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IMPACT OF URBAN WASTEWATER DISCHARGES IN THE WATER QUALITY OF RIA FORMOSA

A CASE STUDY OF FARO-OLHÃO URBAN WASTEWATER TREATMENT PLANT

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Thesis submitted to the university of Algarve as a request to obtain the degree of Master in Marine and Coastal Systems

Work conducted under supervision of: Prof Dr. Alexandra Cravo

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Declaração de autoridade de trabalho:

Declaro ser o(a) autor(a) deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

Universidade do Algarve, 08 de outubro de 2020

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Resumo

A Ria Formosa é uma lagoa costeira situada na costa sul de Portugal com elevada importância social e económica para a região que representa o maior produtor nacional de amêijoa *Ruditapes decussatus* (*ca*. 90%). Este sistema está sob diversas pressões antrópicas, incluindo descargas de águas residuais urbanas que comprometem a qualidade da água. Este estudo, estando integrado no projeto CONPRAR, visa avaliar o impacto da recente ETAR Faro-Olhão (antiga Faro Nascente) na qualidade da água, especialmente na proximidade de viveiros de bivalves, utilizando uma abordagem química e bacteriológica. As ETAR Faro Nascente e de Olhão foram desativadas em outubro e foi implementada no mesmo local de Faro Nascente a nova ETAR de Faro-Olhão que trata os afluentes das duas ETAR então desativadas e parte de S. Brás de Alportel aumentando o efluente diário da ETAR (~ 14 000 m^3 /dia). Esta usa um novo tratamento terciário (NEREDA). A amostragem de água foi realizada mensalmente ao longo do gradiente espacial da ETAR (2000 m) para duas secções da área de estudo no canal de descarga da ETAR (Esteiro da Garganta). Os dados obtidos mostram uma melhoria da qualidade da água ao longo do gradiente de dispersão do efluente para as variáveis químicas e bacteriológicas. Foram avaliados três períodos, setembro-outubro 2018, antes da desativação das duas ETAR de Faro Nascente e Olhão Poente, novembro de 2018 - abril de 2019, 6 meses após a implementação da nova ETAR Faro-Olhão e maio de 2019. Observou-se que o período novembro de 2018 - abril de 2019, foi quando se registou o maior impacto da nova ETAR e quando ocorreu a maior variabilidade associada à não estabilização do tratamento da mesma, a qual foi atingida em maio de 2019. Espacialmente, pelo índice trófico TRIX a maior influência da descarga foi identificada até 750 m para ambas as seções da área de estudo, zona onde não ocorre cultivo de bivalves. A maré desempenhou um papel importante na melhoria da qualidade da água, por promover uma elevada renovação da água, particularmente em preia mar de maré viva. A situação de maré morta e em baixa-mar revelou-se a pior a nível de qualidade de água, devido ao maior tempo de residência e menor renovação de água. Conclui-se assim que face à elevada taxa de troca com o oceano adjacente o impacto da

ETAR é diminuído quando comparado com outros sistemas onde o renovação e circulação são mais restritas.

Palavras-chave: Ria Formosa; *Ruditapes decussatus*; ETAR; nutrientes; *Escherichia coli*

Abstract

Ria Formosa is a coastal lagoon located in the south coast of Portugal, with high social and economic importance to the region representing the largest national producer of the clam *Ruditapes decussatus* (ca. 90%). This system is under several anthropogenic pressures including urban wastewater discharges which compromise its water quality. This study, being integrated in the project CONPRAR, aims to assess the impact of the recent Faro-Olhão WWTP (former Faro Nascente) upon water quality, especially in the vicinity of shellfish beds using a chemical and bacteriological approach. On an initial phase, Faro Nascente and Olhão Poente were decommissioned, and their effluents are treated at the new Faro-Olhão WWTP implemented at the same place of the former Faro Nascente. It uses a new biological treatment (NEREDA) for the population served from Faro, Olhão and a part of S. Brás de Alportel increasing the daily outflow of the WWTP (~14 000 m^3/day). Water sampling was conducted monthly along the spatial gradient of the WWTP (2000 m) for two sections of the study area in the discharge channel of the WWTP (Esteiro da Garganta). Data shows an improvement of water quality further from the stations closest to the discharge for both chemical and bacteriological variables. Three different periods studied: September-October 2018, corresponding to the period before the deactivation of both WWTP's; November 2018 – April 2019, six months after the implementation of the new Faro-Olhão WWTP and May 2019. It was noticed that the period November 2018 – April 2019 was associated to the greatest impact of the WWTP, when values were highest and most variable, also associated to an non stable biological treatment, which, stability was achieved in May 2019. Spatially, the impact of the discharge was noticeable until 750 m for both sections of the study area (TRIX) where bivalve farming does not occur. The tide had an important role in the improvement of water quality by promoting a great water renewal, particularly during flood at spring tides. Low water (neap tides) was therefore associated to the worst-case scenario due to higher residence times and less water renewal. In sum, due to the high rate of water exchange with the adjacent ocean, the impact of the WWTP was decreased when compared to other coastal systems where water renewal and circulation are more restricted.

Keywords: Ria Formosa; *Ruditapes decussatus*; WWTP; nutrients; *Eschericha coli*

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Chapter 1 – Introduction

1.1 Problematic of urban wastewater discharges

The world's coastal zones are under extraordinary and increasing pressure from human activities. While coastal zones represent less than 20% of the land surface, these provide several services, representing a major food source, a focus of transport and industrial development, a location for most tourism and an important repository of biodiversity and ecosystems (Ramesh et al., 2016). Intensive urban and industrial development as well as agricultural practices near coastal areas resulted in an increasing input of nutrients to near shore ecosystems all over the world (Cabaço et al., 2008), with deleterious effects upon water quality.

Urban wastewater discharges are among the anthropogenic pressures causing the most deleterious effects on water quality of coastal systems and are considered one of the most significant threats to coastal environments worldwide. Problems associated to the disposal of urban wastewaters have become a major problem due to the increase in human population and urbanization. The effects of pollutants and contaminants from sewage effluents on the receiving water body are multiple and these depend on the volume and composition of the effluents and on the mixing and dilution with seawater (Owili, 2003).

Urban wastewater can be characterized according to its physical, chemical and biological composition. The substances that are more important to assess concerning the impact of urban wastewater discharges in the water quality of a receiving water body are, biodegradable organic compounds, pathogenic organisms, nutrients (specially nitrogen and phosphorus), suspended solids, priority pollutants, refractory organic matter and metals (Tchobanoglous et al., 2004).

One of the main factors that leads to the degradation of water quality in coastal systems is the discharge of massive amounts of dissolved nutrients from wastewater (enriched mostly in nitrogen and phosphorus) in the receiving water bodies, which, depending on the system's hydrodynamic can lead to eutrophication. This process at its final stage could also lead to the partial or complete depletion of dissolved oxygen from the water (UNESCO, 2009). The excess of nitrogen and phosphorus in the water together with light availability can over stimulate algae growth leading to the development of algal blooms (Reed et al., 2016). During the day, phytoplankton conducts photosynthesis responsible for the increase of dissolved oxygen in the water, while during the night a supplementary amount of dissolved oxygen is used in the respiration process from primary producers (Tchobanoglous et al., 2004). When the bloom ends, and the algae start to die and settling onto the bottom, organic matter will be additionally accumulated there. Afterwards, bacteria will decompose this organic matter consuming oxygen, resulting in further hypoxic or even anoxic conditions in the bottom layers of the water body. Some algae blooms can be toxic and lead to a development of harmful algal blooms (HABs). Toxic algae if consumed by filter feeders can be accumulated in edible resources such as bivalves which, ultimately represents a risk/threat for human health.

Besides the nutrients (mainly in Nitrogen and Phosphorus forms), organic matter enrichment of the receiving water body can also impact the water quality. Particulate matter contained in wastewater can increase the turbidity, hence, decreasing the photosynthetic capacity of algae. Also, suspended matter represents a greater amount of organic matter input which can be mirrored in oxygen consumption to decompose this organic matter.

Along with those compounds, urban wastewater can also promote bacterial contamination/pollution. Even after treatment, with a reduction of 99.9% of bacterial loads, urban wastewater can contain important amounts of pathogenic bacteria (Tchobanoglous et al., 2004). Pathogenic organisms transported through wastewater can transmit infectious/contagious diseases (Tchobanoglous et al., 2004). This can happen directly through the ingestion of water from nearby areas to the discharge point of urban wastewater or indirectly through consumption of raw organisms or not properly cooked that accumulate such pathogenic organisms.

1.2. Lagoon systems and impact of urban wastewater discharges

Coastal lagoons are semi-enclosed water bodies connecting to the adjacent sea through one or more inlets. Coastal lagoons usually occur on low-lying coasts and usually run parallel to the coast, often, being longer than wide. Coastal lagoons occupy about 13% of the coastal areas around the world as they are present in every continent except in Antarctica. In Europe, coastal lagoons occupy *ca*. 5 % of the coastline (Barnes, 1980). Most coastal lagoons in southern Europe are contained within the Mediterranean basin with a microtidal regime. However, the Atlantic lagoons located at the south-west of France and those of the western Iberian Peninsula are under a mesotidal regime (Newton and Mudge, 2003).

Based on water exchange with the coastal adjacent ocean, coastal lagoons, according to Kjerfve (1986, 1994), can be classified into three different geomorphic types. Coastal lagoons can either be “chocked”, “restricted” or “leaky”. “Chocked” lagoons consist of a series of interconnected water bodies with a single inlet or channel connecting to the adjacent ocean (Kennish and Paerl, 2010). Patos lagoon, located in Brazil, is one example of a “chocked” lagoon. “Restricted” lagoons consist of lagoons with two or more channels or inlets connecting to a wider, expansive water body usually parallel to the shore (Kennish and Paerl, 2010). Barnegat Bay-Little Egg Harbor Estuary in New Jersey (USA) is one example of a “restricted” lagoon. “Leaky” lagoons have multiple entrance channels or inlets connecting to an adjacent parallel water body (Kennish and Paerl, 2010). Ria Formosa located in South Portugal is one example of a leaky lagoon.

Coastal lagoons are productive systems with high ecological and economic importance to local and regional communities (Falcão and Vale, 1990; Newton et al., 2003; Mudge and Duce, 2005) and are susceptible to human activities and now ranked among the most heavily impacted ecosystems on Earth (Kennish and Paerl, 2010). Water quality problems in coastal lagoons are generally linked with land-use and watershed development. Land-use point, and non-point source pollution inputs and groundwater contamination inflow are the main sources causing degradation of water quality in coastal lagoons (Stanhope et al., 2009). Since coastal areas are inhabited by over 60% of the human population, sewage related problems have become significant (Owili, 2003) as mentioned before.

These problems are magnified in shallow, restricted microtidal lagoons where low water circulation and renovation occurs and the residence time of contaminants and pollutant increases. Verano lagoon, located in Italy is one of many examples. This lagoon connects to the Adriatic Sea by two artificial channels, exchanging water and sediments during each tidal cycle. Its hydrodynamic cycle is influenced by several driving forces and the water residence time inside this lagoon is long, about ~1.3 years (Specchiulli et al., 2008). This lagoon receives a freshwater input of approximately 87 000 m^3 day^{-1} with a high organic matter content (Villani et al., 2000; Spagnoli et al., 2002) which, associated to its elevated water residence time lead to problems related to eutrophication. Mar Menor, a Mediterranean lagoon located in the Spanish Mediterranean coast, is another example that exhibits the effects of high nutrient inputs. With its low water exchange associated with a microtidal regime, the lagoon has changed from its oligotrophic conditions to be relatively eutrophic, providing ideal growth conditions for two allochthonous jellyfish species, which affects tourism (Velasco et al., 2006). Mesotidal lagoons, due to their higher tidal exchanges promote a greater water renewal than microtidal lagoons. Also, the number of permanent connections to the adjacent water body, allows greater volumes of water exchange and hence, greater water renewal, such, is the case of Ria Formosa, where eutrophication issues are not particularly evident, except close the inner areas (Cravo et al., 2015)

1.3. Ria Formosa characteristics

Ria Formosa (Fig.1) is a shallow multi-inlet barrier island system located in south-western Europe, in the south of Portugal. The humid area of this lagoon covers about 100 km^2, of which, one third corresponds to saltmarshes (Falcão and Vale, 1990), while its watershed covers an area of 745 km^2 (Ferreira et al., 2012). At mean sea level, the lagoon covers a flooded area of 49 km^2, ranging from 16 to 84 km^2 for low and high water of equinoctial spring tides (Andrade 1990). This lagoon is the largest wetland with international recognized importance in southern Portugal and as such considered in the Ramsar convention. This system was classified as a Nature Reserve in the 70’s but its protection status was risen to Natural Park in 1987, due to increasing need to control tourism and urbanistic pressure (Newton et al., 2003). This barrier island system spreads along 55 km (E-W) of coastline between Ancão (Loulé) and Manta Rota (Vila Real de Santo António), and only about 6 km (N-S) at its widest point. Within its limits, Ria Formosa forms a lagoon system where a vast area of saltmarshes, islets divided by a complex network of channels (Newton, 1995) are protected by a strong sand barrier inland, forming two peninsulas (Ancão and Cacela) and five barrier islands, which are: Ilha da Barreta (western most limit); Ilha da Culatra; Ilha da Armona; Ilha de Tavira and Cabanas (eastern most limit; Newton, 1995). Six inlets promote exchanges of water with the adjacent Atlantic Ocean, which are from west to east: Barra do Ancão; Barra do Farol; Barra da Armona; Barra da Fuzeta; Barra de Tavira and Barra do Lancém (Newton, 1995). According to its hydrodynamic, the lagoon can be divided in three sub embayments: western, middle and eastern embayment (Salles et al., 2005). The volume of water inside the lagoon at mean sea level is about 92×10^6 m^3 ranging from 33×10^6 m^3 to 168×10^6 m^3 with most of the tidal exchange occurring predominantly through the Faro-Olhão and Armona inlets (Salles et al. 2005). From those, Faro-Olhão has a larger contribution as an inflow pathway, whereas the remaining inlets contribute more as outflow pathways (Duarte et al., 2005; Jacob et al., 2019; Pacheco et al., 2008; Pacheco et al., 2011).

The physical, chemical and biological properties in coastal lagoons are often controlled by circulation that affect the residence time (Malhadas et al., 2010; Oliveira et al., 2011) and in consequence, the chemical and biological processes (Fabião et al., 2016). Tides, wind and morphology of coastal lagoons can affect water exchanges between lagoons and the adjacent ocean, as well as the transport of nutrients, sediments and organisms exchanged between the lagoon and the adjacent coast (Canu et al. 2003; Roselli et al. 2013). Transport of contaminants and pollutants from point and non-point sources located along the margins is also controlled by physical processes (Carafa et al. 2006) which can have repercussions in the water quality and ecology of the system (Fabião et al., 2016). A three-dimensional circulation model coupled with a particle tracking model applied in Ria Formosa to simulate discharges in the main wastewater treatment plants (WWTPs) showed that the wind influences significantly the transport, by affecting the residual circulation, with distinct effects depending on the wind direction (Fabião et al., 2016). Nevertheless, the tide represents one of the main drivers controlling dispersion of matter within the lagoon with tidal effect decreasing in the inner areas of the lagoon (Cravo et al., 2015). From the modelled discharges of the WWTPs, Faro Nascente WWTP particles tend to remain longer inside the domain, with 40-80% of the particles discharged by this WWTP remaining in the domain after 20 days (Fabião et al., 2016), whereas, the residence time is shortened in areas close to the inlets and main channels (Duarte et al, 2005; Fabião et al, 2016). The discharges from this WWTP are shown to be more dispersed within the western sector of Ria Formosa, where there is a complex interconnectivity between the main channels of the lagoon.

The Ria Formosa has semi diurnal tides, with a mesotidal regime and has an average tidal range of 2 m ranging from 1.5 and 3.5 m (Jacob et al., 2012, 2019) in contrast to the lagoons located in the Mediterranean basin which are microtidal (Basset et al., 2006). Due to lack of relevant freshwater inputs and the strong tidal influence, this system is vertically well mixed (Jacob et al., 2012; Loureiro et al., 2006; Newton and Mudge, 2003; Pacheco et al., 2011). In this system, the tidal prism is greater than the residual volume of the lagoon (Andrade, 1990), suggesting high water renewal and low residence times inside the lagoon mainly at the main channels (Tett et al., 2003). Due to the strong permanent connection with the adjacent ocean through six inlets, this lagoon can renew up to 75% of the water each semi-diurnal tidal cycle (Tett et al., 2003). However, inner areas of Ria Formosa, where water renewal is smaller, tend to be more susceptible to anthropogenic pressures (Barbosa, 2010; Cravo et al., 2015).

As inside the Ria Formosa there are different circulation patterns and different pressures of human activities affecting its water quality, there was a need to distinguish within this system different water bodies (WB) (Ferreira et al., 2005, 2006). Agência Portuguesa do Ambiente (APA), within the scope of the Water Framework Directive (WFD; 2000/60/CE), with the objective to attain a Good Ecological Status classified Ria Formosa water in five different water bodies. Ria Formosa-WB1 (4.7 km^2 – APA, 2015) corresponds to the Ancão basin at the western end of the lagoon. Ria Formosa-WB2 (33 km^2) corresponds not only to the most inner part of Ria Formosa but also to the weakest hydrodynamic sector and most influenced by anthropogenic pressures from the main cities Faro and Olhão, where the main WWTP discharges their effluents. Ria Fomosa-WB3 (30.8 km^2) is characterized by larger exchanges of water through Faro-Olhão inlet. Ria Formosa-WB4 (10.7 km^2) comprises Armona and Fuzeta inlets and is one of the areas with lowest anthropogenic pressures (APA, 2012). Ria Formosa-WB5 (8.8 km^2) corresponds to the area surrounded by Tavira until the eastern edge of Ria Formosa, which is characterized by lower salinities due to the influence of a freshwater source, the Gilão River (Newton and Mudge, 2003). However, such freshwater input is negligible when compared with the volume of seawater entering the system during flood (Cravo et al., 2012; Botelho et al., 2015).

Given the importance of Ria Formosa and the discharged nutrient loads associated to WWTP's effluents its inner areas are considered as sensitive waters to eutrophication (DL 149/2004) that transposed the DL 157/97. Moreover, as Ria Formosa is the main national producer of bivalves according to national legislation (DL 236/98), that transpose the European Shellfish Directive (EEC, 1979), Ria Formosa is also classified as "shellfish waters" in the areas where shellfish beds exist.

The lagoon is of high socio-economic importance to the region mainly due to shellfish related activities, which involve directly and indirectly about 10,000 people. The bivalve harvesting area comprises *ca.* 500 ha with approximately 1500 shellfish beds and produces almost 90% of the clam production of Portugal (Serpa et al., 2005; DRPASul, 2006). The harvest of this resource decreased abruptly in the late 90s, barely reaching 2500 tons in 2010 (DGRM, 2014). This decline in shellfish production was then associated with the deterioration of water quality attributed to the increase in anthropogenic pressures, uncontrolled economic development, and sewage discharges over this system (Bebianno, 1995; Mudge and Bebianno, 1997). Nevertheless, water quality has been improved relatively to the 80-90's, not only due to the operational start of the

Wastewater treatment plants (WWTP) but also due to actions of dredging of main channels and inlets (Cravo et al., 2015, 2018).

1.4. Importance of the living resources of Ria Formosa

During the XX century, Portugal produced oysters mainly for exportation, being France, one of the most interested in the Portuguese oysters, *Crassostrea angulata* (APA, 2017). By that time, Tagus and Sado estuaries, constituted the largest natural banks of Portuguese oyster in Europe, being this species also very abundant in southern Portugal (APA, 2017). Between 1962 and 1971, about 7,500 tons of this valuable resource was being exported annually (Crassosado, 2016). In 1974, the production of Portuguese oyster was abandoned, due to massive mortality caused by an infection through an iridovirus in the gills (APA, 2017; Baptista, 2007), which, was later related to increasing pollution of the water due to urban and industrial development (APA, 2017). To attenuate the effect of the extinction of the Portuguese oyster production, was introduced, in Portugal, the pacific oyster, *Crassostrea gigas*. Nowadays, the main oyster producers in southern Portugal, Algarve, produce mostly pacific oyster coming from English or French maternities (APA, 2017). Given the favorable conditions of the lagoon system of Ria Formosa for shellfish farming, this activity acquired a great economic importance (APA, 2017) for the region, although, in its origins it was practiced essentially by fisherman, as a complement to the family income. In the last decades, shellfish farming has been constituted as a strategic activity (Magalhães, 2006). Nowadays, shellfish farming gives work to a significant part of the active population in the surrounding lagoon space (ca. 10,000 people) and generates a major source of income, with sales growth in recent years, possibly associated with the increase and diversification of the products (Ferreira et al., 2012). The production of pacific oyster represented, in 2010, 26 % of the total production in Portugal (Ferreira et al., 2012). In the last few years, APA refers that there is a significant increase of requests of transmission of titles of use relating to nurseries, usually for new holders who intend to dedicate to the production of oyster in nurseries historically dedicated to the production of clams *Ruditapes decussatus* (APA, 2017).

Besides the production of pacific oyster, part of inter-tidal sandflats in Ria Formosa are used for clam *Ruditapes decussatus* farming (Botelho et al., 2015) which, also represented a main source of income to the region, reaching an annual production of 5000 ton year $^{-1}$ in the 90's, representing 90% of the regional production (APA, 2017).

Given the ecological and social-economical value of Ria Formosa, in particular, its importance of bivalve harvesting, it is imperious to protect and preserve this system, which motivates the development of CONPRAR Project (ref: MAR-01.04.02-FEAMP-0003).

1.5. The WWTP impact upon the water quality of Ria Formosa

Sewage treatment plants started to operate in the main cities surrounding the Ria Formosa by the late 80s – early 90s and even though treated domestic sewage discharges continue to represent one of the main threats upon water quality of this lagoon (Cravo et al., 2015). The resident population of the main cities (Faro, Olhão and Tavira) accounts to about 130 000 inhabitants, (PORDATA, 2018) with an estimated daily sewage discharge in the early 2000s of about 10 000 m^3, 4 500 m^3 and 5 500 m^3 respectively (Cravo et al., 2015). Demographic enhancement during summer months due to increased touristic activity leads to higher discharges of treated urban waste waters into the lagoon (Martins et. al, 2006).

Until October 2018 there were five main WWTP located at the Ria Formosa lagoon system: two in Faro city, Faro Nascente (FN); Faro Noroeste (FNO); two in Olhão city, Olhão Nascente (ON); Olhão Poente (OP) and one in Tavira city (T),. Cravo et al. (2015) evaluated the impact of these five WWTP in terms of water quality including the trophic conditions during 2001-2002. In that study, Faro Nascente WWTP revealed to be the most problematic in terms of water quality, with poor quality until 1050 m from the discharge point on the Eastern channel, where natural bivalve banks existed. This fact was associated with the influence of the highest volume of daily effluent discharge (~10 000 m^3/day), together with restricted hydrodynamic conditions promoted by the physiography and shallowness of both channels around this WWTP discharge point, particularly in the eastern channel. Therefore, this valuable coastal system demands an updated water quality assessment to understand its susceptibility to sewage contamination, particularly in the vicinity of shellfish bed areas (Cravo et al., 2015).This study was repeated 5 years later (2006/2007) and an overall water quality improvement was found, despite the water quality around this WWTP was still the worst (Cravo et al., 2017).

The suitability of bivalve production areas (BPAs) is directly related to the quality of the waters where the living resources grow (Son and Fleet, 1980). Bivalves, as filter feeding organisms, can accumulate contaminants from the waters where they grow, as previously mentioned.

Adequate legislation for safeguarding consumers can minimize the risk of shellfish microbial contamination (Almeida et al., 2012). In Europe, the Directives 2006/113/CE and 2004/41/CE provide guidelines to control the levels of microbial organisms in the water and in shellfish (Almeida et al., 2012). In Portugal, the inspection and monitoring of BPAs were made currently by IPMA (Instituto Português do Mar e da Atmosfera).

The levels of the fecal bacteria of *Escherichia coli* in bivalves from this lagoon were high during the 90s, associated with discharges of untreated wastewaters (Almeida et al., 2012). In 1994 only 54% of the effluents in the south coast of Portugal were treated, the remaining was discharged untreated to the environment, representing one of the main sources of contamination to the lagoon (Cachola and Campos, 2006; Mudge and Bebianno, 1997). Nowadays, the wastewater treatment was ameliorated in Ria Formosa and the effluents suffers secondary or even tertiary treatment before being discharged.

Almeida et al. (2012) conducted a study to assess the microbial contamination in bivalves from the Ria Formosa lagoon over 20 years, between 1990 and 2009, where it was shown that the highest level in bivalve contamination occurred in proximity to the WWTP of Faro Nascente, confirming Cravo et al. (2015, 2017) results. When enteric bacteria from the effluents enter the receiving waters, these are affected by multiple stressors controlling their growth and dispersal in the water. Almeida et al. (2012) concluded that during spring and specially summer, the volume of effluents of the main WWTPs increase due to the increased pressure of tourism. However, the effect of increased temperature, solar radiation (germicide effect) and salinity shows to be responsible for the reduction of the microbial contamination during these seasons.

