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Unravelling the effects of treated wastewater discharges on the water quality in a coastal lagoon system (Ria Formosa, South Portugal): Relevance of hydrodynamic conditions

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ABSTRACT

This study aimed to assess the influence of treated wastewater disposal on Ria Formosa coastal lagoon (South Portugal), the largest national producer of bivalve mollusks. Water quality was evaluated at two areas under different wastewater loads and hydrodynamic conditions, using physico-chemical variables, bacterial indicators of contamination, chlorophyll-a concentration, phytoplankton abundance and composition. Samples were collected monthly, between October 2018 and September 2019. Minor influence of effluent discharge was detected at the eastern Olhão area, exposed to stronger hydrodynamics and higher wastewater load than the northwestern Faro area (ca. 2–4-fold total nitrogen and phosphorus). The lower load weakly flushed area showed a poorer water quality, up to 500 m from the discharge point, more marked during the spring-summer period. The intensity, persistence, and spatial extent of the wastewater footprint, lower for the highest-loading area, reflected the role of local hydrodynamic conditions, modulating the influence of wastewater discharge on lagoonal water quality.

1. Introduction

Coastal lagoons represent one of the most productive ecosystems on Earth, with high ecological and economic value (Kennish and Paerl, 2010). These ecosystems support high biodiversity levels and biological production, functioning as nursery grounds for the early life stages of many species (Gooch et al., 2015), and providing other critical goods and services (e.g., fishing, aquaculture, tourism; see Newton et al., 2018). Despite their importance, the degradation and loss of coastal ecosystems have been globally increasing over the last decades, due to a multitude of local anthropogenic impacts and climate-change pressures acting on coastal ecosystems and their catchment areas (Barbier et al., 2008; He and Silliman, 2019; Newton et al., 2020). These human pressures include the disposal of treated and untreated urban wastewater into natural coastal systems, that usually impairs the quality of receiving water bodies (Kennish and Paerl, 2010; Newton et al., 2014). Wastewater discharges increase the availability of inorganic nutrients, overstimulate the growth of aquatic primary producers, including macroalgae and phytoplankton, thus potentially promoting the occurrence of harmful algal blooms, HABs (Reifel et al., 2013; Glibert et al., 2018a,b; Glibert and Burkholder, 2018; Lapointe et al., 2018; Adams et al., 2020). Increased inputs of organic matter driven by wastewater discharges may additionally promote the (respiratory) activity of heterotrophic prokaryotes, leading to oxygen depletion and, ultimately, eutrophication of the receiving water bodies (Lajaunie-Salla et al., 2017; Pérez-Ruzafa et al., 2019; Renzi et al., 2019). Wastewater discharges have been also reported to induce alterations in ecosystem food web structure and metabolism, for both exposed (Howard et al., 2017; Kudela et al., 2017) and confined coastal systems (Yuan et al., 2010; Leruste et al., 2016; De Wit et al., 2017; Pérez-Ruzafa et al., 2019; Renzi et al., 2019; Taylor et al., 2020). Furthermore, disposal of untreated and treated wastewaters can also promote the contamination of natural coastal systems with faecal material and associated pathogenic microbes (e.g., viruses, bacteria; Mozetič et al., 2008), that can negatively impact edible aquatic resources, namely at shellfish harvesting waters, human health, and socio-economic activities (Biancani et al., 2012; Pandey et al., 2014; Rodrigues and Cunha, 2017; Florini et al., 2020).

The impacts of wastewater discharges on natural receiving coastal ecosystems depend on effluent loading and composition. However, location of discharges and natural ecosystem traits and processes effectively shape ecosystem responses and vulnerability to wastewater discharges, at multiple spatial-temporal scales (Laws et al., 1999; Pérez-

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Ruzafa et al., 2019; Malone and Newton, 2020). As for other shallow confined coastal systems, located at the interface between land and the ocean, biogeochemical processes in coastal lagoons are strongly influenced by hydrodynamic conditions and hydrologic balance (Kjerfve, 1994; Martins et al., 2001; Newton et al., 2014). Thus, water quality in the vicinity of areas receiving wastewater discharges is effectively controlled by channel morpho-physiography, meteorological and oceanographic forcing, tidal dynamics, and water circulation and retention patterns, being more impaired in weakly-flushed areas (Yin and Harrison, 2007; Xu et al., 2011; Zhou et al., 2014; Ruiz-Ruiz et al., 2016; Wang et al., 2018). However, biological control processes, such as predation on primary producers, are also relevant (Pérez-Ruzafa et al., 2019).

As other coastal lagoon systems worldwide, Ria Formosa (RF) is a shallow coastal lagoon system (SE Portugal) that supports high biodiversity and biological production (Barbosa, 2010; Newton et al., 2020). This well mixed mesotidal system is responsible for about 90% of the Portuguese bivalve production, with an annual production of ca. 3000 tons (Serpa et al., 2005), an ecosystem service with high socio-economic relevance, involving ca. 10,000 people (Cravo et al., 2015). RF has been receiving effluents from several wastewater treatment plants (WWTP) since the beginning of the 90's. These discharges represent a relevant nutrient source (Malta et al., 2017), and have impaired water quality (Bebianno, 1995; Newton et al., 2003; Cravo et al., 2015) and bivalve microbiological quality in specific areas of this lagoon (Martins et al., 2006; Almeida and Soares, 2012; Botelho et al., 2015; Cravo et al., 2015). More recently, some WWTPs discharging into the RF have upgraded their treatment technologies (Cravo et al., 2018), but the impact of these alterations was not yet fully assessed (see Jacob et al., 2020). This information is crucial for improving wastewater management strategies (e.g., Biancani et al., 2012; Machado and Imberger, 2012), promoting the condition of shellfish farming grounds, that intrinsically depend on water quality.

In this context, this study aims at assessing the impacts of two main urban WWTPs on the water quality of the RF, and their variability over an annual cycle. The WWTPs are located next to two main cities (Faro and Olhão), at the northwestern Faro area (FNW), and eastern Olhão area (OE). The WWTP effluents are discharged in areas in the vicinity of shellfish farming grounds, subjected to different loading and hydrodynamic conditions (FNW: lower load/flushing; OE: higher load/flushing). Water quality was evaluated considering three components: physico-chemical variables, bacterial indicators of faecal contamination, and phytoplankton assemblages, including harmful taxa. Based on previous in situ- (Cravo et al., 2015, 2018; Ruiz-Ruiz et al., 2016) and model-

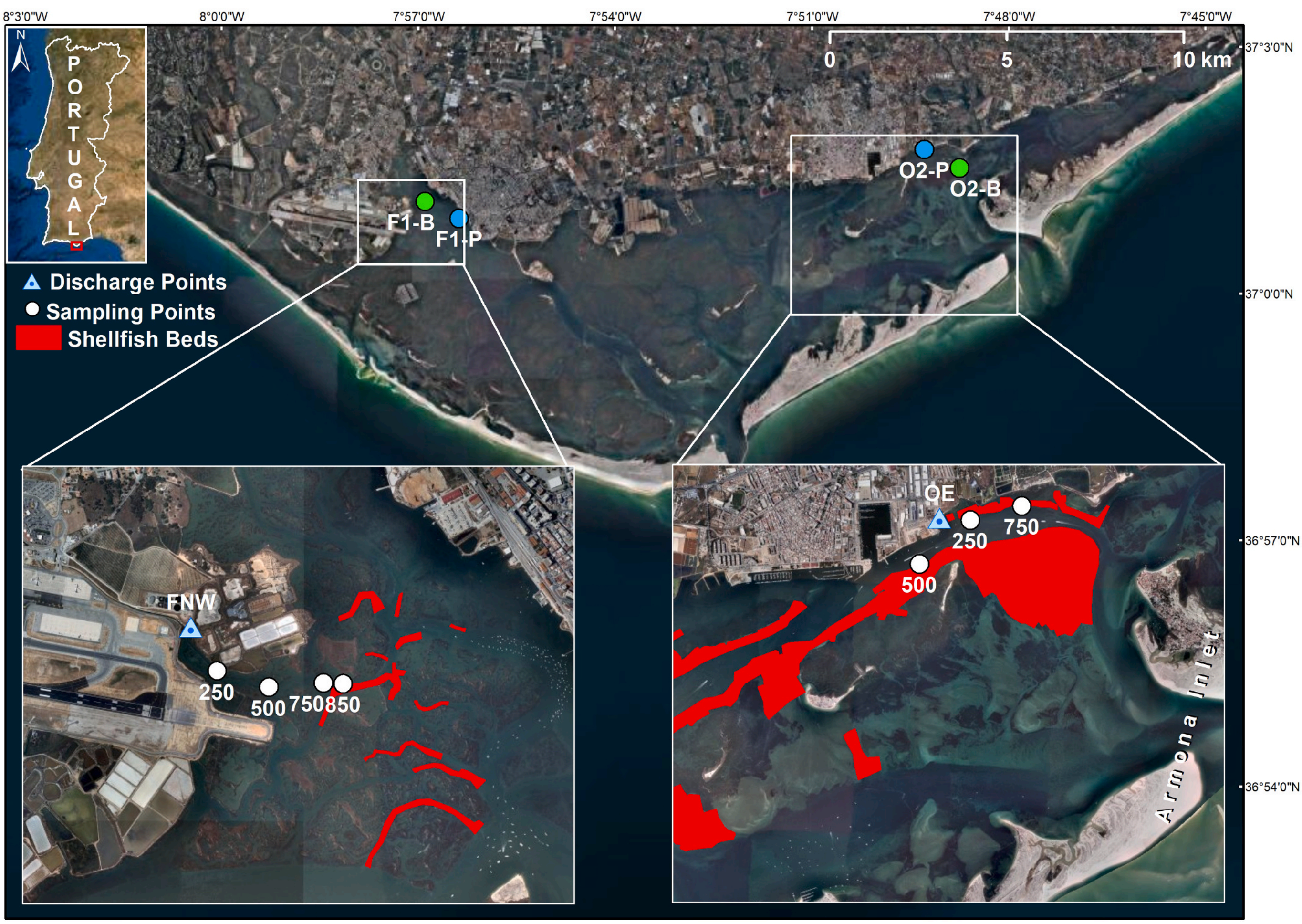

Fig. 1. Map of the Ria Formosa coastal lagoon, and location of the two study areas, under the influence of the wastewater treatment plants (WWTP) of Faro Northwest (FNW; lower left panel) and Olhão East (OE; lower right panel). For each study area, the WWTP effluent discharge point (DP) is indicated by a blue triangle, sampling stations are denoted by white circles, with numbers in white representing distance (in meters) from the discharge point, and areas covered by shellfish beds are shown in red. Approximate location of stations "Faro 1" (F1) and "Olhão 2" (O2), included in the national monitoring program of bivalve mollusks (Portuguese Institute of Sea and Atmosphere, IPMA), are also shown in the main panel. Blue circles (F1-P and O2-P) represent stations used for the analysis of harmful phytoplankton, and green circles (F1-B and O2-B) denote stations used for the analysis of bacterial indicators of faecal contamination and phytoplankton toxins in bivalves (IPMA). Location of shellfish beds provided by Agência Portuguesa do Ambiente. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

based studies (Martins et al., 2006; Veríssimo et al., 2019), we hypothesize that the intensity and spatial extent of the treated wastewater footprint will be higher at the area under a weaker hydrodynamic regime (FNW).

