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Impacts of Decommissioning and Upgrading Urban Wastewater Treatment Plants on the Water Quality in a Shellfish Farming Coastal Lagoon (Ria Formosa, South Portugal)

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ABSTRACT

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Ria Formosa is a productive coastal lagoon, located on the south coast of Portugal, and represents the largest national producer of shellfish bivalves (*ca.* 90% production). This ecosystem is subjected to various anthropogenic pressures, including the discharge of urban wastewater treatment plants (UWWTP), which impacts the lagoon water quality. This study aimed to assess the impact of alterations in the functioning of two UWWTP on the water quality of Ria Formosa, based on chemical variables, phytoplankton composition (including potential harmful species) and faecal contamination. During the period September 2018 - October 2019, water sampling was conducted along dominant longitudinal gradients of the effluent dispersion from the discharge point (1-2 km), for two sites: a decommissioned (OP) and a modified (FO) UWWTP. After modification, the later started receiving a higher influent volume (ca. 40%), under an innovative technology system (biological treatment in aerobic granular sludge). Based on chemical water quality variables, phytoplankton and indicators of faecal contamination, a significant improvement along the longitudinal gradient from the discharge point was observed after OP decommissioning. This improvement was fast, being detected two months after decommissioning, positively affecting areas used as shellfish farming grounds. However, distribution patterns of bacteriological indicators and regular shellfish harvesting interdictions suggested an alternative source of faecal contamination after OP decommissioning. At FO, both chemical variables and bacteriological indicators of faecal contamination revealed a slower improvement, only six-months after the UWWTP alteration. Before that, increased and highly variable ammonium, chlorophyll *a* concentration, phytoplankton abundances and *Escherichia coli* densities, revealed an unstable phase. Overall, a lower water quality at FO in respect to OP reflected not only a higher effluent volume but also more restricted water circulation for the former.

ADDITIONAL INDEX WORDS: *Ria Formosa, bivalves, sewage impact, wastewater treatment plants.*

INTRODUCTION

Coastal lagoons occupy about 13% of the world's coastal areas, have high ecological and economic value but are amongst the most endangered ecosystems on Earth, including by sewage discharges (Kennish and Paerl, 2010). Water quality problems associated with wastewater disposal may occur due to: (a) increased inputs of organic matter that promote microbial decomposition, oxygen depletion, and ultimately eutrophication of the receiving waters; (b) increased amounts of inorganic nutrients which can over stimulate algal growth and promote harmful blooms (Glibert *et al*., 2018); and (c) contamination by pathogenic bacteria that affects edible filter feeder resources and human health (Metcalf and Eddy, 1995). Ria Formosa coastal lagoon (Figure 1), a shallow multi-inlet mesotidal system located along the south Portuguese coast, represents the major Portuguese producer (90%) of clams *Ruditapes decussatus*. Clam farming covers *ca.* 100 km^2, reaching an annual production of *ca.* 2,000 tons (INE, 2007). This valuable resource greatly depends on water quality, which has been threatened by wastewater disposal (Cravo *et al.*, 2015). Until recently, five major urban wastewater treatment plants (UWWTP) discharged their effluents into Ria Formosa. Recently, during November 2018, some UWWTP suffered profound alterations: the Olhão Poente UWWTP (hereafter OP) was decommissioned and its effluents were driven into Faro-Olhão UWWTP (hereafter FO). FO then started receiving the influents of both Faro and Olhão cities, but water treatment was upgraded to an innovative technology system, based on biological treatment in aerobic granular sludge (Nereda® system). Presently, the effluent discharges at FO increased in proportion to the equivalent population served, from *ca.* 10,000 to 14,000 m^3.s^{-1}. This study aims to assess the impacts of OP decommissioning