In Ria Formosa, also blooms of toxic phytoplankton species may occur. Blooms of the diatom *Pseudo-nitzschia* spp. occurred more frequently during spring and summer seasons (Brito et al., 2012). These blooms resulted in interdictions of bivalve harvest due to Amnesic Shellfish Poisoning (Brito et al., 2012) caused by the toxins produced by that species. Maximum levels of another phytoplankton species, the dinoflagellate *Dinophysis acuta*, in August 2009, was responsible by the presence of DSP (Diarrheic Shellfish Poisoning) toxins in shellfish, resulting in the interdiction of bivalve capture in Ria Formosa (Brito et al., 2012). So, it is important to understand if these blooms are associated with the increase of nutrients by effluents discharge or driven from the adjacent coast.

1.6. The “new” Faro-Olhão Urban Wastewater Treatment Plant

Recently, in late 2018, in order to improve the water treatment of the waters discharged in the Ria Formosa FN and OP WWTP were decommissioned. The influents from the former FN and OP using biological treatment of aerobic ponds are now are jointly treated in a new WWTP called Faro-Olhão, which started to operate in the last days of October 2018. It presents an upgraded treatment, located at the original place of FN.

The currently Faro-Olhão wastewater treatment plant has a biological tertiary treatment through activated sludge in a prolonged aeration regime. The feed of the reactors is discontinuous of Sequencing Batch Reactor (SBR) type, through NEREDA® technology (aerobic granular sludge), with the secondary effluent disinfected by UV light, before being discharged in the Ria Formosa (Águas do Algarve, 2017). This new WWTP is designed (lifetime) to treat a maximum flow of 28 149 m^3/day and to serve a population of 113 200 inhabitants that also include the population of S. Brás de Alportel. In the initial stage (first 6 months) of operational period, discharge must comply a reduction of:

- Biochemical Oxygen Demand 70%

- Chemical Oxygen Demand 75%
- Total Suspended Solids 90%
- and as Ria Formosa is considered sensitive waters to eutrophication a maximum value of *Escherichia coli* 300 ufc/100 mL

After the settlement of the biological treatment following the first starting year, the discharge limits are as follows:

- Biochemical Oxygen Demand 25 mg/L O_2
- Chemical Oxygen Demand 125 mg/ L O_2
- Total Suspended Solids 35 mg/L
- *Escherichia coli* 300 ufc/100 mL

As the flow discharged from the new Faro-Olhão WWTP is augmented (about 40%) but conversely, a new advanced treatment has been applied, this represents a challenge in terms of water quality. It is then crucial to better understand the impact of this major WWTP upon the water quality of Ria Formosa, mainly close the clam beds that are so important for the economy of the Ria Formosa.

1.7. Objectives

Considering the Ria Formosa as an important area of production of bivalves and as a system under pressure of treated urban wastewater discharges, within the scope of the project CONPRAR (funded by the Programa Operacional Mar2020, ref MAR-01.04.02-FEAMP-0003), the main goal of this work aims at analyzing the impact of effluent discharges by the recently implemented Faro-Olhão WWTP on the water quality of Ria Formosa at the initial stage of treatment. The specific objectives can be identified as:

a. To evaluate spatially the extent of the influence of WWTP discharge down to the zones of existing natural banks or clam beds

b. To evaluate the dilution effect caused by tidal influence, by comparison of results collected at both low and high water

c. To evaluate the effect of extreme tidal range by comparing the results achieved in spring tides *vs.* neap tides

d. To apply a trophic Index to achieve a “big picture” in terms of trophic conditions and/or eutrophication trends.

e. To assess the temporal evolution of the water quality by comparison of recent results from Faro Nascente WWTP just before the implementation of the new treatment (September and October 2018) with the results from the new Faro-Olhão WWTP from November 2018 to May 2019 and with historical data from the period 2001-2007 as well as a comparison with other lagoonal systems within an international context.

To accomplish these goals, monthly/fortnightly field surveys were performed from September 2018 (end of summer) to May 2019 (beginning of Spring). During each field campaign an *in situ* characterization was done by using a multiparametric probe EXO2 (YSI) together with surface water samples collection to determine nutrients, chlorophyll *a* (as a proxy of phytoplankton density), suspended solids and *Escherichia coli* as an indicator of microbiological contamination.

Chapter 2 – Material and Methods

2.1. Description of the study area

The study area, located in the Ria Formosa (Figure 1) consists in two branches/channels around the effluent’s discharge point located westerly (W) and easterly (E) section to assess the spatial impact of the effluents on the study area. The eastern section encompass five sampling points (FN 250 E, FN 500 E, FN 750 E, FN 1250 E and FN 2000 E) while the western section of the channel contains six sampling points (FN 250 O, FN 500 O, FN 750 O, FN 1250 O, FN 1750 O and FN 2000 O). The station FN2000 O located in the main channel, the Faro Channel, was selected as a reference station, devoid of direct influence from the WWTP while FN 2000 E and FN 1750 O are located in shellfish bed areas to assess it there is impact of the WWTP. In Figure 2, the red polygons indicate where there are shellfish production areas.

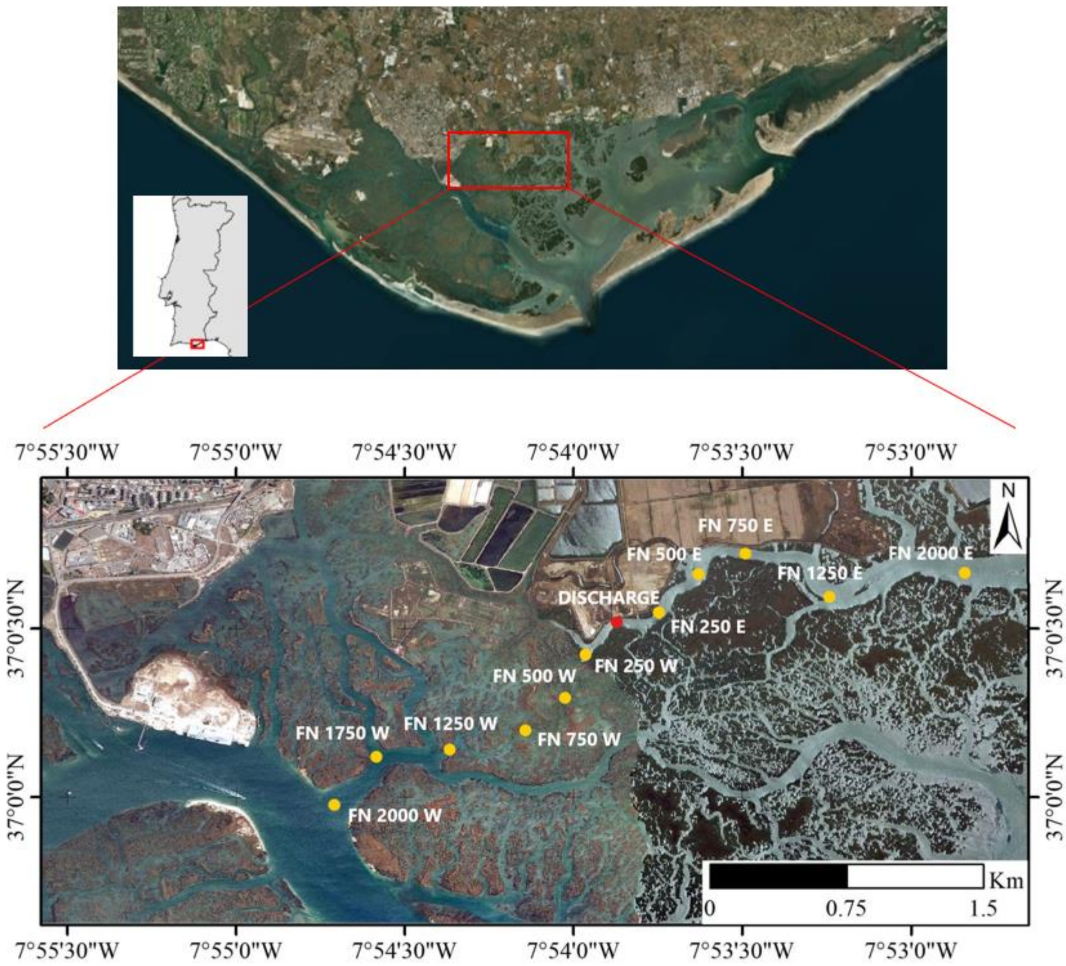

Figure 1. Top: Location of the study area. Bottom: Identification of the yellow sites in yellow and the discharge point in red.

The study area is also contained within WB2, in the innermost part and most weak hydrodynamic sector of all the lagoon (Figure 2) These characteristics together with the anthropogenic pressures from the main cities of Faro and Olhão makes of this water body the most vulnerable amongst the others, regarding nutrient, algal blooms and bacterial contamination.

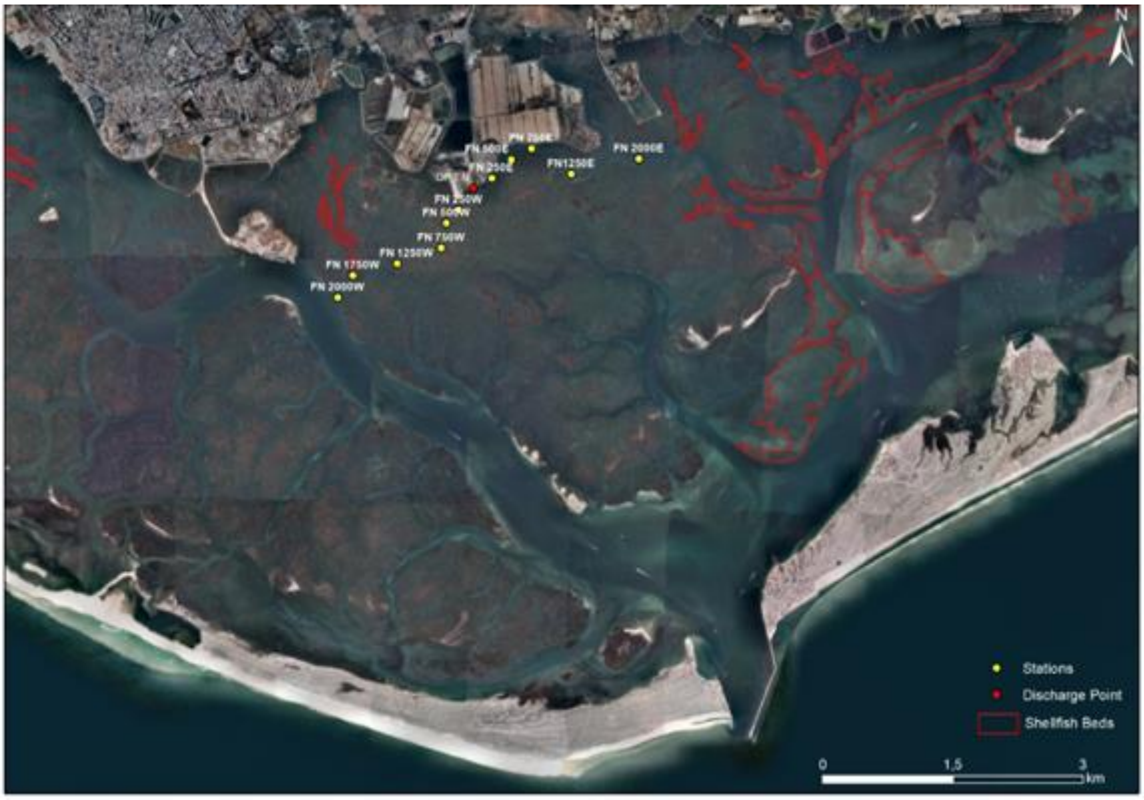

Figure 2. Study area. Sampling points are represented in yellow and the discharge in red. Highlighted in red polygons are the shellfish banks existing in the vicinity of the study area.

2.2. Sampling

A total of 10 campaigns took place in the described study area, from September 2018 to May 2019. In September 2018 (end of summer) and October Faro-Olhão WWTP was not yet operational. On September 2nd campaigns were performed during Spring and Neap tides, to evaluate the effect on the tidal range on the variability of the water quality. Afterwards, from October 2018 to March 2019, during a less problematic period in terms of contamination and development of phytoplankton blooms, sampling frequency diminished to monthly, but conducted during neap tides conditions, when the water quality can become worst by the increase of residence time of water inside the Ria Formosa. For April and May 2019, due to the potential of phytoplankton blooms during spring sampling frequency returns to twice a month under Spring and Neap tides.

In each campaign, during tidal peaks, at low and high-water periods, surface (20-30 cm) *in situ* measurements were conducted for temperature, salinity, dissolved oxygen concentration and in % of saturation and pH using a YSI EXO2 multiparametric probe. Along with that, water samples were also collected at the surface of the water column (20-30 cm) in pre-cleaned polyethylene flasks to determine the concentration of nutrients (ammonium, nitrate, nitrite, phosphate, and silicate), suspended solids, chlorophyll-α (chl-α) and suspended solids including its organic matter fraction. In addition to evaluate the microbiological contamination water samples at three different distances from the discharge point (FN 250 W, FN 250 E, FN 750 W, FN 750 E, FN 1750 W and FN 2000 E) were collected underwater in sterilized flasks to determine the abundance of *Escherichia coli.* These samples were kept in closed containers under cold conditions (ca. 5 ºC) until immediate process at an external laboratory.

2.3. Laboratorial Treatment

The samples collected from the study area were processed at CIMA laboratory of Chemical Oceanography at the University of Algarve. The procedure for the determination of every parameter is described in the following sections.

2.3.1. Nutrients

Nutrients are dissolved inorganic compounds essential for the growth of phytoplankton. Due to this, its availability and limitation control the phytoplankton growth (Laufkötter et al., 2015).

To determine the concentration of nutrients, the water samples were filtered through cellulose acetate Gelman filters with 0.45 µm porosity. The filters were previously cleaned with distilled water and dried at 100ºC for one hour. After filtration, the samples were frozen under -20ºC. The determination of nutrients followed spectrophotometric methods described in Grassholf et al. (1983), based on calibration curves, with a coefficient of correlation (*r*)> 0.99. For each nutrient, specific wavelengths were selected, and standard solutions were used to create the calibration curves in a range of the expected results. After reading the absorbance of every sample and standard solution in triplicates, the concentration in the samples was calculated using the following Equation 1:

$$\text{Concentration of X nutrient} = \frac{\text{abs}-\text{a}}{\text{b}}, \quad (1)$$

where:

abs – absorbance of the sample;

a – origin y intercept of the calibration curve;

b – slope of the calibration curve

Ammonium (NH_4^+)

To determine the concentration of ammonium, alkaline citrate was added to the samples, followed by addition of a phenol solution with sodium nitro prussiate. At final an oxidant solution (Dichloroisocyanuric acid) was added and samples were put in dark conditions for a period between 8 and 24 h. Afterwards its absorbance was measured according to the spectrophotometric method by selecting a wavelength of 630 nm to determine the concentration of the blue compound indophenol formed.

Nitrite (NO_2^-)

The determination of the concentration of nitrite in the samples was based on the process adapted from Shinn (1941) to the seawater by Bendshmeider & Robinson (1952). The base of this principle is the reaction of Griess, which, is a very sensitive reaction, but not affected by other seawater components. Nitrite, under acid conditions (pH 1.5 – 2.0) reacts with an aromatic amine (RHN_2) to form a diazo compound, which, is afterwards complexed with another amine

($ArNH_2$) to form an azo mixture. The concentration of nitrite is determined by the formation of a nitrogen compound after the addition of sulfanilamide and N- (1-naphtyl) ethylenediamine bichloride. Absorbance of the samples are read under a wavelength of 540 nm.

Nitrate (NO_3^-)

The method to determine this ion is through the reduction of nitrate to nitrite using a cadmium column. To ensure a good reduction capacity (> 95%), the column was first treated with a copper sulphate solution ($CuSO_4$). The efficiency of this process is assured by the metal used in the column and by the pH of the solution (NH_4Cl was used as a buffering solution). After the sample reduction in the cadmium column, the analysis of nitrite is identical to nitrate. Its absorbance is measured at wavelength of 540 nm using a spectrophotometer. When the concentration of nitrite is measured, the concentration of nitrate is obtained by subtracting its concentration relatively to nitrite concentration.

Phosphate (PO_4^{3-})

The concentration of the phosphate ion was determined in acidic medium (H_2SO_4) containing the molybdate and potassium antimony tartrate ion as a catalyst. The formed phosphomolybdic acid as a yellowish color. By the addition of ascorbic acid, the latter compound is reduced to molybdenum blue. The reaction is accelerated by addiction of potassium antimony tartrate. The absorbance read at 880 nm wavelength using a spectrophotometer, makes possible to determine the concentration of this nutrient.

Silicate (SiO_4^{4-})

The method used to determine the concentration of this nutrient was based on the formation of silico-molybdic acid (which exhibits a faded yellow coloration) and phosphorus and arsenic complexes, in which, the samples were treated with a solution of ammonium molibdate. Afterwards, a reducing solution with ascorbic acid and oxalic acid is added and forms silico-molybdic complexes decomposing some of the phosphorus and arsenic complexes that were formed. For last, the absorbance of the final solution which exhibits a blue coloration is measured in a molecular absorption spectrophotometer at a selected wavelength of 810 nm.

2.3.2. Suspended solids

Suspended solids are non-soluble particles that are retained in a filter with a specific porosity (0.45 µm) when filtering the water samples. To determine this parameter, duplicate samples (0.5 L) where gently homogenized and filtered through cellulose acetate Gelman filters with 0.45 µm porosity previously labelled, cleaned with distilled water, dried under 100°C for one hour and weighted and cooled in a desiccator to remove any humidity. After filtration, the filters were rinsed with distilled water to remove excess salt that could interfere with the weight of the filters. The filters were then put to dry in an oven at 100°C again for one hour. Afterwards, the filters were placed in a desiccator before its weight was measured. This way, it is possible to obtain value of Total Suspended Solids (TSS) by subtracting the final weight of the filter with the initial weight of the filter divided by the filtered volume expressed in L. To obtain the concentration of TSS the following Equation 2 was used:

$$\text{Total Suspended Solids (mg/L)} = \frac{FinalW - InitialW}{\text{FilteredV}} \text{ x } 10^3, \quad (2)$$

where:

$Final_W$ – Weight after the sample was filtered and the filter was dried (g);

$Initial_W$ – Weight before the filtration of the sample (g);

$Filtered_V$ – Filtered sample volume (L).

2.3.3. Organic matter and fixed fraction of the Suspended Solids

In order to determine the concentration of the volatile (organic) and fixed (inorganic) of total suspended solids, samples were gently homogenized and 200 mL of sample was filtered through glass fiber filters (0.7 µm) previously labelled, cleaned with distilled water, dried under 100°C for one hour and pre combusted at 450°C for 4 h in a furnace, cooled in a desiccator and weighted. After filtration, the filters were rinsed with distilled water to remove salts that could interfere with the weight of the filters. The filters were then put onto an aluminum foil support. The support was taken to a furnace where the filters have undergone combustion at 450°C for 4h. After the filters cooled down in the desiccator, the weight of the filters were measured again and with this, it is possible to obtain the value of the organic matter fraction by subtracting the final weight of the filter to the initial weight of the filter before the furnace, following the described Equations 3 and 4 bellow.

$$Volatile\ Suspended\ Solids\ (mgL^{-1}) = \frac{(Pfinal - Pfurnace)}{Vfiltered} \times 10^3$$

(3)

$$Fixed\ Suspended\ Solids\ (mgL^{-1}) = \frac{(Pfurnace - Pinitial)}{Vfiltered} \times 10^3$$

(4)

P_{final} – weight of the filter after 105ºC at the stove (g);
$P_{furnace}$ – weight of the filter after combustion under 450ºC in the furnace (g);
$P_{initial}$ – weight of the filter before filtration (g);
$V_{filtered}$ - volume of sample filtered (L).

2.3.4. Chlorophyll *a* and Phaeopigments

The concentration of chlorophyll *a* can be related to the biomass of photosynthetic organisms, since it is a pigment present in all algae, being used as a proxy for its biomass (Lorenzen, 1967).

To determine the concentration of chlorophyll *a* and phaeopigments, duplicate samples (0.75 L) were gently homogenized to avoid breaking the cells and filtered (as described in the previous section 2.3.2.) through a Whatman glass fiber GF/F of 0.7 µm porosity under low light conditions and pressure to avoid degradation of the pigments. Afterwards, the filters were folded and wrapped in aluminum foil, labelled and frozen at -20°C until the samples were analyzed. After this procedure, each filter was placed in a test tube previously wrapped in aluminum foil. To the test tube was added 5 mL of refrigerated acetone (90%) and the filters were grinded using a glass rod, which helps to break down the filter and promote the release the chlorophyll *a* into the solvent (acetone). After grinding of the filters, it was added more 5 mL of refrigerated acetone and the tubes were put in a freezer for 24 h. In the following day, the tubes were placed in a centrifuge for 10 minutes at 3600 rpm. The supernatant solution was transferred to a cell in a spectrophotometer where its absorbance was read at both wavelength of 665 and 750 nm. The obtained values correspond to the concentrations of chlorophyll *a* plus phaeopigments. A correction of a reading of 750 nm was conducted to eliminate any turbidity from the filter. After, 100 µL of hydrochloric acid (HCl) at 10% was added to the cell to obtain only the absorbance of phaeopigments. The absorbance was read at both wavelength of 665 and 750 nm. The correction of 750 nm was read again after acidification of the samples.

The expressions used to determine the concentration of chlorophyll *α* and phaeopigments are as followed in the Equations 5 and 6:

$$\text{Chlorophyll } a\ (\mu g.L^{-1}) = 26.7 \times (Ba - Aa) \times V \times L^{-1} \times p^{-1}, \quad (5)$$

$$\text{Phaeopigments } (\mu g.L^{-1}) = 26.7 \times (1.7 \times Aa - Ba) \times V \times L^{-1} \times p^{-1}, \quad (6)$$

where:

Ba – absorbance (abs.) at 665 nm corrected from abs. at 750 nm before (B) acidification;

Aa – absorbance (abs.) at 665 nm corrected from abs. at 750 nm after (A) acidification;

V - Volume of used acetone (mL);

L – Volume of the sample (L);

P – thickness of the spectrophotometer cell (cm)

2.3.5. *Escherichia coli* concentration

The method to determine the concentration of *E. coli* in the collected samples was through the Most Probable Number (MPN) by AQUAEXAM Laboratory. In this method, 100 mL of sample was poured in a Quanty-Tray with a specific reagent for *E. coli* and Total Coliforms. The tray was sealed and later was incubated for 24 hours. In the next day, the number of positive wells that exhibited fluorescence in the tray were counted and the concentration of both *E. coli* and Total Coliforms was estimated.

2.4. Trophic Index (TRIX)

The Trophic Index (TRIX) established by Vollenweider et al., (1998) was applied for all campaigns (10), aggregating different parameters. This index aggregates data from four key water quality parameters: chlorophyll a (Chl-a; µg/L), the absolute deviation from dissolved oxygen saturation (%DO), dissolved inorganic nitrogen (DIN; µg/L) and soluble reactive phosphorus (SRP; µg/L). These parameters where used in the Equation 7 as followed:

$$\mathrm{TRIX} = \frac{\log(Chl\ a\ x\ |100 - \%\ DO|\ x\ DIN\ x\ SRP) - (-1.5)}{1.2} \tag{7}$$

The obtained values in a scale from 0 to 10 can be set in a classification scale that allows to analyze the trophic state of the system:

Very good (Oligotrophic) [0–4[, Good (Oligotrophic to Mesotrophic) [4–5[, Moderate (Mesotrophic) [5–6[and Poor (Eutrophic) [6–10].

2.5. Statistical Analysis

The first statistic approach to the data was to check if the data followed a normal or a non-normal distribution. To do this, the data was divided in two different sets, one with all the data from low water and high water and other with both neap and spring tides data from September 2018 and May 2019. The data was divided in these two different sets so that differences between high and low water could be assessed as well as differences between neap and spring tides. For the data showing a normal distribution, a Paired t-test was used, otherwise, data sets with non-normal distribution, a paired Wilcoxon test was applied, using a confidence level of 95%. To study the spatial extension of the impact of the WWTP an ANOVA / Wilcoxon test was used between the sampling points. In order to have a more holistic approach of the temporal evolution of the impact of the discharge, one-way ANOVA or a Wilcoxon test was used for three periods considered, one from September to October, another from November to April and the month of May. Additionally, to better understand how the studied variables correlate between them, correlation matrices were applied for low and high water under spring and neap tides conditions using the Pearson critical values. Lastly, to understand which variables best explain the variability of the results, a principal component analysis (PCA) was made using the data for temperature, salinity, pH, DO, SS, NH_4^+, NO_3^-, PO_4^{3-}, SiO_4^{4-} and *E. coli* relative to low water (LW) when the impact of the effluents discharge in the receiving waters is maximum.

Chapter 3 – Results

3.1. Meteorological setting

For the duration of the sampling, surface air temperature (Table 1) was highest in the month of September (24.4 °C) while in January the lowest air temperatures were recorded (12.2 °C). Wind direction was predominant from the north (N) quadrant with slight variations to NW and NE (data not shown).

The pattern on rainfall was uneven with several fluctuations throughout the sampling. During the sampling period, rainfall was scarce, ranging from 0.1 mm accumulated in May 2019 to 67.4 mm accumulated in November 2018.

