Diana Patrícia Silva

Water quality of the Ria Formosa at the vicinity of Almargem Urban Wastewater Treatment Plant and shellfish beds

Universidade do Algarve
Faculdade de Ciências e Tecnologia

2020

Diana Patrícia Silva

Water quality of the Ria Formosa at the vicinity of Almargem Urban Wastewater Treatment Plant and shellfish beds

Marine and Coastal Systems Master

Work under the supervision of:

Professor Doctor Alexandra Cravo

(Assistant professor, Universidade do Algarve)

Universidade do Algarve

Faculdade de Ciências e Tecnologia

2020

Water quality of the Ria Formosa at the vicinity of Almargem Urban Wastewater Treatment Plant and shellfish beds

Declaration of authorship of the work

I declare to be the author of this work, which is original and unprecedented. Consulted authors and works are rightfully cited in the text and are included in the list of references.

Faro, 02 of December of 2020

Diana Patrícia Silva

Acknowledgements

To Professor Dr. Alexandra Cravo, scientific advisor for this dissertation, thank you for all the support, scientific knowledge you have given me and opportunities you have given me along this path. It was certainly a pleasure to have you as an advisor and to work with you. Thank you for helping me find a passion in chemical oceanography, for supporting and advising me. You truly are an inspiration for me, academically and in life. Thank you so much for everything.

To Cátia, André and Flor, for all the transmitted knowledge, all the laughs, help and support all the way from the beginning to the end of this master thesis. Thank you for putting up with me and my unwillingness to clean the sampling bottles and macerating the chlorophyll filters, and 'forcing' me to do it anyways.

To my friends Daniela, Maria and Rita, for always supporting me, even when they did not understand my life decisions. Thank you for not letting me give up on my education and for believing in my potential, sometimes more than I believed myself. All in the same scientific area, but in very different fields, supporting each other.

To my best friend, Rita, and her family, that treated me like one of them. For not letting me give up and leave everything I worked for behind. Thank you for supporting me even though there more than 360 km separating us, and we only get to see each other twice a year.

To my mom and brother, for the unconditional love and support, for everything that had to be sacrificed for me to have a higher education and be the first in my family to have a bachelor and a master's degree. For believing that I could become a 'ocean doctor' as I wanted to be when I was 5 years old, for all the "even if everything does not work how you want, we will figure something out, even if you fail, you can always come back home". For bearing with the distance, the financial and health struggle that these 5 years was to our family. For being proud of me, even though you do not understand anything that I am doing/working on.

Thank you to all, for all the help, support and comprehension in this journey! It would have been extremely hard or even impossible without you.

Abstract

Coastal lagoons are very productive and economically important ecosystems that suffer several anthropogenic pressures, including from urban wastewater treatment plants (UWWTP) discharges. This last pressure can induce nutrient enrichment and development of primary producers (toxic or not), microbiological contamination of the water or even eutrophication that ultimately will have impact on the organisms living nearby and on their salubrity.

Ria Formosa lagoon is a coastal lagoon on the Portuguese south coast responsible for over 90% of the Portuguese shellfish production, however along its extension it suffers influence from several UWWTP discharging in this system, some of them in in the vicinity of the production areas. Since in Tavira there is an important area of shellfish production close to the Gilão estuary it is important to determine the impact of the Almargem UWWTP upon the water quality of the receiving waters and to evaluate if there is any influence of it on the shell production area.

The main goals of this work were: i) to determine the spatial extent of the influence of the Almargem UWWTP along the gradient of dispersal of the effluents in the receiving waters, potential impact on the bivalves production area, and the compliance of data with the applicable legislation; ii) to determine the dilution effect within the study area associated with the daily tidal cycles; iii) to determine the effect of different tidal ranges upon the water quality; iv) to determine the seasonal influence of the sewage discharge impact upon the water quality; v) to assess the temporal evolution of the chemical and microbiologic contamination of the water quality in the study area and vi) to calculate a trophic index (TRIX) in the study area, defining a baseline for Almargem UWWTP.

The results showed that the Almargem UWWTP clearly influence the water characteristics till 750 m downstream from the UWWTP discharge. In August and February the *E. coli* concentration was above the limit imposed for the discharge license, both by increased anthropogenic pressure and land runoff after a rainfall event, respectively. The TRIX index varied between eutrophic and oligotrophic conditions, with a lower water quality upstream in the Almargem channel until 1000 m during the summer months. The highest impact on the water quality was registered during low water of neap tides, associated with a higher residence time. The tidal effect and the water renewal are able to decrease contamination, helping to the improve the water quality as observed at 1750 m, considered the reference station and in consequence caused no impact over the shellfish production area. In this area the microbiological contamination recorded suggest that there were external sources at the Gilão low estuary. However, for most of the sampling period, the salubrity of bivalves controlled by IPMA was of class B that can be harvested and sent to depuration, not but negatively affect their production.

Keywords: Coastal lagoons, Ria Formosa, Urban Wastewater Treatment Plants, Water Quality, Shellfish beds

Resumo

As lagoas costeiras são ecossistemas muito produtivos e economicamente importantes e por isso são altamente povoadas e como tal sofrem várias pressões antrópicas. Por serem áreas muito produtivas, as lagoas costeiras são fortemente utilizadas para a aquicultura, em particular para a produção de bivalves. Os impactos das pressões podem ser causados por fontes não pontuais, como escoamento agrícola, ou fontes pontuais, tais como as descargas de estações de tratamento de águas residuais urbanas (ETAR). Esta última pode induzir ao enriquecimento de nutrientes e crescimento de produtores primários (tóxicos ou não), contaminação microbiológica da água ou mesmo eutrofização que acabará por afetar os organismos que vivem nas proximidades e a sua salubridade. Contudo, a extensão destes impactos depende dos padrões de circulação de água e da hidrodinâmica do sistema.

A Ria Formosa é uma lagoa costeira localizada no sul de Portugal altamente produtiva e responsável por mais de 90% da produção nacional de bivalves, mas que ao longo da sua extensão sofre a influência de descarga de efluentes de várias de ETAR. Dada a importância da Ria Formosa, as ETAR mais importantes para este sistema contam com um tratamento secundário dos resíduos e desinfeção final do efluente por radiação ultravioleta. Destas destaca-se a ETAR de Almargem, que serve maioritariamente a população de Tavira. Devido ao reduzido número de estudos realizados nesta área da Ria Formosa com intuito de compreender os impactos da ETAR na qualidade da água, incluindo em simultâneo a caracterização química, fitoplanctónica e microbiológica em áreas de produção de bivalves, é muito importante estabelecer a situação atual e compreender a extensão espacial e variabilidade temporal da influência da descarga desses efluentes nesta zona da Ria Formosa. Tavira representa 10% da produção de bivalves da Ria Formosa e tendo em conta que essa área de produção de bivalves se situa no baixo estuário do rio Gilão é importante determinar não só qual a influência descarga da ETAR de Almargem na qualidade das águas recetoras, mas também se tem alguma influência sobre a qualidade da água na área de produção de bivalves.

Os principais objetivos deste trabalho foram: i) determinar a extensão espacial da influência da descarga da ETAR de Almargem na qualidade de água ao longo do gradiente de dispersão dos efluentes nas águas recetoras, o potencial impacto na área de produção de bivalves e a conformidade dos dados com a legislação aplicável; ii) determinar o efeito de diluição na área de estudo associado aos ciclos diários das marés, baixa-mar, quando a influencia da descarga é máxima em comparação com preia-mar, quando a influência da renovação da água do oceano durante a enchente é máxima; iii) determinar o efeito da variação da altura de marés, maré viva (maior taxa renovação da água) e maré morta (maior tempo de residência; iv) determinar a influência sazonal no impacto do escoamento de esgoto na qualidade da água desde o verão - junho de 2019 (quando a influência turística é máxima e os problemas de qualidade da água podem ser agravados) até o inverno -

fevereiro de 2020; v) avaliar a evolução temporal considerando a contaminação química desde 2001 a 2002 e a contaminação microbiológica de 1990 a 2009 da qualidade da água na área de estudo; vi) calcular um índice trófico (TRIX) na área de estudo, comparando-o com os dados históricos de Tavira e definindo a situação de referência para a descarga da ETAR de Almargem.

Os resultados mostraram que a descarga da ETAR de Almargem representa claramente uma fonte de nutrientes e contaminação microbiológica, influenciando as características da água até 750 m a jusante da descarga da ETAR em comparação com a estação de referência a 1750 m da descarga. Aqui as concentrações são baixas e típicas da Ria Formosa devido à alta renovação da água proveniente da entrada de Tavira e à mistura durante a enchente em cada ciclo diário de maré.

Em condições favoráveis de luz solar, temperatura e disponibilidade de nutrientes observou-se o um crescimento fitoplanctónico e de macroalgas no canal de Almargem, com foi observado em julho, altura em que os nutrientes diminuíram acentuadamente pelo consumo dos produtores primários. Nessa altura a qualidade da água expressa pelo índice trófico (TRIX) diminuiu. Em agosto e fevereiro, a concentração do indicador de contaminação fecal *Escherichia coli* ultrapassou o limite imposto pela licença de descarga, valores causados potencialmente por aumento turístico e pelo escoamento superficial após um evento de chuva, respetivamente. O índice trófico (TRIX) variou entre condições eutróficas e oligotróficas, com menor qualidade da água a montante no canal da Almargem até 1000 m, durante os meses de verão, devido ao aumento do fosfato e da elevada concentração de oxigénio dissolvido. Este aumento de fosfato sugere uma entrada desproporcional de fosfato em relação ao azoto, também confirmada por uma diminuição na relação N:P, que também pode sugerir desorção de fósforo nos períodos de temperatura mais elevada.

As piores condições em termos de impactos da descarga da ETAR foram observadas durante a baixa-mar nas marés vivas nos meses de verão, quando a coluna de água tem menos profundidade levando a uma diminuição da capacidade de diluição. Contudo, em preia-mar de maré viva, a renovação da água é máxima capaz de diminuir drasticamente a contaminação, ajudando a melhorar a qualidade da água, sem efeito na estação de referência (1750 m) nem causar impacto na área de produção de bivalves, junto do baixo estuário do rio Gilão. Nesta área, sem influência da ETAR que foi desativada em 2007, a contaminação microbiológica dos bivalves foi em fevereiro, segundo registos do IPMA, sugerindo que existem fontes externas de contaminação microbiológica próxima às áreas de produção de bivalves. Estes valores foram inclusivamente semelhantes aos registados de 1990 a 2009 (quando a ETAR de Tavira ainda estava operacional). Na maioria dos momentos de avaliação da salubridade dos bivalves realizados pelo IPMA a qualidade dos bivalves tem permanecido, globalmente, na classe B, em que os bivalves podem ser apanhados, e depurados antes de serem comercializados para consumo humano, sem afetar negativamente a produção dos mesmos.

A descarga da ETAR de Almargem demostra ter menor impacto nas águas recetoras quando comparados com outras zonas da Ria Formosa, nomeadamente no local de descarga da ETAR de Faro-Olhão, pois a ETAR de Almargem apresenta um menor caudal, maior renovação da água em cada ciclo de maré semidiurno. Quanto à qualidade de água, esta é relativamente semelhante quando comparas com outras lagoas costeiras, tais como a Ria de Aveiro, Ria de Vigo e Mar Menor.

Index

Figure index

Table index

Abbreviation index

ABS – absorbance

aD% - Absolute deviation from oxygen saturation

ASP – Amnesic Shellfish Poisoning

AZP – Azaspiracid Poisoning

C – Carbon

Chl *a* – Chlorophyll *a*

DIN – Dissolved Inorganic Nitrogen

DIP – Dissolved Inorganic Phosphorous

DL – Decree Law

DSP – Diarrhetic Shellfish Poisoning

E. coli - *Escherichia coli*

EC – European Council

Entero – Enterococcus

EU – European Union

FC – Faecal Coliforms

f_{sal} – salinity factor

HW – High Water

IPMA – Instituto Português do Mar e da Atmosfera

LW – Low Water

MPN – Most Probable Number

N – Nitrogen

NH_4^+ - Ammonium

NO_2^- - Nitrite

NO_3^- - Nitrate

NSP – Neurotoxic Shellfish Poisoning

NT – Neap Tide

O_2 – Oxygen

OHI – Overall Human Influence

P – Phosphorus

PCA – Principal Component Analysis

PO_4^{3-} - Phosphate

PSP – Paralytic Shellfish Poisoning

S – Salinity

SDG – Sustainable Development Goals

Si – Silicon

SiO_4^{4-} - Silicate

SS – Suspended Solids

SST – Sea Surface Temperature

ST – Spring Tide

T – Temperature

TC – Total Coliforms

TDL - Theoretical Dilution Line

TRIX – Trophic Index

UN – United Nations

UV – Ultraviolet

UWWTP – Urban Wastewater Treatment Plant

1. Introduction

1.1 Importance of coastal lagoons

Coastal lagoons are aquatic shallow ecosystems, parallel to the coast that connect the continent with the adjacent ocean by one or several entrances or inlets. These represent 13% of the coastal areas worldwide and are shallow having less than 5 m depth, usually ranging from 1-3 m depth (Kjerfve, 1994). Coastal lagoons can be influenced by distinct contribution of river input, wind stress, tides, precipitation-evaporation balance. So, depending on their characteristics, location and climate conditions they can exhibit salinity variance, ranging from hypersaline to fresh water, or different tidal regimes, from macrotidal (>4 m) to microtidal (<2 m) (Kjerfve, 1994). These ecosystems can trap sediments and organic matter, in addition to the enrichment in nutrients coming from land and their shallow water column, which allows sun light to penetrate till the bottom making coastal lagoons highly productive (Kjerfve, 1994).

In Europe this type of ecosystems represents about 5% of the coast (Barners, 1980; Kjerfve, 1994), mostly concentrated along the shores of the Baltic, Black, Caspian and Mediterranean seas (Whitfield, 2011). Most of them are characterized by a microtidal regime (tidal range lower than 2 m) like encountered in Venice Lagoon (Italy), Thau Lagoon (France) in the Mediterranean Sea, but there is also coastal lagoons under mesotidal regime as found in Europe on the Atlantic coast such as the Oder lagoon (Poland-Germany), Ria de Aveiro and Ria Formosa (Portugal) (Razinkovas et al., 2008).

Coastal lagoons have a high biological value for aquaculture and fisheries, as they provide shelter and serve as a nursery for fishes, birds, molluscs, crustaceans, among others. Thus, these ecosystems contribute to a large income of the regional economies, including fisheries, aquaculture, salt extraction, sand extraction, shipping, transportation, electric power generation and many more economic activities (Whitfield, 2011). This socio-economic value led to their occupation and along with the tourism increase made an accelerated urbanization of the coastal areas, which lead to an increase of the anthropogenic pressure in these environments (Kjerfve, 1994; Lloret et al., 2008) and one of the most impacted aquatic ecosystems (Whitfield, 2011). This impact can include habitat alteration, contamination, and/or water quality degradation, mainly caused by point and non-point sources of pollution/contamination and groundwater contaminant inputs (Whitfield, 2011). From those urban wastewater treatment plants has a central role in the contamination of the coastal lagoons. However, the water quality and contamination of the lagoons depends not only on the anthropogenic pressures but also on the lagoon water circulation and dispersal, water exchange with the ocean and residence time of the water (Kjerfve, 1994).

1.2 Urban wastewater treatment plants (UWWTP) impact on coastal lagoons

Urban wastewater treatment plants can have various types of water treatments, such as primary treatment, secondary and tertiary treatment, including disinfection. The primary treatment uses physical processes like sedimentation to remove dissolved and suspended solids (Gay, 1990). The secondary treatment consists in the usage of chemical and biological processes to remove organic matter. This way most solids are removed but not significant amount of nutrients (Mcnulty, 1977; Metcalf & Eddy, 1995). To have a better removal of pathogenic organisms the effluents can be disinfected using ultraviolet radiation or ozonation (Metcalf & Eddy, 1995).

Urban wastewater treatment plants can cause major impacts on water quality of the coastal zones, since effluents enriched in nutrients and organic matter could lead to eutrophication and phytoplankton blooms, potentially toxic (Cloern, 2001). According to Nixon (1995), eutrophication is "an increase in the rate of supply of organic matter to an ecosystem", a high loading of organic matter coupled with a high entrance of macronutrients (phosphorous and nitrogen) in coastal areas results in the increase of primary production (Statham, 2012). With phytoplankton blooms and/or potential development of harmful algae and increase of heterotrophic bacterial activity undesirable disturbance to the balance of organisms' present can occur. One of the problems could be the decrease of the oxygen levels that ultimately may lead to a potential death of organisms (Cloern, 2001).

In addition, effluents from UWWTP can also have indirect impacts on the human health due to microbiological contamination either by direct contact with the water (recreational use) or by consumption of edible resources, like shellfish (Cravo et al., 2015). Sites near the discharge points at inner areas, of low depth and restricted circulation and limited hydrodynamics (that avoid mixing and dilution with adjoining seawater) are more susceptible to eutrophication problems, phytoplankton blooms and growth of microorganisms, pathogenic to human health.

