Global and regional potential of wastewater as a water, nutrient and energy source. Nat. Res. Forum 44, 40–51. https://doi.org/10.1111/1477-8947.12187.
R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/ (accessed 08/01/2020).
Ramírez-Morales, D., Perez-Villanueva, M.E., Chin-Pampillo, J.S., Aguilar-Mora, P., Arias-Mora, V., Masís-Mora, M., 2021. Pesticide occurrence and water quality assessment from an agriculturally influenced Latin-American tropical region. Chemosphere 262, 127851. https://doi.org/10.1016/j.chemosphere.2020.127851.
Rato, A., Joaquim, S., Matias, A.M., Roque, C., Marques, A., Matias, D., 2022. The impact of climate change on bivalve farming: combined effect of temperature and salinity on survival and feeding behavior of clams *Ruditapes decussatus*. Front. Mar. Sci. 9, 932310. https://doi.org/10.3389/fmars.2022.932310.
Reyes-Velarde, P.M., Alonso-Rodríguez, R., Domínguez-Jiménez, V.P., Calvario-Martínez, O., 2023. The spatial distribution and seasonal variation of the trophic state TRIX of a coastal lagoon system in the Gulf of California. J. Sea Res. 193, 102385. https://doi.org/10.1016/j.seares.2023.102385.
Rodrigues, M., Rosa, A., Cravo, A., Jacob, J., Fortunato, A.B., 2021. Effects of climate change and anthropogenic pressures in the water quality of a coastal lagoon (ria Formosa, Portugal). Sci. Total Environ. 780, 146311. https://doi.org/10.1016/j.scitotenv.2021.146311.
Rosa, A., Cardeira, S., Pereira, C., Rosa, M., Madureira, M., Rita, F., Jacob, J., Cravo, A., 2019. Temporal variability of the mass exchanges between the main inlet of ria Formosa lagoon (southwestern Iberia) and the Atlantic Ocean. Estuar. Coast. Shelf Sci. 228, 106349. https://doi.org/10.1016/j.ecss.2019.106349.
Rosa, A., Cravo, A., Jacob, J., Correia, C., 2022. Water quality of a southwest Iberian coastal lagoon: spatial and temporal variability. Cont. Shelf Res. 245, 104804. https://doi.org/10.1016/j.csr.2022.104804.
Said, A., Sevens, D.K., Sehlke, G., 2004. An innovative index for evaluating water quality in streams. Environ. Manag. 34 (3), 406–414. https://doi.org/10.1007/s00267-004-0210-y.
Salas, F., Teixeira, H., Marcos, C., Marques, J.C., Pérez-Ruzafa, A., 2008. Applicability of the trophic index TRIX in two transitional ecosystems: the mar Menor lagoon (Spain) and the Mondego estuary (Portugal). – ICES. J. Mar. Sci. 65, 1442–1448.
Salmond, N.H., Wing, S.R., 2022. Sub-lethal and lethal effects of chronic ammonia exposure and hypoxia on New Zealand bivalve. J. Exp. Mar. Biol. Ecol. 549, 151696. https://doi.org/10.1016/j.jembe.2022.151696.
Sampaio, E., Santos, C., Rosa, I.C., Ferreira, V., Pörtner, H-O., Duarte, C.M., Levin, L.A., Rosa, R. 2021. Impacts of hypoxic events surpass those of future ocean warming and acidification. Nat. Ecol. Evol. 5, 311–321. doi:https://doi.org/10.1038/s41559-020-01370-3.
Saraiva, S., Pina, P., Martins, F., Santos, M., Braunschweig, F., Neves, R., 2007. Modelling the influence of nutrient loads on Portuguese estuaries. Hydrobiologia 587, 5–18. https://doi.org/10.1007/s10750-007-0675-9.
Scanes, E., Byrne, M., 2023. Warming and hypoxia threaten a valuable scallop fishery: A warning for commercial bivalve ventures in climate change hotspots. Glob. Chang. Biol. 29 (8), 2043–2045. https://doi.org/10.1111/gcb.16606.
Seiler, L.M.N., Fernandes, E.H., Siegle, E., 2020. Effect of wind and river discharge on water quality indicators of a coastal lagoon. Reg. Stud. Mar. Sci. 40, 101513. https://doi.org/10.1016/j.rsma.2020.101513.
Shah, K.A., Joshi, G.S., 2017. Evaluation of water quality index for river Sabarmati, Gujarat. India. Appl. Water Sci. 7, 1349–1358. https://doi.org/10.1007/s13201-015-0318-7.
Silva, C., Ferreira, J.G, Bricker, S.B., Del Valls, T.A, Martín-Díaz. M.L., Yáñez, E., 2011. Site selection for shellfish aquaculture by means of GIS and farm-scale models, with an emphasis on data-poor environments. Aquaculture 318, 444–457. doi:https://doi.org/10.1016/j.aquaculture.2011.05.033.