Regarding the rainfall felt in the 7 days period previous the sampling, in October the highest 7-day accumulated was recorded (10.2 mm) and zero was found for several sampling events (Table 1).

Table 1. Total accumulated rainfall (Total AR), 7-day accumulated rainfall (7-day AR) and Mean Air Temperature (°C) for all sampling months. Data recorded at the IPMA (Instituto Português do Mar e Atmosfera) Faro airport meteorological station (ref. 08554; 37.01657778 °N; 7.971952778 °W).

	Total AR	7-day AR		Air Temperature
Sep	1.2	Sep NT	0	24.4
		Sep ST	1.2	
Oct	61.8	Oct NT	10.2	19.6
Nov	67.4	Nov NT	9.9	15.4
Dec	5.9	Dec NT	5.1	13.8
Jan	1.6	Jan NT	0	12.2
Feb	29.6	Feb NT	0	13.6
Mar	4.9	Mar NT	3.5	16.0
Abr	33.3	Apr NT	6.1	14.5
		Apr ST	0.2	
May	0.1	May NT	0	20.5
		May ST	0	

3.2. Water quality impairment

Salinity, pH, dissolved oxygen (DO%), nutrients, chlorophyll *a*, suspended solids, organic matter content and *Escherichia coli* (*E. coli*) taken from September 2018 to May 2019, at both low and high water, covering neap and spring tides are shown from Figures 3 to 18. A spatial gradient for the studied variables was evident from the discharge point of Faro-Olhão WWTP to both East and West sections of the study area with the lowest variability at the furthest stations from the WWTP (FN 2000 W and FN 2000 E). It was also evident a difference between the values of the studied variables between the east and west sections with the east section showing to be the most affected by the discharge, for both conditions of low water and high water.

To detect if differences occurred due to the implementation the new Faro-Olhão WWTP, 3 different were considered for the various parameters shown in Table 2, as mentioned at section 2.5.

Table 2. Mean values of the three defined periods considering the segment 750 W to 750 E from the study area for temperature (Temp), salinity (Sal), pH, dissolved oxygen (O2), percentage of saturation (%O2), total suspended solids (SS), chlorophyll a (Chl a), ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}), silicate (SiO_4^{4-}), ammonium to total inorganic nitrogen ratio (NH_4^+: NIT), nitrogen to phosphorus ratio (N:P) and nitrogen to silicate ratio (N:Si).

Period	Temp	Sal	pH	[O2]	%O2	SS	Chl a	NH4+	NO3-	NO2-	PO43-	SiO44-	NH4+:NIT	N:P	N:Si
September-October	24.7	33.2	7.8	5.6	82.4	34.5	10.9	109.6	7.3	5.5	7.7	30.1	87.4	23.3	4.9
November-April	17.4	31.6	7.8	6.1	71.7	31.9	28.5	163.3	7.6	5.2	13.4	31.3	92.2	17.7	7.3
May	24.0	32.1	7.9	5.5	79.6	23.4	8.5	100.2	7.4	7.7	28.7	31.0	85.1	5.2	4.3

3.3. Temporal and spatial variability

Water temperature (Figure 3) at the study area reflects the seasonal atmospheric variation with higher values in the warmer months and lower in the colder months. The minimum value (9.8 ºC) was recorded in January (HW) at the station FN 250 W during neap tide conditions, while the maximum value (28.3ºC) was registered at the same station in September (LW) during spring tide conditions.

Water temperature was, generally, significantly higher ($p<0.05$) during low water phase than at high water. This might be due to the shallowness of the water column during this tidal condition that more easily can get warmer that when the water column is deeper at high water. This may explain why the stations closest to the discharge register higher temperature values. However, it is important to remark that water temperature closer to the discharge can be influenced by the discharge itself. It is important to highlight that temperature measurements can be influenced by the time of the day when the temperature was measured. However, no spatial significant differences were found between sections neither for low water or high water ($p>0.05$) nor between the reference station and the remaining ones ($p>0.05$). For temperature, no evident changes were found after October when the new WWTP start to operate (Table 2), since its variability is mostly dependent on the seasonal cycle.

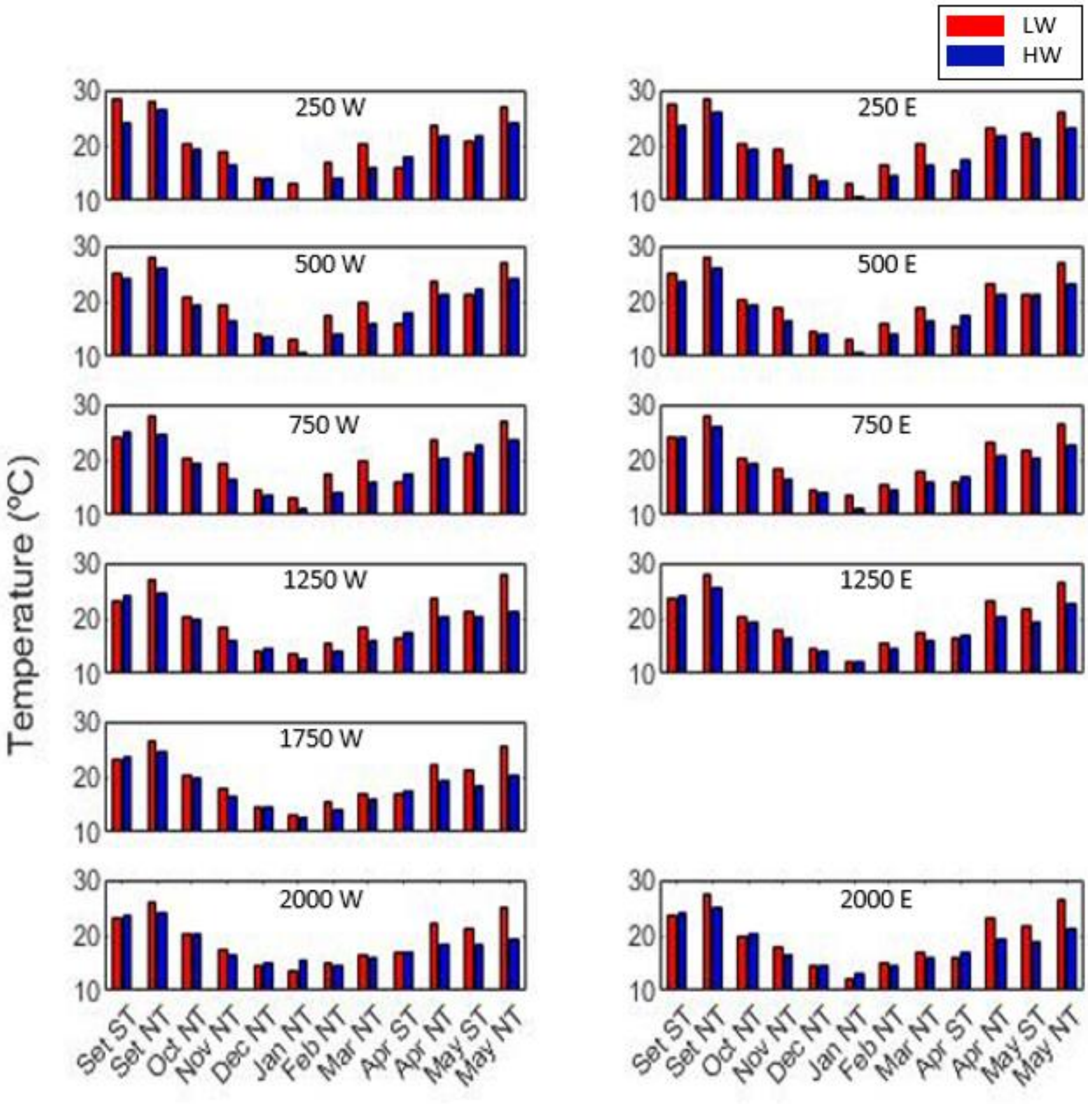

Figure 3. Monthly variation of temperature (°C) for low water (LW) and high water (HW) at both west (left) and east (right) sections of the study area. From top to bottom, stations are organized from 250 m to 2000 m to the discharge. The horizontal axis represents the sampling months and the correspondent tide phase (ST – Spring Tide; NT – Neap Tide).

For salinity (Figure 4), the lowest values were registered during low water at the stations closest to the discharge for both sections of the study area. This is explained by the proximity of the stations to the freshwater discharge from the WWTP.

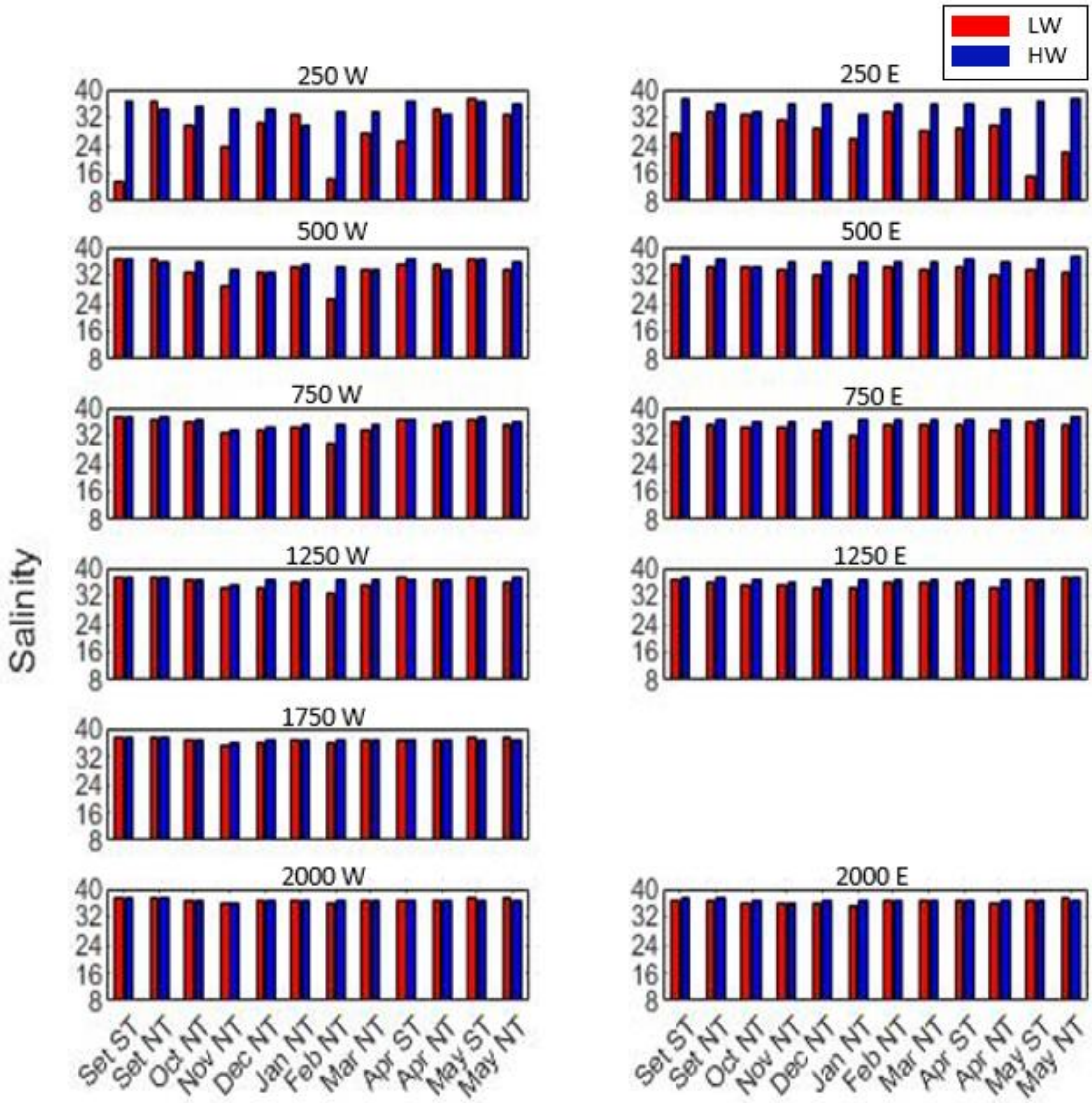

Figure 4. Monthly variation of salinity for low water (LW) and high water (HW) at both west (left) and east (right) sections of the study area. From top to bottom, stations are organized from 250 m to 2000 m to the discharge. The horizontal axis represents the sampling months and the correspondent tide phase (ST – Spring Tide; NT – Neap Tide).

Spatially, salinity increase with the increase of distance from the discharge point and during high water the spatial variability was relatively small showing the increase of seawater contribution during flood ($p<0.05$). The lowest salinity (13.55) was recorded at FN 250 W while the maximum salinity (37.14) was recorded at FN 1750 W, higher than the mean value for the reference salinity (36.61) despite no significant differences were found between the west and east sections of the study area.

From September 2018 to April 2019, at 250 m from the discharge for both sections, several values were < 30, whereas, at high water all values were > 30 for both low and high water. Salinity, globally, was significantly higher during high water ($p<0.05$) than at low water, mostly from 250 to 1250 m from the discharge for both sections.

Salinity at low water, at the eastern channel was slightly lower than in the western channel despite no significant differences were found between both sections ($p>0.05$). During this tidal condition significant differences ($p<0.05$) in salinity occur from the discharge down to FN 750 E in comparison with the reference station (FN 2000 W). The same does not happen for high water, where no significant values were found in comparison to the reference station ($p>0.05$). After Faro Nascente WWTP decommission (October), significative lower values ($p<0.05$) were found for November-April (31.6) than for September – October (33.2), (Table 2).

Regarding pH (Figure 5), values at the closest stations from the discharge point were globally below 8 for both sections of the study area, where variability is the highest. However, the minimum pH value (7.62) was registered in September at the station FN 750 E during low water of spring tide. The values increased with the distance from the discharge point, with the maximum (8.32) registered at the station FN 2000 E during neap tidesimilar ($p>0.05$) to those values measured at the reference station (FN 2000 W), depicting a mean of 8.05.

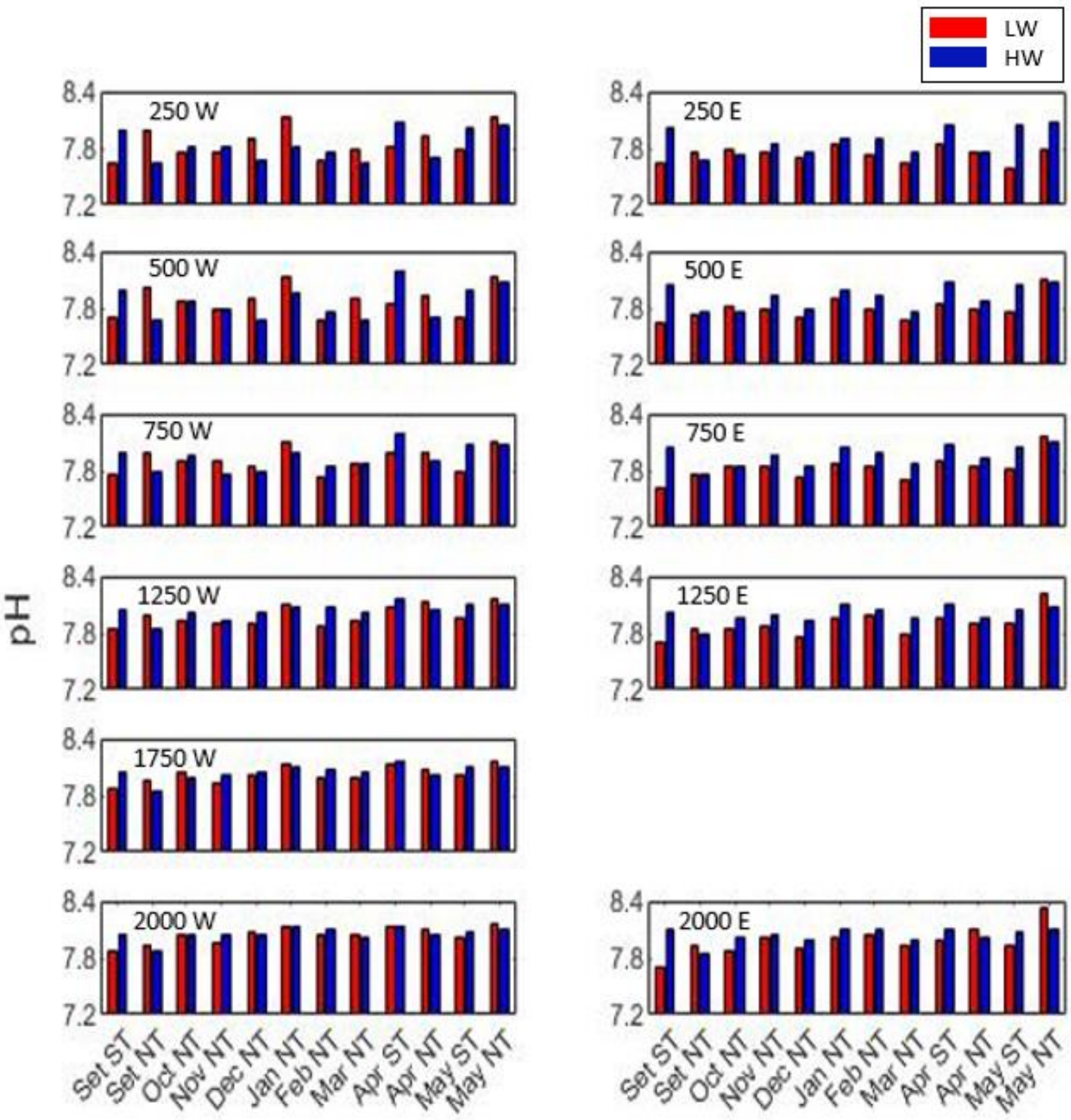

Figure 5. Monthly variation of Ph for low water (LW) and high water (HW) at both west (left) and east (right) sections of the study area. From top to bottom, stations are organized from 250 m to 2000 m to the discharge. The horizontal axis represents the sampling months and the correspondent tide phase (ST – Spring Tide; NT – Neap Tide).

At high water, pH values were significantly higher than those of low water ($p<0.05$), due to the contribution of renewed coastal water entering the Ria Formosa during flood, with typical values close to 8.

During low water, at the east section, pH values are significantly lower than to those obtained in the west section ($p<0.05$), with mean values of 7.85 and 7.94, respectively. For high water, the east section registered a relatively higher mean value of pH (7.96) than the west section, however. no significant differences ($p>0.05$) were found between both sections.

Significant lower pH values were observed in the east section down to 750 E ($p<0.05$), whereas, for high water, significant higher values ($p<0.05$) were found for 250 E and from 250 W to 500 W.. Temporally (Table 2), the mean value for May (7.9) were significantly higher ($p<0.05$) than between September – October, which are similar from the period November – April ($p>0.05$).

DO (mg/L) (Figure 6) shows lower values close to the discharge point, increasing gradually to the stations further down for both east and west sections. This variable depicts a clear trend of higher values in the colder months and lower values in the warmer months. The minimum absolute value for oxygen concentration (3.09 mg/L) was registered in May at the station FN 250 E during low water of spring tide whereas the maximum value (11.32 mg/L) was registered in the same month at the station FN 2000 E during low water of neap tide.

With the implementation of the new Faro-Olhão WWTP (from last days of October) there was ca. 40% increase of the daily flow to about 14 000 m^3/day. Immediately afterwards, in November dissolved oxygen values decreased in comparison to September at FN 250 E and FN 250 W ($p<0.05$). A decrease was also observed during February to April and May (spring tide). During the remaining months dissolved oxygen was generally higher, despite DO also depend on the sampling hour.

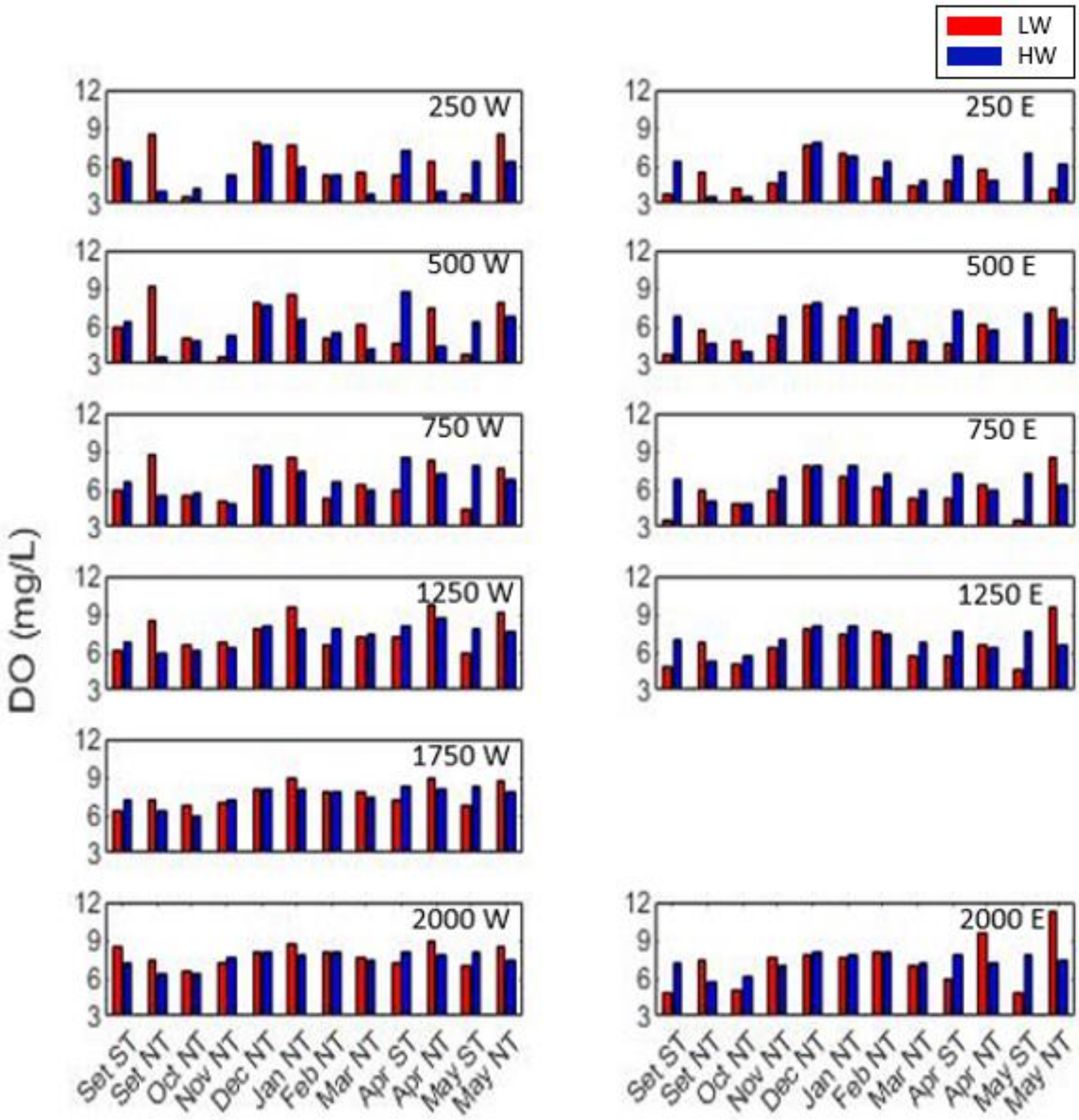

Figure 6. Monthly variation of dissolved oxygen (mg/L) for low water (LW) and high water (HW) at both west (left) and east (right) sections of the study area. From top to bottom, stations are organized from 250 m to 2000 m to the discharge. The horizontal axis represents the sampling months and the correspondent tide phase (ST – Spring Tide; NT – Neap Tide).

Between high water and low water, due to the high variability there were no significant differences ($p>0.05$). Yet, no values related to hypoxia (O_2 < 2 mg/L) were observed during all the sampling period. In May, at the station FN 2000 E during low water of neap tide, oxygen concentration reaches a value of 11.32 mg/L much higher than the correspondent station at 2000 W (reference station), where it was 8.55 mg/L.

For low water there was significant lower dissolved oxygen in the east section than in the west section ($p>0.05$) while for high water, no significant differences were recorded between both sections ($p>0.05$). Spatially, higher values of DO ($p<0.05$) were registered from 250 E to 750 E in comparison to the reference. The same was not verified for high water. In this situation, significant higher values ($p<0.05$) were registered at 250 E and from 250 W to 500 W.

Temporally (Table 2), significantly higher values were found November – April ($p<0.05$) while values between September – October and May were similar ($p>0.05$). Nevertheless, it is important to remind that dissolved oxygen concentration depends also on temperature and salinity.