1.3 Water Policy for water protection

Considering all the impacts and potential risk that the increasing of population has in the coastal areas specially concerning water contamination, there was a need to implement legislation and water policy, not only regionally or nationally but also on a wider and broader spatial scale. In this context, more recently, in 2015, the United Nations (UN) set up a set of 17 Sustainable Development Goals (SDG) in order to achieve a "better and more sustainable future for all" by 2030. All these SDG are broad and interdependent and are measured with indicators. Within each SDG there are specific targets. Regarding the impact of wastewater discharges on the marine waters two SDG play a key role: the 6th – "Clean water and sanitation" and the 14th – “Life below water”. Within the SDG 6 the important targets are the 6.3, related to improving the water quality by reducing the pollution, eliminating dumping, minimizing the release of chemicals and decreasing the amount of untreated wastewater discharge, and the 6.4, which targets the increase of the water-use efficiency in all sectors and ensure the sustainability of the resources. In the SGD 14 the most relevant targets are 14.1, aiming the prevention and reduction the marine pollution, 14.2., of sustainably managing and protecting the marine and coastal systems, and 14.A., concerning the increasing of scientific knowledge in order to improve the ocean health (United Nations, 2019).

Portugal within the European Union, beyond the Water Framework Directive (EU, 2000) must also comply with the Wastewater Directive (The European Parliament and the Council of the European Union, 1991) that was implemented to protect the environment from the adverse effects of the above mentioned wastewater discharges along with Directives 2006/113/EC concerning the water quality required for shellfish, and 2004/41/EC, concerning food hygiene and health conditions for the production and sale of certain products of animal origin intended for human consumption, were implemented. The Directive 2006/113/EC focus on the waters considered important to protected and/or improved in order to sustain life and growth of shellfish and, therefore, good products for human consumption. Also, this directive a set of parameters and a minimum sampling frequency is established to quantify the water quality of the water bodies. In Portugal there is also the Decree Law 236/98 (Diário da República, 1998) that transpose European legislation establishing quality standards, criteria, and objectives in order to protect the aquatic environment and improve the quality of the waters according to its main uses as well as DL 149/2004 that transpose the DL 152/97 and Directive 91/271/EEC of 21 May 1991 concerning urban waste-water treatment.

1.4 Impact of effluents discharge on shellfish/bivalves’ grounds

Shellfish grows in dense beds along coastal ecosystems, such as inshore estuaries and coastal lagoons, with high primary productivity (Lees, 2000). Bivalves are a nutritive food because due to its high-quality animal protein content (Oliveira et al., 2011). They are consumed worldwide and with minimal processing and handling, it is usually consumed lightly cooked or even raw (Lees, 2000). However, due to the over catching and overexploitation of this type of ecosystems, the natural stocks were declining and there was a need for human production of artificial shellfish beds (Oliveira et al., 2011).

Shellfish filter feeder organisms filter the surrounding water to retain their food, mainly phytoplankton and zooplankton but they also filter viruses, bacteria and inorganic matter (Lees, 2000). If the surrounding water is receiving effluent from a UWWTP discharge it can suffer deleterious effects due to their impact on the water quality. Beyond the potential low levels of dissolved oxygen due to organic matter accumulation and decomposition that can impair the survival of organisms including shellfish/bivalves, these organisms can concentrate the contaminants present in the water, including pathogenic bacteria, harmful microorganisms like toxigenic phytoplankton and microplankton that can develop on environments enriched in nutrients (Almeida and Soares, 2012). If shellfish accumulate pathogenic bacteria and/or toxins, human health could be affected by their direct consumption, since this type of organisms have a poor handling and are barely cooked, that may not be enough for safe consumption (Almeida and Soares, 2012; Lees, 2000). Raw or light cooked shellfish are associated with marine algae toxins that cause problems such as Paralytic Shellfish Poisoning (PSP), Diarrhetic Shellfish Poisoning (DSP), Neurotoxic Shellfish Poisoning (NSP), Amnesic Shellfish Poisoning (ASP) or/and Azaspiracid Poisoning (AZP) (FAO, 2004).

Biotoxins can cause health problems for humans, especially those produced by dinoflagellates and diatoms. Unpredicted blooms of these microalgae are associated with these biotoxins and are heat resistant, meaning that even if the shellfish is cooked properly, they can still be unsafe for consumption (Oliveira et al., 2011). With the increasing of population in coastal areas the vulnerability and exposure of shellfish to human and industrial contaminants increase as well (Lees, 2000).

Like in other coastal lagoons, the bivalve production in the Ria Formosa is very important, representing the major national centre of bivalves harvesting. Within this system there are also several major UWWTP discharging their effluents, which may impair theses edible resources. Until recently, Ria Formosa had five major UWWTP, two in Faro, two in Olhão and one in Tavira (Cravo et al., 2015), all of them with secondary treatment. Presently, Faro Noroeste, Faro-Olhão, Olhão Nascente and Almargem UWWTP have disinfection on the final stage of treatment of their effluents by UV.

1.5 Ria Formosa as a major system of bivalves production

Ria Formosa is a shallow coastal lagoon located in the south of Portugal (Figure 1), with 55 km of width, an average depth of only 2 m, and a tidal range of 1.3 m during neap tides and 2.8 m during spring tide (Newton and Mudge, 2003). Ria Formosa has a semidiurnal tidal regime, is well mixed without the presence of stratification either by salinity difference or temperature (Newton and Mudge, 2003). It has 5 barrier islands, 6 inlets, 5 small rivers and 14 streams but most of them dry during the summer months. Ria Formosa has only one major input of freshwater, the Gilão river in Tavira (Newton and Mudge, 2003) with an average annual flow of 7.7 x 10^7 m^3/year (Serpa et al., 2005). There is a high water renewal inside de lagoon, with 50-70% of the water being exchanged daily with the Atlantic Ocean. Ria Formosa is considered to be a marine environment with a salinity value around 36 (Mudge et al., 2008), because of the high water renewal and the low rainfall (400-600 mm/year, mainly from November to February) (Serpa et al., 2005) and is recognised as “coastal water” by the Water Framework Directive of the European Union (Bettencourt et al., 2004). Ria Formosa is located in the Atlantic coast but it has a Mediterranean climate, characterized by hot and dry summers and warm and wet winters, with an air temperature average of 25º C in summer and 12º C in winter (Newton and Mudge, 2003).

Figure 1.1. Location of Ria Formosa, South of Portugal.

Due to the exceptional characteristics, Ria Formosa is recognized as a Wetland Reserve with international importance in the Ramsar Convention in 1980. This ecosystem is also a protected area, Special Protected Zone (The European Parliament and the Council of the European Union, 1979), and it is classified as a Natural Park (78000 ha) since 1987 (Diário da República, 1987; Newton and Mudge, 2003). The Ria Formosa drainage basin supports many industries specially the food industry, mainly livestock and extensive and intensive agriculture using fertilizers and pesticides (Serpa et al., 2005). Ria Formosa itself is important in the salt extraction, fisheries and for aquaculture industries (Newton and Mudge, 2003). In the other hand, due to the nature and climate, the Algarve, and specially Ria Formosa region, receives a large touristic leading to a population increase in the summer months (June to September). All this can be related to a deterioration in the water quality of Ria Formosa, linked to an oxygen decrease and increase of dissolved inorganic nutrients concentration (Barbosa, 2010; Newton, et al., 2003).

Ria Formosa is a very productive ecosystem with an average primary production of ~1400 g C/m^2/year (Barbosa, 2010). It has various types of habitats that allows Ria Formosa to be an excellent place for spawning and as a nursery for several species, specially to bivalves (about 100 km^2) due to the sheltering conditions (Almeida and Soares, 2012). It provides shelter and food for fishes, molluscs, crustaceans and birds (Barbosa, 2010). Ria Formosa's aquaculture is mainly composed by bivalve shellfish (41%), where 2100 tons of the clam *Ruditapes decussatus* is harvested in 2010 along with 183 tons of oyster in the intertidal zone (Ferreira, et al., 2013). In this area, bivalves from different species (clams, cockles and oysters) can be grown together in a mixed culture at shellfish lease sites, however in this region the production consists of monocultures of clam where a small area is reserved for the oyster monoculture (Ferreira, et al., 2013).

The decrease of the shellfish population/production has a reflection on the regional economy, especially in the main harvesting centres such as in Olhão (75%), Faro (10%) and Tavira (10%), where the production of the clam species of *Ruditapes decussatus* corresponds to about 90% of the total national production (Serpa et al., 2005). Due to the potential contamination of the water quality of the Ria Formosa, shellfish depuration is required before the marketing and consumption of the farmed products. Bivalve producers in Ria Formosa often claim that the mortality and the reduction in the production is associated with the discharges from the UWWTPs (Ferreira, et al., 2013). According to previous studies in the late 90's the shellfish production decreased from 3-7 kg/m^2 to 0.5 kg/m^2 associated with water quality problems (Mudge and Bebianno, 1997), in a period when the level of water treatment was incipient or in the beginning.

1.6 Objectives

The main goal of this work is to evaluate the spatial and temporal impact of effluents disposal from the Almargem urban wastewater treatment plant at Tavira upon the chemical water quality in the vicinity area down to 1750 km from the discharge point, in the period from June 2019 to February 2020, within the scope of CONPRAR project. Moreover, bacteriological and phytoplanktonic acquired data will be complemented by information made available on IPMA website for Tavira shellfish production area, where potential risks for the shellfish and human health may occur. To evaluate the potential contribution of other sources of contamination from the Gilão estuary, on the water quality the shellfish beds of Tavira, three additional sampling stations at the Gilão low estuary were considered.

The specific objectives are:

- To determine the spatial extent of the influence of the Almargem UWWTP upon the water quality of the vicinity area along a gradient of dispersal of the effluents and potential impact on the bivalves production area, considering the applicable legislation.

- To determine the dilution effect within the study area in the vicinity of the UWWTP, at low water conditions when the influence from the effluents discharge is maximum in comparison to high water when the influence from the water renewal from the ocean during the flood is maximum.

- To determine the effect of tidal range upon the water quality of the study area, at spring tide (highest water renewal and tidal range) *versus* neap tide (lowest water renewal and tidal range).

- To determine the seasonal influence of the effluents discharge upon the water quality of the study area from summer 2019 (when the touristic influence is maximum and water quality problems could be aggravated) to winter 2020.

- Assess the temporal evolution of chemical water quality in the study area by comparing data from this study with those from 2001 to 2002 (Cravo et al., 2015) and 2005 to 2006 (Cravo et al., 2018) and from 1990 to 2009 for microbiological contamination (Almeida and Soares, 2012).

- To calculate trophic index (TRIX) in the study area and evaluate the temporal evolution of this trophic index with previous studies and define a baseline for Almargem UWWTP that can be used for future studies since there is no contemporaneous data for this trophic index available.

2. Methods

2.1 Sampling Area

Within the CONPRAR project scope, monthly water sampling collection was conducted from June 2019 to February 2020, twice a day, during both low and high water, at 6 specific points (light blue dots in Figure 2) along a gradient of dispersal from the Almargem UWWTP. To these points, 3 (dark blue dots in Figure 2) additional ones were considered in order to evaluate the spatial and temporal evolution in the water quality in the estuarine channel where potential contamination sources, other than the Almargem UWWTP can affect the bivalve's production areas.

Figure 2.1. Location of Ria Formosa and of the sampling sites, in light blue the sampling points belonging to CONPRAR project, in dark blue the extra 3 sampling points, the red line indicates shellfish beds and in red stars the urban wastewater treatment plant discharge point, on the west the discharge point of the old UWWTP (Db) and on the east the discharge point of the Almargem UWWTP (Da).

Surface water samples (20-30 cm of the water column) were collected fortnightly to cover spring and neap tides, from June to September of 2019, in summer months when the contamination problems can be aggravated, and monthly during neap tides (when the water renovation is longer and the impact of sewage influence higher) from October 2019 to February of 2020, along the dispersal gradient from the Almargem UWWTP and in the shellfish production area (Figure 2).

In each sampling point *in situ* measurements of temperature (º C), salinity, pH and dissolved oxygen (both in concentration and percentage of saturation) concentration were performed using a multiparametric probe EXO 2-YSI. Also, at each point water samples, as indicated in Table 1 were taken in 2 polyethylene bottles of 1 L each to further chemical analysis in the laboratory, and at specific points water samples of 50 mL were collected for microbiological characterization analysis (Table 1).

Table 1. Water collection made at each point.

Sampling point code	In situ / chemistry	Microbiology
T250S	X	X
T500S	X	
T750S	X	X
T1250S	X	
T1500S	X	
T1750E	X	X
TD1	X	
TD2	X	
TD3	X	

2.2 Filtration

The water samples collected were processed and analysed in the laboratory using specific filtration methods.

- **Nutrients and suspended solids**

The samples to determine the nutrient concentrations were filtrated into two replicates of 500 mL, using cellulose acetate filters (Gelman) of 0.45 µm of porosity. These filters were previously, identified, washed with distilled water, dried in a drying oven at 100 ± 5º C for one hour and weighted, that were used to determine the suspended solids concentration. The filtered sample was frozen at - 20º C for further analysis of nutrients. The filters for suspended solids concentration determination were washed with distilled water and dried in the oven for one hour at 100 ± 5º C. After cooling down in the desiccator, the filters were weighted again.

- **Chlorophyll *a* (chl *a*) and phaeopigments**

To quantify the chl *a* and phaeopigments, the water samples were separated into two replicates of 750 mL each and filtrated using a glass fibre filter (GF/F) of 0.7 µm of porosity in low luminosity and reduced pressure (<200 mm Hg), in order to reduce the degradation of the phytopigments and avoid the phytoplanktonic cells rupture. After the filtration, the filters were saved inside aluminium foil, identified and frozen at - 20º C to further analysis.

2.3 Laboratory Analysis

2.3.1 Chlorophyll *a* (Chl *a*)

The chlorophyll *a* concentration is used as a proxy to determine the total phytoplankton biomass and was quantified trough spectrophotometric methods, based on Lorenzen method (1967). Knowing the percentage of chlorophyll *a* and phaeopigments it is possible to understand is the phytoplankton population is young or old. If the ratio between the chlorophyll *a* and the total phaeopigments is higher than 0.5 the phytoplankton population is young (Lorenzen, 1967).

This method consists in measuring the absorbance of the samples at 665 nm and 750 nm of wavelength. After filtration, each filter was inserted in a test tube of 14 mL, saved from the light with aluminium foil and identified. Afterward, 5 mL of acetone at 90% was added and the filter was macerated with a glass rod with an irregular end. When having a homogenous solution, more 5 mL of acetone at 90% was added and the test tubes were kept in the cold for 8 to 24 hours. After this period, the tubes were centrifuged for 10 minutes at 4000 rotations per minute. After centrifugation, the supernatant was collected and inserted in a 1 cm cell for measuring the absorbance of 665 nm and 750 nm in the spectrophotometer. The value of absorbance corresponds to the value of chlorophyll *a* and the phaeopigments. After the previous steps were repeated after adding 150 µL of hydrochloric acid (HCl, 1N) in order to measure the phaeopigments absorbance. When all the values of absorbance were available the equations 1 and 2 were applied to calculate the chlorophyll *a* and phaeopigments concentration, respectively.

(1) $\text{Chl } a \ (\mu g / L) = 26.7 \times (A_{665b} - A_{665a}) \times V \times L^{-1} \times p^{-1}$

(2) $\text{Phaeopigments } (\mu g / L) = 26.7 \times (1.7A_{665a} - A_{665b}) \times V \times L^{-1} \times p^{-1}$

A_{665b} – absorbance at 665 nm before acidification
A_{665a} – absorbance at 665 nm after acidification
V – volume (mL) of acetone
L – volume (mL) of the sample filtrated
p – thickness (cm) of the cell used in the spectrophotometer

2.3.2 Suspended Solids

After filtration, the filters were washed with distilled water, dried in the oven for 1h at ± 100º C and weighted. The total concentration of suspended solids is obtained using the equation 3.

$$\text{(3)} \qquad SS\ (mg/L) = \frac{(Wf - Wi)\ x\ 1000}{V}$$

Wf – final weight, after filtration (g)
Wi – initial weight, before filtration (g)
V – filtrated volume (L)

2.3.3 Nutrients

Nutrient concentration was determined using spectrophotometric methods of molecular absorption based on calibration curves, where the wavelength used are specific for each nutrient. For each nutrient, a calibration curve was prepared in order to range from the minimum value (blank solution) and the maximum value of concentration. The methods used were described by Grasshoff (1983), in which each sample is analysed in three replicates of 5 mL for each nutrient and adding the corresponding reagents in the respective volumes.

To calculate the final nutrient concentration, the equation get from the calibration curve (4) was used with a correlation coefficient *(r)*> 0.99.

$$\text{(4)} \qquad Concentration = \frac{Abs - a}{b}$$

Abs – absorbance value for the sample
a – y intercept
b – slope of the straight line

- **Ammonium (NH_4^+)**

To determine the concentration of ammonium, the sample needs to be subjected to a phenolic alkaline citrate intermedium in the presence of sodium nitroprusside, that acts as a catalyser. For this the samples had to be saved from the light at environmental temperature. The concentration was determined by the blue compost of indophenol formed in the presence of ammonium by spectrophotometry of molecular absorption at 630 nm. This could be done by putting 5 mL of each sample in a test tube, add 150 µL of alkaline reagent, 150 µL of phenol solution and 150 µL of oxidant solution, the tubes were tapped and homogenised to accelerate the reaction. The tubes were kept away from light and at environmental temperature for 24h. After, the absorbance was measured in the spectrophotometer at 630 nm wavelength.

This method is affected by the salinity, as the ammonium absorbance decreases by salt interference, so the concentration values given buy the spectrophotometer must be corrected (5) by the salinity factor (f_{sal}) correspondent (Table 2) of the salinity value of the sample.