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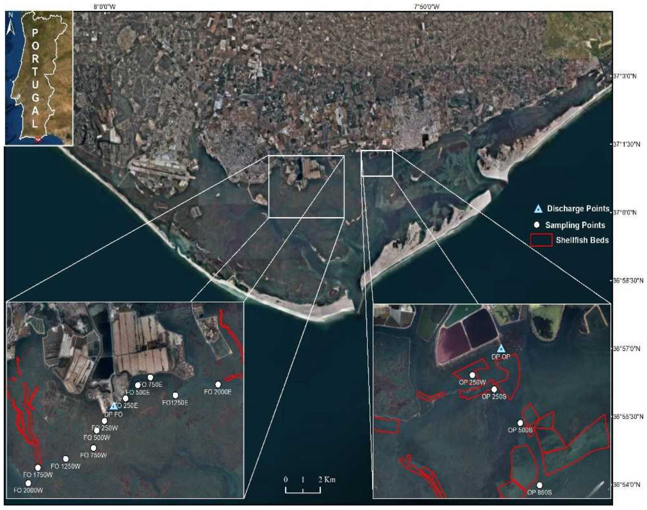

Figure 1. Map of the Ria Formosa coastal lagoon and location of the two studied urban wastewater treatment plants (UWWTP), Faro-Olhão (FO) and Olhão Poente (OP). For each UWWTP, the discharge point, sampling stations and areas covered by shellfish beds are shown.

and FO alteration on the water quality of Ria Formosa lagoon, particularly in areas covered by shellfish beds. In this context, both chemical variables, phytoplankton composition, including potentially harmful species, and faecal contamination were considered.

METHODS

Water sampling was conducted during the period September 2018 - October 2019, along longitudinal gradients of the effluent dispersion from the discharge point (up to 1-2 km), for both OP and FO (Figure 1). At FO, two sections (hereby designated as E and W) were sampled, comprising five and six stations, respectively, with bivalve beds located over the farthest stations (1,750W and 2,000E). At OP, four stations were sampled, all covering areas of bivalve beds. Sampling was undertaken monthly during the period October 2018 - March 2019 (neap tides only), and fortnightly from April to September 2019 (both spring and neap tides), at both low and high tide. For this study, only data collected during the most critical tidal stage in respect with UWWTP impacts, low water neap tide, were explored. Furthermore, physical-chemical variables included only key indicative variables of sewage discharge (salinity, dissolved oxygen, ammonium) in this study.

Surface water temperature, salinity, pH and dissolved oxygen (DO) were measured *in situ* using a multiparametric probe YSI (EXO 2). Surface water samples (*ca*. 20-30 cm) were collected for the analysis of inorganic nutrients (ammonium, nitrate, nitrite, phosphate and silicate) chlorophyll *a*, phytoplankton abundance and species composition, and indicators of faecal contamination.

Nutrients and chlorophyll *a* concentration (Chl*a*), used as a proxy for total phytoplankton biomass, were measured by spectrophotometric methods (Grasshoff *et al*., 1983 and Lorenzen, 1967, respectively). Samples for phytoplankton abundance and species composition were preserved with Lugol's solution immediately after collection, settled in sedimentation chambers, and observed at 400x magnification using inversion microscopy (Zeiss Axio Observer A1), according with Utermohl (1958). Thus, morphologically inconspicuous pico- and nanophytoplankton (*e.g.*, pico-sized cyanobacteria, picoeukaryotes) were not evaluated.

Bacteriological quality assessment included most probable number (MPN) estimation for *Escherichia coli* (EC) and enterococci (E) by Quanti-Tray method, using Colilert and Enterolert systems (IDEXX Laboratories, Westbrook, Maine), following manufacturer (IDEXX) guidelines. After suitable incubation, the number of positive wells were used to estimate MPN, using the provided IDEXX MPN charts. Inhibition by high salt content was avoided with a minimal sample dilution of 1:4 for Enterolert and 1:10 for Colilert, thus raising the lower detection limits of the methods to *ca.* 4/100 mL for enterococci and 10/100 mL for *E. coli.*

For each UWWTP, differences in chemical and faecal indicators between low- and high-tide, and between spring and neap tide were tested using the signed rank Wilcoxon test. For each UWWTP, differences in chemical and biological variables across sampling stations and between sampling periods were evaluated using the Kruskal-Wallis one-way analyses of variance on ranks, and pair-wise comparisons using the Dunn's test. For temporal variability, data were binned into four different periods: September - October 2018 (before alterations on OP and FO functioning); November 2018 - April 2019; May – August 2019; and September – October 2019. When required, statistical comparisons for bacteriological data were based on paired t-tests, after normalization through logarithmic transformation. All statistical analyses were considered at a 0.05 significance level.

RESULTS

Results of this study were divided into three sections: (i) environmental setting; (ii) phytoplankton-related variables; and (iii) bacterial indicators of faecal contamination.