Following the DO (mg/L) distribution, lower values of dissolved oxygen saturation (%DO; Figure 7), were observed at the stations near the discharge point of the WWTP, increasing, further down from this point. During high water, the minimum value of %DO (64%) was at FN 250 W while at low water the values decreased, recording a minimum of 36%. There, regardless this area is not considered a shellfish area, the values were below that minimum threshold for shellfish waters, to avoid deleterious effects on the biota according to national legislation (MAV = 60%; DL 236/98 – Diário da República, 1998) that transpose the EU legislation for shellfish waters (Directive 2006/113/CE). Shellfish beds in the study area are located further down to 1250 m East and 1750 m West from the discharge point, where values were greater than this imposed threshold. The maximum value (142 %) was registered at FN 500 W during low water. Variability at low water was larger than at high water, however, there were no significant differences ($p<0.05$) between both tidal conditions. Between sections, at low water the % of saturation was significantly different ($p<0.05$), being higher at the west section. However, during high water no significant differences were found between both sections of the study area ($p>0.05$). Spatially, as expectedly, the trend for %DO of saturation followed the same trend as for DO concentration.

For the three periods considered (Table 2), the minimum mean value was found in the period between November and April (72%) while the maximum was in September-October (82%), before the implementation of the new Faro-Olhão WWTP, however, no significant differences were observed between these two periods.

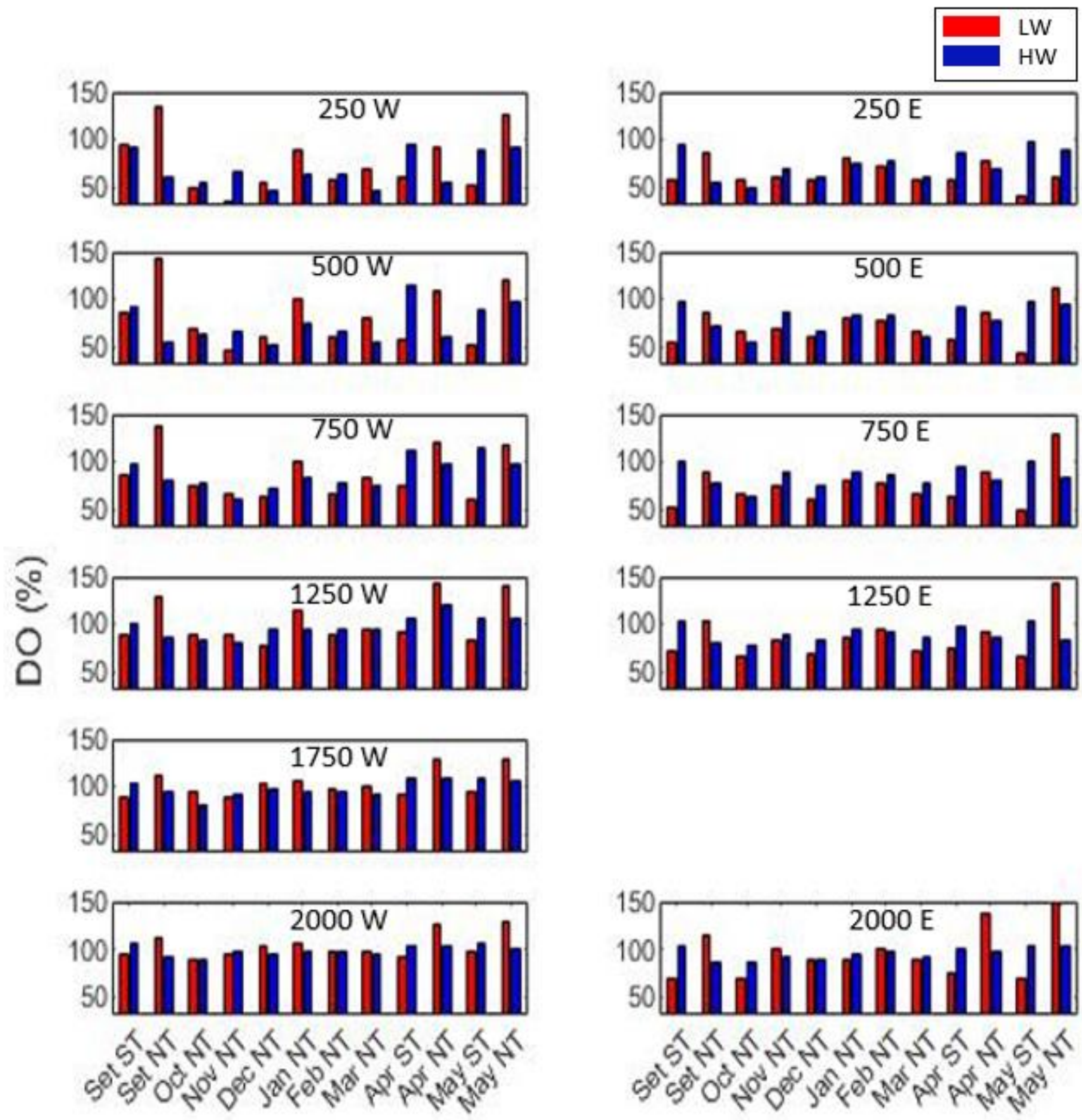

Figure 7. Monthly variation of percentage of saturation (%) for low water (LW) and high water (HW) at both west (left) and east (right) sections of the study area. From top to bottom, stations are organized from 250 m to 2000 m to the discharge. The horizontal axis represents the sampling months and the correspondent tide phase (ST – Spring Tide; NT – Neap Tide).

Chlorophyll *a* (Figure 8) registers very high concentrations at the stations closer to the discharge (up to 250 fold higher than the mean value of the reference) point for both sections of the study area, with greater concentrations in the west section until 1250 m. The maximum concentration (352.4 µg/L) was registered during low water in March at the station FN 250 W (neap tide), whereas several non-detectable values (<0.3 µg/L) were registered at different stations mainly during high water, at the stations further away from the discharge point.

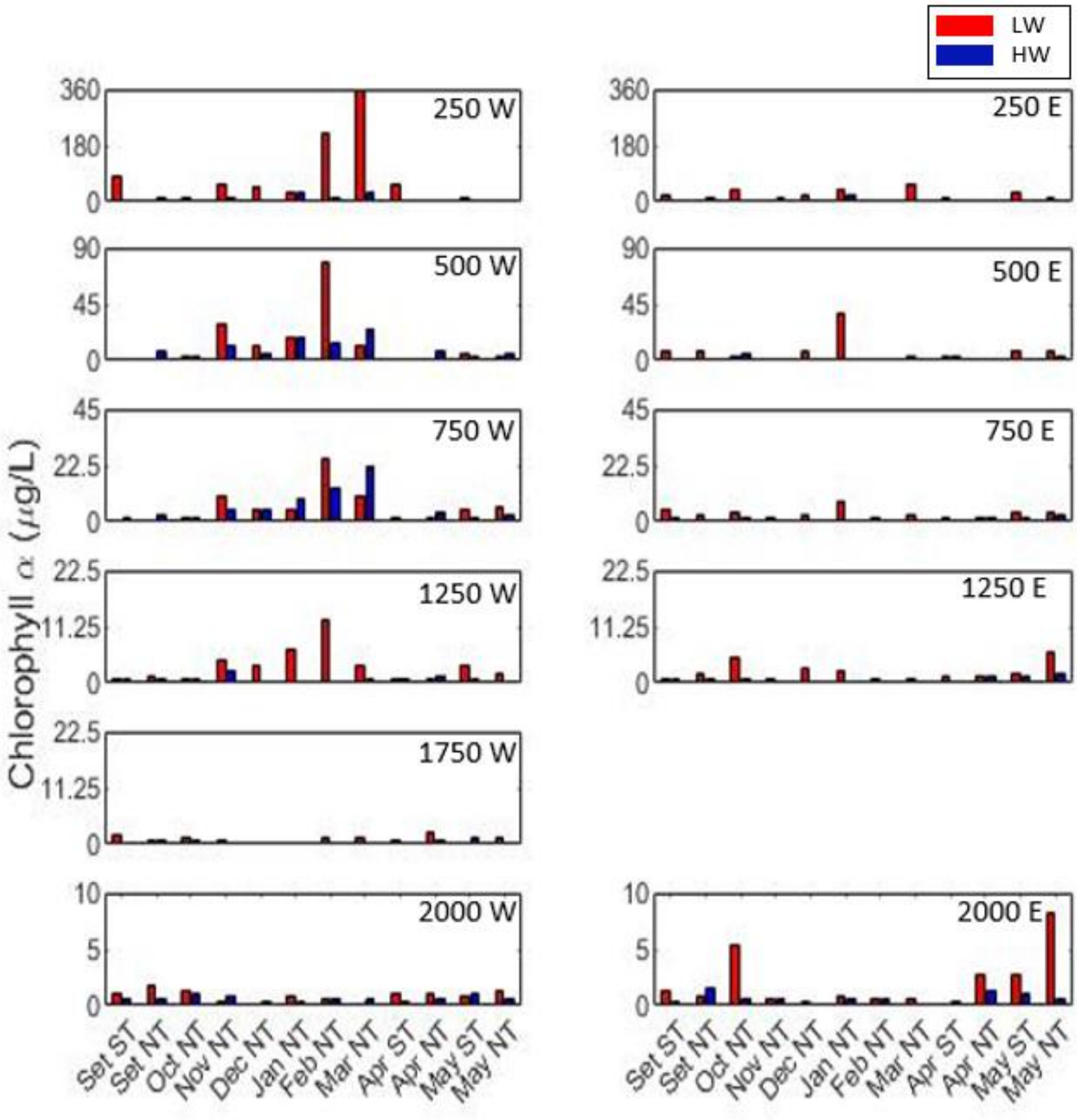

Figure 8. Monthly variation of chlorophyll a (µg/L) for low water (LW) and high water (HW) at both west (left) and east (right) sections of the study area. From top to bottom, stations are organized from 250 m to 2000 m to the discharge. The horizontal axis represents the sampling months and the correspondent tide phase (ST – Spring Tide; NT – Neap Tide).

Concentration decreased with increasing distance from the discharge point where at the furthest sites the values were similar to those of the reference station (FN 2000W). Temporally, in the east section, generally, chlorophyll *a* was lower in the winter (December) and higher during spring months - April and May, while, in the west section, this trend was not evident down to 1750 m. Considering the three periods (Table 2), from September – October, November – April and May, no significant differences were found between these three periods for chlorophyll α ($p>0.05$). In May, at the station FN 2000 E during low water (neap tide) there is a chlorophyll peak (8.31 ug/L) significantly higher than at the reference station (2000 W – 1.25 ug/L),which was reflected by the peak on dissolved oxygen (Figures 6, 7).

During low water, chlorophyll *a* concentrations were significantly higher ($p<0.05$) than those during high water. Globally, values during low water increased up to 4-fold relatively to high water. Between sections, at high water chlorophyll a was significantly higher ($p<0.05$) at the west section whereas for low water concentrations were similar between both sections ($p>0.05$). Spatially, concentrations were significantly higher in the transect 500 E – 500 W in comparison to the reference station ($p<0.05$). For high water, significant higher values ($p<0.05$) are found for the transect 250 W – 750 W.

Total suspended solids (TSS) (Figure 9) also showed higher concentrations at the nearest stations to the discharge decreasing gradually with increasing distance from it towards both ends of the channel. At most cases, total suspended solids concentration during low water were significantly higher than those of high water ($p>0.05$) and increased almost up to 2-fold.

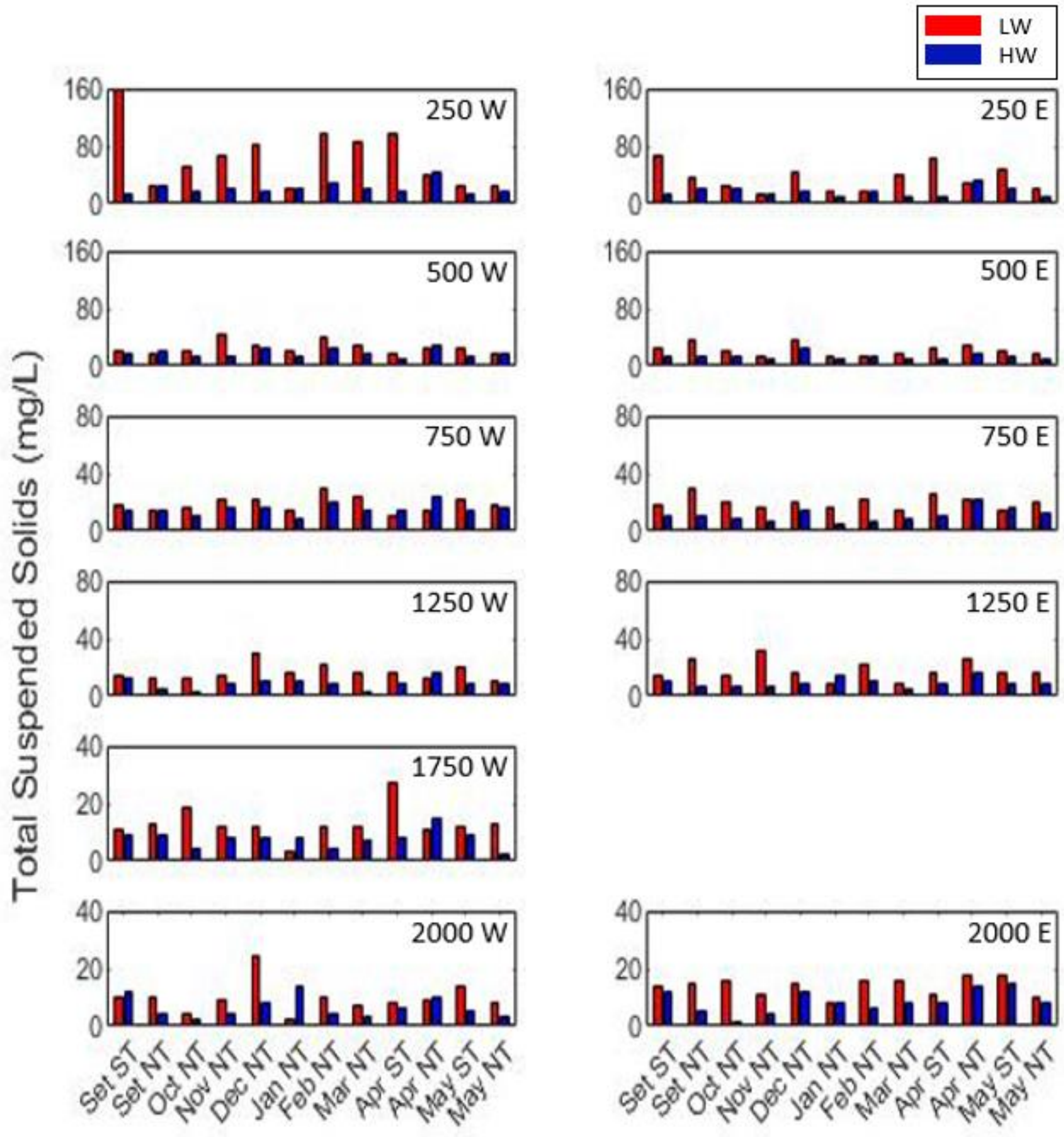

Figure 9. Monthly variation of total suspended solids (mg/L) for low water (LW) and high water (HW) at both west (left) and east (right) sections of the study area. From top to bottom, stations are organized from 250 m to 2000 m to the discharge. The horizontal axis represents the sampling months and the correspondent tide phase (ST – Spring Tide; NT – Neap Tide).

However, in some cases, such as in April during neap tide, values during high water were higher than during low water at all stations of the west section along with FN 250 E. The same, happened in January at some stations at the eastern section (FN 1250 E and FN 2000 E) and at some of the western section (FN 1750 W and FN 2000 W).

For total suspended solids (TSS) at the reference station (FN 2000 W), in general, was lower than those registered in the opposite station on the east section (FN 2000E). Spatially, at low water total suspended solids concentrations were significantly higher ($p<0.05$) in the west sector. At high water, the west section also presents higher suspended solids, however, no significant differences were found between both two sections ($p>0.05$). The minimum value was recorded at the station FN 2000 E in October at high water (neap tide) with a value of about 1.4 mg/L. Maximal values are increased up to 3 to 6 fold (LW) relatively to the mean value at the reference. For this variable, significant higher values ($p<0.05$) were found from the station 750 E until the station 500 W (750 E – 500 W) at low water in comparison to the reference station. For high water, the station 250 E and the transect 250 W – 750 W were significantly higher ($p<0.05$) relatively to the reference. For the 3 periods considered, no significant differences were observed ($p>0.05$).

For TSS, national legislation (DL 236/98 – Diário da República) establishes, for shellfish waters, that this parameter when resulting from a discharge, should never exceed in more than 30% the content measured in the waters not affected by the discharge. In the present case the reference station (FN 2000 W) recorded a mean value of about 7 mg/L. The maximum value registered of about 160 mg/L value was recorded at the station FN 250 W in September at low water (spring tide) which is much higher than this threshold, regardless this is not a shellfish bed area. Shellfish beds are located at 1750 W and 2000 E, where TSS never exceeds the discharge limit (35 mg/L) established by APA for the discharge license.

Organic matter percentage (OM) (Figure 10), unlike other variables displays global higher values further from the discharge, with a maximum value of 92.6 % (2.64 mg/L) in December (neap tide) during high water at FN 2000 E. Overall, values at low water are significantly lower ($p<0.05$) than high water with correspondent mean values of 47.7% (1.37 mg/L) and 69.1 % (1.97 mg/L), respectively. Spatially, significant differences ($p<0.05$) were found between the station FN 1750 W (closest to the reference) and FN 2000 E, which, implies a large dispersion of organic matter in the study area. Values on the east section were, globally, higher when comparing to the west section at both low and high water. At high water, significant higher values were found, with a correspondent mean value of 77.1 % (2.18 mg/L). However, no significant differences were found (p>0.05) for low water. Temporally, there is an evident decrease since February at all sampling stations for both low water and high water at both sections.

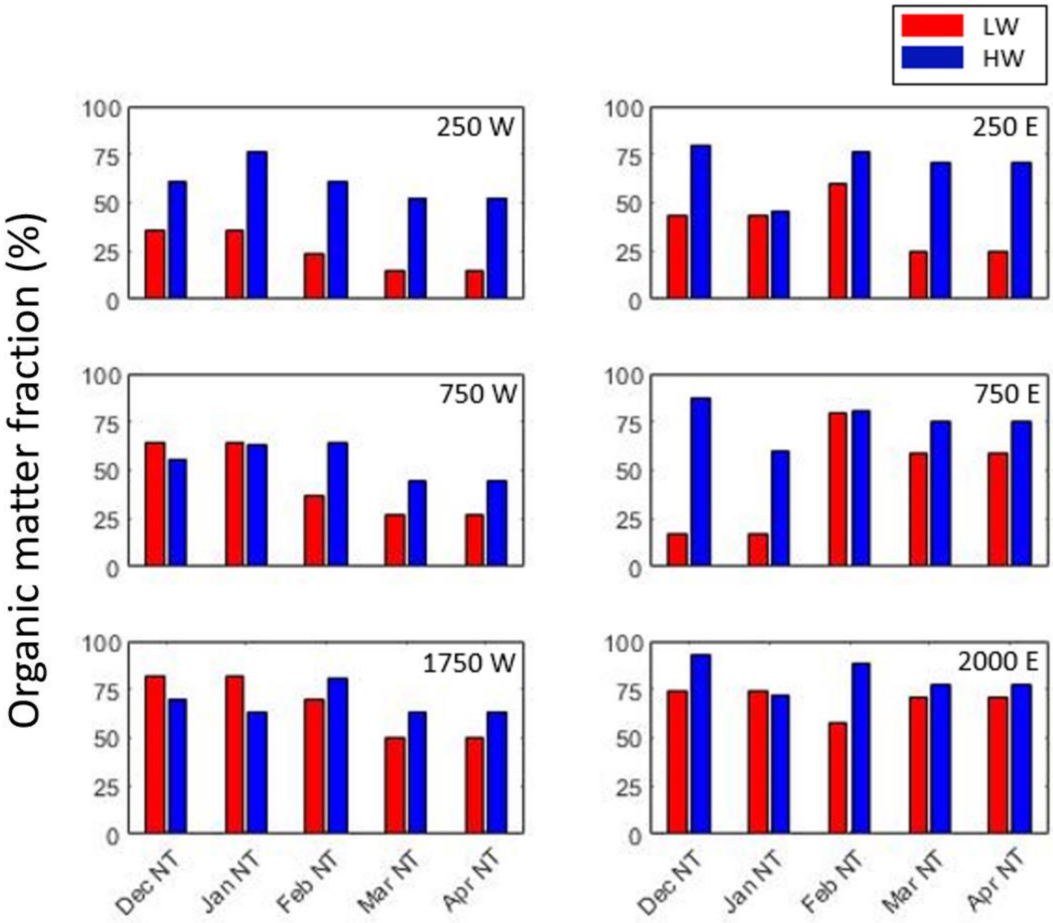

Figure 10. Monthly variation of organic matter fraction (%) for low water (LW) and high water (HW) at both west (left) and east (right) sections of the study area. From top to bottom, stations are organized from 250 m to 1750 m to the west and 250 to 2000 m to the east section. The horizontal axis represents the sampling months and the correspondent tidal phase (NT – Neap Tide).

Nutrients ($NH4^+$, NO_3^-, NO_2^-, PO_4^{3-}, SiO_4^{4-}) show similar distribution along both sections with significantly concentrations higher at low water than at high water ($p<0.05$). The concentration for all nutrients decreases with increasing distance from the discharge point in both sections. Globally, the concentration in the eastern section of the channel were significantly higher than in the western section during low water ($p<0.05$), while no significant differences were found at high water for all nutrients ($p>0.05$), between both sections except for nitrate and nitrite.

Ammonium (Figure 11) was the dominant nutrient of nitrogen and showed higher values at the closest stations to the discharge point in both sections of the study area, decreasing towards both ends of the channels. The highest concentration (240.9 µM) was found at the station FN 250 E in March 2019, during low water in neap tide, a value that is approximately 200-fold higher than the mean value registered at the reference station (1 µM). The minimum (0.06 µM) value was recorded at this station FN 2000 W at high water during neap tide conditions also in March 2019. At low water, ammonium was highest the east section than at the west section. Even though, no significant differences were found between the two sections ($p>0.05$). Globally, between low and high water, significant differences were found with overall higher values at low water ($p<0.05$) with values increased up to 2-fold during low water. At high water the west section registered higher concentrations than at the east section despite no significant differences were found between them ($p>0.05$) as it was the case of September, October, December and April at several stations until 750 m from the discharge. Significant differences ($p<0.05$) were found between the three periods (Table 2). The period from November to April registered the highest mean value (163.3 µM) whereas the lowest was obtained in the month of May (100.2 µM).

Spatially, the concentrations between the stations 750 W and FN 750 E were significantly higher ($p<0.05$) comparing to the reference station like at high water.

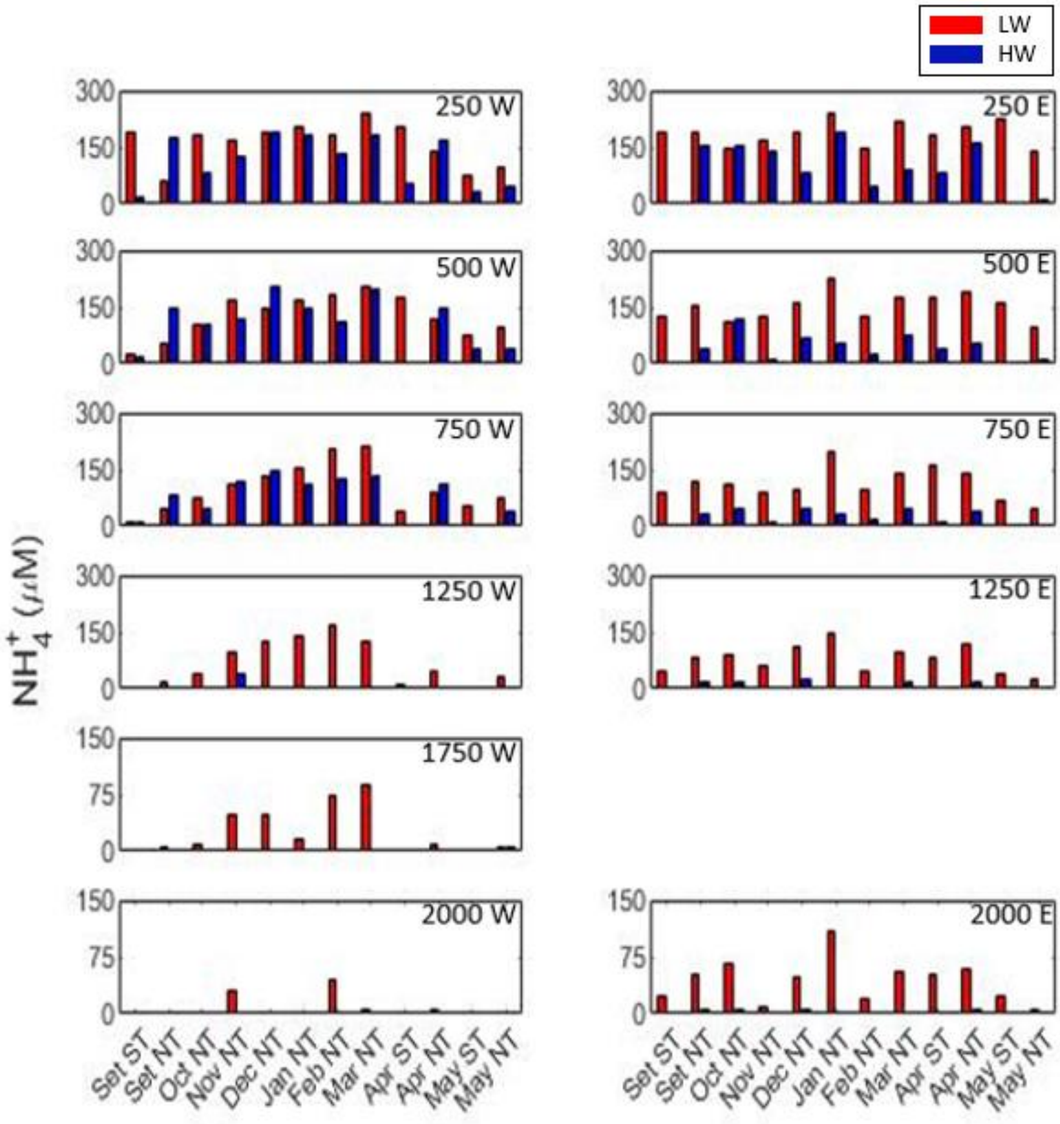

Figure 11. Monthly variation of ammonium (µM) for low water (LW) and high water (HW) at both west (left) and east (right) sections of the study area. From top to bottom, stations are organized from 250 m to 2000 m to the discharge. The horizontal axis represents the sampling months and the correspondent tide phase (ST – Spring Tide; NT – Neap Tide).