(5) $[NH_4^+]_{real} = [NH_4^+] \times f_{sal}$

Table 2. Salinity values and correspondent f_{sal} for ammonium.

Salinity	0-8	11	14	17	20	23	27	30	33	36
f_{sal}	1.00	1.01	1.02	1.03	1.04	1.05	1.06	1.07	1.08	1.09

- **Nitrites (NO_2^-)**

The determination of nitrites concentration is based on the Griess reaction, where the nitrites react with aromatic amines forming a diazo compound that is complexed with other amines forming an azo mixture. The quantity of azo formed is proportional to the initial concentration of nitrites and the absorbance of the final solution is measured by spectrophotometry at 540 nm wavelength of molecular absorbance.

For the quantitative analysis, 5 mL of each samples was added to a test tube, then 0.2 mL of sulphanilamide solution letting it to react for 10 minutes, then added 0.2 mL of N- (1-naphthyl) ethylenediamine bichloride, homogenised and waiting 30 minutes. After the absorbance was measured in the spectrophotometer.

- **Nitrates (NO_3^-)**

The method to determine the nitrates concentration is based on the reduction of nitrites to nitrates using a cadmium column. For this process, the column was activated using copper sulphate ($CuSO_4$) to guarantee a > 95% of reduction. Ammonium chloride (pH = 8.5) was used as a buffer solution, so a 5 mL was added to test tubes with 5 mL of the sample before passing the solution on the cadmium column. After the reduction, the samples received the same treatment as the nitrite samples. Since the samples suffered a dilution of 1:2, the concentration of nitrate needed to be calculated (5).

(5) $[NO_3^-]_{real} = [NO_3^-]_{measured} - ½[NO_2^-]$

- **Phosphates (PO_4^{3-})**

Phosphate concentration was determined in acid medium (H_2SO_4), which contains molybdate (Mo_6^+), ascorbic acid (reducing agent) and antimony ion (Sb_3^+) as catalyst, giving rise to phosphomolybdic acid. This yellow compound was reduced to molybdenum blue by the action of ascorbic acid.

To use this method, 5 mL of the sample was added to a test tube along with 150 µL of mix reagent and 150 µL of ascorbic acid, homogenising and after wanting 20 minutes the absorbances were measured in the spectrophotometer at 880 nm.

- **Silicates (SiO_4^{4-})**

Silicate concentration is determined by the formation of silico-molybdate acid when the sample reacts with an ammonium molybdate solution. By reacting with ammonium molybdate under acidic conditions, the sample forms silica, phosphorus and arsenic molybdate complexes. The acidic conditions were given by adding 150 µL mix reagent and letting it react for 10 minutes. Afterward, by adding the reducing solution consisting of ascorbic acid and oxalic acid (100 µL of each) and waiting for 30 minutes, the silico-molybdate acid complexes will be reduced and at the same time some traces of phosphorus molybdate and arsenic are decomposed. As there are several salts in seawater, they decrease the absorbance read from the molecular absorption spectrophotometry, in estuarine waters the variability of dissolved salts is big, so it is necessary to multiply the concentration obtained by the salinity factor (f_{sal}) (Table 3) (6).

(6) $[SiO_4^{4-}]_{real} = [SiO_4^{4-}] \times f_{sal}$

Table 3. Salinity values and correspondent *f*sal for silicates.

S ‰	5	9	14	20	25	30	35
f_{sal}	1.02	1.04	1.06	1.09	1.11	1.13	1.15

2.4 Calculation of Nutrients ratios

The proportion between nitrogen (N), phosphorous (P) and silica (Si) is almost constant in plankton (Johnson, 2010; Frigstad et al., 2011). The ratio 106C:16N:1P (Redfield, et al., 1963) represents the atomic proportion in which inorganic matter is assimilated by primary producers.

The molar ratios were calculated between nitrogen (ammonium + nitrates + nitrites) and phosphorous, and between nitrogen and silica. The ratio N:P should be 16:1, and if the values are different the limiting nutrient for phytoplanktonic productivity can be inferred. If the ratio is lower than 16:1, N is the limiting nutrient, if the opposite happens means that P is the limiting nutrient (Davidson et al., 2012). Silica is assimilated in the same proportion as nitrogen, so the ratio N:Si is 1 (Turner, 2002), if silica is the limiting nutrient it can impact the competition between diatoms and other species (Statham, 2012).

2.5 TRIX Calculation

To assess water quality an indicator was used, the trophic index (TRIX) (Vollenweider et al., 1998) which integrates water quality variables: chlorophyll a, oxygen, inorganic dissolved nitrogen compounds and dissolved inorganic phosphorus (7).

(7) $TRIX = (\log(chl\ a \times aD\% \times DIN \times DIP) + 1.5) / 1.2$

Chl *a* – Chlorophyll *a* concentration (µg/L)
aD% – Absolute deviation from oxygen saturation (%)
DIN – Dissolved inorganic nitrogen (µg/L)
DIP – Dissolved inorganic phosphorous (µg/L)

The classification of the waters considering the TRIX index was the one adopted by Penna et al., 2004, where the index was dimensioned into a 0-10 scale (Table 4).

Table 4. TRIX index scale based on Penna, Capellacci and Ricci, 2004.

Trophic Scale	State	Conditions
<4	Excellent	Oligotrophic
[4-5[	Good	Mesotrophic
[5-6[	Medium	Eutrophic
[6-10]	Poor	Hypertrophic

2.6 Complementary information

2.6.1 Microbiological Data

To quantify the faecal coliforms (FC) it was used the IDEXX Quanti-Tray/2000, that was designed to quantify bacteria of 100 mL samples. For each specific bacterium there is a specific set of reagents to add to the samples then incubated for 48h in a precision bath at 44.5 ± 0.02º C. After the number of positive large and small wells were counted and using a Most Probable Number (MPN) table it was possible to know the most probable number of bacteria (Clesceri, et al, 1998). The *E. coli* was quantified (per 100 mL) after adding reagent and being incubated. Every well was counted (large and small) if they showed a positive result. These data were provided by CONPRAR project.

2.6.3 Information on bivalves salubrity

The data used relative to phytoplankton, microbiology and biotoxins on bivalves values were retrieved from IPMA website (https://www.ipma.pt/pt/bivalves), along with meteorological conditions.

2.7 Statistical Analysis

All the data acquired were submitted to statistical analysis using the software RStudio. Normal distribution was tested to establish the use parametric or non-parametric tests.

To understand if the tidal range have a significant effect on the water quality, a paired t-test (or equivalent non-parametric, Wilcoxon) was used to compare data from neap tide and spring tide. The same was done to evaluate the effect of water renewal during a semi tidal cycle, to see if there are differences between the low tide and high tide. To see if there a significant spatial and temporal variability, a One-Way ANOVA was applied to the datasets that presented a normal distribution and a Kruskal-Wallis test to the non-normal distributed datasets. All these tests were done with a confidence interval of 95%.

To analyse the correlation between the variables of each sampling campaign, the Spearman coefficient (non-parametric data) or Pearson coefficient (parametric data) will be used, using a 95% confidence level.

In order to understand the global variability at Almargem a principal component analysis (PCA) was applied. This analysis allowed to understand the relationships between the different environmental variables and the grouping of the different campaigns and sampling stations, based on the factors that best explain the variance of the results.

3. Results

3.1 Temporal and spatial variability

The *in situ* parameters measured at all the sampling stations along the UWWTP discharge channel and at the Gilão low estuary, during the sampling period for both tidal peak conditions (low and high water) are represented in Figure 3.1. The station T1750E outside the Almargem channel was considered as a reference station, without direct influence of effluents dispersal.

The water temperature (Figure 3.1.) was variable during the campaigns, slightly higher upstream, gradually decreasing downstream and relatively lower during high water. However, there were no significant spatial differences ($p > 0.05$), meaning that the temperature values for the different sampling points were similar along the sampling period. The values showed to be similar ($p > 0.05$) between the Almargem and the Gilão channels. Along time there were significant differences of temperature ($p < 0.05$). Values at low water for September were similar with those of July but significantly higher from the others. In the other summer months, the values were similar but significantly lower than in July, both at low and high water. The highest value of 30.49º C was recorded at T250S in July during low water. The months from June and August showed significant higher values during low water than at high water. The lowest values were similar in December, January and February, with the lowest of 12.04º C found at T1000S in January. In January and December temperature values were significantly different from the months between June and October, during both low and high water.

Salinity (Figure 3.1.), unlike the temperature, showed a tendency for decreasing upstream along Almargem and Gilão channel, particularly at low water, when, generally, the values were lower. Globally, salinity was highest during summer months and lowest during the winter ones. There was an extreme low value in February at the point T250S (16.09), but, in general, the salinity ranged from 24.75 in June (T250S) and 37.35 in September (T1750E), Significant differences ($p < 0.05$) were found throughout the months and the sampling stations. The sampling points T250S and T500S were similar ($p > 0.05$) at low water and significantly lower than the other stations, while T500S was significantly lower ($p < 0.05$) than T1750E, considered the reference stations. Stations on the Gilão channel were not significantly different ($p > 0.05$) from the reference. The values of salinity for the TV points were much more similar between them but still significantly different ($p < 0.05$) temporally and spatially, showing that the stations from TV150N to TV900N were significantly different and that January and February had significant differences with all the other months.

Concerning the pH (Figure 3.1.), the values were slightly alkaline, ranging from 7.84 in November at T250S and 8.95 in July, both at T250S and values for low water were similar but higher to those for high water. Generally, the values were similar within the survey ($p > 0.05$), but significantly different ($p < 0.05$)

between campaigns. The values were higher in summer months (June, July and August) than in winter months (November, December and January). But the values for high water in January were significantly lower than the summer months. The values at TV150N and TV400N were similar to those found at the reference, but at TV900N the values were lower.

The oxygen concentration (mg/L) in the water (Figure 3.1.) was variable along time and space with the highest values found at low water. Generally, it ranged from 4.4 mg/L (T500S in September) and 14.20 mg/L (T500S in June), excluding the extremely high value of 23.3 mg/L found in July at T250S. Correspondently, the oxygen saturation (%), in general, ranged from 60% and 208%, in T500S of September and T250S of August, respectively. However, extremely high values (> 300%) were registered in July during low water on the neap tide. There were significantly differences ($p < 0.05$), concerning both spatial and temporal variation. Usually, values were significantly higher closer to the discharge point (T250S and T500S) in comparison with the reference station (T1750E) and Gilão low estuary points (TV150N, TV400N and TV900N), particularly during summer months (June and July). However, the lowest values were also recorded in September and July. During winter, values were less variable, close to saturation both at the reference station and Gilão low estuary points.

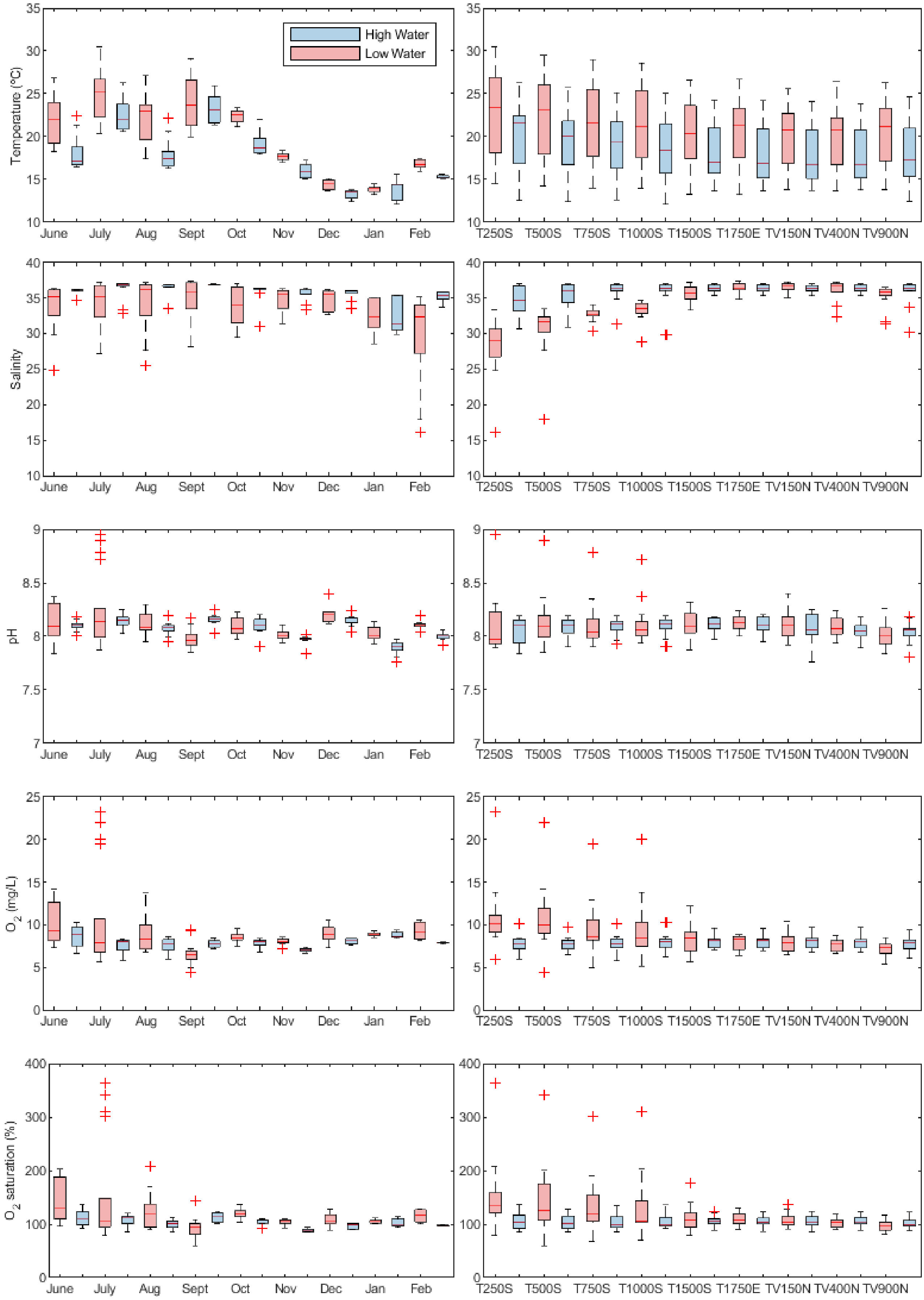

Figure 3.1. Temporal and spatial variability of temperature (ºC), salinity, pH, oxygen concentration (mg/L) and oxygen saturation (%) for both low and high water. Boxplots represent the 25% and 75% percentiles (bar), the extremes (minimum and maximum), the median (red line) and the crosses represent the outliers.

Generally, the nutrients, represented in Figure 3.2., show higher concentrations upstream gradually decreasing downstream, and higher than at the Gilão low estuary. The pattern of variation of the nutrients was opposite to the variation of the salinity, meaning that higher values of nutrient concentration corresponded to lower salinity values and the lower values of nutrient concentration corresponded to higher values of salinity. It is also noticeable that there were differences in the concentration values between high water and low water situations (detailed in the section 3.2).

Concerning ammonium (NH_4^+), the values ranged from 0.08 µM at T1500S in June to 106.63 µM at T250S in August (Figure 3.2.). There were significant differences between campaigns and between sampling points ($p < 0.05$). These differences can be seen between August during low water, when the values had a peak and the winter months (November, December and January) with the lowest concentration values. August also showed significantly higher values (0.05) than in June and July. Spatially, the station T250S had significant differences with all the other stations besides T500S , meaning that the values were higher upstream, closer to the UWWTP discharge point, in comparison with the refence points and the Gilão low estuary sampling stations.

Nitrate (NO_3^-) and nitrite (NO_2^-) concentrations, represented in Figure 3.2., as ammonium, show significant differences ($p < 0.05$) between the stations, where T250S had differences with all the other stations except T500S. This last station also showed significant higher concentrations ($p < 0.05$) than T1500S, the reference (T1750E) and the sampling points from the Gilão low estuary except TV900N, T750S also show significantly higher concentrations ($p < 0.05$) than the reference - T1750E. Temporally, nitrite was similar along months ($p > 0.05$), despite the values were higher in summer than in winter. Nitrate showed significant differences ($p < 0.05$) between June and January. The values of nitrate varied between 0.03 µM in July (T750S) and 22.51 µM in October (T250S). Nevertheless, the highest median values were recorded in February and January. The values of nitrite varied between not detectable in June and 11.94 µM in August (T250S).

For phosphate (PO_4^{3-}) (Figure 3.2.), the values ranged from 0.01 µM (T1750E of October) and 38.02 µM (T250S of September), presenting significant differences between sampling stations ($p < 0.05$), but not between the months ($p > 0.05$), despite generally higher in summer than in winter. The values follow the same pattern of distribution as the previous nitrogen nutrients, where the most upstream stations (T250S and T500S), closer to the discharge point had significant higher concentrations ($p < 0.05$) when compared with the other stations, .

The silicate (SiO_4^{4-}), Figure 3.2., present the same spatial distribution pattern as the other nutrients, significantly higher ($p < 0.05$)at T250S and T500S than at all the other stations. The Gilão low estuary sampling stations show still significantly difference ($p < 0.05$) with T1000S. The values ranged from 0.19 µM at T1500S in October, and 49.56 µM at T250S in September, with no significant differences ($p > 0.05$)

between the months. Nevertheless, the highest median values were recorded in February and January, like for nitrate.