Environmental Setting

Considering the key physical-chemical variables indicative of sewage influence, salinity, DO and ammonium (NH_4^+), significant differences were detected between high and low tide, both for OP ($p<0.05$) and FO ($p<0.0001$). During low tide, differences between neap and spring tides were also significant ($p<0.05$), showing a global decline in water quality during the former stage. Comparisons between the two UWWTP, under low neap tide, revealed higher NH_4^+ and lower salinity and DO at FO in respect to OP ($p<0.01$; see Figures 2 and 3).

Spatially, during low water neap tides, at OP (Figure 2), a gradient of salinity and NH_4^+ values was observed, decreasing with increasing distance from the discharge point. Yet, no differences in DO were detected between sampling stations. At FO (Figure 2), a clear effect of dispersal and dilution was observed with increasing distance from the discharge point, for salinity, NH_4^+ and DO. Discharge impact was noticeable up to 750 m from the discharge point, in respect to the 2,000W station, where maximum salinity and minimum NH_4^+ values were detected. Globally, the eastern sector stations revealed a higher impact of sewage dispersal, with lower salinity and DO, and higher NH_4^+ than the western sector.

Temporally (Figure 3), at OP, higher variability was observed during September - October 2018 (before OP decommissioning), for all variables. For subsequent periods, salinity increased, reaching maximum values during the last study period (September 2019). DO values, usually >100% (supersaturation), reached minimum values (*ca*. 70%) during September 2019. NH_4^+ showed a decline after OP decommissioning, which persisted throughout

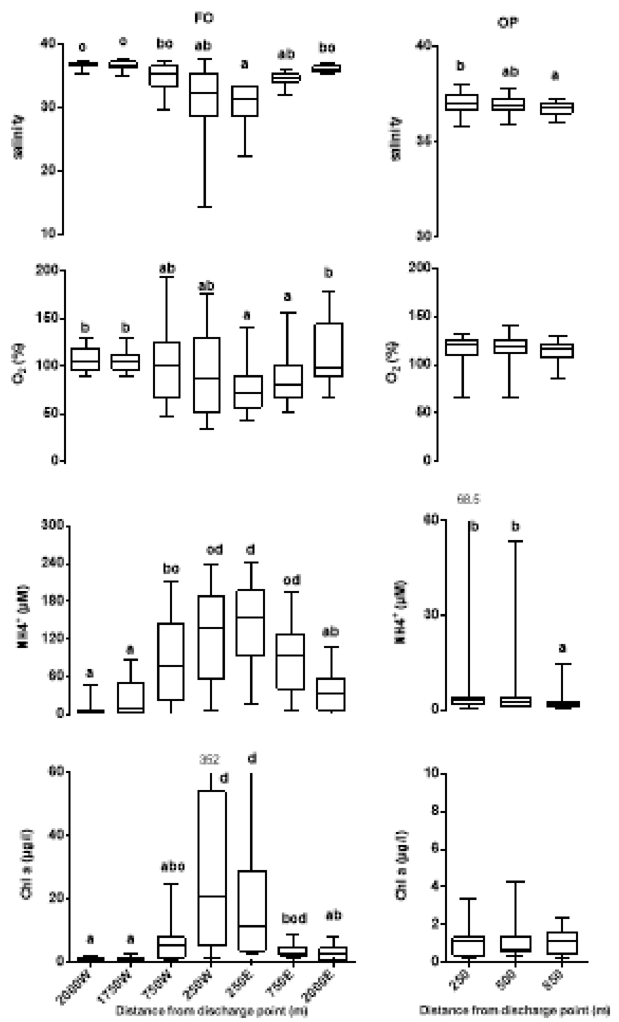

Figure 2. Spatial distribution of salinity, dissolved oxygen (O2, %), ammonium (NH4+) and chlorophyll-a concentration (Chla) at FO and OP UWWTPs, as a function of the distance from the discharge point. Median values are represented by the lines within the boxes, 25th to 75th percentiles are denoted by box edges, and minima and maxima values are depicted by the error bars. For each UWWTP, different letters over the bars denote significant differences between stations ($p<0.05$).