Nitrate (NO_3^-) (Figure 12) showed the lowest concentrations recorded between nutrients. The maximum concentration was 12.8 µM reached at the station FN 250 W in May 2019 at low water during spring tide and the minimum 0.06 µM for the station FN 750 W in the month of April 2019 at high water during spring tide. Relatively to the reference, maximal values were increased up to 20-fold. Globally, nitrate also shows significantly higher ($p<0.05$) concentrations during low water than during high water, with an increase of almost 2-fold.

For both sections of the study area at low water, nitrate shows a gradual decrease towards both ends of the channel. Concentrations were significantly higher between 750 W and FN 1250 E ($p<0.05$) when compared with the reference station, whereas for high water, significant higher values ($p<0.05$) where observed for the stations 500 W to 500 E. Within the three defined periods (Table 2), no significant differences were found ($p>0.05$), where mean values oscillate between 7.35 and 7.63 µM.

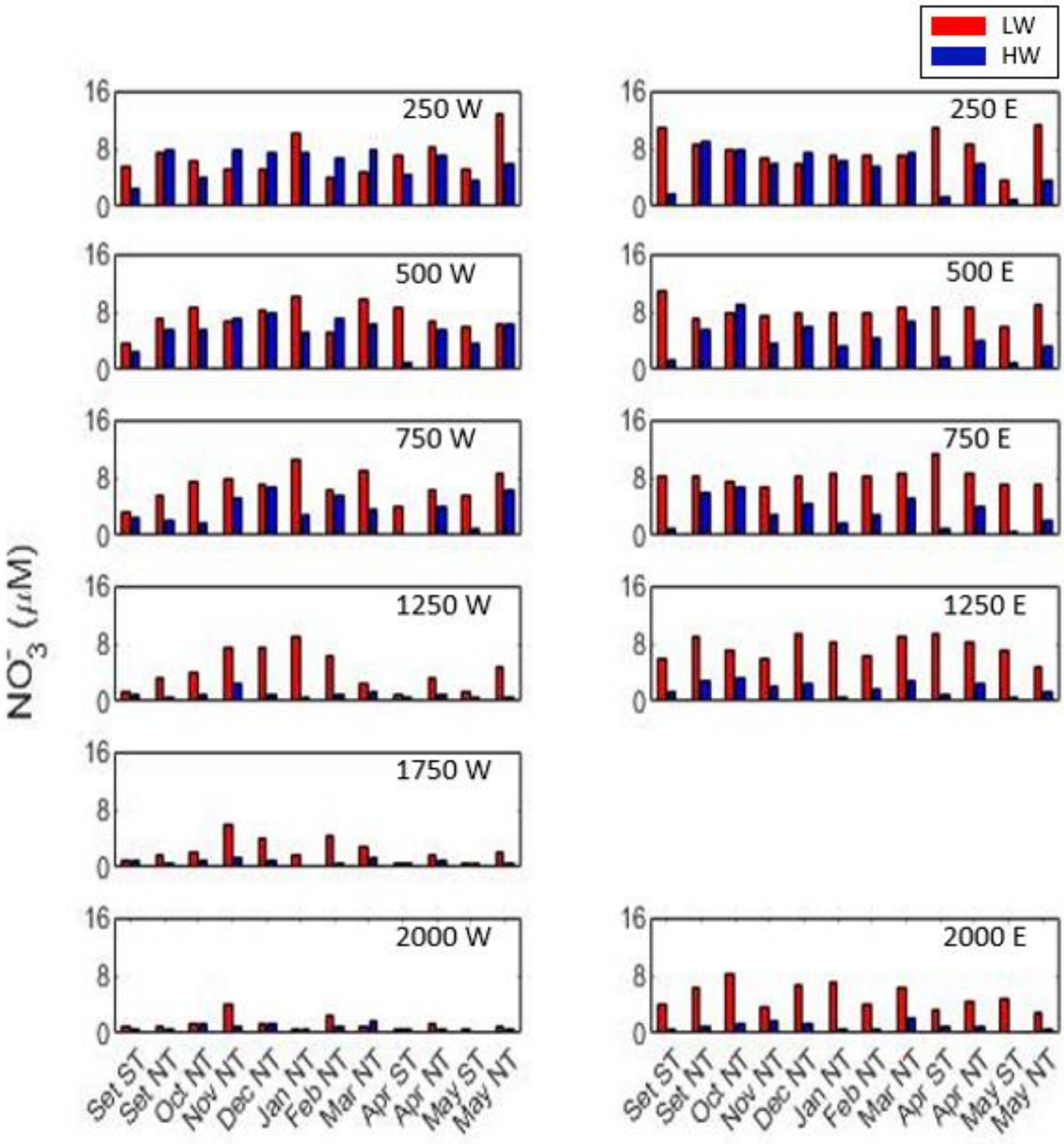

Figure 12. Monthly variation of nitrate (µM) for low water (LW) and high water (HW) at both west (left) and east (right) sections of the study area. From top to bottom, stations are organized from 250 m to 2000 m to the discharge. The horizontal axis represents the sampling months and the correspondent tide phase (ST – Spring Tide; NT – Neap Tide).

Nitrite (NO_2^-) (Figure 13) showed a similar distribution as nitrate, with the highest values registered at the stations closest to the discharge point, decreasing further away from the discharge. The maximum (12.85 µM) concentration was registered in September 2018 and May 2019 at FN 250 E and FN 250 W respectively, whereas the minimum (0.05 µM) was registered in May at the reference station during low water (neap tide). Maximal values showed to be 10 up to 12-fold higher than the values at the reference station. For this nutrient, May was the month with the highest value at low water until FN 1250 W, whereas such is not observed in the east section, with significant differences between the two sections ($p<0.05$). However, the same does not happen for high-water ($p>0.05$). Significant higher values were found for low water, ($p<0.05$) with a mean value almost up to 4-fold higher than high water. In the stations closest to the discharge (FN 250 E and FN 250 W) from September to December nitrate depicts a decreasing trend, with a slight increase from January to May. However, no significant differences ($p>0.05$) were detected in the three defined periods (Table 2), even though, the period of May registers the higher mean value of all three (7.72 µM).

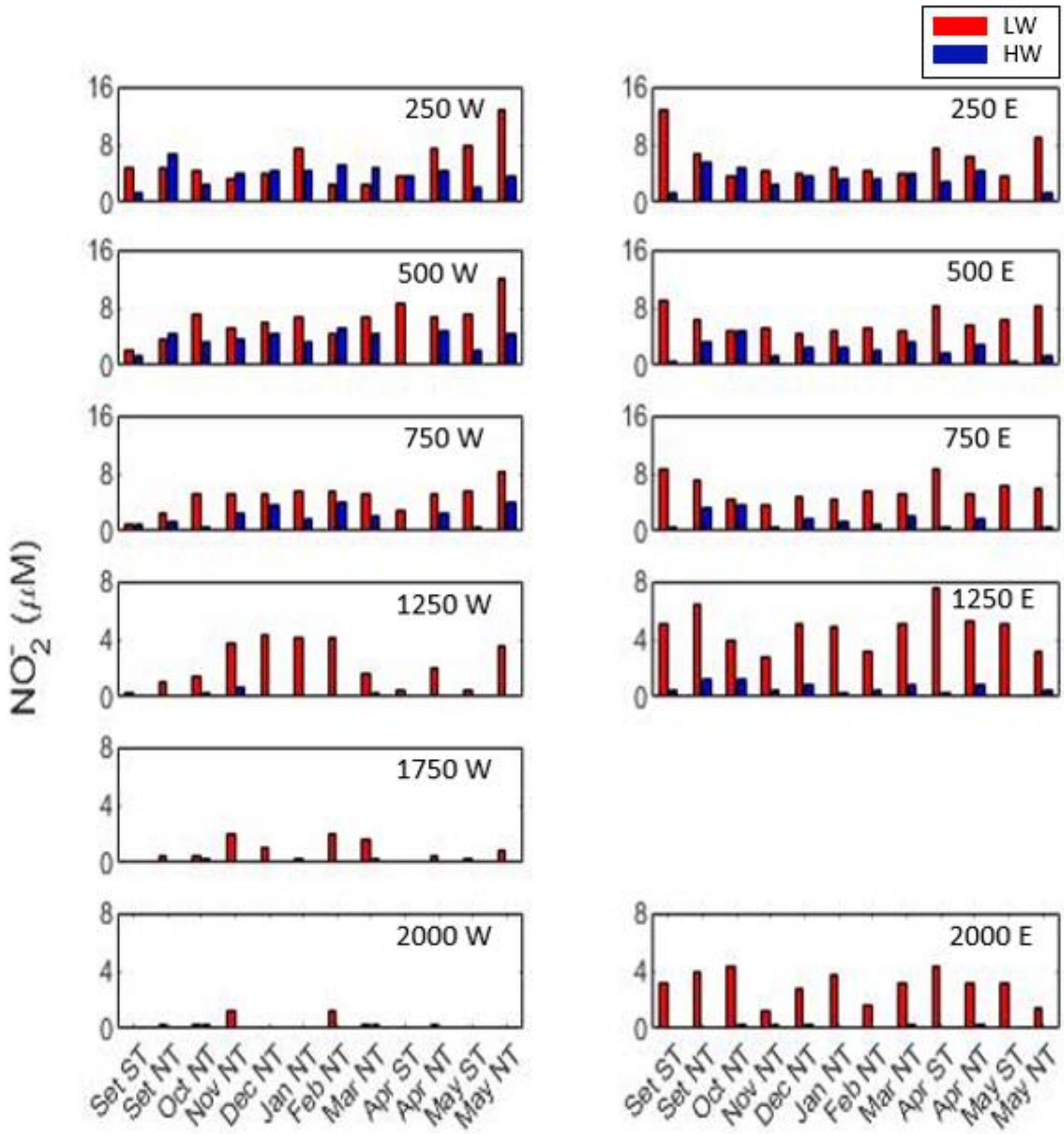

Figure 13. Monthly variation of nitrite (µM) for low water (LW) and high water (HW) at both west (left) and east (right) sections of the study area. From top to bottom, stations are organized from 250 m to 2000 m to the discharge. The horizontal axis represents the sampling months and the correspondent tide phase (ST – Spring Tide; NT – Neap Tide).

NH_4^+: TIN ratio was calculated to understand the contribution of the dominant form of nitrogen, by dividing ammonium by the sum of the three compounds of nitrogen. For the studied period, low water values were always above 74% for the stations located on the east section, showing the predominance of ammonium. At the western section, values were similar despite at the reference station values were lower, registering a minimum value of 62%.

Higher NH_4^+: TIN occur in the stations closest to the discharge, diminishing gradually towards both ends of the channel during low water. For high water, NH_4^+: TIN values are in general lower for all stations. In comparison to low water, the reference station registers the lowest value obtained (3%) in March during neap tide conditions and values did not exceed the ratio of 73%.

For both low water and high-water conditions, NH_4^+: TIN does not depict any evident temporal trend although, significantly higher values were found at low water in comparison to high-water ($p<0.05$). When comparing both sections of the study area, no significant differences were found ($p>0.05$) at both high and low water. Within the three defined periods, the period of May registered the lowest mean value out of the three periods (85%), whereas the 6 months period after the decommission of the WWTP (November-April) has the higher value (92%).

Phosphate concentrations (PO_4^{3-}) (Figure 14) decrease rapidly downward from the discharge point both during both low water and high-water conditions for both sections of the study area. Maximum concentration (72.83 µM) was registered in May 2019 during low water (neap tide) while the minimum (0.06 µM) close to the reference station, at FN 1750 W during low water (neap tide) in February. Phosphate values at the stations closer to the discharge were increased up to 70-fold relatively to the mean value registered at the reference (LW). Phosphate concentrations were significantly higher ($p<0.05$) during low water than at high-water, being approximately 3-fold higher at LW than HW. However, no significant differences were found between the two sections ($p>0.05$), despite relatively higher at the eastern section for both low water and high-water conditions.

Unlike nitrate and nitrite, significant differences ($p<0.05$) were found for phosphate between the three defined periods (Table 2), where the higher mean values was obtained for the period of May (28.7 µM), followed by November-April and September-October, respectively (13.4 and 7.7 µM). However, the second period was not significantly different from the third ($p>0.05$). Spatially, significant differences were found until 750 m for both sections from the discharge in comparison to the reference station ($p<0.05$), whereas, at high water, significant higher values ($p<0.05$) where found until 750 m for the west section and until 500 m at the east section.

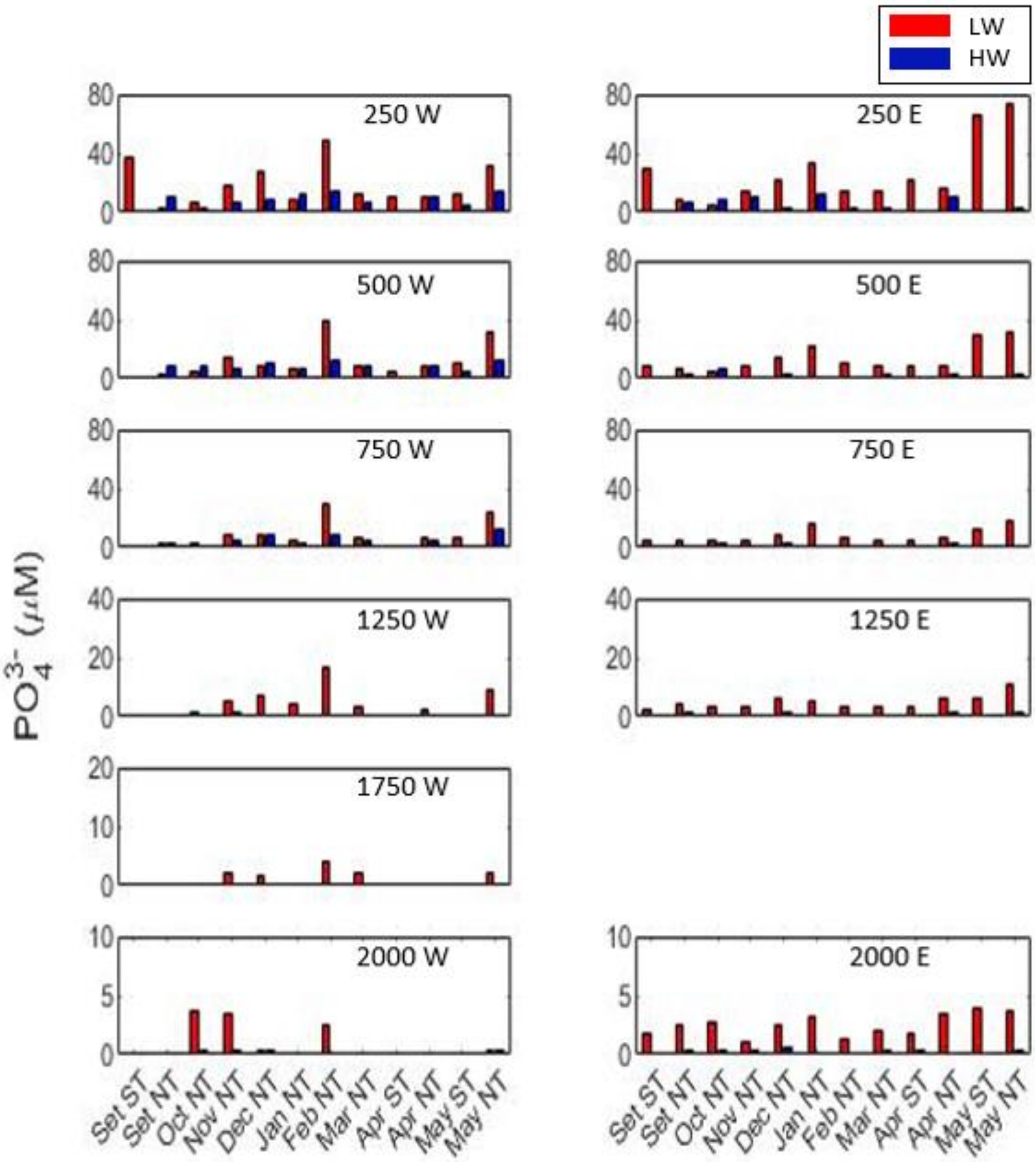

Figure 14. Monthly variation of phosphate (µM) for low water (LW) and high water (HW) at both west (left) and east (right) sections of the study area. From top to bottom, stations are organized from 250 m to 2000 m to the discharge. The horizontal axis represents the sampling months and the correspondent tide phase (ST – Spring Tide; NT – Neap Tide).

Silicate (SiO_4^{4-}) (Figure 15) concentrations also decreased rapidly downward from the discharge point both in low and high-water conditions. The maximum concentration (138.1 µM) was recorded in September at the station FN 250 W at low water during spring tide while the minimum (1.1 µM) was registered in January at the reference station at high-water during neap tide. Maximal values of silicate were up to 20-fold higher than the mean value for the reference at LW. In most cases, significantly higher values ($p<0.05$) are registered during low water than during high-water, being 2-fold higher at LW. However, no differences were found between the two sections of the study area ($p>0.05$) for both low water and high water, as found for phosphate. In addition, no significant differences were found ($p>0.05$) between the three periods (Table 2), with mean values fluctuating between 30 and 31 µM. Spatially, significant differences ($p<0.05$) were found in comparison to the reference station until 750 m (750E and 750W) from the discharge for both sections. However, for high water, significant higher ($p<0.05$) were found until 500 m from the discharge (east) and down to 750 m for the west section, like for phosphate.

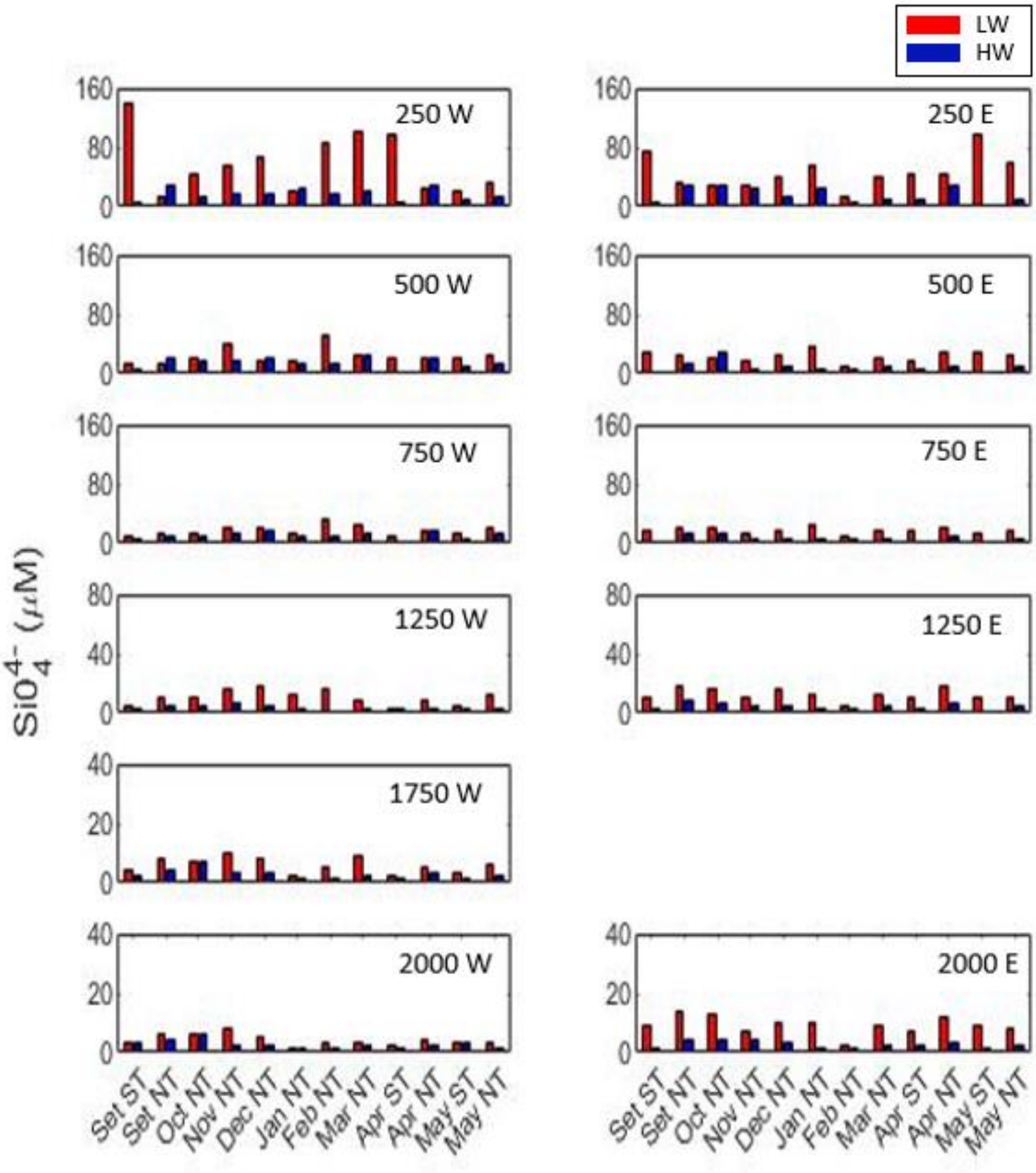

Figure 15. Monthly variation of silicate (µM) for low water (LW) and high water (HW) at both west (left) and east (right) sections of the study area. From top to bottom, stations are organized from 250 m to 2000 m to the discharge. The horizontal axis represents the sampling months and the correspondent tide phase (ST – Spring Tide; NT – Neap Tide).

Nutrient ratios were calculated to understand which nutrient (N, P or Si) is the limiting nutrient.

Nitrogen to Phosphorus (N:P) ratio (Figure 16) was calculated for both low water and high-water, showing significant higher values of N:P ($p<0.05$) for low water than in high water. For both low and high water the eastern section shows no significant differences ($p>0.05$) when compared with the western section. Globally, except for the reference, at most months the N:P ratio exceeded 16, showing the P as the limiting element even at the stations far away from the discharge point, suggesting an increase of nitrogen nutrients. At the reference station the N:P ratio was generally lower than 16 and in October the lowest N:P ratio (1.22) was registered. However, in September and from January to April at low water values higher than 16 were measured while in May a global decrease was observed at the overall stations, leading to N to be the limiting element, as typically found in coastal waters.

Globally, N:P ratio did not depict any evident temporal trend. Even though, significant differences ($p<0.05$) were found between the three defined periods (Table 2), where, the periods September – October and May show the highest (23) and lowest mean N:P ratio (5), respectively.