The Redfield ratios of N:P and N:Si are represented in Figure 3.3. The highest values of N:P ratio, 50.65 in low water and 60.87 in high water, correspond to the point TV900N in January that is the most upstream sampling point located in the Gilão low estuary. The N:P ratio show significant differences between campaigns and stations ($p < 0.05$), where the station TV900N is different from all the other stations. TV400N was also different from the stations T250S and T500S. February and January showed similar values ($p > 0.05$) and January had significant differences with all the other months . The values reveal that nitrogen was the limiting element (N:P < 16) in the majority of the sampling points, except TV900N showing most of the samples with the ratio N:P > 16, meaning that there the limiting element was phosphorous. Concerning the N:Si ratio, July had the highest values for this ratio, meaning that the element limiting was the silicon, specially at the upstream stations in the Almargem channel (T250S, T500S and T750S; with values of 34.03, 28.63 and 12.68 respectively), while for the rest of the months and stations, the values stayed closer to the equilibrium (N:Si = 1). For the N:Si ratio, there were no significant differences ($p > 0.05$) concerning the spatial or temporal variability.

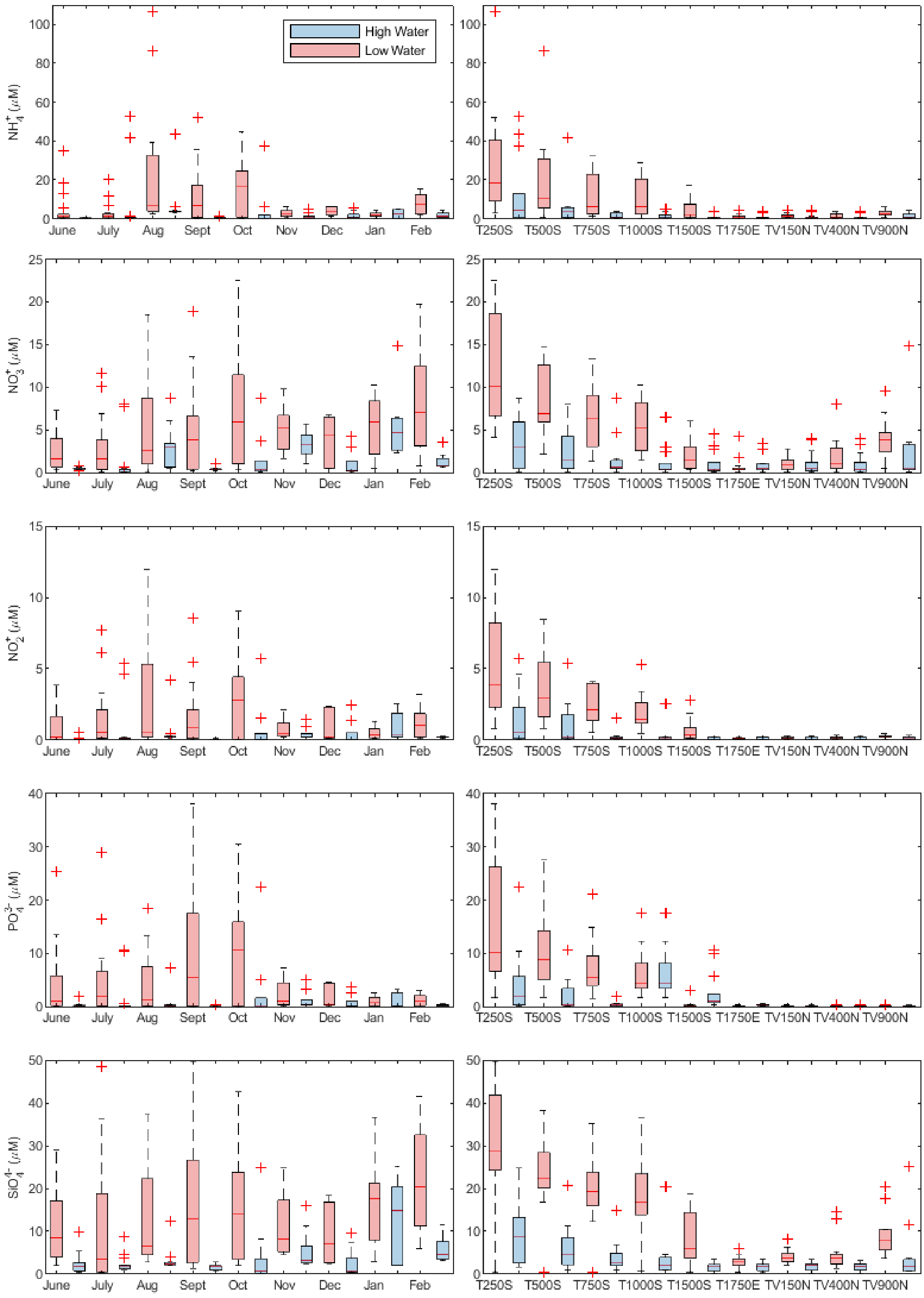

Figure 3.2. Temporal and spatial variability of ammonium (NH_4^+,μM), nitrates (NO_3^-,μM), nitrites (NO_2^-,μM), phosphates (PO_4^{3-},μM) and silicates (SiO_4^{4-},μM) concentrations. Boxplots represent the 25% and 75% percentiles (bar), the extremes (minimum and maximum), the median (red line) and the crosses represent the outliers.

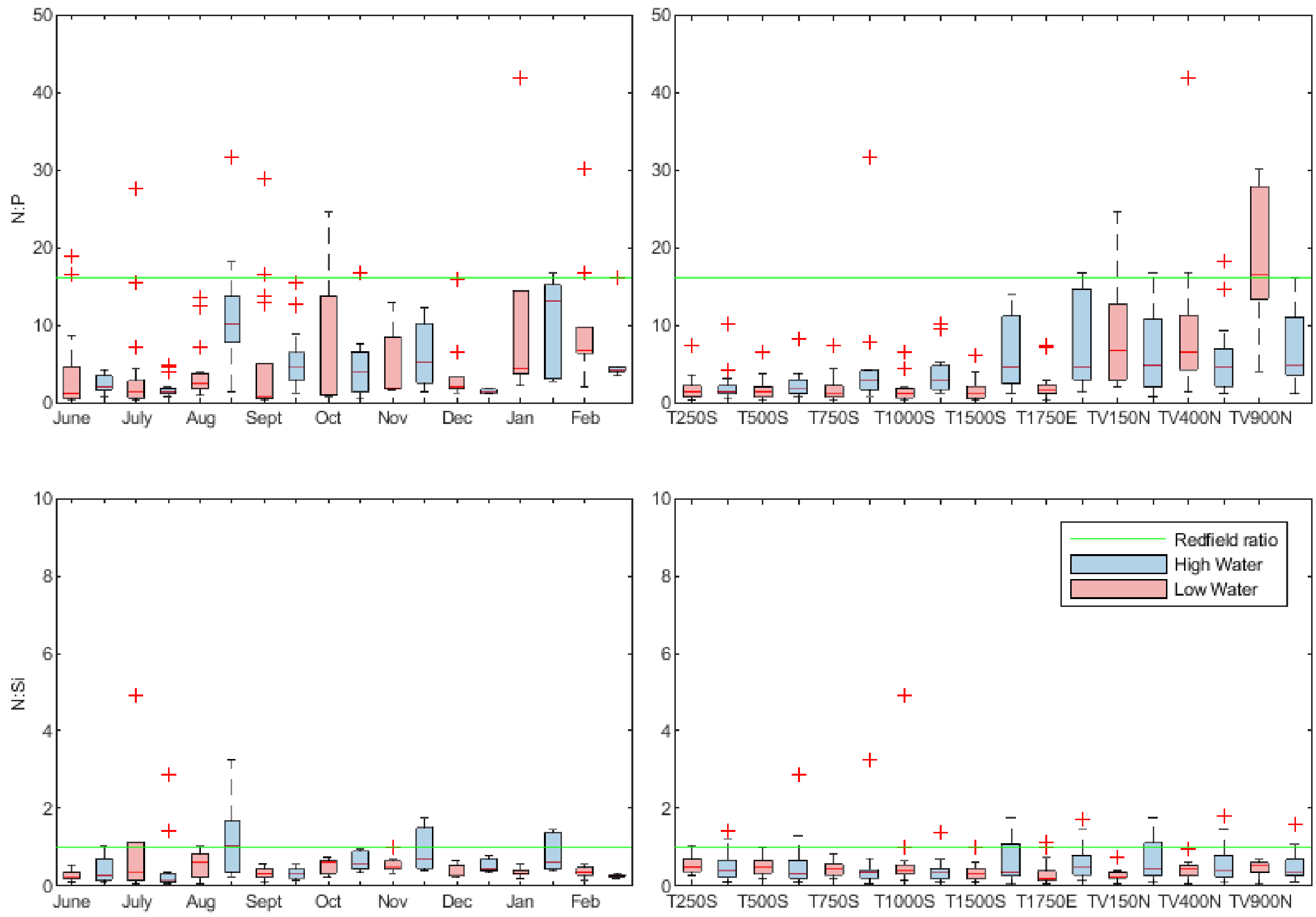

Figure 3.3. Temporal and spatial variability of N:P and N:Si ratios. The green line represent the Redfield ratios, N:P = 16 and N:Si = 1. Boxplots represent the 25% and 75% percentiles (bar), the extremes (minimum and maximum), the median (red line) and the crosses represent the outliers.

In relation to chlorophyll *a* (Figure 3.4.), the lowest values were observed in winter, in particular in November and December when some stations showed no detectable values. In summer (July) the chlorophyll *a* concentration reached 141.07 µg/L at T500S. These values showed that there were significant differences between the months ($p < 0.05$), with July having significantly higher values than the other months. However there were no significant differences between the stations within the same month ($p > 0.05$).

The phaeopigments concentration (Figure 3.4.), showed a maximum value of 18.46 µg/L in T750S in July despite mainly ranging between not detectable values and 2.85 µg/L at the station T250S in November. The range is not particularly large, and the values did not show significant differences between the months or between the stations ($p > 0.05$).

In order to evaluate the state of the phytoplanktonic population, a ratio between the chlorophyll *a* and the total pigments (sum of active chlorophyll *a* and phaeopigments) was calculated. Generally, the values were above 50%, indicating a dominance of young phytoplanktonic populations. The values showed temporally significant differences ($p < 0.05$) between December and all of the other months and between

January and August, but not spatially ($p > 0.05$), meaning that between stations there were more younger populations in summer than in winter, while in winter the phytoplanktonic populations were older.

The suspended solids concentrations (Figure 3.4.) registered the maximum value of 31.87 mg/L at T500S in August and the minimum value of 0.21 mg/L at T1500S also in August. The concentration of suspended solids did not show significant differences between the sampling stations ($p > 0.05$), but show significant differences between the months, with values in July, August and September significantly higher ($p < 0.05$) than in November, December, January and February. Suspended solids concentrations in August was also significantly higher ($p < 0.05$) than in October and June significantly higher ($p < 0.05$) than in January.

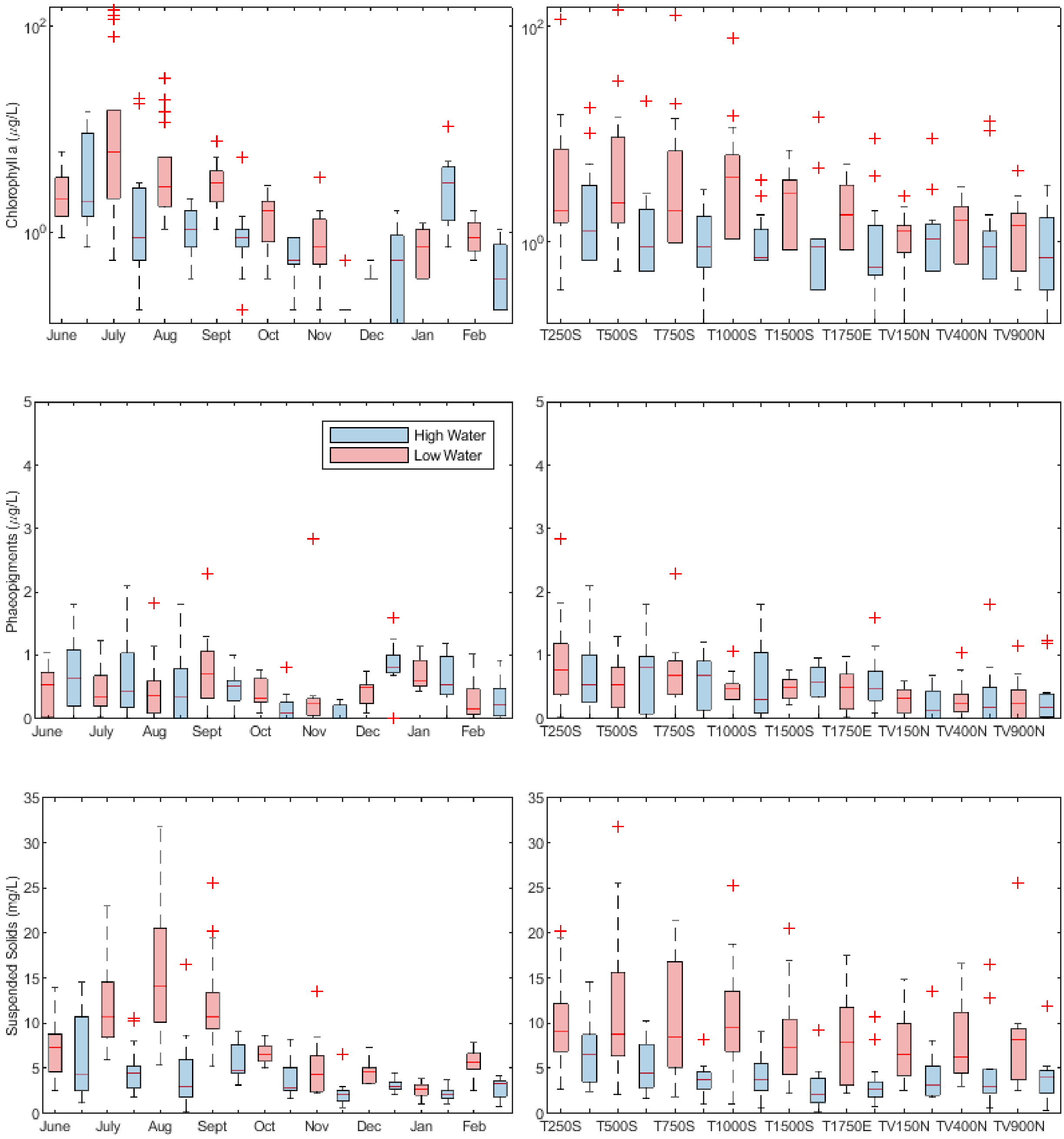

Figure 3.4. Temporal and spatial variability of chlorophyll *a* (µg/L), phaeopigments (µg/L) and suspended solids (mg/L). Boxplots represent the 25% and 75% percentiles (bar), the extremes (minimum and maximum), the median (red line) and the crosses represent the outliers.

To evaluate the microbiological contamination of the Almargem UWWTP, the total coliforms, *Escherichia coli* (*E. coli*) and enterococcus were measured at T250S, T500S, T1500S and T1750E at low water and at T250S, T500S and T1750E at high water. The results (Figure 3.5.) show that the total coliforms presented higher values in the summer months and closer to the UWWTP discharge point, with the maximum value > 24196 MPN/100 mL in August and September, both at T250S, and the minimum values < 10 MPN/100 mL in several months, mainly at T1500S and T1750E, the reference station out of the channel, where the effluents are discharged. Like for nitrate and silicate the highest median value was recorded in February. For the *E. coli*, the values followed the same spatial pattern as total coliforms, with higher values at the stations upstream, showing the highest value at T250S in August with a value of 9208 MPN/100 mL and minimum values were below 10 MPN/100 mL in several stations and months. In August, especially at high water, it is visible that at the T250S the values exceeded the limit of 2000 MPN/100 mL given for the discharge license for this UWWTP (Águas do Algarve, 2019). Like for total coliforms, the highest median value was recorded in February Enterococcus ranged from lower than 4 MPN/100 mL in several stations and months and 688MPN/100 mL at T250S in February. The enterococcus values show a different distribution pattern, being higher in winter months, particularly in January and February. All these parameters show no significant differences ($p > 0.05$) between the months, but significant differences ($p < 0.05$) between the sampling stations with values at T250S significantly higher than at T750S and T1750E, while values between the stations T750S and T1500S were similar ($p > 0.05$).