2. Material and Methods

2.1. Study area

Ria Formosa is a shallow multi-inlet coastal lagoon, located along the south Portuguese coast (Fig. 1), and separated from the ocean by six inlets. RF is a euryhaline mesotidal, well mixed system, with several main channels and a branched system of creeks (see Barbosa, 2010). Tides are semi-diurnal, with a mean tidal range of around 2 m. A large fraction of the lagoon water volume (ca. 50–75%) is exchanged with the ocean on a daily basis, with higher exchange rates during spring tides in comparison to neap tides (Mudge et al., 2008). The coastal region adjoining the lagoon is subjected to regular upwelling events, more frequent from March to October, that impact the outer sections of the RF lagoon, and may extend at least ca. 6 km upstream the lagoon inlets (Barbosa, 2010; Cravo et al., 2014).

Four main WWTP currently discharge their effluents into the RF, and this study assessed the impact of two urban WWTP, located next two main cities (Faro and Olhão), discharging effluents at the northwestern Faro area, and eastern Olhão area. For both WWTP, wastewater treatment includes primary physical treatment, followed by secondary biological treatment using activated-sludge, and final ultraviolet disinfection. The main characteristics of the effluents discharged by the two WWTP, and lagoon receiving areas, FNW and OE, are shown in Table 1. FNW area receives domestic-derived treated wastewater with relatively reduced loads. At this area, WWTP effluents are discharged into a shallow creek-channel (<1 m, at low tide), at an average discharge rate of ca. 4000 m^3 day^{-1} (Table 1). In contrast, OE area receives domestic and industrial-derived treated wastewater, mostly derived from auto and boat mechanics, laundry and a canning company, with higher mean loads of total nitrogen (ca. 2×) and total phosphorus (ca. 4×), in respect with FNW. At OE area, WWTP effluents are discharged into a deeper and wider main channel, connected with a major dominant inlet (Armona inlet; see Fig. 1), at average discharge rate of ca. 2000 m^3 day^{-1} (Table 1). The areas surrounding the discharge point of both WWTPs, FNW and OE, include shellfish harvesting grounds, with larger production areas at OE (Fig. 1).

Table 1
Characteristics of the effluents of urban wastewater treatment plants (WWTP) discharging in the two Ria Formosa study areas: northwestern Faro (FNW) and Eastern Olhão (OE), during the period October 2018–September 2019. Information provided for each WWTP/study area include types of effluents, equivalent served population, effluent discharge rate, effluent loads (mean, minimum and maximum values, n = 12) for total nitrogen, total phosphorus, and total suspended solids, and main features of the WWTP-effluent receiving ecosystem areas. All information (mean, minimum and maximum values, n = 12), except the features of receiving ecosystem areas, were provided by Águas do Algarve (AdA), the water supply company (see Fig. 1 for WWTP location).

	FNW	OE
Types of effluent	Domestic effluents	Industrial and domestic effluents
Equivalent served population (hab eq.)	ca. 26,000	ca. 18,000
Effluent discharge rate (m^{-3} d^{-1})	4174 (3860–4840)	2145 (1770–2675)
Total nitrogen load (kg N d^{-1})	35 (15–70)	64 (45–102)
Total phosphorus load (kg P d^{-1})	5 (3–15)	22 (15–27)
Total suspended solids load (kg g^{-1})	16 (8–24)	23 (6–41)
Features of effluent receiving ecosystem areas	Narrow and shallow (<1 m) creek-channel at low tide, with channel width and depth rapidly increasing with distance from the effluent discharge point. Weak hydrodynamic conditions. Water residence time (inner lagoon areas): >2–3 days (Mudge et al., 2008) up to 11 days (Duarte et al., 2008). FNW effluent residence time: ca. 9 days (Fabião et al., 2016).	Main channel (Marim Channel), larger and deeper than at FNW, connected with one major inlets of the Ria Formosa lagoon system (Armona Inlet). Strong hydrodynamic conditions. Water residence time (main channels): <1 day (Martins et al., 2003; Duarte et al., 2008). OE effluent residence time: ca. 7 days (Fabião et al., 2016).

2.2. Sampling strategy

Water sampling was conducted along longitudinal transects of the effluent dispersion from the WWTP discharge point, for both study areas (Fig. 1). At FNW, four stations were sampled, at 250 m (FNW 250), 500 m (FNW 500), 750 m (FNW 750), and 850 m (FNW 850) from the discharge point, with the shellfish beds located around the farthest sampling station (FNW 850). At OE, three stations were sampled, located on eastern and western sections, at 250 m (OE 250), 500 m (OE 500), and 750 m (OE 750) from the discharge point, also close to shellfish beds. According with previous studies (Cabaço et al., 2008; Cravo et al., 2015, 2018), the furthest stations from the discharge points (FNW 850 and OE 750), located in main channels, are sufficiently apart from the effluent dispersal influence. Sampling was undertaken monthly during the period between October 2018 and September 2019 (neap tides), at both low tide and high tide. Phytoplankton was sampled only during neap low tide. Thus, the most critical tidal stage concerning the water quality (neap low tide) was covered for all variables. For the analysis of physico-chemical variables and bacterial indicators of faecal contamination, sampling frequency was intensified from April to September 2019, covering both neap and spring tides for each month.

The impact of effluents derived from WWTP on RF water quality was evaluated using: (a) selected chemical variables commonly used as proxies for wastewater discharge (salinity, dissolved oxygen, ammonium, and phosphate; e.g., Xu et al., 2011; Zhou et al., 2014); (b) abundance of *Escherichia coli* and enterococci, common members of the gastrointestinal consortia of humans and other warm-blooded animals, thus used as indicators of environmental faecal contamination (e.g., Boehm and Sassoubre, 2014); and (c) phytoplankton abundance, biomass, and species composition, including potentially harmful taxa (see Tweddle et al., 2018).

Water temperature, salinity, dissolved oxygen, and pH were measured in situ, using a YSI EXO 2 multiparametric probe. Surface water samples (ca. 20–30 cm) were collected for the analysis of dissolved inorganic macro-nutrients, suspended solids, bacterial indicators of faecal contamination, Chl-*a*, and phytoplankton abundance and composition.

2.3. Water physico-chemical variables and indicators of faecal contamination

Water samples for nutrient quantification were filtered using a vacuum pump, through 0.45 μm membrane filters (cellulose acetate, Pall Corporation), and frozen at −20 °C until analysis. Suspended solids were determined following the gravimetric method described in APHA (2017), Method 2540D, after sample filtration (250–500 mL) through membrane filters (nominal porosity 0.45 μm; cellulose acetate, Pall Corporation). Nutrient (ammonium, nitrate, nitrite, phosphate, and silicate) concentrations were analyzed using spectrophotometric methods and calibration curves, as described by Grasshoff et al. (1999), using an Evolution 200 Thermo Scientific spectrophotometer. Detection limits were 0.1 μM for nitrate and ammonium, 0.05 μM for silicate, 0.03

μM for phosphate and 0.02 μM for nitrite. The Marine Nutrient Standards Kit (OSIL) was used as reference material to ensure accuracy. Relative errors for nutrient concentrations were smaller than 2.5%, and precision ranged between 1% for silicate, nitrite, and phosphate, and 2% for nitrate and ammonium. The concentration of DIN was calculated as the sum of nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+).

E. coli and enterococci were assessed by the Quanti-Tray method, using the defined-substrate assays Colilert and Enterolert systems, respectively, from IDEXX Laboratories (Westbrook, Maine). Samples were diluted to minimize inhibition by high salt content, prior to the addition of reagents; minimal sample dilutions of 1:4 and 1:10 were used for Enterolert and Colilert, respectively. Quanti-trays were sealed, incubated for 18 to 22 h at 35 ± 0.5 °C (*E. coli*) or 24 to 28 h at 41 ± 0.5 °C (enterococci), and then analyzed according with the manufacturer guidelines. Trays were examined side by side with their respective comparators for distinguishing threshold positive results from negative results, that were subsequently used to estimate Most Probable Number (MPN), using the provided IDEXX MPN charts (e.g., Schang et al., 2016). Lower detection limits were ca. 4 MPN/100 mL for enterococci, and 10 MPN/100 mL for *E. coli*. Additionally, information on the concentration of faecal coliforms in shellfish species harvested from wild populations (*Mytilus* spp. and *Polititapes aureus* Gmelin) and aquaculture (*Ruditapes decussatus* Linnaeus, and *Crassostrea gigas* Thunberg), measured at different RF lagoon shellfish production areas in the vicinity of FNW ("Faro 1") and OE ("Olhão 2", see approximate locations in Fig. 1) study areas, during the regular monitoring program undertaken by the Portuguese Institute of Sea and Atmosphere (IPMA) was also used. This information was retrieved from IPMA public database, for the period October 2018–September 2019 (IPMA, 2018a, 2019a).

2.4. Phytoplankton

Samples for determination of Chl-a were filtered through glass-fiber filters (GF/F, Whatman, nominal pore size = 0.7 μm), and frozen at −20 °C prior to analysis. After cold extraction with 90% acetone, pigment extracts were analyzed spectrophotometrically as described by Lorenzen (1967). Detection limit for Chl-a was 0.2 μg L^{-1}. Chl-a was used as a proxy for total phytoplankton biomass.