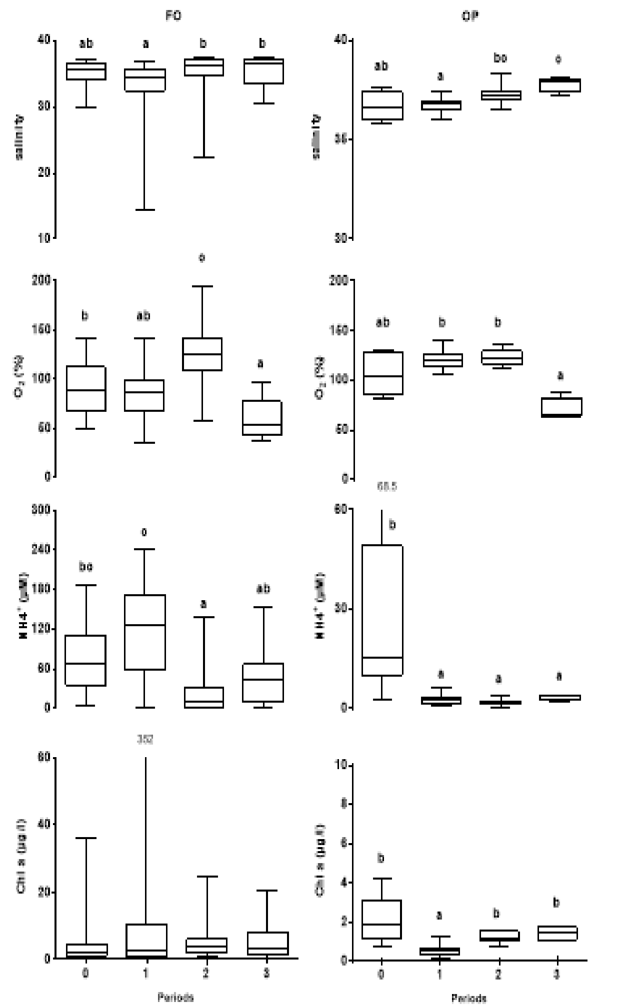

Figure 3. Variability of salinity, dissolved oxygen (O2, %), ammonium (NH4+) and chlorophyll-a concentration (Chla) at FO (left panels) and OP (right panels) UWWTPs during four study periods. Period 0: September – October 2018; Period 1: November 2018 – April 2019; Period 2: May – August 2019; Period 3: September – October 2019. Decommissioning of OP and remodeling FO were undertaken during late October 2018 (after period 0). For box-plot details, see caption of Figure 2.

subsequent periods. At FO (Figure 3), all variables showed higher variability during November 2018 - April 2019 period, immediately after FO alteration. DO values, down to 35%, were well below the minimum allowable value for shellfish waters (60%; Directive 2006/113/EC).

Phytoplankton

Chl*a* (0.1 - 352.4 $\mu g.L^{-1}$) and total phytoplankton abundance ($0.2x10^6$ - $0.2x10^9$ $cells.L^{-1}$) showed higher values at FO than at OP ($p<0.01$). Freshwater chlorophytes and colonial cyanobacteria represented the dominant phytoplankton groups at FO and OP, respectively. Several potentially harmful taxa were detected, including toxigenic diatoms (*Pseudo-nitzschia* spp.), raphydophytes (*e.g.*, *Heterosigma* cf. *akashiwo*), dinoflagellates (*e.g.*, *Dinophysis acuminata Gymnodinium catenatum*, *Lingulodinium polyedra*), and cyanobacteria (*Anabaena* sp., *Oscillatoria* spp./*Planktothrix* spp.).

In respect to spatial variability, no significant differences in Chl*a* (Figure 2) or phytoplankton abundance (Figure 4) were detected along sampling stations for OP. By contrast, at FO, significant gradients of decreasing values with increasing distance from the discharge point ($p<0.01$) were observed for both Chl*a* (Figure 2) and chlorophyte abundance (Figure 4).