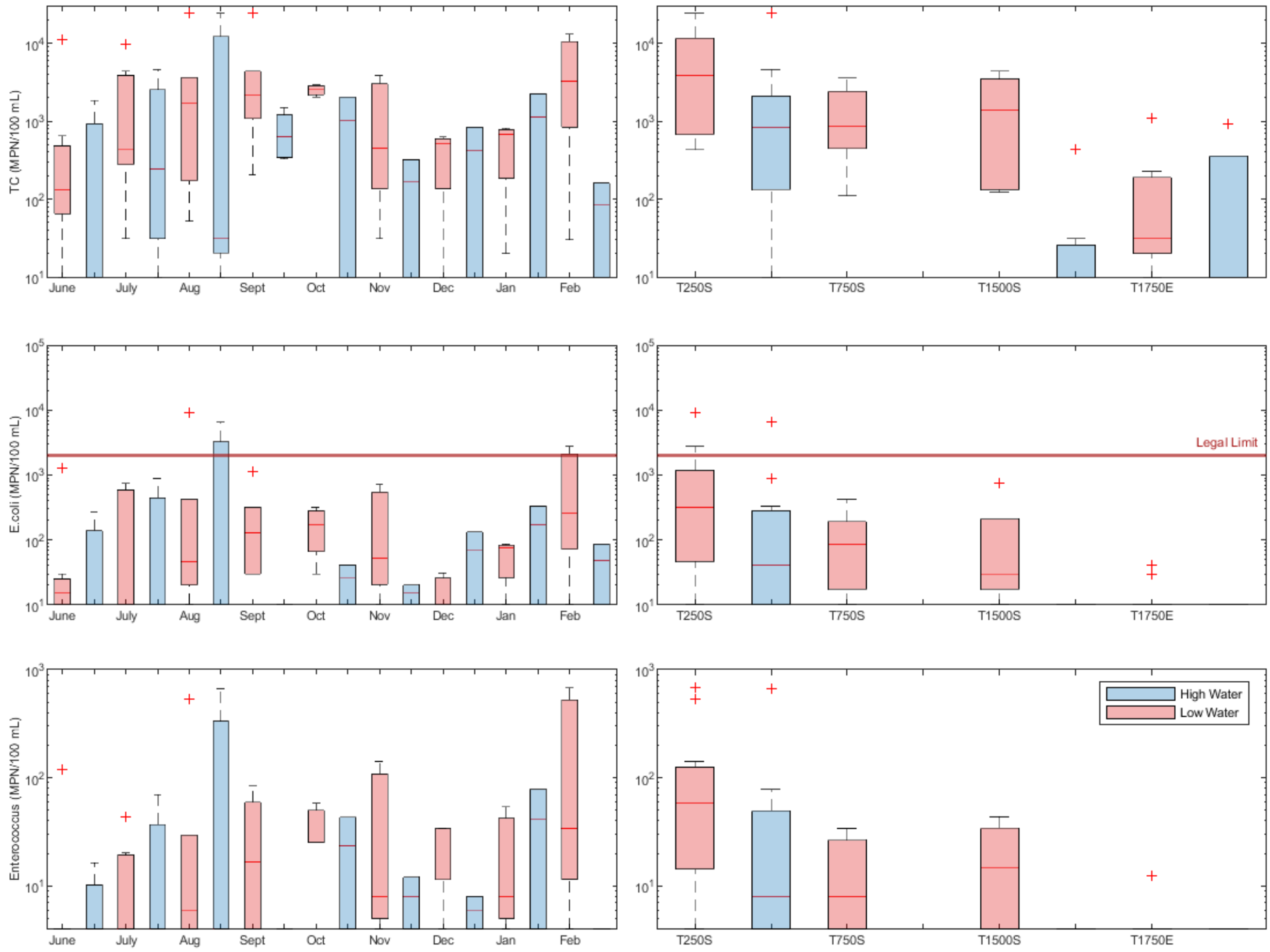

Figure 3.5. Temporal and spatial variability of total coliforms (TC, MPN/100 mL), *Escherichia coli* (*E.coli*, MPN/100 mL) and Enterococcus (MPN/100 mL). The red line represents the legal limit of faecal coliforms that can be discharged in order to maintain the quality parameters. Boxplots represent the 25% and 75% percentiles (bar), the extremes (minimum and maximum), the median (red line) and the crosses represent the outliers.

3.2 Tidal variability

In order to study the influence of the tide on the variability of the parameters, the campaigns were conducted in both low water and high water on the same day. Moreover, for the summer months (from June to September) when the potential growth of phytoplankton blooms are more plausible two campaigns were conducted in the same month under extreme tidal height conditions, one during spring tide (highest tidal range) and other during neap tide (lowest tidal range). These results are represented in the Figures 3.6. - 3.10. The results were separated into T points in yellow, correspondent to the points from the Almargem channel where the UWWTP effluents are discharged, and TV points in green, correspondent to the points from the Gilão low estuary.

The results for the *in situ* measurements are represented in Figure 3.6. The temperature showed significant differences ($p < 0.05$) between low and high tide, with higher values at low water both in T and TV points, and values at the low Gilão estuary were lower than at the Almargem area. There are also significant differences ($p < 0.05$) between the neap and spring tide, with higher values recorded during neap tide for both study areas but not between both areas.

For the salinity, the values were significatively lower ($p < 0.05$) during low water only at the T points. There were no significant differences during high water either between TV points or between those and T points. The values are also significant different ($p < 0.05$) between tides, with the values for the neap tide of the T and TV points lower than for spring tides.

Concerning the pH, the values showed no significant differences ($p > 0.05$) between the low and high water. However, there are significant differences between neap and spring tides ($p < 0.05$). The T points on neap tides had higher values than on spring tides.

Concerning the oxygen concentration and its percentage of saturation, the maximum and minimum values were detected at low water, even though was significantly higher than at high water for both T and TV points. Between neap tide and spring tidal conditions, values in neap tide were significant higher ($p < 0.05$) than in spring tides for both T and TV points. Regarding the dissolved oxygen percentage of saturation values at TV points were significantly similar at T for both spring and neap tides.

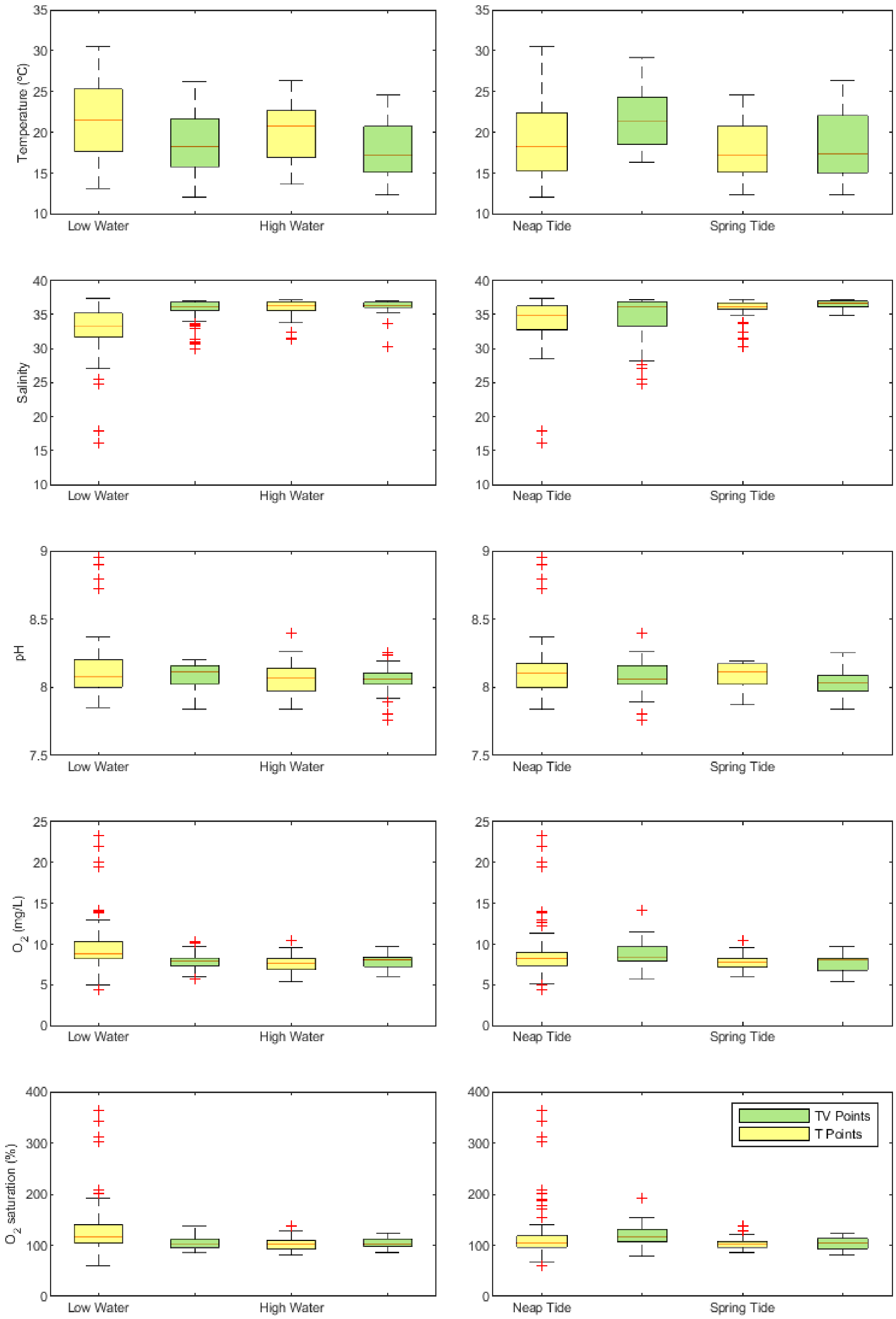

Figure 3.6. Tidal variability of temperature (ºC), salinity, pH, oxygen concentration (mg/L) and oxygen saturation (%). The yellow boxes correspond to the T points and the green boxes correspond to the TV points. Boxplots represent the 25% and 75% percentiles (bar), the extremes (minimum and maximum), the median (red line) and the crosses represent the outliers.

The nutrient variability is represented in Figure 3.7. For the ammonium (NH_4^+), the values were significantly higher ($p < 0.05$) during spring tide on the T points as well as the TV points. The TV points at neap tide also showed to be significantly higher concentrations ($p < 0.05$). Concentrations at low water were significantly higher than at high water, for both T and TV points and ammonium at T points were higher than at TV. The other nutrients despite recording different magnitudes had a similar behaviour between low and high water and between spring and neap tides meaning that for the low water nutrient concentration at the T points were higher than all the other tidal conditions.

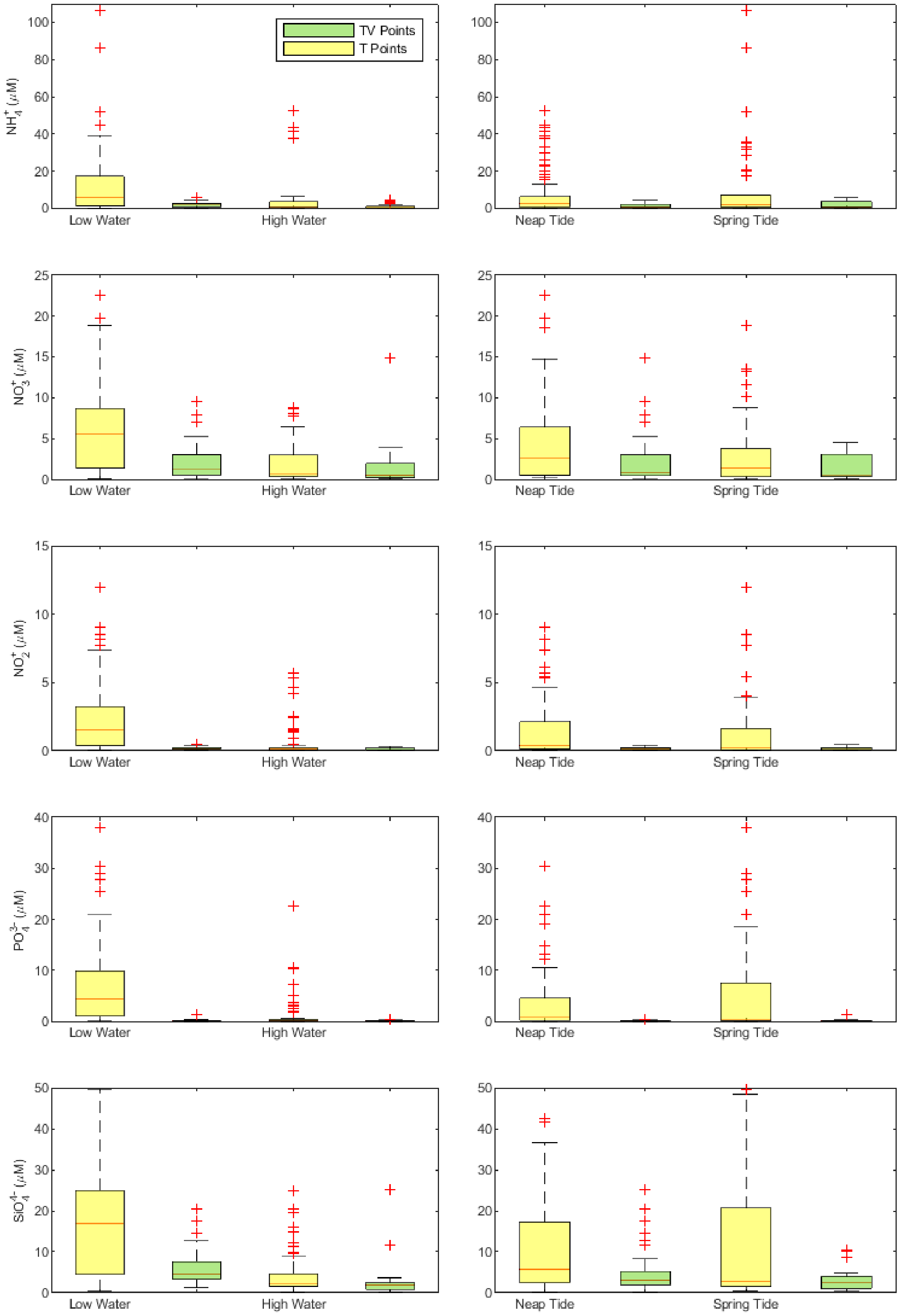

Figure 3.7. Tidal variability of ammonium (NH_4^+,µM), nitrates (NO_3^-,µM), nitrites (NO_2^-,µM), phosphates (PO_4^{3-},µM) and silicates (SiO_4^{4-},µM) concentrations. The yellow boxes correspond to the T points and the green boxes correspond to the TV points. Boxplots represent the 25% and 75% percentiles (bar), the extremes (minimum and maximum), the median (red line) and the crosses represent the outliers.

The ratios between the nutrients (Figure 3.8.), showed that the N:P ratio had a larger range on neap tides, reaching the value of 50.65 at low water and 60.87 at high water. The values also showed that there were significant differences ($p < 0.05$) between low water and high water, with significatively higher values ($p < 0.05$) at TV points than at T sampling points for both low water and high water. Oppositely, at T points values for the low water are significatively lower ($p < 0.05$) than at high water. The values for the neap tide and spring tide situations, also presented significant differences ($p < 0.05$), showing that the values for the T points, both in neap and spring tide, were lower than the values at the TV points. Globally, as mentioned before, the values were below the Redfield ratio (N:P = 16), showing that the limiting element was the nitrogen. For the N:Si ratio, the values presented some extreme values of 12.68, 28.63 and 34.03 at low water on neap tides. The values showed no significant differences ($p > 0.05$) between low water and high water conditions or between neap and spring tides, for both T and TV sampling stations. Similar to the N:P values, the N:Si values were generally lower than the Redfield ratio (N:Si = 1), confirming that nitrogen was the limiting element.

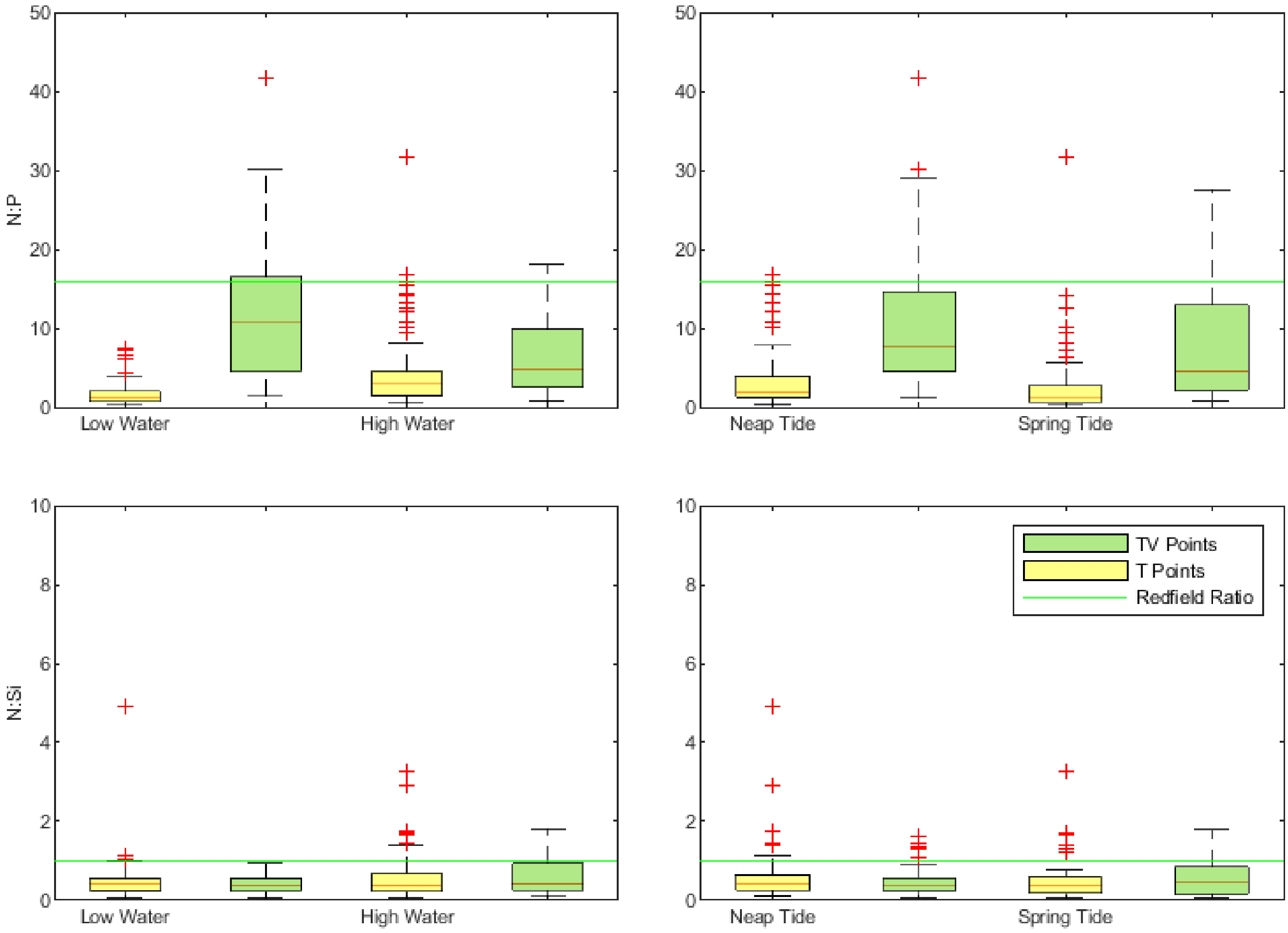

Figure 3.8. Tidal variability of N:P and N:Si ratios. The green line represent the Redfield ratios, N:P = 16 and N:Si = 1. Boxplots represent the 25% and 75% percentiles (bar), the extremes (minimum and maximum), the median (red line) and the crosses represent the outliers.