Samples for phytoplankton abundance and species composition were preserved with Lugol's solution immediately after collection, settled in sedimentation chambers (10 mL, 25 mL, or 50 mL), and observed using inversion microscopy (Zeiss Axio Observer A1), according with Utermöhl (1958). Only plastidic cells, including both auto- and mixotrophic taxa, were included in the analysis. Larger less abundant phytoplankton *taxa* (e.g., *Ceratium* Schrank, *Dinophysis* Ehrenberg, *Proboscia* Sundström) were inspected in the entire bottom of the chamber, at 100× magnification. Smaller microphytoplankton *taxa* (ca. 20–50 μm) and morphologically conspicuous nanophytoplankton (ca. 2–20 μm) were enumerated in randomly selected microscopic fields, at 400× magnification. The use of inverted microscopy precluded the analysis of picophytoplankton (e.g., cyanobacteria and eukaryotes <2 μm) and morphologically inconspicuous nanophytoplankton (2–20 μm; see Barbosa, 2006; Domingues et al., 2015, 2017a, 2017b). A minimum of ca. 400 cells in total were enumerated per sample and, assuming a random distribution of phytoplankton cells in the chamber, counting precision was 10% (Andersen and Thröndsen, 2004).

Phytoplankton was identified to the lowest possible taxonomic level, using morphological characteristics and taxonomy reference literature for marine (Schiller, 1937; Cupp, 1943; Dodge and Hart-Jones, 1982; Tomas, 1997; Kraberg et al., 2010) and freshwater (John et al., 2011; Wehr et al., 2014). As for most of the *Pseudo-nitzschia* spp. cells, identification to species level is not possible using light microscopy, they were assigned to two groups (see Fehling et al., 2006; Danchenko et al., 2019): *Pseudo-nitzschia delicatissima* group (cells with linear shape and width <3 μm), and *Pseudo-nitzschia seriata* group (cells with lanceolate shape and width > 3 μm). Taxonomic and nomenclatural information was updated according with the AlgaeBase database (Guiry and Guirry, 2021).

The classification of observed phytoplankton taxa as potentially harmful was based on the IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (Moestrup et al., 2009), AlgaeBase (Guiry and Guirry, 2021), and other references (Kraberg et al., 2010; Shumway et al., 2018). Additionally, our dataset was complemented with information on the abundance of toxigenic phytoplankton species, measured at two RF lagoonal shellfish production areas, in the vicinity of FNW ("Faro 1") and OE ("Olhão 2"; see approximate location in Fig. 1) study areas, and the nearest coastal production area (L8), from the regular monitoring program undertaken by IPMA. This information was retrieved from IPMA public database, for the period October 2018–September 2019 (IPMA, 2018b, 2019b). The regulatory alert (trigger) levels and interdiction levels for the abundance of different HAB-taxa used by IPMA regular monitoring program are included in the supplementary material (see Table S4 caption). Information on phycotoxin concentrations in selected bivalve species for these three shellfish production areas was also retrieved from IPMA during the same period (IPMA, 2018c, 2019c).

2.5. Meteorological variables

Daily rainfall precipitation, total daily surface radiation (kJ m^{-2}), and hourly surface wind speed and direction, measured at Faro land-based meteorological station, were provided by IPMA to characterize FNW and OE study areas. No information on freshwater flow into the western sub-embayment of the Ria Formosa was available in public databases (see Barbosa, 2010) for the study period.

Additionally, ocean surface wind data, indicative of dominant oceanographic conditions over the RF adjacent coastal waters (upwelling versus downwelling), were also retrieved from the Advanced Scatterometer installed on-board MetOp-B meteorological satellite (ASCAT-B). Daily ASCAT-B wind direction and intensity, at 0.25° spatial resolution, in both descending and ascending modes (Products: KNMI-GLO-WIND_L3-OBS_METOP-B_ASCAT_25_ASC_V2 and KNMI-GLO-WIND_L3-OBS_METOP-B_ASCAT_25_DES_V2), were retrieved from Copernicus Marine Service (http://marine.copernicus.eu/). Daily data for a spatial domain comprised between 36.54°–37.7° N and 8.30°–7.45° E (offshore 50 km from the coastline) was averaged, for the period October 2018–September 2019.

2.6. Statistical analysis

Basic statistics (mean, standard deviation, standard error), correlation coefficients, and statistical tests were performed using Statistica 6.0® software package. Basic assumptions for parametric analyses, data normality and variance homogeneity, were tested with Shapiro-Wilk and Levene's tests, respectively. Parametric approaches were used for physico-chemical variables, whereas non-parametric approaches were used for bacterial indicators of faecal contamination and phytoplankton. All statistical analyses were considered at an $\alpha = 0.05$ level.

Spatial variability patterns for different variables were inspected using comparisons between each study area (FNW versus OE), and across stations, within each study area (FNW 250–FNW 850, and OE 250–OE 750). For physico-chemical variables, differences between FNW and OE, considering the whole dataset, were tested using a t-student test. For indicators of faecal contamination and phytoplankton, differences between FNW and OE areas were tested using signed rank Wilcoxon test. For each study area and variable (chemical, bacteriological, phytoplankton), differences across stations were evaluated using the non-parametric Friedman analysis of variance by ranks, assuming dependence along the longitudinal transects (Sokal and Rohlf, 1995). For each study area (FNW or OE), differences in physico-chemical variables and indicators of faecal contamination between low-tide and high-tide (October 2018–September 2019), and between spring and neap tide

(April–September 2018) were tested using a paired *t*-test, and signed rank Wilcoxon test, respectively (Sokal and Rohlf, 1995).

The strength of monotonic relationships between chemical and meteorological variables was assessed using Pearson correlation coefficients. Correlations between chemical, bacteriological, meteorological, and phytoplankton were assessed using Spearman rank correlation coefficients. Meteorological data (rainfall, total surface radiation, and wind speed) were averaged over a 7-day period prior to each sampling date.

3. Results

Basic statistical information collected at FNW and OE study areas, including average, standard-deviation, minimum, and maximum values (Table S1–Table S3), and the list of identified phytoplankton taxa (Table S4) are summarized in the supplementary material. For physico-chemical variables, Chl-a (Table S1) and bacterial indicators of faecal contamination (Table S2), information for both low-tide and high-tide is also provided. Phytoplankton abundance and composition (Table S3) is available only for neap low tide, considered the most critical tidal stage regarding RF water quality. Basic statistical information for ancillary data is also included as supplementary material (Table S5).

This section is organized into three subsections. The first (Section 3.1) uses the complete data set for each study area (FNW and OE) to address overall differences in water quality between FNW and OE, and the effects of tidal stage. Next, the impacts of treated wastewater discharge on the water quality of Ria Formosa consider the spatial distance from the discharge point (Section 3.2), and the effect of inter-annual variability (Section 3.3), for both FNW and OE. This information will be used to understand how far and when the effluent discharges have a measurable influence on water quality in RF.

3.1. Contrasting study areas and tidal stages

Considering the complete data set collected for each study area (all stations), highly significant differences were detected between FNW and OE for most variables, except dissolved oxygen saturation (Table S1). FNW exhibited lower salinity and pH, and higher water temperature, and concentration of nutrients, suspended solids, and dissolved oxygen ($p < 0.01$; Table S1). At FNW, mean ammonium and phosphate concentrations were about 70 and 15-fold higher, respectively, in respect to OE. The abundance of *E. coli* and enterococci was also higher at FNW ($p < 0.01$; Table S2), with mean values ca. 20-fold and 30-fold higher, respectively, in respect to OE. Values below detection levels (bd) were often observed at the later study area. The ratio between *E. coli* and enterococci was also higher and more variable at FNW in respect with OE (average ± 1SE: 19.2 ± 6.2 versus 6.7 ± 1.1; data not shown).

Chl-a, a proxy for phytoplankton biomass, was higher at FNW ($p < 0.01$), with mean (3.3 μg L^{-1} versus 1.0 μg L^{-1}) and maximum (33.4 μg L^{-1} versus 3.0 μg L^{-1}) values ca. 10-folder higher than at OE (Table S1). Phytoplankton, identified at the genus or species level, comprised a total of 48 taxa and 100 taxa at FNW and OE, respectively, including several potentially HAB-forming taxa (see Table S4). In comparison with FNW, increased species richness at OE was a result of a higher number of diatom taxa (23 versus 48) and dinoflagellate taxa (6 versus 37; see Table S4). Phytoplankton composition showed marked differences between study areas. Higher relative and absolute abundances of chlorophytes, cyanobacteria, and benthic diatoms were detected at FNW, and higher abundances of cryptophytes and planktonic diatoms were detected at OE ($p < 0.01$; Table S3; see also Fig. 3). At a more specific level, higher abundances of the chlorophyte *Scenedesmus* Meyen ($p < 0.01$), cyanobacteria order Oscillatoriales, and dinoflagellate *Kryptoperidinium foliaceum* Lindemann ($p < 0.05$) were detected at FNW, while higher abundances of the potentially harmful diatom *Pseudo-nitzschia* spp. were detected at OE ($p < 0.01$; Table S3). No differences between FNW and OE were observed for other specific phytoplankton taxa (data not shown).

Considering all stations combined, significant differences between low-tide and high-tide were detected for most physico-chemical variables and indicators of faecal contamination, more evident at FNW (Tables S1 and S2). At FNW, water temperature ($p < 0.05$), dissolved oxygen, nutrient concentrations, and *E. coli* and enterococci were significantly higher, while salinity was lower ($p < 0.01$), during low-tide in respect with high-tide. At OE, water temperature, nutrient concentrations ($p < 0.05$) and *E. coli* ($p < 0.05$) showed higher values during low-tide, in comparison with high-tide. For this area, no differences in salinity, pH, dissolved oxygen, and enterococci were observed between low-tide and high-tide (Tables S1 and S2).

The influence of tidal range on physical-chemical variables and faecal indicators was inspected during the period April–September 2019 (Table S1). At FNW study area, water temperature was higher, and salinity, pH, dissolved oxygen saturation and silicate were lower during neap tides, in comparison with spring tides ($p < 0.05$). At OE, water temperature, pH, Chl-a, and phosphate were higher, and suspended solids and nitrate concentration were lower during neap tides, in respect with spring tides ($p < 0.05$). No differences between neap and spring tides were detected for other variables (data not shown).