Phytoplankton temporal variability patterns over the two UWWTPs were also different. At OP, significant differences in Chl*a* (Figure 3), cyanobacteria and phytoplankton abundance (Figure 5) were detected between periods ($p<0.05$). Higher variability was observed, for all variables, during September -October 2018 (before OP decommissioning), and minimum values were detected in the following November 2018 - April 2019 period. In subsequent periods, both Chl*a* and cyanobacteria

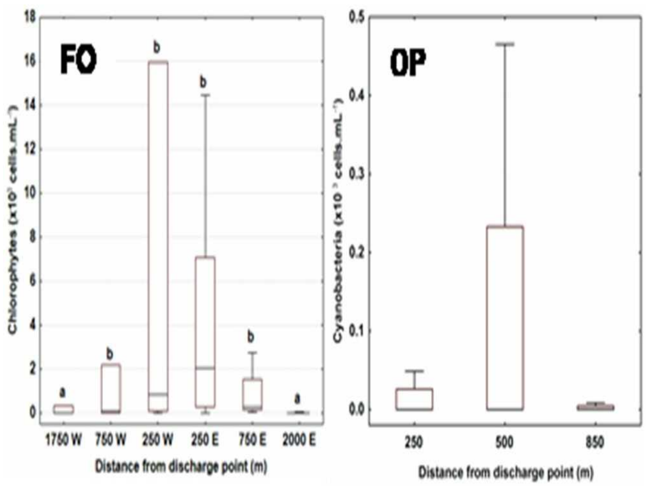

Figure 4. Spatial distribution of the abundance of dominant phytoplankton groups, chlorophytes and cyanobacteria, at FO and OP UWWTPs, as a function of the distance from the discharge point. Median values are represented by the lines within the boxes, 25th to 75th percentiles are denoted by box edges, and non-outlier limits are depicted by the error bars. Extreme values were omitted for clarity. For each UWWTP, different letters over the bars denote significant differences between stations ($p<0.05$).

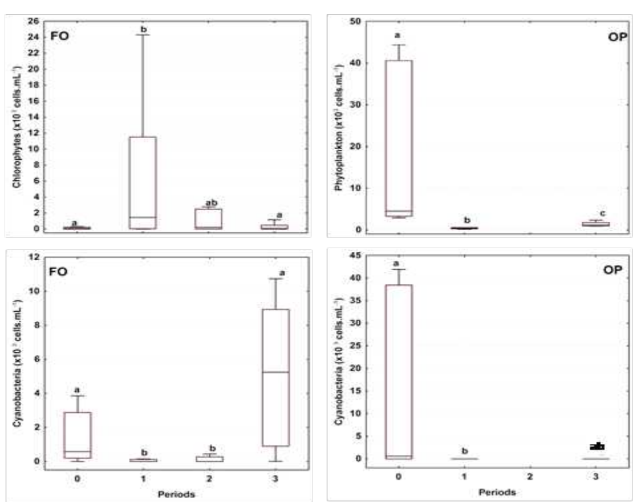

Figure 5. Variability of the abundance of different phytoplankton groups at FO (left panels) and OP (right panels) UWWTPs during four study periods (see caption of Figure 3 for period definition). Note: no data available for OP during period 2. For box-plot details, see caption of Figure 4.

increased to median values statistically similar to those before OP decommissioning, whereas total phytoplankton abundance reached significantly lower values.

At FO, all phytoplankton-related variables showed higher variability during November 2018 - April 2019 period. Chl*a* (Figure 3) and total phytoplankton abundance were similar between study periods. However, chlorophytes ($p<0.05$) and cyanobacteria ($p<0.01$) showed significant but contrasting patterns, with maxima and minima, respectively, during intermediate study periods

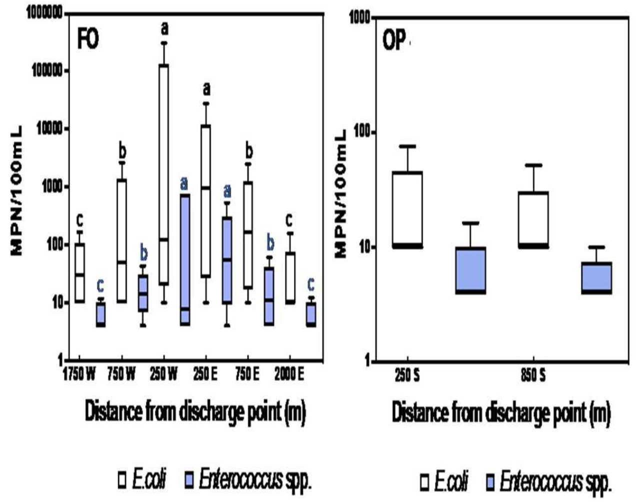

Figure 6. Spatial distribution of *Escherichia coli* (*E. coli*) and *Enterococcus* spp. at FO and OP UWWTPs, as a function of the distance from the discharge point. Median values are represented by the line within the boxes, 25th to 75th percentiles are denoted by box edges, and minima and maxima values are depicted by the error bars. For each UWWTP, different letters over the bars denote significant differences between stations ($p<0.05$).