In the Figure 3.9. is represented the chlorophyll *a*, phaeopigments and suspended solids concentrations. The chlorophyll *a* concentration was maximum at the T stations, especially at low water. These values were significantly ($p < 0.05$) higher than at high water as well as when compared with TV points for both low and high water. Concerning the concentrations at neap and spring tides, the values did not show significant differences ($p > 0.05$). For the phaeopigments, the results show no significant differences ($p > 0.05$) due to high variability among results. The suspended solids had the minimum and the maximum values at spring tides, at high and low water respectively, at the T points. The values demonstrated to be significantly lower ($p < 0.05$) at high water, for both T and TV points, and at neap tides than in spring tides.

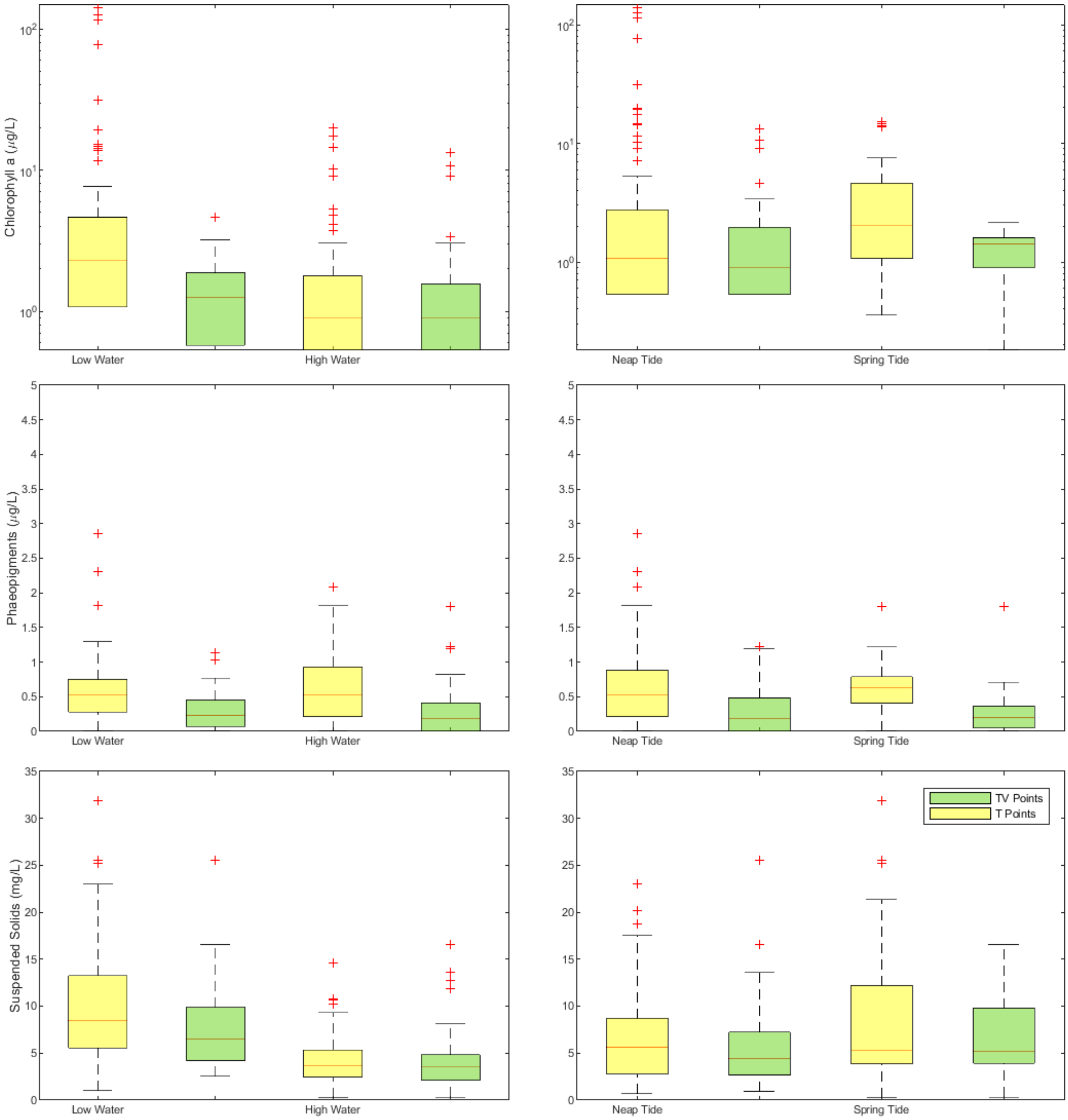

Figure 3.9. Tidal variability of chlorophyll a (µg/L), phaeopigments (µg/L) and suspended solids (mg/L). Boxplots represent the 25% and 75% percentiles (bar), the extremes (minimum and maximum), the median (red line) and the crosses represent the outliers.

The tidal variability for the microbiological contamination parameters is represented in the Figure 3.10. Since there were no microbiological contamination analysis for the TV stations, only T sampling points were represented. The values for the total coliforms (CT) were higher during low water particularly at neap tide but no significant differences ($p > 0.05$) occurred between low water and high water or between neap and spring tidal situations. The Enterococcus and the *Escherichia coli* had a similar tidal distribution for both situations low and high water and between spring and neap tides.

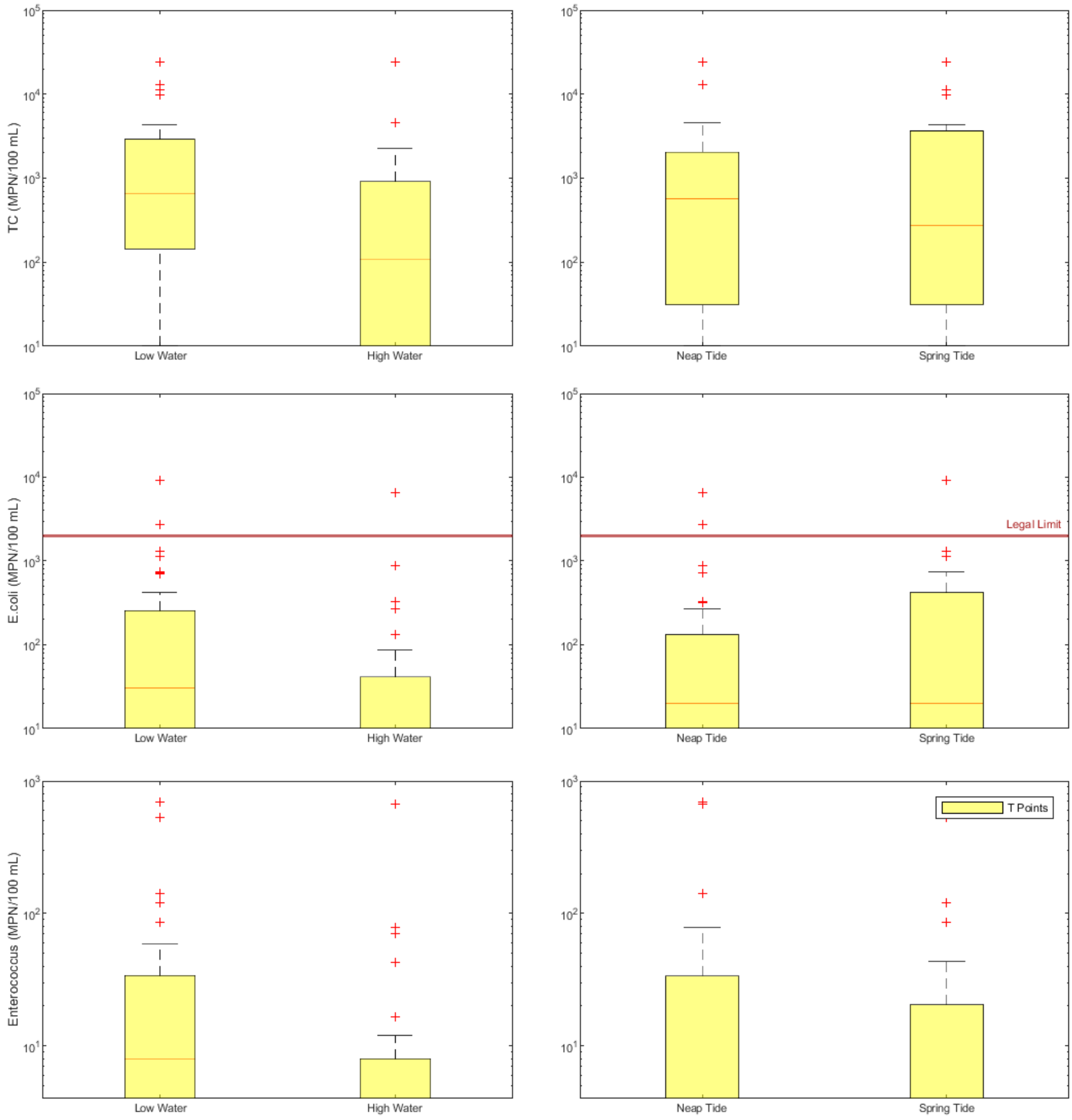

Figure 3.10. Tidal variability of total coliforms (TC, MPN/100 mL), Escherichia coli (*E.coli*, MPN/100 mL) and Enterococcus (MPN/100 mL). The red line represents the legal limit of faecal coliforms that can be discharged in order to maintain the quality parameters. Boxplots represent the 25% and 75% percentiles (bar), the extremes (minimum and maximum), the median (red line) and the crosses represent the outliers.

3.3 Behaviour of nutrients during mixing with seawater

It is possible to determine the behaviour of dissolved compounds, nutrients in particular, along the estuary using the theoretical dilution line (TDL; Liss and Burton, 1976) and if these behave conservatively to calculate their concentration in the freshwater source (S = 0). In the present study, the points from the Almargem channel (T points) were separated from the Gilão estuary points (TV points) because these have two different freshwater sources: one the Almargem stream together with the UWWTP and the other the Gilão River.

As seen in the Figure 3.11., the nutrients along the Almargem channel that showed to be more conservative are silicate and nitrate, while ammonium, nitrite and phosphate were still conservative. In February at the T250S and T500S, where salinity was lower than 20, the concentrations of nutrients were below the theoretical dilution line, which may result from a relevant consumption from the phytoplankton population and/or some adsorption to suspended particles. In addition, there is also a few points where the salinity was between 25-30 in July and August that had much higher concentration of ammonium, nitrate and phosphate on the T250S and T500S station, suggesting a peak driven from the effluents discharged, causing a higher deviation from the mixing line. Removing these odd points apart from the theoretical mixing line and assuming a conservative behaviour of the mixing waters it can be calculated the concentration of the nutrients at the pure freshwater source where S = 0. The estimated concentrations are about 133 µM for silicate, 101 µM for ammonium, 48 µM for nitrate, 21 µM for nitrite and 68 µM for phosphate.

For the Gilão estuary points (TV), nitrate, nitrite and silicate were conservative. Also estimating the concentrations of these nutrients at the Gilão River, assuming S = 0, it is observed that silicate and nitrate were relatively similar with Almargem stream. Silicate was about 20% lower at Gilão River, with 111 µM whereas nitrate (58 µM) was about 20% higher there. However, nitrite at Gilão River having 1 µM was much lower than at Almargem (21 µM).

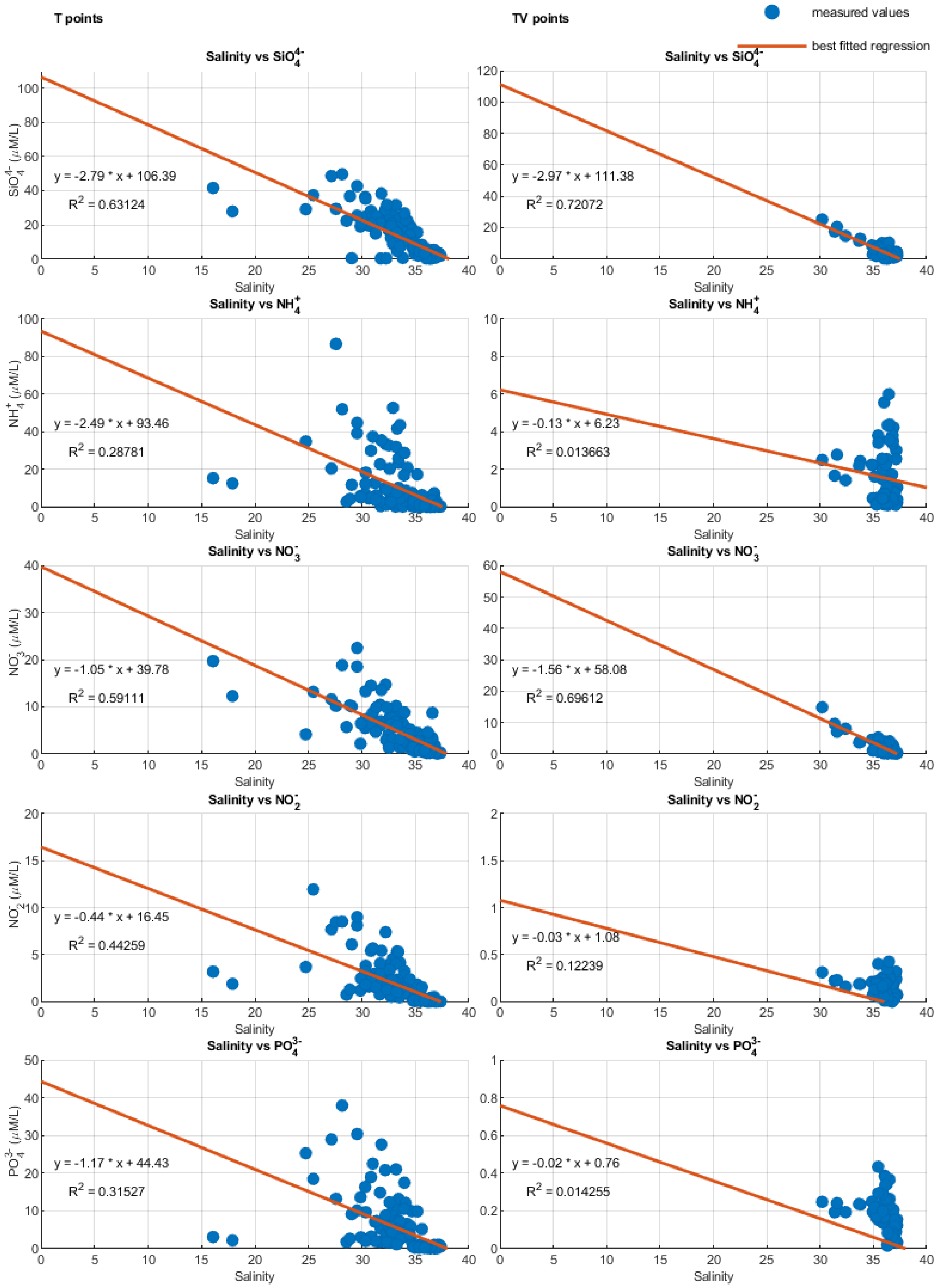

Figure 3.11. Theoretical dilution line correspondent to silicates (SiO_4^{4-}), ammonium (NH_4^+), nitrates (NO_3^-), nitrites (NO_2^-) and phosphates (PO_4^3), divided into T points and TV points, with the respective equation and r^2.

3.4 TRIX index calculation

To evaluate the trophic state of both study areas, the TRIX index which integrates the nitrogen and inorganic phosphorous concentration, chlorophyll *a* and absolute deviation of the oxygen saturation was applied to the data of all the sampling stations for every month . The classification of the TRIX index varies from oligotrophic conditions (TRIX = [0 – 4[) or excellent quality state and hypertrophic conditions (TRIX = [6 – 10[) or poor quality state, as established by Penna et al. (2004). This index was presented to depict the trophic status for both Gilão low estuary and Almargem channel for each sampling point and along the months, separated in low water and high water situations (Figure 3.12.) and different tidal phases (neap tide and spring tide) (Figure 4.13).