3.2. Influence of effluent discharges on the water quality of Ria Formosa: wastewater spatial "footprint" at contrasting lagoon areas

3.2.1. Faro Northwest (FNW) study area

The variability in water chemical variables, bacterial indicators of faecal contamination, Chl-a, and abundance of specific phytoplankton groups along the longitudinal transect is summarized in Fig. 2. Overall, the influence of treated wastewater disposal at FNW was evident for most studied variables, and the nearest and furthest stations from the discharge point showed statistically different values for most variables. Additionally, most variables, including salinity, ammonium, phosphate (Fig. 4), *E. coli*, enterococci (Fig. 5), Chl-a, and key phytoplankton taxa (Fig. 6), generally showed higher variability at stations near the discharge point in respect with furthest stations.

Water salinity increased progressively along the longitudinal transect, reflecting the mixing of the low-salinity effluent with the lagoon

VARIABLES	STATIONS			
	FNW 250	FNW 500	FNW 750	FNW 850
Salinity	a	ab	bc	c
Dissolved Oxygen (%)	a	ab	b	ab
Ammonium	a	ab	b	c
Phosphate	a	ab	bc	c
Escherichia coli	a	ab	bc	c
Enterococci	a	ab	bc	c
Chlorophyll-*a*	a	ab		b
Cyanobacteria	a	ab		b
Oscillatoriales	a	ab		b
Chlorophytes	a	ab	bc	c
Scenedesmus spp.	a		ab	b
Benthic Diatoms	a	ab		b
Cryptophytes	a		ab	b
Plastidic Dinoflagellates	a	ab	c	bc
Kryptoperidinium foliaceum	a	ab	b	

Fig. 2. Summary diagram representing the variability in water chemical variables, bacteriological indicators of faecal contamination, chlorophyll-a concentration, and abundance of specific phytoplankton groups along a longitudinal transect from the effluent discharge point at Faro Northwest study area (FNW), during the period October 2018–September 2019. Notation used for each station represents the distance, in meters, from the discharge point (FNW 250–FNW 850 m). For each variable, different letters denote significant differences between FNW stations ($p < 0.05$), and warm and cold colors indicate significantly higher and lower values, respectively. Variables that showed no differences across FNW stations are not included in the diagram.

water (Fig. 2). Salinity was negatively correlated with dissolved oxygen and nutrient concentrations ($p < 0.05$, n = 48). Dissolved oxygen concentration and saturation (Table S1) showed lowest values closest to the effluent discharge point, progressively increased up to FNW 750, with a relative decrease at FNW 850 (Fig. 2). At the later station, oxygen saturation levels measured during April, August, and September 2019 (Fig. 4) were even lower than the regulatory limits for shellfish production waters (Minimum Admissible Value, MAV = 70%, Portuguese Decree-Law 236/98). Dissolved oxygen saturation was positively correlated with pH and wind intensity ($p < 0.05$, n = 48).

Concentrations of ammonium, phosphate, *E. coli* and enterococci showed a progressive decline towards furthest stations (Figs. 4 and 5). All nutrient concentrations were positively correlated with each other ($p < 0.05$, n = 48). The abundance of *E. coli* detected at FNW overpassed the regulatory concentration of faecal coliforms imposed for effluent discharge licencing into this confined lagoon area, classified as sensitive area under the Urban Wastewater Treatment Directive, 91/271/EEC (300 MPN/100 mL, Environmental Portuguese Agency), in 50% of samples. *E. coli* and enterococci were strongly positively correlated with each other and with ammonium, phosphate, total suspended solids ($p < 0.01$), and negatively correlated with dissolved oxygen ($p < 0.05$, n = 48).

Chl-a also showed a progressive decrease with increasing distance from the discharge point (Fig. 2). Chl-a was strongly positively correlated with freshwater chlorophytes and benthic diatoms ($p < 0.01$, n = 40), dominant phytoplankton taxa at FNW (see Fig. 3), and water temperature ($p < 0.01$), and negatively related with dissolved oxygen ($p < 0.05$, n = 48). Phytoplankton assemblages (see Fig. 3, and Table S4) at FNW were globally dominated by colonial freshwater chlorophytes (*Hafniomonas* Ettl & Moestrup, *Scenedesmus*, *Chlamydomonas* Ehrenberg, and *Oocystis* Nägeli ex Braun), and benthic diatoms (*Navicula* Bory, *Cylindrotheca closterium* Reimann & Lewin). In terms of spatial variability, chlorophytes, benthic diatoms and cyanobacteria showed a progressive decline with increasing distance from the discharge point (Fig. 2). The abundance of Oscillatoriales (cyanobacteria) and *Scenedesmus* spp. (chlorophyte) also followed this pattern. By contrast, cryptophytes, and the dinoflagellate species *Kryptoperidinium foliaceum* increased towards the furthest stations from the discharge point (Fig. 2). Interestingly, phytoplankton composition at this station, already located in a wider deeper lagoon channel, showed a clear similarity with the OE area (Fig. 3). At FNW, chlorophytes, benthic diatoms, and cyanobacteria were strongly negatively correlated with salinity, and positively correlated with ammonium, phosphate, and silicate, whereas cryptophytes and plastidic dinoflagellates showed opposite relationships with these variables ($p < 0.01$).

At FNW, abundances of potentially toxigenic *taxa* were systematically lower than the alert levels (see Table S4). However, during the study period, the IPMA public database (n = 48 samples) reported several occurrences at "Faro 1" shellfish production are (*Dinophysis* spp.: 8% above alert and interdiction levels; *Lingulodinium polyedra* Dodge: 2% above alert level; and *Gymnodinium catenatum* Graham: 2% above alert).

3.2.2. Olhão East (OE) study area

In contrast with the FNW area, no significant differences across stations were detected for any of the evaluated variables at OE ($p > 0.05$). However, higher variability was generally observed at the station close to the discharge point (see Figs. 3 and 4). At OE, water salinity, dissolved oxygen, nutrient concentrations, *E. coli*, and enterococci (Tables S1 and S2) showed similar values for all stations. The abundance of *E. coli* at OE (see Fig. 5C and Table S2) never exceeded the regulatory concentration of faecal coliforms imposed for effluent discharge licencing into a main lagoon channel, not sensitive to eutrophication (2000 MPN/100 mL, Environmental Portuguese Agency). *E. coli* was positively correlated with nitrite, phosphate, and enterococci, and negatively related with pH, dissolved oxygen saturation ($p < 0.01$) and wind intensity ($p < 0.05$, n = 36). Enterococci showed strong positive relationships with ammonium, nitrite ($p < 0.01$) and total suspended solids ($p < 0.05$), and negative relationships with pH and dissolved oxygen saturation ($p < 0.01$, n = 36).

Phytoplankton assemblages (see Fig. 3, and Table S4) at OE were globally dominated by cryptophytes and colonial planktonic diatoms (*Chaetoceros* Ehrenberg, *Pseudo-nitzschia delicatissima* group, and *Skeletonema* Greville). In contrast with FNW area, abundances of potentially toxigenic taxa (see Table S4) higher than alert levels were observed at OE area (*P. delicatissima* group: 3% samples, n = 1; and *Lingulodinium polyedra*: 10% samples, n = 3) and interdiction levels (*P. delicatissima* group: 20% samples, n = 6) and *Pseudo-nitzschia seriata* group: 3% samples, n = 1). During the study period, the IPMA public database (n = 48 samples) reported a higher number of these occurrences at "Olhão 2" shellfish production area (*Dinophysis* spp.: 17% above alert and interdiction levels; *Lingulodinium polyedra*: 13% above alert; *Gymnodinium catenatum*: 4% above alert).

Chl-a (Table S1) was similar between stations ($p > 0.05$), yet

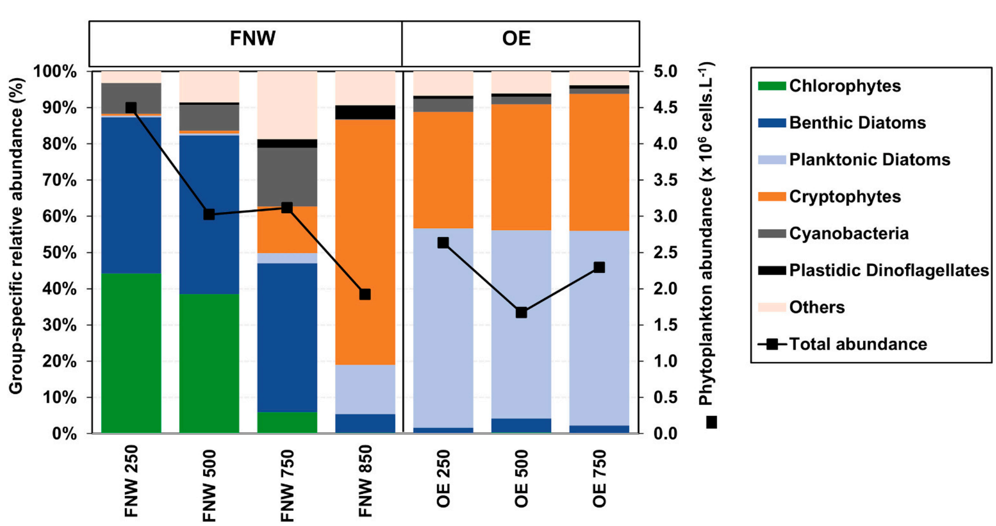

Fig. 3. Mean annual relative contribution of different phytoplankton groups, and total abundance of these groups, along longitudinal transects from the effluent discharge points for Faro Northwest (FNW) and Olhão East (OE) study areas, during the period October 2018–September 2019 of phytoplankton abundance and. The notation used for each station represents the distance, in meters, from the discharge point for both FNW (FNW 250–FNW 850) and OE (OE 250–OE 750).