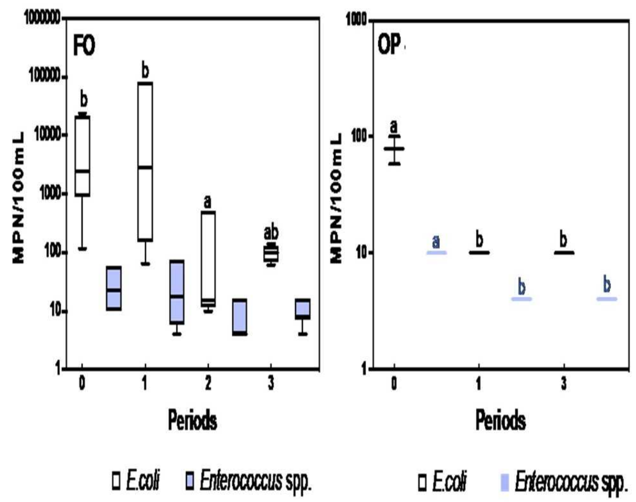

Figure 7. Variability of the abundance *Escherichia coli* (*E. coli*) and *Enterococcus* spp. at FO (left panels) and OP (right panels) UWWTPs during four study periods (see caption of Figure 3 for period definition). Note: no data available for OP during period 2. For box-plot details, see caption of Figure 6.

(November 2018 – August 2019; Figure 5). Thus, no persistent differences were detected between periods before and after FO alteration.

Bacteriological Indicators

Both Wilcoxon's signed rank test and Paired Data t-Test, applied on $\log_{10}$ transformed data, failed to detect differences ($p>0.05$) in the densities of EC and E between neap and spring tides, or between low and high water.

During low water neap tides, at OP (Figure 6), maximum EC and E densities were lower than the legislation limits for shellfish waters (Directive 2006/113/EC). Median values for both indicators corresponded to their lower detection limits, thus impairing the visualization of sewage dilution effects.

Dilution of EC and E contaminated water was evident at FO (Figure 6), with decreasing densities with increasing distances from the discharge point. Lowest EC and E densities were observed at 2,000E, where median values coincided with the lower detection limits. At 750E, median values represented only 22% for EC and 29% for E of the values at 250E. The decline in EC was more pronounced for the western sampling sector than the eastern sector, but E median values showed a transient increase at 750W.

In terms of temporal variability, OP data (Figure 7) showed a pronounced decrease in both EC and E immediately after OP decommissioning, with all values at or under the lower detection limits. For FO, maximum densities of EC, but not E, increased during November 2018 - April 2019, but both EC and E decreased steeply during the following period (Figure 7), reaching values compliant with limits for shellfish waters (Directive 2006/113/EC).

DISCUSSION

Based on chemical water quality variables, phytoplankton and indicators of faecal contamination, a significant improvement along the longitudinal gradient from the discharge point was observed after OP decommissioning. This improvement was fast, being detected two months after decommissioning (data not shown), positively affecting areas used as shellfish farming grounds. Although EC densities were lower than admissible values for shellfish waters, regular shellfish harvest interdictions were reported by the Portuguese Institute of Sea and Atmosphere (IPMA) after OP decommissioning, in shellfish production areas close to station 850 (IPMA, 2018-2019). These interdictions were caused by EC contamination in bivalves above the regulatory threshold imposed by EU legislation (>46,000 MPN/100 g). Considering previously determined clam depuration coefficients (Martins *et al.*, 2006), other origins of faecal contamination for shellfish beds and/or sediments, rather than OP, should be considered.