All the sampling stations from the Gilão low estuary presented oligotrophic conditions (excellent quality state) along the sampling period and for both low and high water, similarly to the conditions found for the reference station (T1750E). The results for the Almargem channel show that the TRIX index is higher, typical of eutrophic waters from station T250S to T750S. Water quality progressively improved downstream, meaning that closer to the UWWTP discharge the water quality is worse when compared to the reference sampling station (T1750E) classified as oligotrophic. Mesotrophic conditions were still observed down to 1500S during low water. The values for the low water situations were, globally, higher than at high water and more variable, showing a higher spatial difference along the gradient of the effluents dispersal. In addition, temporally, the TRIX values were higher in summer and autumn months (June to October), particularly from July to September, when more eutrophic waters were encountered when compared to the winter months, when the waters were oligo-mesotrophic. At low water the values did not present significant differences ($p > 0.05$) between the months but show significant differences ($p < 0.05$) between the sampling stations, with stations from T250S to T1000S significantly different from the stations T1500S and T1750E (the reference point) and from the Gilão low estuary stations. For high water situation the values showed significant differences ($p < 0.05$) between the months and the stations. T250S and T500S stations were different from the station T1500S down to the station TV900N, and January, October and November showed to achieve the worst condition from all the other months.

Comparing globally the values at low water and high water, the water quality at high water was significantly better ($p < 0.05$). However, there was no significant differences ($p > 0.05$) between neap and spring tide values, despite, the lowest water quality was recorded during neap tides, when the residence time is higher than in spring tides.

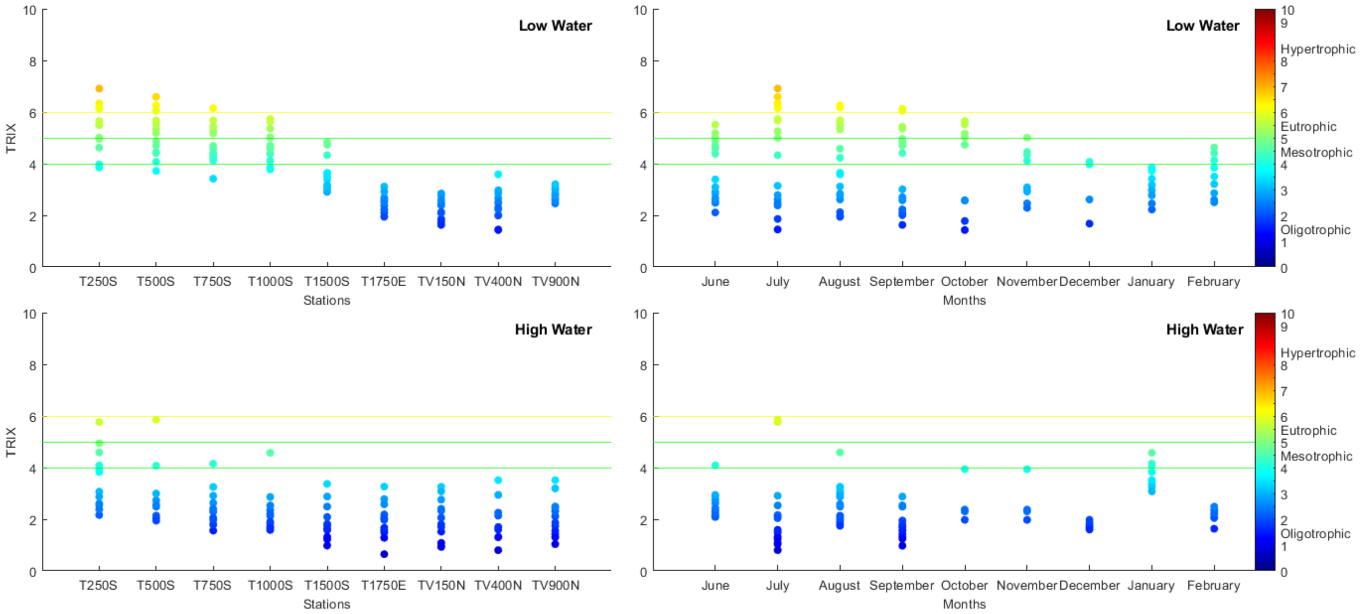

Figure 3.12. Temporal and spatial variability of the TRIX trophic index values, with the colour scale boundaries: light blue for oligotrophic, green for mesotrophic and yellow for eutrophic conditions.

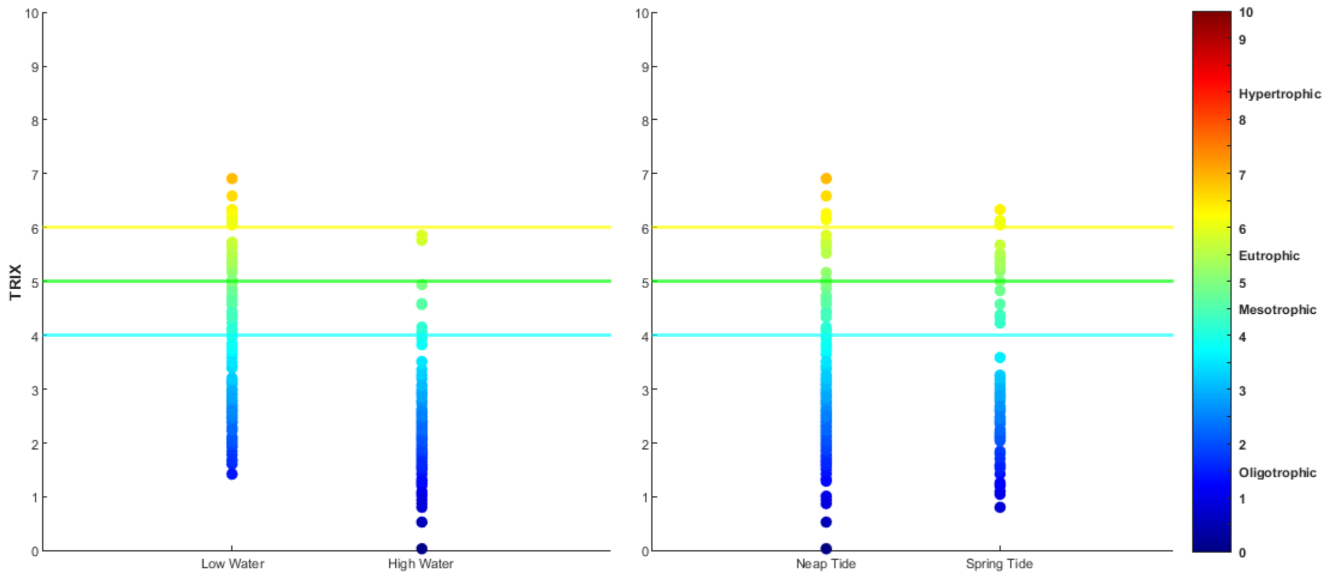

Figure 3.13. Tidal variability of the TRIX index values, with the colour scale boundaries: light blue for oligotrophic, green for mesotrophic and yellow for eutrophic conditions.

3.5 Inter-relationship between variables

A correlation matrix was performed between all the variables for a better understanding of the global behaviour of the studied parameters. The correlation matrix is presented in Table 5, where the highlighted orange cells correspond to the significative correlation between variables ($p < 0.05$), either positive or negative.

Analysing the correlation matrix, it can be observed that the salinity had a negative correlation with the oxygen (concentration and saturation), chlorophyll *a* concentration, all the nutrients and microbiological parameters. This negative correlation shows that the lower the salinity (more freshwater influence) correspond to higher values of those parameters, meaning that the mixing between seawater and freshwater is the main process responsible for that. The higher values for oxygen, chlorophyll *a*, microbiological contamination and nutrients can be found close to the UWWTP discharge point (with lower salinity) and the lowest at the stations closest to the reference station (with higher salinity). The suspended solids and chlorophyll *a* also have a positive correlation between them since phytoplankton make part of the suspended solids. As expected, the chlorophyll *a* (along with the suspended solids) had a positive significant correlation with the oxygen and the temperature meaning that during the summer the suspended solids and chlorophyll *a* were higher producing more oxygen, and correspondently lower during the winter. Concerning the nutrients, these had a positive correlation between them and with the microbiological contamination parameters (CT, *E. coli* and enterococcus) suggesting that all these parameters derive from the same source, particularly from the Almargem UWWTP.

Table 5. Correlation matrix between temperature (Temp), salinity (Sal), pH, dissolved oxygen (O_2), percentage of oxygen saturation (O_2 %), chlorophyll *a* (Chl*a*), suspended solids (SS), ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}), silicate (SiO_4^{4-}), total coliforms (TC), *Escherichia coli* (*E.coli*) and enterococcus (Entero). Highlighted in red is the positive correlations and in yellow the negative correlations.

	Temp	Sal	pH	O_2 (mg/L)	O_2 (%)	Chl*a*	SS	NH_4^+	NO_3^-	NO_2^-	PO_4^{3-}	SiO_4^{4-}	TC	*E.coli*	Entero
Temp	1.00														
Sal	-0.08	1.00													
pH	0.45	0.08	1.00												
O_2 (mg/L)	0.35	-0.37	0.79	1.00											
O_2 (%)	0.53	-0.29	0.84	0.97	1.00										
Chl*a*	0.43	-0.16	0.76	0.78	0.82	1.00									
SS	0.63	-0.26	0.28	0.32	0.43	0.44	1.00								
SiO_4^{4-}	0.36	-0.54	-0.17	0.07	0.12	0.02	0.48	1.00							
PO_4^{3-}	0.20	-0.77	-0.12	0.25	0.22	0.13	0.33	0.67	1.00						
NH_4^+	0.42	-0.66	-0.01	0.31	0.33	0.23	0.50	0.89	0.83	1.00					
NO_3^-	0.43	-0.56	-0.21	0.12	0.16	0.10	0.45	0.70	0.70	0.81	1.00				
NO_2^-	0.15	-0.79	-0.36	0.07	0.02	-0.08	0.34	0.60	0.83	0.73	0.78	1.00			
TC	0.26	-0.59	-0.28	-0.02	0.00	-0.05	0.27	0.76	0.53	0.67	0.59	0.60	1.00		
E.coli	0.12	-0.44	-0.20	-0.03	-0.03	-0.05	0.13	0.73	0.33	0.54	0.28	0.35	0.84	1.00	
Entero	0.02	-0.60	-0.19	-0.04	-0.05	-0.07	0.07	0.54	0.43	0.41	0.20	0.39	0.78	0.86	1.00

3.6 Principal Component Analysis (PCA)

In order to discriminate the factors that better explain the global variability of the data in the study area under influence of wastewater discharge, a Principal Component Analysis (PCA) was applied to the results (logarithmized to avoid effect of different scales of parameters) obtained from the T sampling stations (Almargem channel stations). The parameters used were: salinity (Sal), temperature (Temp), O_2 saturation in percentage (O2Sat), chlorophyll *a* concentration (Chloro), suspended solids (SS), ammonium (NH4+), nitrate (NO3-), phosphates (PO43-), silicates (SiO44-), total coliforms (TC), *Escherichia coli* (*E.coli*) and Enterococcus (Entero) concentrations as shown in Figure 3.14.

The data showed that the main 3 axes explained about 83% of the variance, from which the PC1 has a key role, explaining almost half of the variance (46%). PC2 explained 28% of the variance and the PC3 only 9%. The PC1 (Figure 3.14.) clearly depicts the inverse relationship between the salinity and the nutrients and microbiological contamination, confirming that the mixture of the effluent dispersed from the UWWTP into seawater is the most important process to explain the variance of the data, as shown in the correlation matrix (section 3.4). The highest impact of nutrients was found in August and September 2019. PC2 (Figure 3.14.) is explained by the dissolved oxygen (O_2), chlorophyll *a*, suspended solids and pH highly associated with temperature with maximal values presented in July 2019 at T250S and T750S. This suggests that phytoplankton activity associated to photosynthesis is the driver factor for this variability. PC3 (Figure 3.14.) is explained by the opposite behaviour between temperature, SS (and chlorophyll *a*, to some extent) and percentage of saturation of dissolved oxygen. This indicates that in July 2019 at low water during early morning the lowest oxygen values were associated with the highest suspended solids (that also include phytoplankton and other organic matter content), meaning that the respiration processes has a tertiary role to explain the variance of the data.

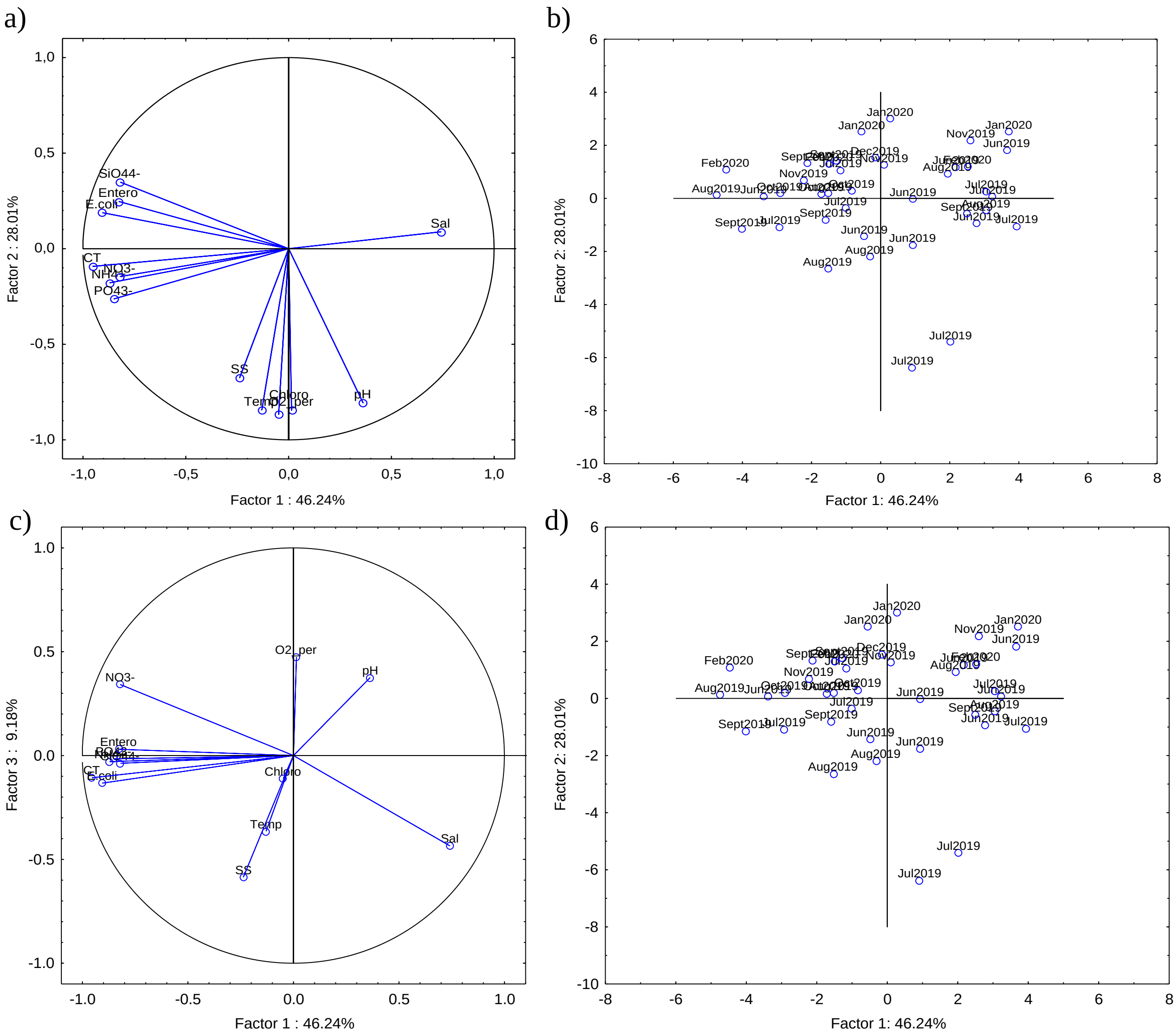

Figure 3.14. Principal Component Analysis (PCA) applied to Almargem study area: a) projection of variables explaining Principal Component 1 and Principal Component 2, b) projection of the cases associated with months of sampling that explain Principal Component 1 and Principal Component 2, c) projection of variables explaining Principal Component 1 vs Principal Component 3, d) projection of the cases associated with months of sampling that explain Principal Component 1 and Principal Component 3, applied to salinity (Sal), temperature (Temp), O_2 concentration (O2_con), chlorophyll a concentration (Chloro), suspended solids (SS), ammonium (NH4), nitrate (NO3), phosphates (PO43), silicates (SiO44), pH, total coliforms (TC), *Escherichia coli* (*E.coli*) and Enterococcus (Entero).

4. Discussion

4.1 Influence of the Almargem UWWTP discharge in the water quality on the Almargem channel

UWWTP discharges may affect the receiving waters in many ways. Some of the most problematic impacts include decrease of the water quality leading to eutrophication, development of phytoplanktonic blooms (sometimes toxic/harmful), and/or microbiological contamination that can lead to indirect impacts on human health and edible resources (Cravo et al., 2015). However, the magnitude of the impacts depends on the volume of the effluents discharged, on the composition of the effluents, the characteristics of the receiving waters including hydrodynamics and circulation patterns. Ria Formosa is located in a highly touristic area, with population enhancement during the summer. It is a shallow mesotidal coastal lagoon with a semidiurnal tidal regime where fortnightly neap and spring tide occur. Under a mesotidal regime, it suffers an important dilution effect every tidal cycle promoted by a great water renewal (*ca.* 75%; Tett et al., 2003), what was confirmed by the water quality improvement expressed by TRIX when high water was considered (Figures 3.12 and 3.13).