Sivaranjani, S., Rakshit, A., Singh, S., 2015. Water quality assessment with water quality indices. Int. J. Biol. Sci. 2 (2), 85–94. https://doi.org/10.5958/2454-9541.2015.00003.1.
Sobral, P., Widdows, J., 1997. Influence of hypoxia and anoxia on the physiological responses of the clam *Ruditapes decussatus* from southern Portugal. Mar. Biol. 127, 455–461.
Sobral, P., Widdows, J., 2000. Effects of increasing current velocity, turbidity and particle-size selection on the feeding activity and scope for growth of *Ruditapes decussatus* from ria Formosa, southern Portugal. J. Exp. Mar. Biol. Ecol. 245 (1), 111–125. https://doi.org/10.1016/S0022-0981(99)00154-9.
Sokal, R.R., Rohlf, F.J., 1995. Biometry: The Principles and Practice of Statistics in Biological Research, 3rd edition. W.H. Freeman and Co., New York.
Swamee, P.K., Tyagi, A., 2000. Describing water quality with aggregate index. J. Environ. Eng. 126 (5), 451–455.
Tiwari, N.K., Gupta, S.D., Swain, H.S., Jha, D.H., Samanta, S., Manna, R.K.,·Das, A.K., Das, B.K., 2022. Water quality assessment in the ecologically stressed lower and estuarine stretches of river ganga using multivariate statistical tool. Environ. Monit. Assess. 194, 469. doi:https://doi.org/10.1007/s10661-022-10007-w.
Tyagi, S., Sharma, B., Singh, P., Dobhal, R., 2013. Water quality assessment in terms of water quality index. Am. J. Water Resour. 1 (3), 34–38. https://doi.org/10.12691/ajwr-1-3-3.
Uddin, G., Nash, S., Olbert, A., 2021. A review of water quality index models and their use for assessing surface water quality. Ecol. Indic. 122, 107218. https://doi.org/10.1016/j.ecolind.2020.107218.
Uddin, M.G., Nash, S., Rahman, A., Olbert, A.I., 2022. A comprehensive method for improvement of water quality index (WQI) models for coastal water quality assessment. Water Res. 219, 118532. https://doi.org/10.1016/j.watres.2022.118532.
Uddin, M.G., Nash, S., Rahman, A., Olbert, A.I., 2023. A sophisticated model for rating water quality. Sci. Total Environ. 868, 161614. https://doi.org/10.1016/j.scitotenv.2023.161614.
US EPA, 1989. Ambient water quality criteria for Ammonia (saltwater). EPA 440/5-88-004, 59 pp.
Utermöhl, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik: Mit 1 Tabelle und 15 abbildungen im Text und auf 1 Tafel. Internationale Vereinigung Für Theoretische Und Angewandte Limnologie: Mitteilungen 9 (1), 1–38. https://doi.org/10.1080/05384680.1958.11904091.
Vaz, L., Sousa, M.C., Gómez-Gesteira, M., Dias, J.M., 2021. A habitat suitability model for aquaculture site selection: ria de Aveiro and rias Baixas. Sci. Total Environ. 801, 149687. https://doi.org/10.1016/j.scitotenv.2021.149687.
Vincenzi, S., Caramori, G., Rossi, R., De Leo, G.A., 2006. A GIS-based habitat suitability model for commercial yield estimation of Tapes philippinarum in a Mediterranean coastal lagoon (Sacca di Goro, Italy). Ecol. Model. 193, 90–104. https://doi.org/10.1016/j.ecolmodel.2005.07.039.
Vollenweider, R.A., Giovanardi, F., Montanari, G., Rinaldi, A., 1998. Characterization of the trophic conditions of marine coastal waters with special reference to the NW Adriatic Sea: proposal for a trophic scale, turbidity and generalized water quality index. Environmetrics 9, 329–357.
Webber, J.L., Tyler, C.R., Carless, D., Jackson, B., Tingley, D., Stewart-Sinclair, P., Artioli, Y., Torres, R., Galli, G., Miller, P.I., Land, P., Zonneveld, S., Austen, M.C., Brown, A.R., 2021. Impacts of land use on water quality and the viability of bivalve shellfish mariculture in the UK: A case study and review for SW England. Environ. Sci. Pol. 126, 122–131. https://doi.org/10.1016/j.envsci.2021.09.027.
Woodward, K.P., Rajan, A., Barber, M.C., Sullivan, E., Richkus, J.A.S., Everett, K.H., Whaley, M.G., 2020. Application of the Canadian Council of Ministers of the environment water quality index to assess and communicate monitoring data from coastal waters in Abu Dhabi. United Arab Emirates. Aquat. Ecosyst. Health 23 (2), 145–153. https://doi.org/10.1080/14634988.2020.1798144.
Zotou, I., Tsihrintzis, V.A., Gikas, G.D., 2019. Performance of seven water quality indices (WQIs) in a Mediterranean River. Environ. Monit. Assess. 191, 505. https://doi.org/10.1007/s10661-019-7652-4.