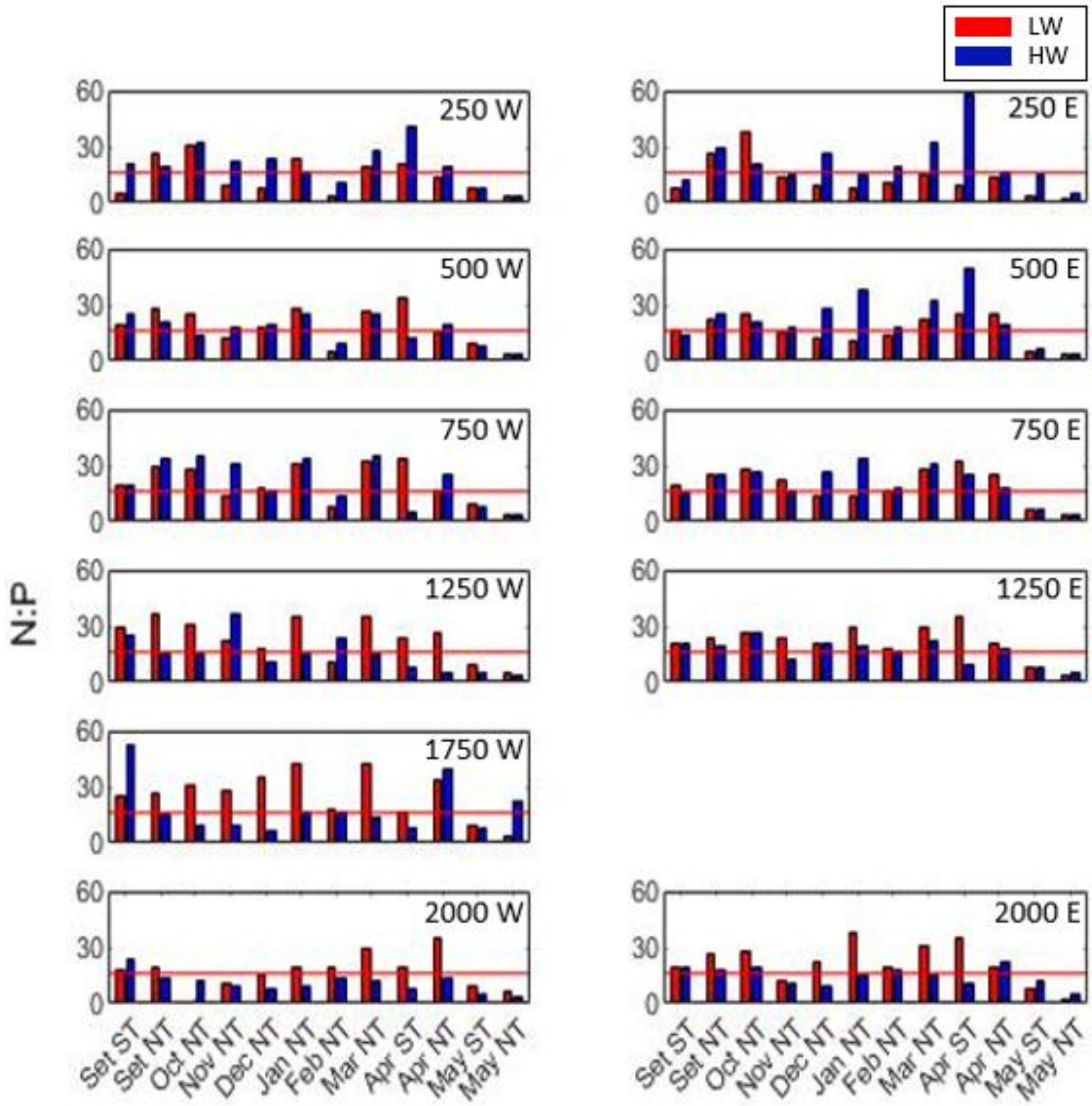

Figure 16. Monthly variation of Nitrogen to Phosphate ratio (N: P) for low water (LW) and high water (HW) at both west (left) and east (right) sections of the study area. From top to bottom, stations are organized from 250 m to 2000 m to the discharge. The horizontal axis represents the sampling months and the correspondent tide phase (ST – Spring Tide; NT – Neap Tide).

The N:Si ratio (Figure 17) was also calculated and it depicts an evident temporal trend with higher values during the winter months (December-February) that can be associated with the increase (of about 40%) of flow discharge and consequently of nitrogen forms of the new Faro-Olhão (FO) WWTP along with some rainfall and runoff influence, particularly increasing the nitrogen. Lower concentrations were observed in September and from April to May that can also be explained by stabilization of the treatment with an evident removal of nitrogen, together with nitrogen consumption by phytoplankton during the autumn and spring months. The typical N:Si ratio for sea water is 1 and values were < 1 for the stations away from the discharge (FN 1750 W, FN 2000 W and FN 2000 E where nitrogen was limiting. However, globally silicate as the limiting nutrient since this ratio is higher than 1, indicating the nitrogen being excessive relative to silicon.

At low water, N:Si ratio is significantly higher than at high water ($p<0.05$), since the effluents discharge, more important in low water, is the source of nitrogen forms, particularly ammonium. Significant higher values ($p<0.05$) were obtained in the east section during low water, however, the same did not happen for high water ($p>0.05$).

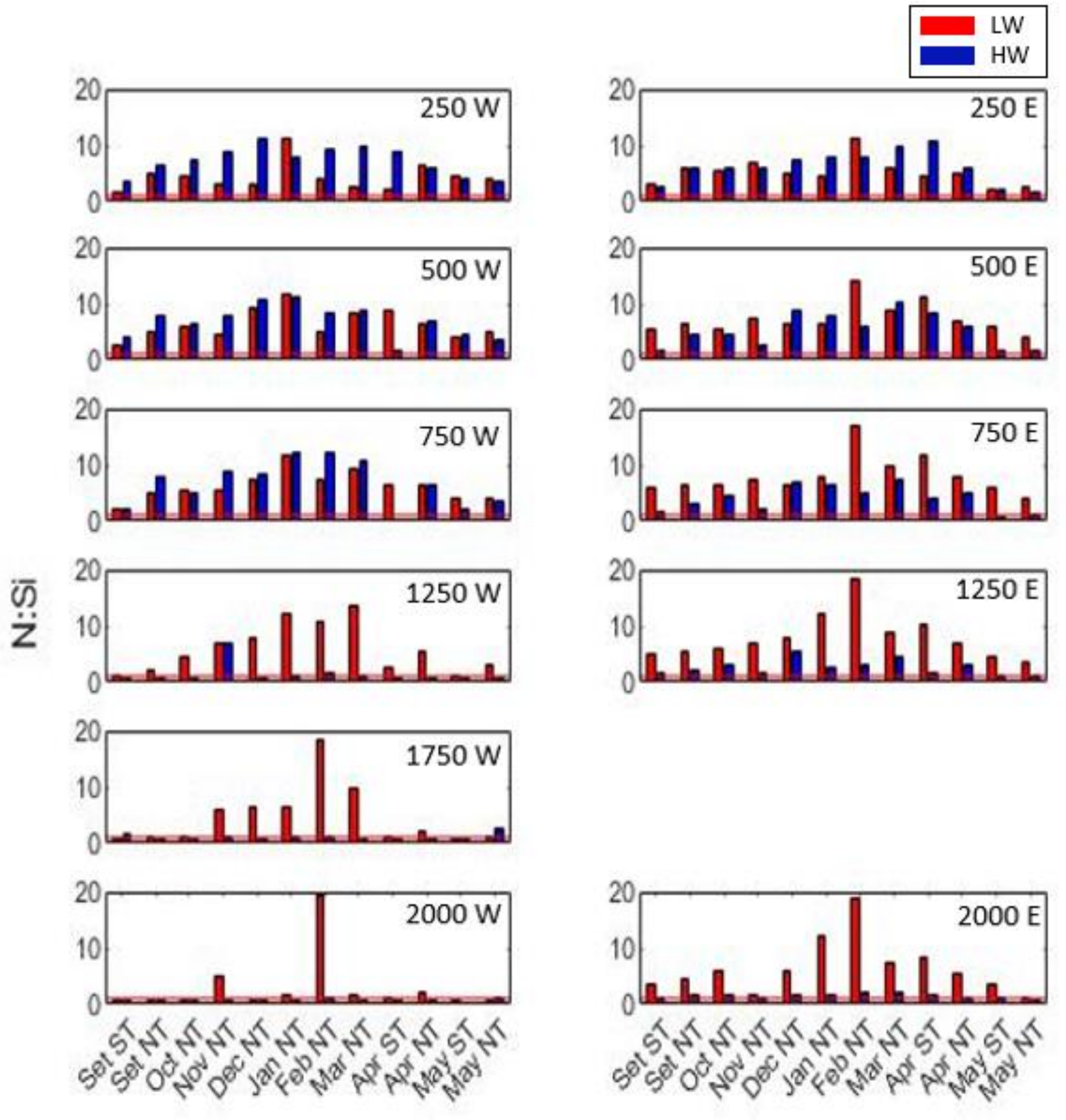

Figure 17. Monthly variation of Nitrogen to Silicate ratio (N: Si) for low water (LW) and high water (HW) at both west (left) and east (right) sections of the study area. From top to bottom, stations are organized from 250 m to 2000 m to the discharge. The horizontal axis represents the sampling months and the correspondent tide phase (ST – Spring Tide; NT – Neap Tide).

For *Escherichia coli* the limit of license for the effluents discharge for the new Faro-Olhão WWTP is 300 MPN/100 mL).

E. coli in this study was determined at less stations than for the chemical parameters (Figure 18). At low water it was determined at the closest (250 E and W), at an intermediate distance (750 E and W) and at the furthest stations where there are clam beds (2000 E and 1750 W). At high water only FN 250 E and FN 2000 E were sampled, based on a previous study that showed that the contamination was more evident at the eastern sector (Cravo et al., 2015).

Data showed, at low water, an evident decrease with increasing distance from the discharge point of the WWTP for both sections. The maximum value for *E.coli* was registered at the station FN 250 W in March during low water at neap tide with a value of 3.11×10^{5} MPN/100 mL, while the minimum was of 10 MPN/100 mL at several stations even at the closest station from the discharge (FN 250 E) in February during low water at neap tide. The maximum value increased up to 1000-fold than the limit of emission (300 MPN/100 mL)

A temporal variability pattern was observed for both sections of the study area. From September to October, there is an overall increase of *E. coli* values at all sampled stations followed by a clear decrease after March at the west section of the study area, despite the low value in January. It is important to remind that previous to the week of October sampling it was recorded the maximum accumulated rainfall (Table 1), which may be responsible for an increase of runoff enriched in organic matter and microbiological contamination in a period when the former WWTP was still operational. Unfortunately, no data exist for the organic matter fraction of TSS. At the east section, a clear decrease is also observed after March, regardless the low value in February. The emission limit is exceeded more often for the stations closest to the discharge, decreasing for the stations further away from the discharge, where at the reference station (FN 2000W) was never exceeded and only twice at FN 2000 E.

At high water, as at low water, an increase is observed from September to October (FN 250 E) followed by an overall decrease from November until May. Even though, the emission limit is exceeded at most months with few exceptions

(September – NT, February, and May – ST). At the station FN 2000 E, *E. coli* values never exceeded the threshold of 300 MPN/100 mL.

For *E. coli*, higher values were observed for low water despite no significant differences were obtained when comparing low water with high-water (p>0.05). Also, no significant differences exist between the sections of the study area (p>0.05) for both low and high-water.

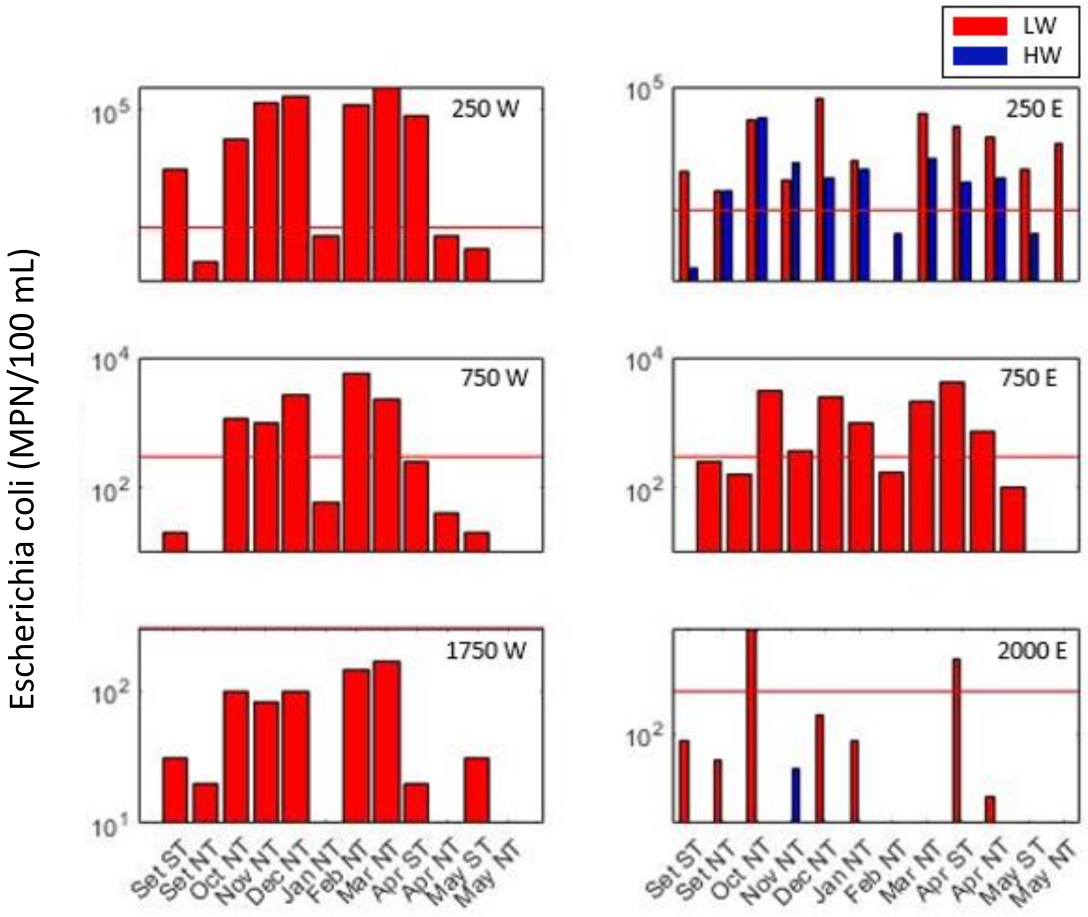

Figure 18. Monthly variation of E. coli abundance (MPN/100mL) for low water (LW) and high water (HW) at both west (left) and east (right) sections of the study area. From top to bottom, stations are organized from 250 m to 2000 m to the discharge. The horizontal axis represents the sampling months and the correspondent tide phase (ST – Spring Tide; NT – Neap Tide). The red line at each chart represents the limit value of emission (300 MPN/100 mL).

3.4. Trophic Index results (TRIX)

A trophic index (TRIX) was also calculated, for both low water and high-water conditions individually and globally for both situations to understand wastewater influence on both sections (east and west) of the study area and the effect of tidal renewal (Table 3).

Table3 – Trophic quality index (TRIX) calculated for the sampling stations individually for low water, high water and globally for both conditions.

	LW	HW	Global
FN 2000 E	3.9	2.2	3.5
FN 1250 E	5.1	3.2	4.5
FN 750 E	5.7	3.9	5.1
FN 500 E	8.8	7.0	8.2
FN 250 E	9.6	8.2	9.1
FN 250 W	7.3	6.1	6.9
FN 500 W	6.3	6.0	6.2
FN 750 W	5.6	5.3	5.5
FN 1250 W	4.4	2.2	3.1
FN 1750 W	3.3	1.5	2.2
FN 2000 W	2.8	1.5	1.8

[0 - 4[	Oligotrophic
[4 - 5[	Oligotrophic to Mesotrophic
[5 - 6[	Mesotrophic
[6 - 10]	Mesotrophic to Eutrophic

For low water, a 'Very Good' (Oligotrophic) trophic state was registered at the extremes of the channels, FN 2000 E and 1750 and 2000 W, an 'Moderate' (Mesotrophic) quality was obtained from FN 1250 to FN 750 E and 750 W. A 'Bad' (Mesotrophic to Eutrophic) quality index is maintained from FN 500 E to FN 500 W, with FN 250 E registering the worst classification with a value of 9.6. For the low water condition, TRIX confirms that the east section is more affected by the wastewater discharge than the west section, where a better overall classification was observed.

At high water, the quality index improved at most stations, except from FN 500 E to FN 250 W where the quality was still classified as 'Bad'. For the remaining stations, a slight improvement is observed.

For the overall sampling encompassing both low and high water, a 'Very Good' trophic state was obtained for the station FN 2000 E whilst, a 'Good' (Oligotrophic to Mesotrophic) and 'Moderate' trophic state was achieved for the stations FN 1250 E and FN 750 E respectively. For the stations FN 500 E and FN 250 E, a 'Bad' status was achieved, the same is observed for the stations FN 250 W and FN 500 W. For the station FN 750 W, the trophic status was achieved the same as the station FN 750 E ('Moderate'). However, a 'Very Good' status was achieved from FN 1250 W to FN 2000 W.

With the results of the global index covering both extreme tidal peaks of low and high water it is possible to infer that the west section of the study area is the least affected from the wastewater discharge, showing an improvement of the trophic quality compared to the east section, where the influence of the wastewater discharge apparently was greater.

3.5. Correlation between variables

The correlation between variables was studied to understand how variables correlate between them. With this objective, a correlation matrix was performed separately for low water and high water using all the studied variables measured *in situ*, nutrients, TSS, chlorophyll *a* (Chl *a*) and *E. coli* for neap and spring tides (Tables 4 and 5, respectively).

Table 4 - Correlation matrix for low water (LW). Green values represent the significant positive correlations while the red ones represent the negative significant correlations.

	Temp	*Sal*	*pH*	*O2 mg/L*	*%DO*	*SS*	*Chl a*	*NH4+*	*NO3-*	*NO2-*	*PO43-*	*SiO44-*	*Ecoli*
Temp	1												
Sal	0.05	1											
pH	0.00	0.49	1										
DO mg/L	-0.03	0.37	0.77	1									
%DO	0.38	0.43	0.79	0.85	1								
TSS	0.07	-0.79	-0.43	-0.30	-0.34	1							
Chl a	-0.06	-0.57	-0.24	-0.17	-0.22	0.63	1						
NH4+	-0.24	-0.70	-0.54	-0.41	-0.53	0.55	0.41	1					
NO3-	-0.04	-0.27	-0.35	-0.30	-0.33	0.18	0.00	0.66	1				
NO2-	0.16	-0.28	-0.32	-0.38	-0.33	0.23	-0.01	0.58	0.86	1			
PO43-	0.09	-0.82	-0.34	-0.30	-0.32	0.50	0.36	0.57	0.33	0.46	1		
SiO44-	0.08	-0.92	-0.49	-0.37	-0.40	0.89	0.66	0.70	0.29	0.33	0.73	1	
Ecoli	0.01	-0.45	-0.17	-0.17	-0.26	0.45	0.72	0.36	0.06	0.07	0.48	0.51	1

Table 5 – Correlation matrix for high water (HW). Green values represent the significant positive correlations while the red ones represent the negative significant correlations.

	Temp	*Sal*	*pH*	*O2 mg/L*	*%O2*	*SS*	*Chl a*	*NH4+*	*NO3-*	*NO2-*	*PO43-*	*SiO44-*	*Ecoli*
Temp	1												
Sal	0	1											
pH	-0.04	0.67	1										
O2 mg/L	-0.38	0.47	0.76	1									
%O2	0.14	0.74	0.90	0.77	1								
SS	0.09	-0.58	-0.55	-0.42	-0.49	1							
Chl a	-0.19	-0.73	-0.50	-0.46	-0.53	0.38	1						
NH4+	-0.18	-0.88	-0.81	-0.60	-0.83	0.66	0.75	1					
NO3-	-0.07	-0.75	-0.82	-0.68	-0.85	0.62	0.56	0.84	1				
NO2-	-0.02	-0.76	-0.79	-0.69	-0.81	0.68	0.60	0.87	0.95	1			
PO43-	-0.06	-0.78	-0.61	-0.50	-0.63	0.65	0.65	0.83	0.82	0.85	1		
SiO44-	0.05	-0.80	-0.80	-0.73	-0.81	0.67	0.64	0.92	0.88	0.89	0.85	1	
Ecoli	-0.02	-0.61	-0.45	-0.56	-0.59	0.33	0.28	0.47	0.48	0.48	0.42	0.50	1

Looking for both matrices, at high water stronger correlations were found possibly due to the stronger mixing effect. For low water (Table 4), temperature showed a positive correlation with %DO, meaning that in warmer months higher values of temperature corresponded to higher levels of dissolved O_2, by enhancement of photosynthesis, whereas temperature correlates negatively with NH_4^+ meaning that the highest concentrations were found in the cooler months (December to February) in the beginning of the operational start of the new WWTP. The opposite was recorded during warmer months, also possibly associated with phytoplankton consumption and higher rates of nitrification. With the remaining variables, temperature does not show any significant correlation. pH, DO and %DO present a significant positive linear correlation with salinity meaning that these parameters showed the highest values far away from the discharge point where the salinity was maximum and the minimum where the freshwater contribution from the effluent was the highest. Values of pH also correlates positively with oxygen, since an increase of dissolved oxygen by photosynthesis removes CO_2 from the water, raising pH (Zang et al., 2010). Moreover pH and dissolved oxygen correlated negatively with all nutrients, Chl *a*, total suspended solids and *E. coli*, meaning that closer to the effluents discharge where these concentrations were higher, pH and O_2 were lower, implying higher availability of organic matter, which degradation leads to a decrease in dissolved O_2. The opposite was observed at the furthest stations from the effluent's discharge. Moreover, salinity was strongly negative correlated with nutrients demonstrating their main source was the effluent.

A positive correlation was also obtained between DO and OM (data not shown) at low water, suggesting that part of the organic matter can correspond to phytoplankton, able to produce dissolved oxygen. However, at high water, OM values are higher and increase further from the discharge which can suggest an additional source of OM to the study area during the flood period.

The positive correlation between TSS, Chl *a*, *E. coli* and nutrients can be ascribed to the presence of treated sewage from the stabilization ponds of the WWTP. As sewage matures in the stabilization ponds, high nutrient concentrations (N and P) can dramatically increase phytoplankton growth. Hence, high Chl *a* close to the discharge can be ascribed to this process. TSS, consists mainly of either organic or inorganic particles, that, in wastewater, can represent part of the particles that were not eliminated during the treatment process, and aggregates of bacteria and phytoplankton makes part of TSS. Chl *a* and TSS was higher closer to the discharge reflecting the direct impact of the wastewater. High concentration of TSS is directly linked to an increase in turbidity, which, decreases the photic layer and may inhibit photosynthesis, supporting that high Chl *a* in the vicinity of the discharge is mostly associated with the phytoplankton grown in the maturation ponds of the WWTP.

Regarding nutrients, just like Chl *a* and TSS, high concentrations can be ascribed to the wastewater, which, is resulting from the treatment of the domestic influents that after decomposition and remineralization provides high concentrations of nitrogen and phosphorus.

The principal component analysis applied for low water data, when the impact of sewage discharge in more evident, allow to identify three main components that explain mostly the variance of the data (75%; Figure 19). PC1 is explained by salinity (Sal) against nutrients (except nitrate), *E. coli*, TSS and

Chl *a*, accounting almost for 50% of the variance of the results. PC2 (15% of the variance) is explained by nitrate in opposite direction of DO percentage of saturation while PC3 (11% of the variance) is explained by temperature against *E. coli* and Chl *a*. Therefore, the key role processes that best explain the variability of the data are: the physical mixing of waters (effluents disposal and seawater) and dilution effect by tidal renewal, followed by the chemical process of nitrification and lastly as a tertiary process the biological inhibition during some summer periods that due to high solar radiation can have a germicide effect upon bacteria that along nutrient consumption may lead to a decrease in chlorophyll *a*.

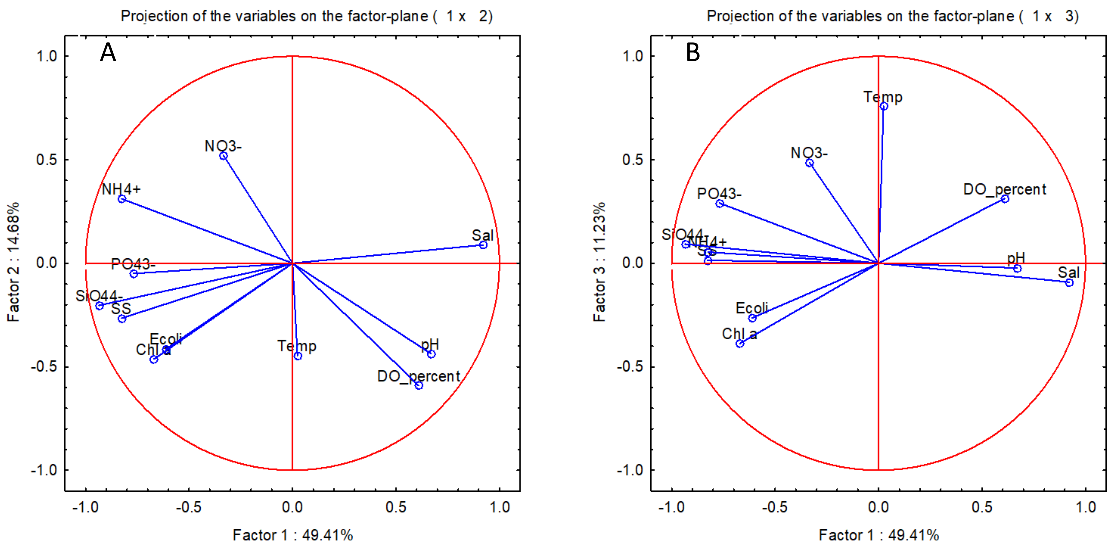

Figure 19. Principal component analysis (LW) for nutrients (NH_4^+, NO_3^-, NO_2^-, PO_4^{3-}, SiO_4^{4-}), temperature (Temp), salinity (Sal), pH, dissolved oxygen (DO_percent), total suspended solids (SS), chlorophyll a (Chl_a) and Escherichia coli *(Ecoli).*

Chapter 4 - Discussion

4.1. Water quality assessment – Spatial and tidal influence

It is well recognized that coastal areas under direct impact of wastewater are prone to nutrient and organic pollution, which, under extreme events can lead to eutrophication (Cabral et al., 2019).