At FO, both chemical variables and bacteriological indicators of faecal contamination revealed an improvement only after May 2019. Before that, due to the increased influent volume (*ca.* 40%), the biological treatment system (Nereda®) was probably not yet stabilized. During November 2018 - April 2019, low DO (undersaturation, down to 35%), along with increased and highly variable NH_4^+, Chl*a*, phytoplankton and chlorophytes abundances and EC densities, revealed an unstable phase. Moreover, the persistent decline in freshwater chlorophytes and simultaneous increase in cyanobacteria after FO alteration could also represent the transition from high to low organic load conditions in the waste stabilization ponds (Amengual-Morro *et al.*, 2012). The future implementation of water disinfection by ultraviolet radiation is expected to decrease faecal contamination of water and shellfish.

Overall, all variables showed lower water quality at FO than at OP. These results reflected not only differences in the magnitude of effluent volumes (*ca.* 2.5-fold higher at FO before OP decommissioning), but also local differences in hydrodynamics. The later could also explain lower water quality for the eastern sector of FO, which could impair shellfish beds next to 2,000E. At OP, less restricted circulation (Cravo *et al*., 2015) and a smaller effluent volume, disposed along a wider channel section, explained the lack of spatial gradients for most variables. Despite differences between FO and OP during low water neap tides, during high tide (particularly in spring tides), water quality at FO and OP farthest stations was similar (data not shown), typical of adjacent coastal waters, due to high water renewal (Cravo *et al*., 2015). However, the similarity of bacterial contamination between low and high water, as well as between neap and spring tides, pointed to other sources of faecal contamination, namely due to sediment resuspension during spring tidal floods.

CONCLUSIONS

OP decommissioning was associated with a general and fast increase in water quality, detected for chemical, phytoplankton and bacteriological indicators, representing thus a benefit for the shellfish production area. Yet, other sources of faecal contamination should be investigated for this UWTP. Alterations in FO functioning induced a slower water quality improvement, due to the period required for Nereda® system stabilization, and will be further monitored.

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LITERATURE CITED

Amengual-Morro, C.; Niell, G.M., and Martínez-Taberner, A., 2012. Phytoplankton as bioindicator for waste stabilization ponds. *Journal of Environmental Management*, 95, S71-S76.

Cravo, A.; Fernandes, D.; Damião, T.; Pereira, C., and Reis, M.P., 2015. Determining the footprint of sewage discharges in a coastal lagoon in South-Western Europe. *Marine Pollution Bulletin*, 96, 197-206.

Directive 2006/113/EC of the European Parliament and of the council of 12 December 2006 on the quality required of shellfish waters.

Instituto Nacional de Estatística, 2007. Estatísticas da Pesca 2006, 2007. 97 pp.

Glibert, P.M.; Al-Azri, A.; Allen, J.I.; Bouwman, A.F.; Beusen, H.W.; Burford, M.A.; Harrison, P.J., and Zhou, M., 2018. Key questions and recent advances on harmful algal blooms in relation to nutrients and eutrophication. In: Glibert, P.M.; Berdalet, E.; Burford, M.A.; Pitcher, G.C., and Zhou, M. (Eds.), *Global Ecology and Oceanography of Harmful Algal Blooms*, Springer, 229-260 pp.

Grasshoff, K.; Erkhardt, M., and Kremling, K., 1983. Methods of Seawater Analysis. Verlag Chemie, New York.

IPMA (Instituto Português do Mar e de Atmosfera, I.P.), 2018–2019. Resultados das Análises de Microbiologia. http://www.ipma.pt/pt/bivalves, accessed on 23 October 2019.

Kennish, M.J. and Paerl, H.W., 2010. Coastal Lagoons: Critical Habitats of Environmental Change. *In*: Kennish, M.J. and Paerl, H.W. (Eds.), *Coastal Lagoons*. CRC Press, pp. 1–16.

Lorenzen, C.J., 1967. Determination of chlorophyll and pheo-pigments: spectrophotometric equations. *Limnology and Oceanography*, 12(2), 343–346.

Martins, F; Reis, M.P.; Neves, R.; Cravo, A.; Brito, A., and Venâncio, A., 2006. *Journal of Coastal Research*, SI 39, 1551 – 1555.

Metcalf and Eddy, Inc., 1995. Wastewater Engineering: Treatment, Disposal and Reuse. McGraw-Hill Ltd., New Delhi.

Utermöhl, H., 1958. Zur Vervollkomnung der quantitativen Phytoplankton-Meyhodik. Mitt Internat Ver Limnol 9, 1-38.