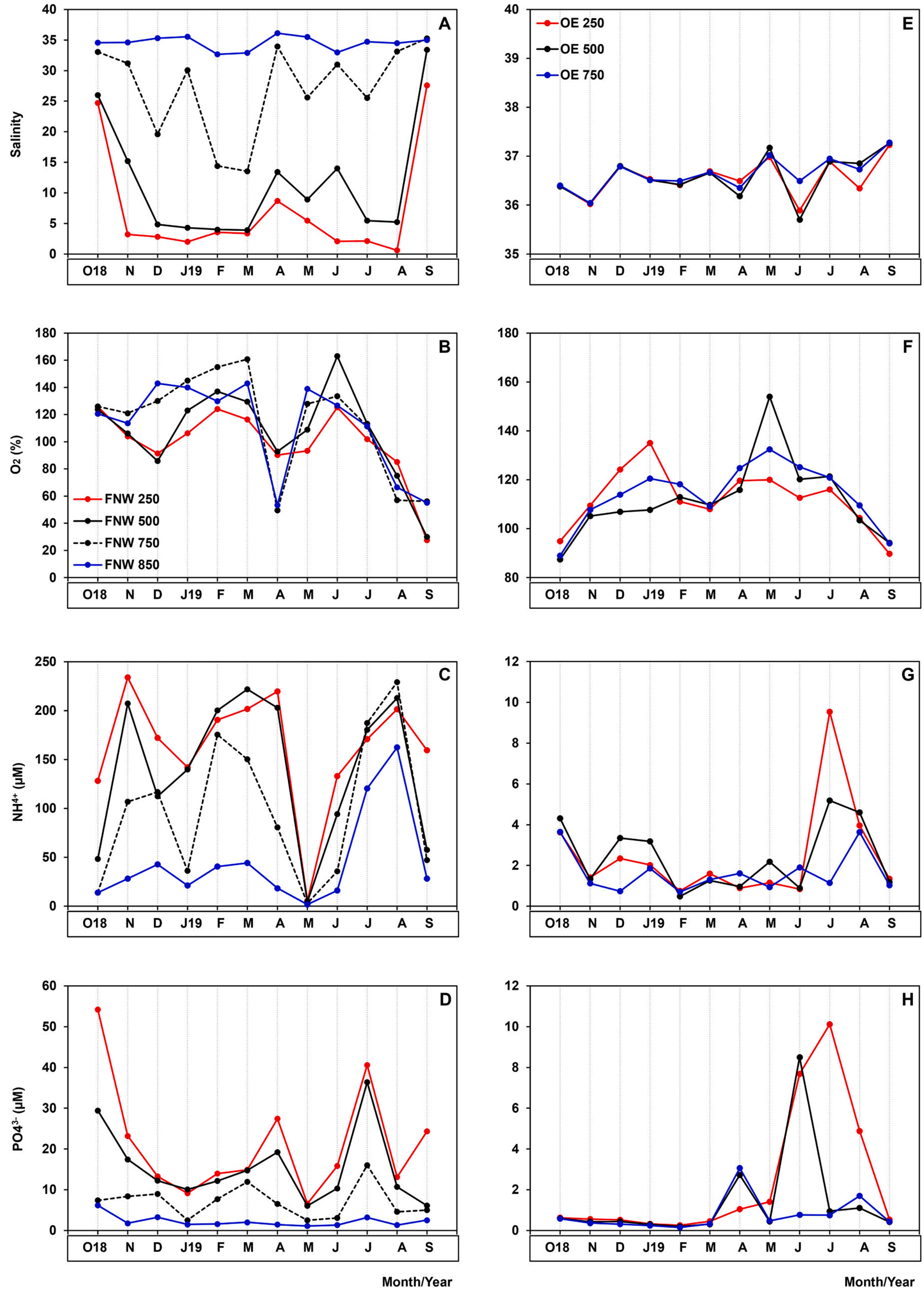

Fig. 4. Monthly variability of physical-chemical variables for different stations along longitudinal transects from the effluent discharge points for Faro Northwest (FNW, left panels) and Olhão East (OE, right panels) study areas, during the period October 2018–September 2019. Panels: (A, E) salinity; (B, F) dissolved oxygen (O_2, %); (C, G) concentration of ammonium (NH_4^+); (D, H) concentration of phosphate (PO_4^{3-}). For some variables, note differences in scale between FNW and OE. The notation used for each station represents the distance, in meters, from the discharge point for both FNW (FNW 250–FNW 850) and OE (OE 250–OE 750).

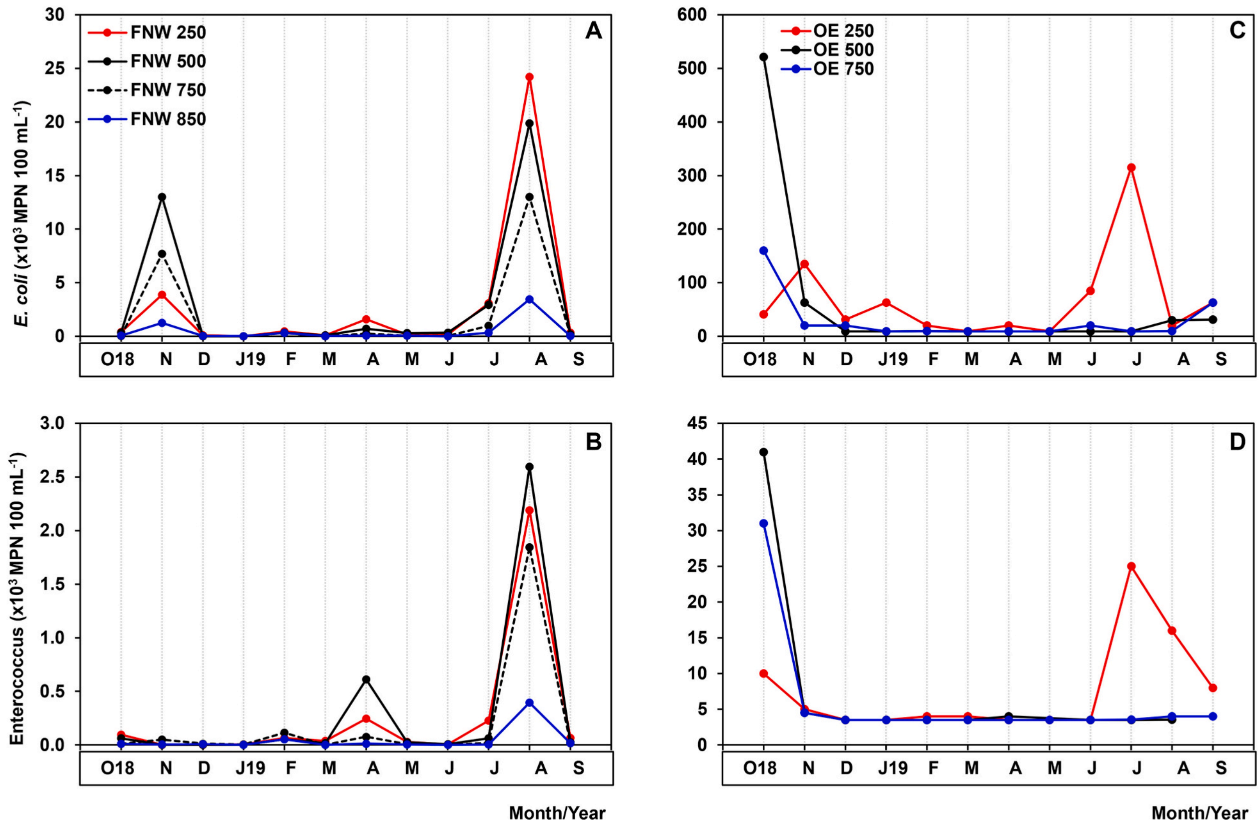

Fig. 5. Monthly variability of the abundance of bacteriological indicators of faecal contamination for different stations along longitudinal transects from the effluent discharge points for Faro Northwest (FNW, left panels) and Olhão East (OE, right panels) study areas, during the period October 2018–September 2019. Panels: (A, C) *Escherichia coli*; and (B, D) enterococci. Note differences in scale between FNW and OE. The notation used for each station represents the distance, in meters, from the discharge point for both FNW (FNW 250–FNW 850) and OE (OE 250–OE 750).

generally more variable at the station close to the discharge point (Fig. 3). Chl-a was strongly positively correlated with cryptophytes and planktonic diatoms ($p < 0.01$, n = 29), dominant taxa at OE (see Fig. 3), water temperature and phosphate, and negatively related with dissolved oxygen and suspended solids ($p < 0.05$, n = 35). Planktonic diatoms at OE were strongly positively correlated with water temperature, and negatively correlated with dissolved oxygen and wind speed ($p < 0.01$). Further, cryptophytes also showed positive but weaker correlations with temperature and nutrient concentrations ($p < 0.05$; n = 29).

3.3. Intra-annual variability of the water quality in Ria Formosa at contrasting lagoon areas under influence of wastewater discharges

Intra-annual variability patterns varied depending on the variable and station considered but, globally, a stronger similarity between stations was detected at the OE area (Figs. 4–6). Over the FNW area, salinity showed lower values in winter and higher during late-summer to early-autumn, at all transect stations except FNW 850 (Fig. 4A). Dissolved oxygen saturation, generally, increased during the autumn-winter period, showing a steep decline from spring to summer period, at all stations (Fig. 4B). Intra-annual patterns in ammonium concentration varied across stations. At the station furthest from the discharge point, ammonium was higher in summer while the nearest stations additionally revealed high sustained (winter-spring) or episodic (autumn) values, that inversely paralleled salinity variability (Fig. 4C). Phosphate concentration was generally higher during early-autumn and summer, for all stations (Fig. 4D). *E. coli* (Fig. 5A) and enterococci (Fig. 5B) showed similar patterns for all stations, with winter minimum values, and notorious peak values in August 2019. The abundance of *E. coli* more frequently overpassed the regulatory concentration of faecal coliforms imposed for effluent discharge licencing during summer, particularly at stations close to the effluent discharge point (see Fig. 5A, and Table S2).