One of the first evidence of organic pollution is oxygen depletion (Diaz and Rosenberg, 1995). For this study, only 17% of the samples in LW were below the threshold of 60% imposed as the minimum available value (MAV = 60%) by national legislation (DL 236/98 for shellfish waters) and no sample corresponded to hypoxic values (> 2 mg. L^{-1}). However, there is no guarantee that during dawn, values did not reach hypoxic levels. Samples that were below the threshold of 60% were close to the vicinity of the discharge, where high concentrations of nutrients and certainly organic matter in decomposition were present. This justifies the negative correlation between %DO and nutrients ($r < -0.63$; Table 5) which along both channels can be associated with remineralization of organic matter as reported by other authors (*e.g.* Cabral et al., 2019). However, relatively high concentrations of NH_4^+ were still observed in the stations further from the discharge in the east section, as shown in Figure 10, implying a great dispersal of organic matter and remineralization along that channel. This can be explained during periods of higher residence time such as neap tides when remineralization processes are intensified (Cabral et al., 2019) and justifies a lower water quality during this tidal condition.

Low DO at the stations closest to the discharge can be associated with high rates of respiration by aerobic bacteria during remineralization (with NH_4^+ release) and phytoplankton (during dawn and dusk). Dominance of NH_4^+ for this study denotes prevalence of low DO in the early morning sampling moments, when respiration is dominant (Newton et al., 2003) and then limits nitrification. It is important to remark that NH_4^+ concentrations were much higher than nitrate, which may support a low efficiency of the nitrification process during the treatment of the sewage due to the high amounts or organic matter that rapidly consumes DO during bacterial respiration. This was more evident mainly in the period when the bacterial biomass was not yet stabilized (November 2019-April 2020). Moreover, some events of rainfall such as those recorded close to October and November sampling moments may also contribute to increased runoff , augmenting organic matter and bacteria in the system close to the WWTP (Cravo et al., 2015), therefore, decreasing DO concentrations and increasing the ammonium values. Accompanied by the increase of N (NH_4^+, NO_3^-, NO_2^-) close to the point of discharge there is also the availability of the others nutrients as observed in PCA (Figure 19A) and Tables 4 and 5. However, when high water was sampled in the afternoon, both channels presented saturated and above saturation levels at most stations corresponding to a period when the photosynthesis surpassed respiration, and availability of nutrients under optimal light radiation stimulate the growth of algae, leading to increased values of DO.

NO_3^- and NO_2^- presenting similar concentrations, can confirm the low rate of the nitrification process. Nevertheless, this process had a secondary role to explain the variance of the results (Figure 19A).

Amongst the two sections of the study area, the most impacted was the eastern section, confirmed both through the statistical analysis and the trophic index (TRIX). This can be explained by the preferential circulation patterns occurring on the eastern section due to channel physiography, which is more restricted on the western section of the study area (Cravo et al., 2015). The channel in the western section, as shown in Figure1, is narrower and shallower where during some periods of low water, this is devoid of water, as reported by Cravo et al (2015). Also, during ebb period, water is driven from Esteiro da Garganta (at the Eastern part of the channel) to Armona inlet (Fabião et al., 2016) whereas, during flood, water runs from Armona inlet to Esteiro da Garganta, pushing the water to the western section of the channel. This fact explains sometimes higher values obtained during high water at the western channel.

Trophic status classification reflects the tidal influence and allows to understand the extension and effect of the discharge on both sections of the channel by integrating four key water quality parameters. It reveals a greater impact of the discharge on the eastern section, with a ‘Moderate’ (mesotrophic) quality until 750 m from the discharge and a ‘good’ (oligotrophic) quality further down 1250 m. Equivalently, at the western section, further down to 1250 m the water quality increased to a ‘very good’ (oligotrophic) status. At low water, due to restricted circulation together with low dilution by mixing with seawater, the impact of the discharge was magnified, presenting a worst trophic quality. TRIX status (Table 2) also showed that that the trophic quality was improved in high water, due to a dilution effect promoted by tidal flushing and mixing with seawater poorer in nutrients and organic matter and enriched on dissolved oxygen. Spatially, regardless the evident impact down to 750 m in both sections, tide represents the key driving mechanism responsible for the amelioration of the water quality within this system along the dispersal gradient and during high water, promoted by the exchanges with the adjacent ocean through its 6 inlets, as reported by various authors devoted to water quality issues of Ria Formosa (e.g. Falcão et al., 2016, Newton et al., 2003, Cravo et al., 2015, 2018) or in other systems (Canu et al., 2003, Tett et al., 2003). This fact is responsible for not founding eutrophication symptoms close to the main channels. However, water quality is not only threatened by nutrient contamination. Wastewater is also a source of pathogenic bacteria which can also compromise water quality even if the treatment is 99% efficient (Cravo et al., 2015). *E. coli* is one major indicator of fresh and marine water quality (Price and Wildeboer, 2017). In this study, *Escherichia coli*, constituting about 90% of Total Coliforms (Hachich et al., 2012) (data not shown), proved to be of great concern, where at most stations, the MRV (maximum recommended value) for bathing waters (500 ufc/100 mL) was exceeded as well as the permitted value for discharge (300 MPN/100 mL) by APA This limit was exceeded several times, even at 2000 E where bivalve banks occur. In this study, *E. coli* was directly correlated to all nutrients and against salinity (Tables 4 and 5) relating all these parameters with the effluent of the WWTP, as confirmed by other authors (Mallin et al., 2000; Ishii and Sadowsky, et al., 2008; Cabral et al., 2019).

At the present study, the highest nutrients, chlorophyll *a*, suspended solids and *E. coli* concentrations registered at both low water and high water were observed mainly during neap tides, due to a higher residence time within the system also promoting longer periods for chemical and biological reactions. The volume of water that enters Ria Formosa in neep tide is about half of that in spring tides, decreasing the dilution effect during mixing seawater and the effluents. Having in mind that wastewater discharges pose a threat to coastal ecosystems, for this study it is important to remark that the deleterious effects of the discharge upon water quality were identified to be amplified during neap tide. Nevertheless, the mesotidal range of Ria Formosa associated to a semi-diurnal tidal regime promotes a great dilution effect and enhanced water renewal (*ca.* 75%) at each tidal cycle (Tett et al., 2003), what was confirmed by the water quality improvement given by TRIX when high water was also considered (Table 3).

4.2. Temporal variability of the wastewater impact on the receiving waters

Temporally, regarding temperature, it followed the seasonal cycle of atmospheric temperature. Closest to the discharge (FN 250 W and 250 E) higher values can also influenced by the shallowness of the channel.

Nutrients such as ammonium, nitrite and phosphorus are good tracers of wastewater. From September to April, high variability was associated for these nutrients for both LW and HW. In the winter months, concentrations of these nutrients along with nitrate can be increased due to runoff from nearby agricultural fields. However, 7-day accumulated rainfall (Table 1) showed no important rainfall volumes that could justify an increase of these nutrients in the study area. Even though, October and November register the highest 7-day AR (10.2 mm and 9.9 mm) which, is compliant with an increase of these nutrients in this period. It is important to remark that after October, the new WWTP started to operate with an increased daily outflow (ca. 14 000 m^3/day in comparison to the former 10 000 m^3/day), which, should also explain the increase in the concentrations of these nutrients. In opposition to nitrogen compounds that decreased in May, phosphate has a sudden increase during this month, which, could be explained partly by desorption from sediments particularly with increasing water temperature (Leote and Epping, 2015). However, during May- Spring tide at low water, phosphate registered a high value (66.4 µM; Figure 14) together with ammonium (225.4 µM; Figure 11) and silicate (98.6 µM; Figure 15) at FN 250 E, which suggest an evident contribution of sewage disposal, and reflected by one of the lowest values of salinity (Figure 4). Therefore, this overall increase in phosphate can be related to an increase in the outflow of the WWTP supplemented by desorption from sediments, to some extent.

Silicate is one good indicator of the presence of treated sewage once its concentration is high in freshwater. Hence, the months that register the highest concentrations of silicate are the ones will correspond to a major increase on the daily flow of the WWTP and/or rainfall as it was the case of October and December samplings.

Chlorophyll *a* was one of the studied variables with the highest variation along the sampling period specially in the west section at the stations closest to the WWTP discharge point. It was highest in the winter months relatively to summer/spring. However, the month of May registered a high value at LW (8.3 µg/L) at the farthest station in relation to the WWTP (FN 2000 E) which, can derive from an external source driven from the coast. A 8-day survey from 21st of May to 29th (data not showed) showed persisting high values of Chl *a* up to 15 mg/m^3 near the coast together with a negative temperature gradient towards the coast (https://worldview.earthdata.nasa.gov/) could indicate upwelling during this time period, therefore, explaining the high Chl *a* and DO values obtained at 2000 E.

Therefore, mostly of the high chlorophyll *a* concentrations found can be directly linked to the discharge of the WWTP, where sunlight and nutrient availability in the maturation ponds led to an increase in phytoplankton growth. The negative correlation observed between DO and Chl a can occurred particularly when sampling was performed during the early morning when phytoplankton used DO in respiration, more evident in the western section, where Chl α is highest. These data also show that during periods of high nutrients availability if light radiation was very strong phytoplankton growth was not promoted. The impact of decommissioning of Faro Nascente and Olhão Poente and upgrading of the new Faro-Olhão WWTP on the water quality of Ria Formosa was evaluated by Jacob et al. (2020) from September 2018 to September 2019. In this study that encompassed the period of the present study, it was observed that DO, NH_4^+, Chl *a* confirmed the highest concentrations and variability during the period November 2018 – April 2019 after the former WWTPs decommissioning. The spatial impact of the WWTP from September 2018 to September 2019 (Jacob et al., 2020), was noticeable down the same distances, down to 750 m from the discharge. For *E. coli*, the same spatial and temporal variability was observed, with an overall improvement after May 2019, where values comply the discharge license (300 MPN/100 mL). This may confirm the stabilization of the new biological treatment (NEREDA) as well as the efficiency of the UV lamps that start to operate in May. Thus, after this month the nutrients decrease (N and P; Figures 11, 12, 13 and Figure 14) can indicates a more efficient removal of those from the bacteria from NEREDA treatment, as well as lower concentrations of SS, Chl *a* and *E. coli*, even with an increase of the daily flow (ca. 40%) from the new WWTP. Vu (2020), accessed the impact of wastewater in Ria Formosa after May 2019, where a drastic decrease in the N:P ratio was observed in Faro-Olhão WWTP, founding values 4-5 times lower than those obtained at the present study. Therefore, this may support that the stabilization in the biological treatment of the WWTP (NEREDA) increased the N removal.

As the assessment of the impact of wastewater over the resource clam *Ruditapes decussatus* is one of the objectives of the project CONPRAR, it is important to highlight if whether this resource is threatened due to the discharge of wastewater. As far as concerning the data obtained during this study, the "plume" of the WWTP further extends down to 750 m for both sides of the channel as previously mentioned, where no bivalve banks occur. Nevertheless, bivalves as filter feeders, bioaccumulate contaminants/ pollutants and pathogenic organisms, which, can pose a threat to human health. In this study, analysis of the concentration of *E. coli* in *Ruditapes decussatus* was not made, since it is done under the responsibility of IPMA (Instituto Português do Mar e Atmosfera) on a monthly basis and information made available in its website (www.ipma.pt). As shown in Figure 21, concentrations of *E. coli* in the bivalve tissues covering the sampling period of this work, on an area close to the 2000 E were higher in February and April (3500 MPN/100g), which matched the high *E. coli* values for the stations closer to the discharge (Figure 18). However, these values decayed rapidly further down from the discharge to values below the thresholds according to national and international legislation (300 MPN/ 100 g). No significant correlation (not shown) was found between data from 2000 E of *E. coli* in the water with these data from the bivalves collected at Olhão 4 zone, in Esteiro da Garganta. Even though, it is important to note that the concentrations of *E. coli* in the water is not representative of that within the tissue content- As mentioned before On this bivalve production area the tissue content was always higher than the limit value for bivalves consumption (230 MPN/100g) – Class A but was never higher that 4600 MPN/100g (dashed line) – Class B which, still allows the catch for depuration, transposition or industrial transformation.

From the data acquired, it is suggested that the WWTP did not directly affect the bivalve banks. Even though, in October NT and April ST during LW at 2000 E, values were above 300 MPN/100 mL (Figure16) where bivalve banks occur, which, was already reported by Martins et al. (2004, 2006). So, apparently part of the excessive values at the shellfish production zone (Olhão 4) can derive also from external sources driven from other areas rather than the FO WWTP. In fact, the adjacent production zone Olhão 3 has been prohibited since March 2019 due to microbiological contamination (www.ipma.pt) and may also contaminate this area through circulation.

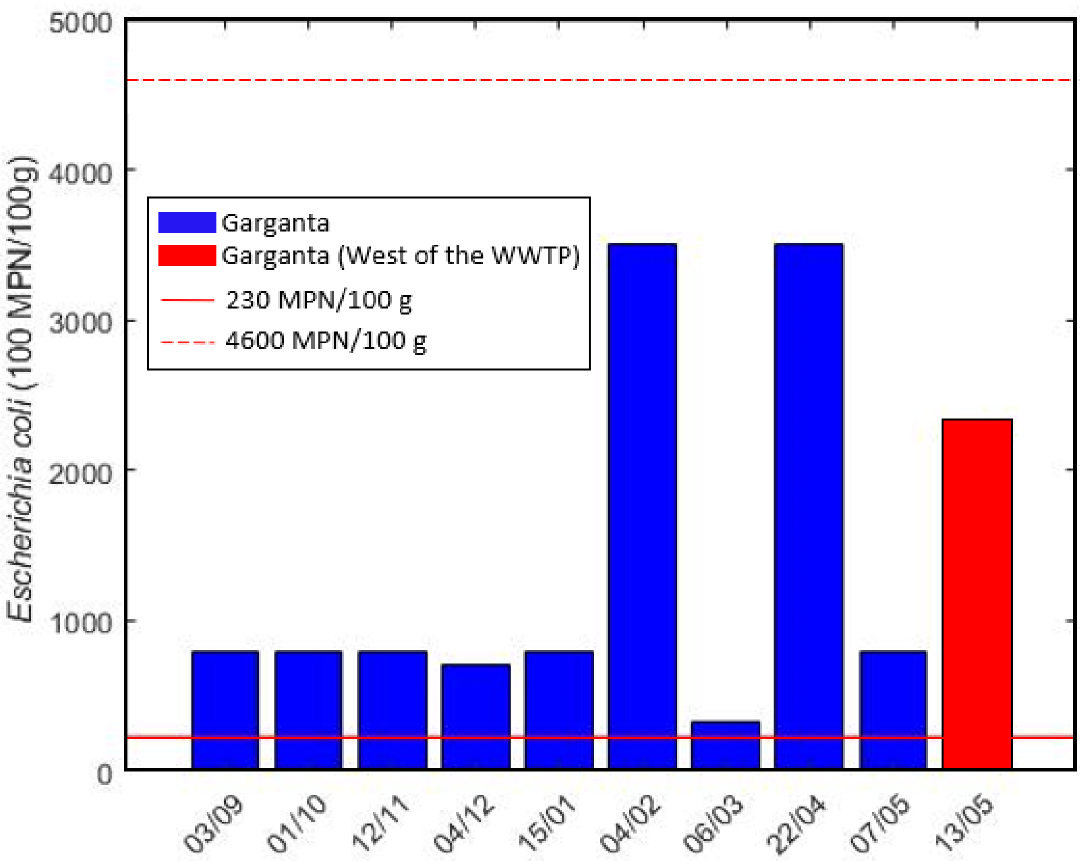

Figure 20. Monthly variation of E. coli *in the tissues of* Ruditapes decussatus *(MPN/100g). The data was retrieved from the monthly reports from IPMA.*

4.3. Comparison with other studies

The Ria Formosa lagoon accounts as an important national bivalve harvesting area, with 75% being harvested in Olhão, 17% in Faro and 8% at Tavira (Cachola, 1986; Cravo et al., 2015). These were the cities with major effluent discharges to Ria Formosa (Cravo et al., 2015). Studies over the impact of wastewater discharges upon water quality, eutrophication and microbial contamination in the Ria Formosa are scarce regardless its major importance as one of the most productive coastal systems in Portugal. From those, the most recent ones are from the authors Pó et al. (2000) that in the beginning of 90's conducted a survey on the microbiological quality of Ria Formosa as recreational waters in a period when the wastewater treatment in this system was just starting. Cabaço et al. (2008) studied the impact of sewage water over seagrass meadows mostly focused on Faro Noroeste WWTP area in the beginning of 2000's. Almeida and Soares (2012) assessed the microbial contamination of bivalves from Ria Formosa over a period of 20 years (1990-2009). Cravo et al., (2015) determined the impact of the five major WWTPs (from Faro, Olhão and Tavira) over water quality for the period 2001-2002, which work was revisited again in 2006-2007 (Cravo et al., 2018). These data will be used for comparison with the present work.

4.3.1. Ria Formosa

Cravo et al. (2015), conducted a study in 2001/02 to evaluate the sewage footprint at the five main STP discharging in the Ria Formosa. In this study was concluded that the WWTPs with more impact over the water quality was Faro Nascente (in the same place of the new Faro-Olhão WWTP). In Table 6 it is performed a comparison between data from this WWTP and the present study in the new FO WWTP. Overall, the present study shows relatively similar values for nutrients (NH_4^+, NO_3^-, PO_4^{3-}, SiO_4^{4-}), chlorophyll α, *E. coli*, N/P ratio and NH_4^+/TIN ratio (Table 6) than in 2001/2002. By that time, the accumulated rainfall (and land runoff) was about 2 to 3 times higher, during late fall and winter also increasing nutrient concentrations in the study area. Yet, concentrations were slightly lower than the highest values found in the present study. This fact can be ascribed to the increase in the outflow of the WWTP in about 40% since November 2018, which augmented the input of nutrients and other contaminants/pollutants to the study area.

Table 6 – Range and mean values for LW and HW for the present study (2018 - 2019) and for Cravo et al., 2015 study (2001/2002) of temperature, salinity (S), pH, dissolved oxygen (DO), percentage of saturation (%DO), ammonium (NH_4^+), nitrate (NO_3^-), ammonium to total inorganic nitrogen ratio (%NH_4^+/TIN), phosphate (PO_4^{3-}), nitrogen to phosphorus ratio (N/P), silicate (SiO_4^{4-}), chlorophyll a *(Chl* a*) and* Escherichia coli (E. coli).

		2018-2019		2001	
		LW	HW	LW	HW
Temperature (°C)	Range	9.8 - 28.3		14.1 - 30.9	
	Mean	**20.0**	**18.5**	**21.8**	**20.7**
S	Range	13.5 - 37.3		19.3 - 36.9	
	Mean	**33.9**	**36.1**	**33.8**	**35.1**
pH	Range	7.6 - 8.3		7.31 - 8.12	
	Mean	**7.9**	**8**	**7.86**	**7.9**
DO(mg/L)	Range	2.9 - 11.3		3.07 - 11.9	
	Mean	6.5	6.6	6.63	6.98
%DO	Range	35 - 173		47 - 188	
	Mean	**86**	**86**	**89**	**94**
NH4+ (µM)	Range	0.1 - 241		n.d - 219.3	
	Mean	**96.2**	**43.3**	**55.6**	**28.3**
NO3- (µM)	Range	0.1 - 12.9		0.08 - 35.8	
	Mean	**5.9**	**2.9**	**5.0**	**3.2**
%NH4/NIT	Range	3.0 - 99.0		0 - 98	
	Mean	**86**	**77**	**79**	**76**
PO43- (µM)	Range	0.1 - 72.8		0.12 - 61.9	
	Mean	**9.2**	**2.7**	**9.5**	**4.3**
N/P Ratio	Range	1.2 - 58.1		2.2 - 33.8	
	Mean	**21.9**	**20.2**	**8.2**	**7.9**
SiO44- (µM)	Range	1.1 - 138.1		2.1 - 134.9	
	Mean	20.8	8.1	23.8	11.7
Chl *a* (µg/L)	Range	n.d - 352.4		n.d - 309.7	
	Mean	**12.2**	**3.2**	**9.7**	**2.6**
E. coli	Range	10 - 3.11E+05		10 - 4.60E+04	
	Mean	**1.85E+04**	**1861**	**1859**	**554**

However, after May the nutrients and *E. coli* concentrations decreased evidently apparently due the stabilization of microbiological treatment (NEREDA) and beginning of disinfection by UV lamps. Nevertheless, TRIX results (Table 3) for the present study show a 'Poor' quality down to 500 m from the discharge and a 'Moderate' quality until 1250 m. Cravo et al. (2015), found that FN exhibited a 'Poor' quality down to 1050 m from the discharge with a daily effluent of ~10 600 m^3/day, which is lower than the current outflow (14 000 m^3/day implying an improvement of the water quality in the study area since the last study. Moreover, this study only includes 9 months, including the period of the operational starting of the new FO WWTP while Cravo et al. (2015), encompasses 21 months (May 2001 to December 2002), being more representative of the global characteristics of the study area for almost 2 years. In 2006/2007, Cravo et al. (2018) revaluated the water quality in the same study area. By that time an improvement of the water quality was observed in comparison to 2001/2002, where the highest impact was found down to 1000 m eastward from the discharge point having a 'Bad' (Eutrophic) classification until 750 m from the discharge, which is still higher than for the present study.

Nutrients and chlorophyll α at the main inlets of other studies (Cravo et al., 2013, 2014 and 2019) are compared with stations closest to the discharge. There, nutrient concentrations reached up to 2-20 times higher than those at the main inlets, away from the influence of sewage, like at 1750 W. For chlorophyll *a*, values were increased up to 2-40 times than values at the inlets where values are relatively similar to those at the reference station (1750 W). This confirms that the reference station has a negligible influence from the WWTP.

Pó (2000) in the late 90's, over a 24-month survey, assessed microbiological pollution in a channel of Ria Formosa, close to Faro city at three different stations, with different degrees of pollution from the discharge of urban effluents, but not so close as in the present study. In this study, a gradient with decreasing values with increasing distance from the influence of the discharge was noticed as verified in the present study. However, the maximum value of coliphages in the former study was 9.2×10^3 cfu/100 mL, 2 orders of magnitude lower than that measured the present work, at the closest stations to the discharge point (10^5).

As microbiological pollution in Ria Formosa is a major problem, especially near areas where bivalve harvesting occurs, Almeida and Soares (2012), evaluated the evolution of faecal contamination in bivalves from Ria Formosa over 20 years (1990 – 2009). In the 90's microbiological pollution was highest but decreased due to increase and improvement of WWTP in the Ria Formosa in the 2000's. In this work, site 5 (including Faro) where Faro Nascente WWTP is located, was the one of the most impacted places. However, such impact was not only related to the WWTP but also to other urban effluents. For this study, *E. coli* content in the intervalvar liquid and tissues of the bivalves was classified with C (>4600 MPN/100 g and ≤46 000 MPN/100 g), which, is higher than the data available from IPMA site during the present study, when, the classification of nearest bivalve production got class B (> 230 MPN/100 g and ≤ 4600 MPN/100 g; Figure 21).

Botelho (2015) assessed nutrient in waters and *E. coli* contamination on clams during few months in between November 2010 and October 2011. In this study, site F (near Faro-Olhão WWTP) was the one registering the high level of contamination. Also, a seasonal variation was noticed, showing that during autumn and winter contamination was enhanced due to heavy rainfall, which agrees with increasing values of *E. coli* during that period at the present study (Figure 20). During summer, site F did not decrease evidently in comparison to other areas, which was attributed to not effective germicide solar radiation to minimise microbiological inputs from the effluents from the WWTP.

Cabaço (2008) conducted a study in Faro Noroeste WWTP on a seasonal basis from July 2001 to May 2002 where the range of concentrations of nutrients is similar to those obtained at the present study. An evident decreasing spatial gradient, with the increase of distance was also found due to the efficient mixing with seawater.

4.3.2. Other lagoonal systems

To compare Ria Formosa with other similar coastal lagoons worldwide, the mean values for LW during NT conditions were chosen were used to reflect the worst-case scenario and the most stressful conditions for Ria Formosa.

Ria de Aveiro lagoon influenced by riverine input from Rio Vouga, is located in northern Portugal, holds considerable regional importance and supports vast activities economically important such as salt-production and aquaculture (Lopes et al., 2007), like Ria Formosa. In the early 2000's, Lopes et al. (2007) evaluated the nutrient dynamics to better understand the seasonal succession of phytoplankton assemblages. For this study, like recorded in Ria Formosa, nutrients registered the highest concentrations during low water along with significant inverse linear relationship with salinity. Nitrate was the predominant form of N (67%), registering the highest concentration (mean 442 µM) as well as phosphate (mean 18 µM) at the station affected by industrial effluents and domestic sewage from a WWTP, similarly to that found in the present study. It was noticeable a seasonal variability, with a strong increase in the concentrations in late autumn to winter followed by a decrease towards spring also affected by the riverine and runoff contribution. In the present case, this trend was not so evident since the FO WWTP was in the initial phase of operation (6-7 months), starting to stabilize the biological treatment after April 2019.

In other coastal lagoons in Europe, most studies assessing the impact of WWTP on lagoon systems do not consider integrative approaches of various types of parameters. These are restricted to either nutrients, DO, Chl α or merely to bacteriological indicators. In addition, in most of these studies, sampling stations are located far from the direct influence of the WWTP discharge, which turns the comparisons difficult. In Europe, most coastal lagoons are located in the Mediterranean and in consequence are microtidal, from which Mar Menor is one example. García-Pintado (2007), studied the input of anthropogenic sources of nutrients into this coastal lagoon under the influence of a WWTP with secondary treatment. In this study, in the vicinity of the WWTP, mean values for NH_4^+ were in the same order of magnitude as of the present study. In Mar Menor lagoon the seasonal variability was evident, particularly during summer months, associated with the population increase that led to an increased outflow from the WWTP, along with agricultural contribution, that was greater than in the present study. Regarding the NO_2^- and NO_3^-concentrations were higher at Mar Menor lagoon.

Perini et al. (2015) evaluated the temporal patterns of *Escherichia coli* distribution in Venice lagoon at two sampling sites (not directly under the influence of direct WWTP discharge) in function of the tidal forcing. Venice lagoon is microtidal with a semidiurnal regime, exchanging water with the Adriatic. These authors noticed that *E. coli* concentrations were in antiphase with the tidal height, recording maximum peaks during LW at both sites, decreasing during HW due to exchange of water from the Adriatic Sea even under a microtidal regime. In this study, *E. coli* ranged from $2.27 \pm 0.19 \times 10^3$ to $1.54 \pm 0.22 \times 10^4$, which values are within a similar magnitude as in the current study. This fact can be due to the microtidal regime in Venice lagoon not promoting an efficient dilution effect. Regardless that, at FO study area higher maximal values were registered ascribed to the direct influence of the WWTP discharge. Also in Venice lagoon Canu (2003) studied the importance of physical forcing of tides upon ecosystem variation through the application of a model, showing an important role of tidal mixing in the improvement in water quality at areas close to urban inputs, as shown in the present work.

Conceição lagoon is a small microtidal lagoon in Brazil with an average depth of 1.7 m covering an area of 9.9 km^2 (Cabral et al., 2019). The lagoon has a microtidal regime with negligible exchanges with the adjacent ocean. Cabral (2019) found symptoms of eutrophication in the lagoon near areas with high dissolved inorganic phosphorus input coming from raw sewage and fertilizers with maximal values higher in comparison to the present study. The faecal coliforms contamination was also evident, attributed to the presence of wastewater (like the present study) and other point sources such as runoff and untreated sewage. Coliform values in this study were higher near urbanized streams reaching values of 1.9×10^3, which, was lower in comparison to those obtained for FO WWTP (10^5).

At Nador coastal lagoon, on the Moroccan coast, in the Mediterranean basin, Oujidi (2020) in 2017 studied the impact of the watershed on the seasonal variation of the water quality of this lagoon. In this study, water quality was classified as bad for NH_4^+ and very bad for PO_4^{3-}, where values were almost up to 2-fold higher than those obtained at the present study particularly during the summer months, in opposition to the present study and studies from Jacob et al. (2020) and Vu (2020). Water quality in Nador lagoon was poor derived from the anthropogenic pressure on the watershed and land runoff. Concerning DO, presence of hypoxia/ anoxia was not found whereas the minimum value (3.84 mg/L) was similar to the one registered at the present study (3.04 mg/L) in the area receiving the effluent from the WWTP. The main driver of water circulation within the lagoon was the wind. Water quality was improved since the opening of the new inlet in 2011, increasing water exchange with the Mediterranean by tidal flush, despite under a microtidal regime. A greater water quality improvement due to tidal effect was evidenced at the present study since this is mesotidal system.

Seitu Lagoon, is a low mesotidal type lagoon located in Malaysia, which connects to the China Sea through a single inlet. This lagoon is under pressure due to agriculture and aquaculture activities. Zainol (2020) in 2017 conducted a study to evaluate hydrodynamics, nutrient concentrations, and phytoplankton biomass under different tidal and monsoonal events. In this study, nutrient concentrations (NH_4^+, NO_3^-, SiO_4^{4-}, PO_4^{3-}) and Chl *a* exhibited higher value during low water, which complies with the present study. High concentrations in NH_4^+ during monsoonal events were ascribed to land runoff from land activities such agriculture. However, the highest mean NH_4^+ (4.58 µM) and NO_3^- (3.96 µM) concentrations at stations close to a sewage disposal area were much lower when compared to the present study. In this study, tidal and seasonal changes of water quality are highly dependent of monsoonal influence. Tidal current was documented to be stronger during floods than ebbs during the dry season, while the opposite was observed during the monsoon. This oscillation can lead to accumulation of contaminants/ pollutants in the system during the dry season. Nevertheless, the nutrients concentrations can be considered low when compared with the present study under wastewater disposal influence.

Regarding the comparison of Ria Formosa (under a mesotidal regime) with other coastal lagoons it is important to remark the high tidal effect, that during flood to high water period promotes a marked water renewal, as already mentioned, able to decrease significantly the contamination along the study area and with higher water quality than in other coastal lagoons worldwide. It is known that summer months due to increase in tourism represents a greater pressure on the coastal areas including Ria Formosa lagoon reflected on the increase of effluents volume discharging on the receiving waters. Unfortunately, during this study, the summer impact of sewage disposal was not assessed but a later study

(Vu, 2020) in the same area including summer months showed an improvement on water quality by: nutrients uptake by phytoplankton and decrease of bacteriological contamination due to increased germicide effect of solar radiation during the summer months as reported by Botelho et al.(2015).

For the studied period, globally, the most deleterious effects occurred until 750 m from the discharge, until where TRIX index classified the area with a ‘Moderate’ (Mesotrophic) quality. Nevertheless, it improved with increasing distance from the discharge, where further down 1250 m, for both low and high water a good (oligotrophic) quality where harvesting areas of the clam *Ruditapes decussatus* exist, suggesting this resource would be less vulnerable from the impact of the WWTP.

Chapter 5 – Conclusions

- Aiming the importance of *Ruditapes decussatus* to the local economy, the discharge of the WWTP was particularly evident until 750 m from the discharge, while, where bivalve banks occur (2000 E), a very good (Oligotrophic) quality index was obtained. Apparently, the water quality classified through TRIX was not compromising the bivalve production areas. However, TRIX does not contemplate microbiological or toxic algae blooms. Data from IPMA at a site close to 2000 E indicated that throughout the sampling period the concentration of *E. coli* in *Ruditapes decussatus* was always above the allowed value for consumption without depuration (> 230 MPN/100 mL). It is important ti consider that this fact might result from an additional source of contamination driven from the adjacent production area (Olhão 3), from where harvesting has been prohibited since March 2019.

- Amongst the two sections of the study area, the East (E) section was the most affected ascribed to preferential circulation between Esteiro da Garganta (E) and Armona inlet.

- Temporally, it was possible to define three different periods during the sampling period: September-October corresponding to the former WWTP with secondary treatment; November-April corresponding to an unstable treatment period due to the operational starting of the new FO WWTP, when highest and most variable values were found; May, characterized by an overall decrease on the nutrients and microbiological concentrations, consequence of the stabilization of the biological treatment (NEREDA) and UV lamps disinfection.

- The impact of the discharge was most noticeable at LW during neap tide (higher residence time), when the highest concentrations of contaminants were recorded. On other hand, due to an effective tidal effect in Ria Formosa, HW of spring tidal conditions was associated to the lowest wastewater impact due to high hydrodynamic conditions, mixing and high renewal, being responsible for amelioration of the water quality of the study area.

- Comparing with historical data, the new FO WWTP improved since the last studies by Cravo et al. (2015 and 2018).

- As bivalve harvesting is one of the main economic sectors of Ria Formosa, its utmost importance must always be considered. Therefore, further long-term monitoring is required to understand and monitor the impacts of the new Faro-Olhão WWTP over this sensitive resource.

Chapter 6 – Bibliographic references

Águas do Algarve, (2019). *ETAR de Faro/ Olhão.* Retrieved from https://www.aguasdoalgarve.pt/content/etar-de-faroolhaodata

Almeida, C., & Soares, F. (2012). Microbiological monitoring of bivalves from the Ria Formosa Lagoon (south coast of Portugal): A 20years of sanitary survey. *Marine Pollution Bulletin*, *64*(2), 252–262.

Andrade, C. (1990). O ambiente de barreira da Ria Formosa (Algarve-Portugal). Tese de Doutoramento. Faculdade de Ciências de Lisboa, Lisboa. 645p.

Baptista, F.M. (2007). Assessment of the aquacultural potential of the Portuguese oyster *Crassostrea angulata*. Dissertação de doutoramento em Ciências do Meio Aquático. Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Porto. 245p.

Barbosa, A. B. (2010). Variability of Planktonic Microbes in a Mesotidal. *Coastal Lagoons: Critical Habitats of Environmental Change*, 335–366.

Barnes, R. S. K. (1980). Coastal Lagoons. Cambridge University Press, Cambridge, pp106.

Basset, A., Sabetta, L., Fonnesu, A., Mouillot, D., Do Chi, T., Viaroli, P., Giordani, G., Reizopoulou S., Abbiati, M. & Carrada, G.C. (2006). Typology in Mediterranean transitional waters: new challenges and perspectives. Aquatic Conservation: *Marine and Freshwater Ecosystems, 16*, 441–455.

Bebianno, M.J. (1995). Effects of pollutants in the Ria Formosa lagoon. Sci. Total Env. 171, 107-115.

Botelho, M. J., Soares, F., Matias, D., & Vale, C. (2015). Nutrients and clam contamination by *Escherichia coli* in a meso-tidal coastal lagoon: Seasonal variation in counter cycle to external sources. *Marine Pollution Bulletin*, *96*(1–2), 188–196.

Brito, A. C., Quental, T., Coutinho, T. P., Branco, M. A. C., Falcão, M., Newton, A., Icely, J., & Moita, T. (2012). Phytoplankton dynamics in southern Portuguese coastal lagoons during a discontinuous period of 40 years: An overview. *Estuarine, Coastal and Shelf Science*, *110*(January), 147–156.

Cabaço, S., Machás, R., Vieira, V., & Santos, R. (2008). Impacts of urban wastewater discharge on seagrass meadows (*Zostera noltii*). *Estuarine, Coastal and Shelf Science*, *78*(1), 1–13.

Cabral, A., Bercovich, M. V., & Fonseca, A. (2019). Implications of poor-regulated wastewater treatment systems in the water quality and nutrient fluxes of a subtropical coastal lagoon. *Regional Studies in Marine Science*, *29*, 100672.

Cachola, R.A. & Campos, C.J.A. (2006). Redefinição das zonas de produção de bivalves nos sistemas lagunares do Algarve no âmbito do programa de monitorização microbiológica. *Relat. Cient. Téc.Inst. Invest. Pescas Mar*, Nº 31.

Cachola, R. & Lima, C. (1984). Qualidade da água e dos recursos vivos da costa algarvia (verão 1984). *Relatórios do Instituto Nacional de Investigação das Pescas, 38*, 1-15.

Canu, D.M., Solidoro, C. & Umgiesser, G., (2003). Modelling the responses of the Lagoon of Venice ecosystem to variations in physical forcings. *Ecological Modelling 170*, 265-289.

Carafa, R., Marinov, D., Dueri. S., Wollgast, J., Ligthart, J., Canuti, E., Viaroli, P. & Zaldívar, J.M. (2006). A 3D hydrodynamic fate and transport model for herbicides in Sacca di Goro coastal lagoon (Northern Adriatic). *Mar Pollut Bull 52*(10):1231–1248.

Crassosado (2016). Estado atual da ostra-portuguesa (*Crassostrea angulata*) no estuário do Sado, ameaças e oportunidades para a sua exploração como recurso. Projeto Crassosado, Rel. final, financiado pela Portucel S.A. 98p.

Cravo, A., Cardeira, S., Pereira, C., Rosa, M., Alcântara, P., Madureira, M., Rita, F., Luis, J. & Jacob., J. (2014). Exchanges of nutrients and chlorophyll a through two inlets of Ria Formosa, South Portugal, during Coastal upwelling events. *Journal of Sea Research, 93*, 63-74.

Cravo, A., Fernandes, D., Damião, T., Pereira, C., & Reis, M. P. (2015). Determining the footprint of sewage discharges in a coastal lagoon in South-Western Europe. *Marine Pollution Bulletin, 96*, 197-209.

Cravo, A., Ferreira, C., & Jacob., J., (2018). Water quality improvement in Ria Formosa since the early 2000? In: Sanitation Approaches and Solutions and The Sustainable Development Goals. Editors: J. Saldanha Matos and M. João Rosa. EWA – European Water Association, APESB – Associação Portuguesa de Engenharia Sanitária e Ambiental.

DGRM, (2014). Plano estratégico para aquicultura portuguesa 2014-2020. Governo de Portugal. Ministério da agricultura e do mar.

Diaz, R.J., Rosenberg, R., (1995). Marine Benthichypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. *Oceanogr. Mar. Biol. 33*, 245–303.

DRPASul (Direcção Regional das Pescas e Aquicultura do Sul), (2006). Volume II - Caracterização e Diagnóstico. Pescas no Sul Diagnóstico. 1976/2006: 30 anos ao serviço das pescas no Algarve, 18 pp.

Duarte, P., & Azevedo, B. (2005). Hydrodynamic Modelling of Ria Formosa (South Coast of Portugal) with EcoDynamo University Fernando Pessoa , Centre for Modelling and Analysis of Environmental Systems January 2005. January.

Fabião, J., Rodrigues, M., Fortunato, A.B., Jacob, J., & Cravo, A. (2016). Water exchanges between a multi-inlet lagoon and the ocean: the role of forcing mechanisms. *Ocean Dynamics, 66*, 173-194.

Falcão, M., & Vale, C. (1990). Study of the Ria Formosa ecosystem: benthic nutrient remineralization and tidal variability of nutrients in the water. *Hydrobiologia*, *207*(1), 137–146.

Falcão, M., Fonseca, M., Serpa, D., Matias, D., Joaquim, S., Duarte, P., Pereira, A., Martins, C., & Guerreiro, J. (2003). Development of an Information Technology Tool for the Management of European Southern Lagoons under the influence of river-basin runoff, EVK3-CT-20022-00084 (DITYY Project).

Ferreira, J. G., Bettencourt, A., Bricker, S. B., Marques, J. C., Newton, A., Nobre, A., Salas, F., Silva, M. C., Simas, T., Soares, C. V, Stacey, P., Vale, C., & Wolff, W. J. (2005). *Water Framework Directive – Transitional and Coastal Waters Proposal for the definition of water bodies*. 38.

Ferreira, J. G., Nobre, A. M., Simas, T. C., Silva, M. C., Newton, A., Bricker, S. B., Wolff, W. J., Stacey, P. E., & Sequeira, A. (2006). A methodology for defining homogeneous water bodies in estuaries - Application to the transitional systems of the EU Water Framework Directive. *Estuarine, Coastal and Shelf Science*, *66*(3–4), 468–482.

Ferreira, J.G., Saurel, C., Nunes, J.P., Ramos, L., Lencart e Silva, J.D., Vazquez, F., Bergh, Ø., Dewey, W., Pacheco, A., Pinchot, M., Ventura Soares, C., Taylor, N., Taylor, D., Verner-Jeffreys, J., Baas, J., Petersen, J.K., Wright, J., Calixto, M., Rocha, M. (2012). Framework for Ria Formosa Water Quality, Aquaculture, and Resource Development, Lisbon, Portugal, 110p.

Hachich, E., Di Bari, M., Christ, A., Lamparelli, C., Ramos, S. and Sato, M. (2012). Comparison of thermotolerant coliforms and *Escherichia coli* densities in freshwater bodies. *Brazilian Journal of Microbiology*, *43*(2), pp.675-681.

Ishii, S., & Sadowsky, M. J. (2008). *Escherichia coli* in the environment: Implications for water quality and human health. *Microbes and Environments*, *23*(2), 101–108.

Jacob, J., Correia, C., Torres, A.F., Xufre, G., Matos, A., C. Ferreira, Reis, M.P., Caetano, S., Freitas, C., Barbosa, A.B. and Cravo, A., (2020). Impacts of decommissioning and upgrading urban wastewater treatment plants on the water quality in a shellfish farming coastal lagoon (Ria Formosa, South Portugal). In: Malvárez, G. and Navas, F. (eds.), *Global Coastal Issues of 2020*. Journal of

Coastal Research, Special Issue No. 95, pp. 45–50. Coconut Creek (Florida), ISSN 0749-0208.

Jacob, J., & Cravo, A. (2019). Recent evolution of the tidal prisms at the inlets of the western sector of the Ria Formosa, south coast of Portugal. *Regional Studies in Marine Science*, *31*, 100767.

Kennish, M.J., & Paerl, H.W. (2010). Coastal Lagoons Critical Habitats of Environmental Change.

Kjerfve, B. (1986). Comparative oceanography of coastal lagoons. Pp. 63–81, In: D. A. Wolfe (ed.), *Estuarinevariability*. New York: Academic Press.

Kjerfve, B. (1994). Coastal lagoons. Pp. 1–8, In: B. Kjerfve (ed.), *Coastal lagoon processes*. Amsterdam: Elsevier.

Leote, C., Epping, R.H.J., (2015). Sediment–water exchange of nutrients in the Marsdiep basin, western Wadden Sea: phosphorus limitation induced by a controlled release? *Cont. Shelf Res. 92*, 44–58.

Loureiro, S., Newton, A., & Icely, J. (2006). Boundary conditions for the European Water Framework Directive in the Ria Formosa lagoon, Portugal (physico-chemical and phytoplankton quality elements). *Estuarine, Coastal and Shelf Science*, *67*(3), 382–398.

Magalhães A., Vicente, M., Pestana, R. (2006). Guia de Boas Práticas em Moluscicultura. Ed. Animação local para o desenvolvimento e criação de emprego na Ria Formosa, Olhão. 154 pp.

Malhadas, M.S., Neves, R., Leitão, P.C. & Silva, A. (2010). Influence of tide and waves on water renewal in Óbidos Lagoon, Portugal. *Ocean Dyn, 60*, 41–55.

Mallin, M.A., Williams, K.E., Esham, E.C., Lowe, R.P., 2000. Effect of human development on bacteriological water quality in coastal watersheds. *Ecol. Appl., 10*(4), 1047–1056.

Martins, F., Reis, M. P., Neves, R., Cravo, A. P., Brito, A., & Venâncio, A.

(2004). Molluscan Shellfish Bacterial Contamination in Ria Formosa Coastal Lagoon:AModellingApproach. *Journal of Coastal Research SI SI*, *39*(39), 1551–1555.

Mudge, S. M., & Bebianno, M. J. (1997). Sewage contamination following an accidental spillage in the Ria Formosa, Portugal. *Marine Pollution Bulletin*, *34*(3), 163–170.

Mudge, S. M., & Duce, C. E. (2005). Identifying the source, transport path and sinks of sewage derived organic matter. *Environmental Pollution*, *136*(2), 209–220.

Newton, A. (1995). *The water quality of Ria Formosa lagoon, portugal*. School of Ocean Sciences, PhD Thesis, University of North Wales, Bangor.

Newton, A., Icely, J. D., Falcao, M., Nobre, A., Nunes, J. P., Ferreira, J. G., & Vale, C. (2003). Evaluation of eutrophication in the Ria Formosa coastal lagoon, Portugal. *Continental Shelf Research*, *23*(17–19), 1945–1961.

Oliveira, A., Rodrigues, M., Guerreiro, M., Fortunato, A.B., & Bruneau, N. (2011). Impact of inlet morphology on the 3D water renewal and residence times of a small coastal stream. *J Coast Res Spec Issue 64*,1555– 1559.

Owili, M. A. (2003). Assessment of Impact of Sewage Effluents on Coastal Water Quality in Hanfarfjordur, Iceland. Kenya Marine and Fisheries Research Institute.

Pacheco, A., Williams, J. J., Ferreira, Ó., Garel, E., & Reynolds, S. (2011). Applicability of sediment transport models to evaluate medium term evolution of tidal inlet systems. *Estuarine, Coastal and Shelf Science*, *95*(1), 119–134.

Perini, L., Quero, G. M., Serrano García, E., & Luna, G. M. (2015). Distribution of *Escherichia coli* in a coastal lagoon (Venice, Italy): Temporal patterns, genetic diversity and the role of tidal forcing. *Water Research*, *87*, 155–165.

Pó, L., Rheinheimer, G., & Borrego, J. J. (2000). Microbiological pollution of Ria Formosa (South of Portugal). *Marine Pollution Bulletin*, *40*(2), 186–193.

Price, R., & Wildeboer, D. (2017). *E. coli* as an Indicator of Contamination and Health Risk in Environmental Waters. *Escherichia Coli* - Recent Advances On Physiology, Pathogenesis And Biotechnological Applications.

Serpa, D., Jesus, D., Falcão, M., Cancela da Fonseca, L., (2005). Ria Formosa ecosystem: socio-economic approach. *Relatórios Científicos e Técnicos IPIMAR*, *28*, 50 pp.

Ramesh, R., Chen, Z., Cummins, V., Day, J., D'Elia, C., Dennison, B., Forbes, D. L., Glaeser, B., Glaser, M., Glavovic, B., Kremer, H., Lange, M., Larsen, J. N., Le Tissier, M., Newton, A., Pelling, M., Purvaja, R., & Wolanski, E. (2015). Land-Ocean Interactions in the Coastal Zone: Past, present & future. *Anthropocene*, *12*(January), 85–98.

Reed, M. L., Pinckney, J. L., Keppler, C. J., Brock, L. M., Hogan, S. B., & Greenfield, D. I. (2016). The influence of nitrogen and phosphorus on phytoplankton growth and assemblage composition in four coastal, southeastern USA systems. *Estuarine, Coastal and Shelf Science*, *177*, 71–82.

Roselli, L., Cañedo-Argüelles, M., Costa Goela, P., Cristina, S., Rieradevall, M., D'Adamo, R., & Newton, A. (2013). Do physiography and hydrology determine the physico-chemical properties and trophic status of coastal lagoons? A comparative approach. *Estuarine, Coastal and Shelf Science*, *117*, 29–36.

Salles, P. (2001). Hydrodynamic controls on multiple tidal inlet persistence. PhD Thesis, Massachusetts Institute of Technology and Woods Hole Oceanographic Institution, 272 pp.

Salles, P., Voulgaris, G. & Aubrey, D. (2005). Contribution of Nonlinear Mechanisms in the Persistence of Multiple Tidal Inlet Systems. *Estuarine Coastal and Shelf Science*, *65*, 475–491.

Spagnoli, F., Specchiulli, A., Scirocco, T., Carapella, G., Villani, P., Casolino, G., Schiavone, P., & Franchi, M. (2002). The lago di Varano: Hydrologic characteristics and sediment composition. *Marine Ecology, 23*, 384-394.

Specchiulli, A., Scirocco, T., Cilenti, L., Florio, M., Renzi, M., & Breber, P. (2008). Spatial and temporal variations of nutrients and chlorophyll a in a Mediterranean coastal lagoon: Varano lagoon, Italy. *Transitional Waters Bulletin*, *2*(4), 49–62.

Son, N.T., & Fleet, G.H. (1980). Behavior of pathogenic bacteria in the oyster, *Crassostrea* commercialis, during depuration, re-laying, and storage. *Applied and Environmental Microbiology, 40*(6), 994–1002.

Stanhope, J. W., Anderson, I,.C., & Reay, W.G. (2009). Base flow nutrient discharges from lower Delmarva Peninsula watersheds of Virginia, USA. *J. Environ. Qual*. *38*,2070–2083.

Tchobanoglous, G., Burton, F., & Stensel, H. (2004). *Wastewater engineering.* Boston: McGraw-Hill.

Tett, P., Gilpin, L., Svendsen, H., Erlandsson, C. P., Larsson, U., Kratzer, S., Fouilland, E., Janzen, C., Lee, J. Y., Grenz, C., Newton, A., Ferreira, J. G., Fernandes, T., & Scory, S. (2003). Eutrophication and some European waters of restricted exchange. *Continental Shelf Research*, *23*(17–19), 1635–1671.

UNESCO, (2009). *Coastal eutrophication: linking nutrient sources to coastal ecosystem effects and management: news2use.*

Villani, P., Carapella, G., Scirocco, T., Specchiulli, A., Maselli, M., Schiavone, R., Spagnoli, F., Marolla, V., Casolino, G., Franchi, M., Schiavone, P., & Deolo, A. (2000). *Progetto Integrato di Recupero e Riqualificazione della Zona Umida della Laguna di Varano*. Technical Report Consorzio ELTCON, Roma, Italy.

Vu, C. (2020). IMPACT OF URBAN WASTEWATER EFFLUENTS UPON THE WATER QUALITY OF RIA FORMOSA. CASE STUDIES OF FARO-OLHÃO

AND OLHÃO POENTE WASTEWATER TREATMENT PLANTS. IMBRSEA Master thesis. UAlg.

Zang, C., Huang, S., Wu, M., Du, S., Scholz, M., Gao, F., Lin, C., Guo, Y. and Dong, Y., (2010). Comparison of Relationships Between pH, Dissolved Oxygen and Chlorophyll *a* for Aquaculture and Non-aquaculture Waters. *Water, Air, & Soil Pollution, 219* (1-4), pp.157-174.