For the FNW area, intra-annual variability patterns in Chl-a varied across stations (Fig. 6A). Maxima values were detected during late-winter (FNW 750) to mid-spring (FNW 850) for the furthest stations from the discharge point, and late-spring to summer for nearest stations, and minima values were observed during winter. Chlorophytes, the dominant phytoplankton group at FNW, also revealed higher abundances during the spring-summer period (Fig. 6B), and peak abundances (May 2019) were concomitant with minimum ammonium concentrations (Figs. 4C and 6B). Benthic diatoms (Fig. 6C), indicators of sediment resuspension, showed different variability patterns across stations, with higher values during late-winter (FNW 750) or late-spring (FNW 250 and FNW 500). At station FNW 850, already located in a wider deeper lagoon channel, phytoplankton seasonality was more strongly controlled by planktonic diatoms and cryptophytes (data not shown), as globally observed for the OE area (see Fig. 6E and F). Over FNW, the abundances of potentially toxigenic phytoplankton *taxa* were systematically lower than the alert levels. However, the IPMA database reported several occurrences for the "Faro 1" shellfish production area (see Fig. 1), mostly during the autumn-winter period (*Dinophysis* spp.: October 2018, December 2018, January 2019, and March 2019; *Gymnodinium catenatum*: October 2018; *Lingulodinium polyedra*: July 2019).

For the OE study area, salinity showed marginally higher values during summer (Fig. 4E). Dissolved oxygen saturation generally increased from autumn to early-winter and declined during the spring-summer period (Fig. 4F). Ammonium concentrations were globally lower during spring and higher in summer (Fig. 4G), and phosphate concentration was generally lower during winter and higher in the

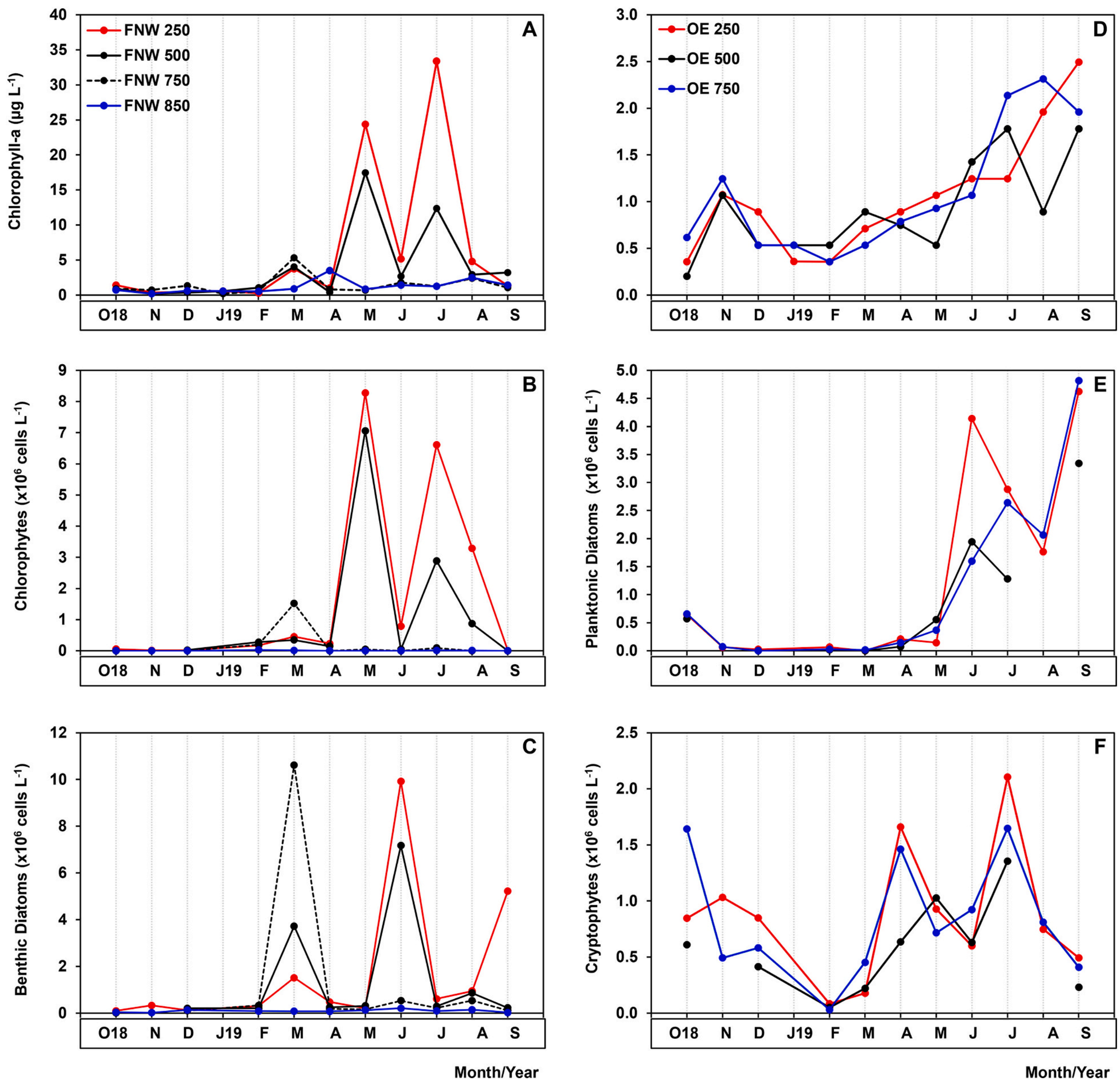

Fig. 6. Monthly variability of phytoplankton biomass and abundance of representative functional groups of phytoplankton different stations along longitudinal transects from the effluent discharge points for Faro Northwest (FNW, left panels) and Olhão East (OE, right panels) study areas, during the period October 2018–September 2019. Panels: (A, D) chlorophyll-a concentration, a proxy for phytoplankton biomass; (B) chlorophytes; (C) benthic diatoms; (E) cryptophytes; and (F) planktonic diatoms. Note differences in scale between FNW and OE. The notation used for each station represents the distance, in meters, from the discharge point for both FNW (FNW 250–FNW 850) and OE (OE 250–OE 750).

spring-summer period (Fig. 4H). *E. coli* (Fig. 5C) and enterococci (Fig. 5D) showed similar intra-annual patterns, with lower values in the winter-spring period, and notorious peak values during October 2018 and July 2019.

Chl-a also showed similar intra-annual patterns, with lower values during the autumn-winter period and higher values in summer (Fig. 6D). Planktonic diatoms, the dominant phytoplankton group at OE, generally paralleled Chl-a patterns (Fig. 6E). Cryptophytes showed less marked variability, reaching relatively high values during all seasons, except winter (Fig. 6F). Several events of potentially toxigenic phytoplankton above alert and interdiction levels (see Table S4 caption) were detected at OE study area. Events above alert levels were associated with *Lingulodinium polyedra* (June–July 2019, max. abundance: 4.3×10^3 cells L^{-1}, and *Pseudo-nitzschia delicatissima* (August 2019, max. abundance: 0.8×10^6 cells L^{-1}). The later HAB group was also associated with events above interdiction levels (June 2019, max. abundance: 3.6×10^6 cells L^{-1}; September 2019, max. abundance: 1.3×10^6 cells L^{-1}). At nearest lagoonal ("Olhão 2") and coastal shellfish production area (L8), these occurrences were also generally detected during late spring–summer period (*Dinophysis* spp.: May–September 2019; *Lingulodinium polyedra*: June–July 2019; *G. catenatum*: October 2018, and June 2019). Yet, maxima HAB abundances were higher at the coastal L8 area (data not shown).

4. Discussion

Our study used multiple water quality determinants for assessing the influence of two main urban WWTPs on the water quality of receiving areas of the RF coastal lagoon. Globally, most variables conventionally used as wastewater indicators showed a higher variability and a lower water quality at FNW in respect with OE area, aggravated during low tide. This fact was apparently reflected in the concentration of *E. coli* in shellfish bivalves, with higher concentrations at the production area closest to FNW, in comparison to OE area (IPMA, 2018a, 2019a). As previously hypothesized, a more intense, and spatially extended wastewater influence was detected at FNW area, subjected to a relatively lower wastewater loading (total nitrogen, phosphorus, and suspended solids) but weaker hydrodynamic regime. These results highlighted the relevance of hydrodynamic conditions as a modulators of ecosystem susceptibility to wastewater discharges. A reduction of the impacts of wastewater discharges under strong flushing conditions was also reported for other receiving coastal systems or areas (Laws et al., 1999; Thompson and Waite, 2003; Yin and Harrison, 2007; Xu et al., 2011; Sebastiá and Rodilla, 2013; Zhou et al., 2014; Ruiz-Ruiz et al., 2016; Wang et al., 2018).

4.1. Wastewater footprints at contrasting Ria Formosa lagoon areas

At OE area, despite under the influence of higher mean nitrogen and phosphorus effluent loads, salinity values close to those usually associated with marine waters (>36), and low concentrations of nutrients, *E. coli*, enterococci, and Chl-a indicated a negligible imprint of wastewater discharge, and an increased influence of coastal water masses. In fact, concentrations of nutrients, dissolved oxygen, and Chl-a at OE were typical of those previously referred for RF main channels, under increased ocean influence (Barbosa, 2010; Brito et al., 2012b; Cravo et al., 2015, 2018; Domingues et al., 2015, 2017a, 2017b). At this area, a more extensive cultured shellfish bivalve beds (ca. 5-fold higher than FNW) could also partially justify a relative reduction in Chl-a (Barbosa, 2010; Brito et al., 2012b; Roselli et al., 2013; Lucas et al., 2016; Pérez-Ruzafa et al., 2019). Further, the dominant phytoplankton functional groups at this area, chain-forming planktonic diatoms, and cryptophytes, were also reported as dominant nano- and microphytoplankton groups in the RF system (Barbosa, 2006; Domingues et al., 2015, 2017a, 2017b) and other lagoonal systems (see Leruste et al., 2016). Despite the common linkage between eutrophication and HABs, the number of potentially toxigenic phytoplankton taxa (see Table S4) and *Pseudo-nitzshia* spp. abundance, associated with the human syndrome amnesic shellfish poisoning, were higher at OE area, not at FNW, reflecting the importation of HAB-forming species from adjacent coastal waters into the RF. This process was also reported for *Dinophysis acuminata* and *D. acuta*, associated with diarrhetic shellfish poisoning, and *Gymnodinium catenatum*, associated with paralytic shellfish poisoning (Barbosa, 2010; Brito et al., 2012b; Botelho et al., 2019).

At OE area, a deeper and wider main channel near one the main RF lagoon inlets (Jacob and Cravo, 2019), high tidal exchange rate promotes rapid advection, mixing, dispersion, and dilution of WWTPs effluents and related constituents, explaining the relative improvement in water quality, in respect with FNW area, and the absence of along transect variability. High exchange of lagoon water and adjacent coastal seawater dilutes and horizontally exports bacterial indicators of faecal contamination (Martins et al., 2006; Cravo et al., 2015; Veríssimo et al., 2019), nutrients (ammonium, phosphate; Newton et al., 2003; Newton and Mudge, 2005; Falcão and Vale, 2003; Cravo et al., 2015), and phytoplankton biomass into the coastal area (Barbosa, 2006, 2010; Cravo et al., 2019; Rosa et al., 2019), being generally responsible for the reduced susceptibility of the RF lagoon to eutrophication (Tett et al., 2003; Newton and Mudge, 2005; Mudge et al., 2008; Domingues et al., 2015, 2017a, 2017b). In contrast, microtidal restricted coastal systems subjected to high nutrient loads generally develop eutrophication symptoms (Tett et al., 2003; García-Pintado et al., 2007; McGlathery et al., 2007; Bricker et al., 2008; Roselli et al., 2013; Newton et al., 2014).

At FNW, narrow and shallow creek-like channels and increased distance to main lagoon inlets restrict water circulation patterns and increase water residence time and lagoon residual circulation patterns, limiting the dispersal of terrestrial inputs and wastewater discharges (e.g., Fabião et al., 2016; Veríssimo et al., 2019). Inner shallow RF areas naturally present higher nutrient concentrations, and higher light intensity in the mixed layer, conditions that globally promote phytoplankton growth and biomass (Chl-a) accumulation (Barbosa, 2010; Domingues et al., 2015). In shallow, inner lagoon domains, water quality can also be affected by naturally intensified benthic-pelagic interactions (e.g., Pérez-Ruzafa et al., 2019). Sediments in inner RF areas have high concentrations of organic matter, that promote benthic respiration (Santos et al., 2004), microphytobenthic algae (Brito et al., 2012a), and are key reservoirs of inorganic nutrients (Falcão and Vale, 2003; Falcão et al., 2009 and references therein). Additionally, sediments are also considered relevant reservoirs for faecal contaminants in shallow ecosystems (e.g., Byappanahalli et al., 2012; Hassard et al., 2016). Thus, resuspension of microbes and detritus deposited on the sediment bed of this shallow weakly-flushed area, and diffusion of nutrients from sediment interstitial water, can then partly justify increased mean concentrations of nutrients, suspended solids, *E. coli*, enterococci, Chl-a, and benthic diatoms at FNW, in respect with OE, even in the absence of wastewater discharges.

However, at the weakly-flushed FNW area, progressive correlated declines in salinity and oxygen concentration, and concurrent increases in nutrient concentrations (ammonium, phosphate), bacterial indicators of faecal contamination (*E. coli*, enterococci), Chl-a, and freshwater chlorophytes, namely *Scenedesmus* spp., showed an evident wastewater footprint, down to 500 m from the discharge point (see Fig. 2). Relatively similar spatial patterns for salinity, nutrients, faecal indicators, Chl-a, and chlorophytes were recently reported for other main WWTP (Faro-Olhão) discharging effluents into a relatively confined RF location (Jacob et al., 2020). However, the effects associated with this WWTP were more intense, affecting areas furthest from the discharge point (up to 750 m), probably due to a 3.5-fold higher effluent discharge rate, in respect with FNW (Jacob et al., 2020). These along transect patterns reflected the passive dilution of wastewater-enriched water masses with water masses from main lagoon channels. However, active processes, including nutrient uptake by microalgae, and natural mortality processes of phytoplankton (Barbosa, 2006; Pérez-Ruzafa et al., 2019) and faecal indicators (Dionísio et al., 2000; Byappanahalli et al., 2012), can further explain relative declines along the longitudinal transect from the discharge point.

The in-depth analysis of specific water quality determinants along the longitudinal transect from the discharge point more effectively demonstrates the influence of wastewater discharge on water quality at FNW. At stations close to the discharge point, namely during low tide, ammonium was the dominant form of inorganic nitrogen, as reported for secondarily treated wastewater due to intense decomposition of organic matter (Caron et al., 2017). Low salinity, and excessive amounts of ammonium and phosphorus were also detected at these stations, as referred for other coastal confined and exposed systems receiving wastewater discharges (Yuan et al., 2010; Xu et al., 2011; Comber et al., 2013; Zhou et al., 2014; Saito et al., 2018). A wide diurnal range in oxygen saturation, from low undersaturated conditions during early morning samplings (spring tides) to high supersaturated conditions during the afternoon (neap tides), was observed at FNW stations close the discharge point, as also reported for other wastewater impacted environments, where anoxia and algal blooms may coexist (Krause-Jensen et al., 1999; Marinov et al., 2007; Viaroli et al., 2008; Bas-Silvestre et al., 2020). Events of low dissolved oxygen concentration (<4 mg L^{-1}), characteristic of oxygen deficiency (Vaquer-Sunyer and Duarte, 2008; OSPAR, 2009), and oxygen saturation below MAV for shellfish producing waters (70%), were observed even at FNW 850.

At stations close to the FNW discharge point, increased concentrations of *E. coli* and enterococci could also denote the influence of wastewater discharges. Indeed, higher concentrations of bacterial indicators of faecal contamination in water (Dionísio et al., 2000) and shellfish bivalves have been referred for restricted areas of this lagoon, particularly under the direct influence of Faro and Olhão cities, usually higher for the former area (Almeida and Soares, 2012; Botelho et al., 2015). Considering the more restricted enteric habitat for *E. coli*, diverse extraenteric habitats of enterococci (e.g., sediments, aquatic, and terrestrial vegetation), and their higher resistance in marine systems (see Byappanahalli et al., 2012), higher ratios of *E. coli* to enterococci at FNW probably indicated a higher relative influence of recent human-derived faecal. Further, at the shallowest stations close to the FNW discharge point, low salinity along high concentration of suspended solids, that minimize the germicide effects of intense sunlight, can also enhance the survival and concentration of coliforms in sediments (Fujioka and Narikawa, 1982; Davies and Evison, 1991; Korajkic et al., 2013). Additionally, faecal inputs from wild animals, specifically birds, commonly foraging on these stations, could also represent alternative sources of bacterial faecal contamination (Pachepskya and Shelton, 2011; Byappanahalli et al., 2012; Hassard et al., 2016).

As frequently reported for other coastal systems receiving wastewater discharges, phytoplankton biomass (Chl-a) declined with distance from the discharge point at FNW area, and maximum Chl-a values at the stations closest to the discharge point (33.4 μg L^{-1}) were higher than values reported for inner and urban-impacted areas of RF lagoon (<10 μg L^{-1}; Barbosa, 2010; Brito et al., 2010). These high Chl-a values may be a result of increased exposure of microalgae to higher nutrient concentrations, long enough to promote their substantial growth (Seubert et al., 2017), and/or direct importation of phytoplankton from the WWTP effluent. In fact, a strong increasing trend in the abundance of freshwater chlorophytes, specifically *Scenedesmus*, and cyanobacteria negatively correlated with salinity, was detected at these stations. Cyanobacteria and chlorophytes are usually considered well adapted to high organic load conditions and low oxygen (Palmer, 1969), and chlorophytes have been reported as relevant taxa in wastewater stabilization ponds and receiving coastal areas (Soler Torres and Del Rio, 1995; Amengual-Morro et al., 2012; Ouali et al., 2015; Leruste et al., 2016; De-los-Ríos-Mérida et al., 2017; Olano et al., 2019). Additionally, the genera *Scenedesmus* and *Navicula* were also classified in the top eight more tolerant genera to organic pollution (Palmer, 1969). Considering most chlorophyte taxa detected at FNW are not considered harmful (see Table S4), increased abundance of chlorophytes does not necessarily imply, per se, a decline in water quality. However, their linkages with different wastewater indicators probably make this group and species interesting candidates to be included in multi-metric indices of water quality for the RF lagoon. The potential value of cyanobacteria (e.g., Vasconcelos and Pereira, 2001), and benthic diatoms as indicators of wastewater influence at FNW is more debatable, since spatial gradients could be also justified by the effects of increased resuspension at the shallowest stations (Brito et al., 2012a), closest to the discharge point. Additionally, in contrast with previous reports for other coastal systems (e.g., Reifel et al., 2013), no evidence of effluent impacts on HAB species were detected at FNW. The euryhaline armoured harmful dinoflagellate *Kryptoperidinium foliaceum*, reported for various estuarine and lagoonal systems (e.g., Kempton et al., 2002; Domingues et al., 2011; Alkawri, 2016), including RF (Brito et al., 2012b), increased with distance from the discharge point, apparently exploring an intermediate inner lagoon domain, between FNW and OE areas.

At the furthest station from the discharge point, already located in a main lagoon channel, dissolved oxygen concentration, Chl-a, and phytoplankton abundance and composition were like those observed at OE (Figs. 3–6), reinforcing the relevance of hydrodynamic conditions as a modulator of ecosystem vulnerability to wastewater discharges. Chl-a values (1.2 ± 0.9 μg L^{-1}) were within the range of values usually reported for RF lagoon (<3 μg L^{-1}; Barbosa, 2010; Brito et al., 2012b; Domingues et al., 2015, 2017a, 2017b; Cravo et al., 2015, 2018, 2020). However, ammonium, nitrate, nitrite, and phosphate concentrations at FNW 850 were still considered elevated in respect with typical values referred for the main RF channels (Barbosa, 2010; Cravo et al., 2015, 2020 and references therein), and winter background values for the Portuguese coastal waters with salinity >35–35.5 (dissolved inorganic nitrogen = 10 μM; phosphate = 0.6 μM; OSPAR, 2005). Additionally, a relatively low mean salinity (34.5) was observed at FNW 850 and, despite the final ultraviolet disinfection of the effluent, *E. coli* concentration surpassed the limit of effluent discharge license at FNW 850 in 33% of samples. Considering filter-feeding shellfish bivalves concentrate faecal indicators from the water (Martins et al., 2006; Campos et al., 2013), our results highlight the need to establish specific measures to protect the quality of shellfish grounds located in the vicinity of FNW 850 (see Fig. 1).

Overall, in comparison with studies undertaken during early 2000's, before the upgrade of wastewater treatment systems (change in secondary treatment from aerobic stabilization ponds to activated sludge with final ultraviolet disinfection; see Cravo et al., 2018), a generalized improvement in water quality was detected. In fact, previous studies report higher concentrations of phosphate, bacterial indicators of faecal contamination, and Chl-a, for both FNW and OE study areas (Cabaço et al., 2008; Cravo et al., 2015). Marked improvements in the water quality of coastal receiving systems, including increases in dissolved oxygen and reductions of nutrient concentrations and Chl-a, were also associated with upgrades in wastewater treatment systems (Carstensen et al., 2006; Xu et al., 2011; Saeck et al., 2013; McMellor and Underwood, 2014; Leruste et al., 2016; Beck et al., 2018; Garnier et al., 2018; Zhong et al., 2019).

4.2. Influence of wastewater discharges on the intra-annual variability in water quality of Ria Formosa lagoon

Coastal lagoons are complex transitional ecosystems and, as such, variability of the water quality along the annual cycle is potentially shaped by the interplay of multiple processes, including of land-driven fluxes (e.g., riverine, submarine groundwater, and wastewater discharges), ocean dynamics, and meteorological forcing, including precipitation and prevailing winds (e.g., Romero-Sierra et al., 2018; Pérez-Ruzafa et al., 2019). Riverine contributions are considered negligible for the study area (Cravo et al., 2020), and were probably minimized during the study period, with an accumulated annual precipitation (ca. 220 mm) typical of dry years (30-year climatological value for Faro: 509 mm; http://www.ipma.pt/en/oclima/normais.clima/). Thus, considering salinity was mostly controlled by wastewater discharge, evaporation rate, and entrance of seawater through lagoon inlets and channels, as reported for other similar systems (e.g., Romero-Sierra et al., 2018), its lower intra-annual variability at the OE area revealed a lower influence of wastewater at this area.

For the strongly flushed OE area, despite increased phosphorus loading between late-spring and summer (data not shown), minor evidence of wastewater discharges was detected on the intra-annual variability patterns of water quality determinants. Maxima concentrations of ammonium, phosphorus (≤10 μM), *E. coli* (<350 MPN 100 mL^{-1}) and enterococci (<30 MPN 100 mL^{-1}), observed at stations closest to the effluent discharge point, during June–July 2019, could reflect an influence of wastewater discharges. However, no further effects of these low magnitude changes in water quality were detected on phytoplankton. Indeed, increases in cryptophytes during spring followed by planktonic diatoms during summer, detected at all OE stations, represent the phytoplankton annual succession pattern previously reported for RF lagoon domains not influenced by wastewater discharges (Barbosa, 2006; Domingues et al., 2015, 2017a, 2017b). Diatoms in the RF are usually strongly limited by light availability and temperature during autumn-winter, with minor nutrient availability during summer, and present summer maxima (Barbosa, 2006; Domingues et al., 2015, 2017a, 2017b). Additionally, considering the location of the OE area, near a main lagoon inlet, summer upwelling events could additionally explain summer Chl-a maxima, dominated by centric diatoms. In fact, persistent strong westerly winds detected during August–September 2019 over the Portuguese southern coast (see Fig. S1), indicative of upwelling-favorable conditions, were associated with high surface Chl-a over this area and period (satellite imagery, data not shown).

The strong connectivity between RF and adjacent coastal waters, with importation of nutrient and phytoplankton rich water masses into the RF lagoon, during coastal upwelling events (Barbosa, 2010; Brito et al., 2012; Cravo et al., 2014, 2019; Domingues et al., 2015; Rosa et al., 2019), also advect HAB-forming taxa into the RF lagoon. This demonstrates that increases in phytoplankton should not be solely attributed to human stressors, namely WWTP discharges. Indeed, maxima abundances of potentially toxigenic dinoflagellates (*Dinophysis* spp., and *Lingulodinium polyedra*), detected during late spring-summer at the OE area and in nearest lagoonal ("Olhão 2") and coastal (L8) shellfish production areas, were higher for the later area (IPMA, 2018b, 2019b). Additionally, the number of days of full or partial bivalve harvest interdiction due to phycotoxins, mostly concentrated during summer (July–September 2019), was lower at the lagoonal production areas ("Faro 1": 45 days; "Olhão 2": 53 days) in respect with the adjacent coastal production area (L8: 300 days; IPMA, 2018c, 2019c). Increased stratification and lower nutrient availability in coastal waters, coupled with the functional traits of dinoflagellates (e.g., motility, mixotrophy, allelopathic interactions; Weithoff and Beisner, 2019), usually promote late spring-summer dinoflagellate blooms (see Glibert et al., 2018b and references therein). However, in coastal upwelling systems, dinoflagellate blooms are usually intercalated with diatom blooms (Smayda and Trainer, 2010; Glibert et al., 2018b). For Portuguese coastal waters, diarrhetic shellfish poisoning-producers usually bloom during spring-summer upwelling relaxation or downwelling phases, while *Pseudo-nitzschia* blooms, as detected at the OE area, are commonly associated with summer upwelling events (e.g., Palma et al., 2010; Moita et al., 2016; Díaz et al., 2019; Danchenko et al., 2019).

For the FNW area, most water quality determinants, pointed to a poorer water quality during the spring-summer period (April–August 2019; Figs. 4–6), more evident at stations close to the discharge point. This pattern can be partially attributed to the marked increase in wastewater concentration and load of total nitrogen during this period (data not shown), a plausible reflection of increased touristic influx. However, for phosphate, a nutrient with a high affinity to adsorb to sediments (Vidal, 1994), relative increases during summer may as well reflect its release from sediments, promoted by higher temperatures and lower dissolved oxygen concentration due to increased remineralization rates (e.g., Jensen et al., 1995; Slomp et al., 1998; Asmus et al., 2000; García-Pintado et al., 2007; Falcão et al., 2009; Leote and Epping, 2015). In contrast with phosphate, nitrate usually show maxima concentrations in the RF during winter, reflecting the importation from adjacent coastal area during the winter convective mixing period (see Krug et al., 2018) and the effect of episodic rainfall events and associated land runoff (Cravo et al., 2015).

The effect of inactivation of faecal indicators by high light intensity during summer (e.g., Byappanahalli et al., 2012) was probably masked at FNW area due to increased effluent loads and/or minimized by higher turbidity levels, especially at the stations closest to the effluent discharge point. Furthermore, the interactions with indigenous aquatic microorganisms (e.g., predation, viral lyses, competition) can also control the distribution of faecal indicators in RF water column (see Byappanahalli et al., 2012; Korajkic et al., 2019). Despite maxima concentrations of faecal indicators in the water at FNW during summer, higher contamination of bivalves was detected during the autumn-winter period, for both "Faro 1" and "Olhão 2" production areas (IPMA, 2018a, 2019a). Previous studies also reported higher contamination of *Ruditapes decussatus* during winter, particularly in association with intense rainfall events and increased land-runoff (Almeida and Soares, 2012; Bettencourt et al., 2013; Botelho et al., 2015). The reduced precipitation during the study period precluded the observation of these intense precipitation events, that increase not only faecal contaminants (Campos et al., 2013; Cravo et al., 2015; Florini et al., 2020).

Phytoplankton biomass (Chl-a) showed maxima during spring-summer period, at both FNW and OE. Unimodal phytoplankton annual cycles with summer maxima, driven by increased light intensity and temperature, are frequently reported for shallow confined coastal areas with relatively high nutrient concentrations (Cebrián and Valiela, 1999), including RF lagoon (Barbosa, 2006, 2010; Domingues et al., 2015, 2017a) and other similar lagoonal systems (Pérez-Ruzafa et al., 2019; Bas-Silvestre et al., 2020). However, at FNW area, spring-summer Chl-a maxima mostly reflected the behavior of freshwater chlorophytes (e.g., *Scenedesmus* spp.), particularly at stations FNW 250 to FNW 750. This functional group, strongly correlated with chemical proxies for wastewater influence (see previous section), was probably stimulated by higher nutrient availability, temperature, and light intensity. In fact, many chlorophyte species have optimal temperature for growth above 25 °C, and a higher affinity for ammonium uptake than diatoms and dinoflagellates (see Leruste et al., 2016, and references therein).

Overall, hydrodynamic conditions then emerged as a relevant modulator of water quality susceptibility to wastewater discharges. This information is crucial for future development or improvement of wastewater management strategies in the FR lagoon and other transitional coastal systems. Adequate location of WWTPs discharge points and definition of effluent discharge rates, and preservation or improvement of natural hydrodynamic processes on receiving coastal systems will promote the condition of biological resources (e.g., shellfish farming grounds), that intrinsically depend on water quality.

5. Conclusions

This study revealed that influences of treated wastewater discharge on the water quality of the RF lagoon system, considering both spatial variability and intra-annual dynamics, were more intense, spatially extended, and persistent for the low-load weakly flushed FNW area in comparison to the high-load strongly flushed OE area. However, at OE area, water quality issues related with toxigenic phytoplankton were aggravated due to ocean-coastal processes, rather than wastewater discharges. For the FNW, a poorer water quality was detected, particularly under low tide. For this area, a progressive increase in salinity with distance from the effluent discharge point, and concurrent declines in nutrient concentrations (ammonium, phosphate), total suspended solids, bacterial indicators of faecal contamination (*E. coli*, enterococci), Chl-a, and freshwater chlorophytes (namely *Scenedesmus* spp.), showed an evident wastewater footprint down to 500 m from the discharge point. A generalized decline in water quality, during late spring to summer, at FNW area was associated with increased wastewater total nitrogen load, probably a result of higher touristic influx over the area.

Globally, our results highlighted the relevance of hydrodynamic conditions as modulators of the response of RF water quality to wastewater discharges. This informative dataset can be used for improving environmental management decisions, related with water, and living marine resources, in the RF coastal lagoon and other transitional coastal systems.

CRediT authorship contribution statement

A. Cravo: Conceptualization, Investigation, Formal analysis, Writing – original draft, Writing- Reviewing and Editing. **A.B. Barbosa:** Investigation, Formal analysis, Writing – original draft, with equal contribution to first author, Writing- Reviewing and Editing. **C. Correia:** Investigation, Writing – original draft. **A. Matos:** Investigation, Writing – original draft. **S. Caetano:** Investigation, Writing – original draft. **M.J. Lima:** Investigation, Formal analysis, Writing – original draft. **J. Jacob:** Investigation, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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.2021.113296.

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