The Almargem UWWTP effluents discharge represents a source of nutrients and microbiological contamination, as confirmed by the negative correlation between the salinity and the nutrient and microbiological contamination parameters (Table 5), also shown in the PC1 (Figure 3.14). As expected, the highest impact was evident at the closest station to the UWWTP discharge point (T250S). There, the concentrations were significantly higher than at the reference point (T1750E), where, for example phosphates in low water of spring tides attained almost 2 orders of magnitude higher than the reference station, and equivalently about 30 times higher in the case of neap tides. For the nitrite, the same comparison show that the values were also almost 2 orders of magnitude higher during low water in spring tides and almost 50 times during low water in neap tides. However, due to the high water renewal and tidal mixing every tidal cycle the nutrient concentrations decrease during flood to high water and gradually with the distance from the discharge point till the reference station outside of the Almargem channel. Nutrients concentration (Figure 3.2) was higher during low water when compared with high water, particularly in the summer months, confirming the influence of the tidal dilution caused by the seawater entrance during the flood, like found by other authors in the same area (Botelho, et al., 2015; Cravo et al., 2015). This was also identified by other authors such as Cabaço et al. (2008) studying the impact of effluents discharge in other areas of Ria Formosa, such as at Faro Noroeste UWWTP, showing that the tidal mixing and water renewal are the key parameters that explain the decrease of contamination from the UWWTP.

From the sampling period only summer months (June to September, when the phytoplankton blooms are more plausible) were characterized for both neap and spring tide to evaluate the impact of different tidal ranges on the variability of the water quality. So, data showed that the highest impact of effluents discharge was found at low water of spring tides due to the reduced dilution of the effluent when the depth of water column of the receiving water is the shallowest. Nevertheless, at high water of spring tides, a better water quality was attained since the volume of water incoming to the Ria Formosa through the Tavira inlet is sufficiently high to dilute the high concentrations found at low water, as clearly observed from the TRIX results (Figure 3.13). Considering both spring and neap tides, the neap tides could represent, a worst condition due to a higher residence time, when the biological processes can be intensified, and a lower dilution effect promoted by the renewed water coming from the ocean.

The influence of the discharge was clearly evident during low water down to 750 m, as shown by the TRIX results (Figure 3.12). It ranged from Eutrophic-Hypertrophic conditions at T250S and T500S stations, to Eutrophic at T750S while for the reference station the classification attained was Oligotrophic. However, in August and September during low water on the spring tides it was Eutrophic till 1000 m. This was due to an evident increase of phosphate during this period suggesting a disproportional input of phosphate in relation to nitrogen coupled with a potential occurrence of desorption of phosphorous from the sediments under high temperatures and low oxygen (Leote and Epping, 2015). In fact, there was a decrease of the N:P ratio, where the values were really low (< 1.5) near the discharge. Nevertheless, the N:P ratio at T1750E (Figure 3.3) increased but was still below the Redfield ratio (N:P = 16), meaning that the nitrogen was the limiting element along the Almargem channel.

The mixing process clearly explains the spatial gradient of the parameters analysed in the Almargem channel (T points), as depicted from PC1 of PCA (Figure 3.14). Using the TDL (Figure 3.11) for the dissolved nutrients, it was found that silicate and nitrate had the most conservative behaviour. However, in July and August the values or ammonium, nitrate and phosphate near the discharge were much higher than the predicted by the TDL suggesting that there an enhancement of concentration by input from the effluents discharge on those months. In July, it was also observed a peak in chlorophyll *a* (attaining values over 100 μg/L) and oxygen saturation over 300% of dissolved oxygen during low water on the neap tide, between T250S and T1000S. These are dissolved oxygen saturation and chlorophyll *a* values approximately 2 and 25 times, respectively higher than the values registered at T1500S or at the same stations for the other months. At the same time, the values for the nutrients were relatively lower due to consumption when compared with the other summer months. Along that section on the channel an algal mat was visible (Figure 4.1) that contributed to further increase the dissolved oxygen concentration beside the phytoplanktonic bloom. This confirms the importance of the photosynthesis on the variability of results, as evidenced by PC2 of PCA (Figure 3.14). The higher values for chlorophyll *a* during these summer months can be explained by light and temperature increase accompanied by nutrient availability that allow the growth of primary producers, as found by other authors in this system (Barbosa, et al., 2010; Cravo et al., 2015, 2018). These extreme values of dissolved oxygen and chlorophyll *a* in the Almargem channel in July and August, especially during low water, also contributed to increase the TRIX index, leading to a decrease of water quality conditions representative of the Eutrophic/Hypertrophic class at site T250S, T500S and T750S.

In July, the importance of respiration and degradation of organic matter was also evident, as shown in PC3 of PCA (Figure 3.14), reflected in low dissolved oxygen saturation and higher SS concentration (that also include phytoplankton) achieved in low water sampled during the early morning. This also contribute to increase the TRIX index values, associated to a decrease of water quality (Figure 3.12).

Figure 4.1. Algal mat found in the Almargem channel on the 25th of July 2019.

The influence of the discharge was also visible on the microbiological contamination parameters (Figure 3.5), particularly during low water, where at the station closer to the discharge the concentrations were significantly higher (almost two orders of magnitude) than at the reference station, especially for the *E. coli*. The Almargem UWWTP has UV disinfection of the final effluent, so it is expected that *E coli* in the receiving waters is lower than the limit of discharge license for faecal coliforms (2000 MPN/100 mL) values (https://www.aguasdoalgarve.pt/content/etar-de-almargem-0). Despite the increase of the population during summer the amount of *E.coli* in the system was not significantly higher, that can be explained not only by the UV disinfection of the final effluent into the UWWTP but also by the increase of temperature and solar radiation that can be a natural disinfectant of the water (Bettencourt, et al., 2013; Dionisio, et al., 2000). However, there were some of values that exceeded this limit as found in August or in February (Figure 3.5). In August, apparently there was an increase of the discharged flow or a less efficient disinfection by UV lamps. In February these exceeding values could also be partly associated with rainfall events (Table 6) and land runoff into the Almargem stream some days before the sampling. The same fact has been found in other microbiological contamination studies conducted in the vicinity UWWTP Ria Formosa after, rainfall events (Almeida and Soares, 2012; Cravo et al., 2015).

Table 6. Precipitation records for the week of the campaigns (data supplied by IPMA).

	2019											2020	
Month	06		07		08		09		10	11	12	01	02
Tidal Phase	NT	ST	NT	ST	NT	ST	NT	ST	NT	NT	NT	NT	NT
Precipitation (mm)	0.2	0	0	0	0	0	0	0	0	1.2	3.8	0.2	0.8

Moreover, even if this section along the Almargem channel is not considered bathing waters it was very frequent to find people bathing or harvesting bivalves along this channel in natural banks. It is also important to remark that several *E. coli* values exceeded the class of sufficient quality for bathing waters in coastal and transitional areas (500 cfu/100 mL; Directive 2006/7/EC). For enterococcus most of the results were below the limit for bathing waters of good quality (200 cfu/100 mL; Directive 2006/7/EC). In the Portuguese decree-law 236/98, the values for the total coliforms in bathing waters should not overpass the 10000 MPN/100 mL as the maximum admissible value. This threshold was passed in the same occasions that the *E. coli* values were the highest. So, considering the microbiological contamination, it means that in the Almargem channel has some contamination that can affect not only the biota but also the people harvesting bivalves or other filter feeder organisms. However, as at the reference station (1750 m) microbial contamination was almost negligeable it suggests that this UWWTP does not affect the main channel and the bivalve production zone in Quatro Águas, as indicated in Figure 3.5.

4.2 Water quality on the Gilão river low estuary and close to shellfish beds grounds

Tavira region is one of the important shellfish production areas in Ria Formosa (10%), producing mainly *Ruditapes decussatus* (Serpa et al., 2005). The shellfish production in this study area is located downstream of the Gilão river, as shown in Figure 1.2.

Analysing the nutrients concentrations (Figure 3.2), the values were significantly higher ($p < 0.05$) during low water when compared with high water, also confirming the tidal influence and water renewal of seawater from the Tavira inlet during the flood period, as previously reported by other authors (Botelho, et al., 2015; Cravo et al., 2015). This led to a dilution of the river discharge decreasing the concentrations in terms of nutrients, suspended solids and chlorophyll *a*. Regardless that, the values were significantly lower when compared with the same tidal conditions in the Almargem channel, at stations down to 750 m. In this study section at the Gilão low estuary the water flows in direction to the Tavira inlet, carrying material along the river and estuary together with contribution from external sources such as land runoff. Some years ago, in this section of the study area, the effluents were discharged from the "old" Tavira UWWTP (decommissioned in 2007 to be substituted by the "new" Almargem UWWTP). This explains that the water quality of the estuary now is less impacted than before and has a water quality similar to that found further down 1500 m from the Almargem UWWTP. Even though at this section, particularly at the upstream station (TV900 N) a high variability of results was observed namely for salinity and nutrients, which reflects the different contribution of freshwater from the Gilão river into the estuary along the tidal cycles. Indeed, during the winter months, the increase in nitrate and silicate was evident and changed the nutrients ratios to values slightly higher than the equilibrium nutrient ratios (N:P = 16 and N:Si = 1). This estuary is surrounded by agricultural fields and since the Gilão river is the main freshwater source, the runoff from these agricultural fields during precipitation periods contributes to the input of phosphate and mainly nitrate from fertilizers, making phosphorous the limiting element (N:P > 16), distorting the optimal ratio (N:P = 16). By that time, the phosphate increase was not so obvious as nitrogen since it has a peculiar behaviour with strong affinity to be adsorbed to particles/suspended matter (Froelich, 1988). For the most conservative nutrients, nitrate estimated in the river (S = 0) from the TDL, can be considered relatively high, achieving almost 60 µM, which is typical of rivers contaminated by nitrate, as found by Correia at al. (2020) in the Arade and Guadiana Rivers and Rodrigues et al. (2020) in Tagus River. Regarding silicate, the value estimated in the river was *ca.* 100 µM, typical of rivers and within the range found from the previously mentioned authors for Guadiana and Arade rivers. Looking in detail for the TDL for this section (Figure 3.11), indeed, the phosphate is the nutrient showing to have a less conservative behaviour, confirming its different chemical behaviour. From TDL it was also observed that in this area nutrients were more conservative than at the Almargem channel. In the estuarine section, ammonium was higher than expected from the mixing line at salinity > 35, which may suggest some external sources of this nutrient at the low estuary.

The tidal mixing is what explain the gradual decrease of nutrient concentration downstream the low estuary, more noticeable for silicate and nitrate concentrations (Figure 3.2). At TV150N (station closer to the shellfish beds) the values are similar to the concentration values found at T1750E ($p > 0.05$) and significantly lower ($p < 0.05$) than the values found at TV900N (station most upstream of the Gilão river low estuary). Even though, the TRIX index (Figure 3.12) attributes a classification of Oligotrophic for these three sampling stations of the low estuary, typical of waters of high quality.

These data also confirms there is not a mixing between the low estuary waters and the Almargem channel, since these two data sets have different characteristics, meaning that the UWWTP discharge does not directly affect the shellfish beds. No microbiological contamination analysis on the water was performed in this area. Monthly data available in the IPMA website about the microbiological contamination is only relative to bivalves, at a place close to TV150N, and reflected in the maps of permission/interdiction of bivalve harvesting, in a perspective of human health protection.

It is important to mention that these edible bivalves are filter feeder organisms and can concentrate bacteria present in the water column (Lees et al., 2000). The interdiction of bivalves harvesting is imposed when the *E. coli* values exceed the 46000 MPN/100 g, according to IPMA classes of health status (Table 7). The values of *E. coli* found on *Ruditapes decussatus* during the sampling campaign at the site monitored at Tavira are shown in Figure 4.2. The green line is the upper limit for the A class (230 MPN/100 g) and the yellow line the upper limit for the B class (4600 MPN/100 g). In June and August the health status of bivalves can be considered of class A, that allows the harvesting and commercialization of shellfish without depuration, while during the winter the health status dropped to class B and, and even to class C in February. The health status in this area is usually B class, as reported in the IPMA report from April 2020. The increase of *E. coli* in winter can be explained by land run-off, as reported at the Almargem channel, due to precipitation (Table 6), carrying organic matter enriched in faecal bacteria. However, the contamination by *E. coli* was not sufficiently high to interdict the shellfish harvesting. It is also important to remark that there are other factors for interdicting the bivalve harvesting such as marine biotoxins, metal contamination and organic contaminants, such as Benzo (a)pyrene. IPMA website also provides data on the phytoplankton population and biotoxins present in the water column. For the time of the sampling the concentration of biotoxins varied and in July, August and September passed the legal limits of 160 µg/kg for lipophilic toxins (853/2004/EC and 786/2013/EU). Some harmful phytoplanktonic species classes, such as Dinophyceae and Bacillariophyceae also passed the warning limits of 200 cells/L and 80000 cells/L, respectively. This was the case of Dinophyceae in the summer months (June to September) and in October and Bacillariophyceae in October, November and January. However, it only passed the interdiction limit on July 17^{th}, 2019. By that time the concentration of Dinophyceae, producer of yiessotoxins and homo-yiessotoxins, doubled the interdiction limit (> 1000000 cells/L). It is important to remark that those groups are typically marine that can reach this study area driven from the adjacent ocean during the flood period, as seen in Figure 4.3 there were higher concentrations of chlorophyll on the south coast of Portugal. Unfortunately, there are no monthly maps available from the IPMA website concerning permissions/interdictions of bivalves harvesting after January 2019.

Table 7. Health Status with the legal limits for shellfish harvesting (adapted from IPMA website).

[1.] Regulations: 854/2004/EC, 1021/2008/EC and 2015/2285/EU.

Health Status	Legal limit[1.] of *E. coli* (MPN/100 g)	Observations
A	≤ 230	The shellfish can be harvested and commercialized for direct human consumption.
B	> 230 and ≤ 4 600	The shellfish can be harvested and sent to depuration, transposition and transformation into an industrial unit.
C	> 4 600 and ≤ 46 000	The shellfish can be harvested and only sent for prolonged transposition or transformation into an industrial unit.
Forbidden	≥ 46 000	The harvesting of shellfish is not authorized.

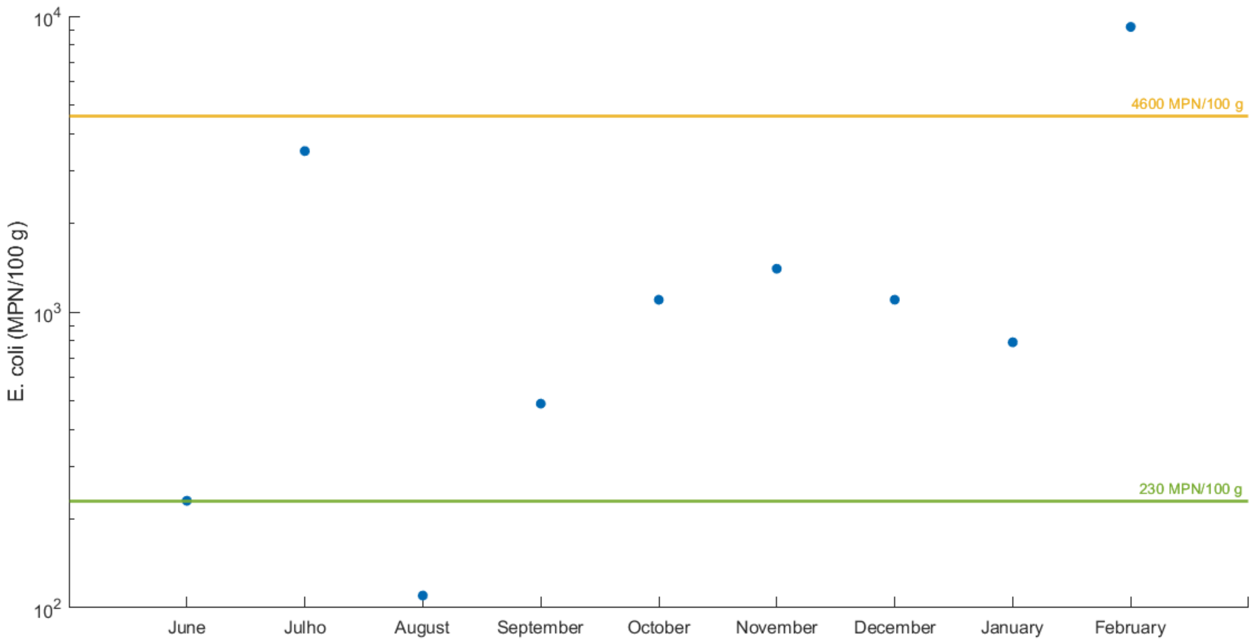

Figure 4.2. *E. coli* values in Ruditapes decussatus from the Gilão river low estuary (data from IPMA).

At Gilão low estuary, near the shellfish production area there is anthropogenic pressure as reflected in the microbiological contamination of the bivalves. In the surrounding area it is located an hotel, restaurants and bars, and a pier with a significant number of boats passing. So, it is not guaranteed that there are no other sources of contamination, especially from the microbiological point of view of the bivalves, which are able to filter water and accumulate contaminants along their live span. Unfortunately, there are no available data relative to these other sources of contamination.

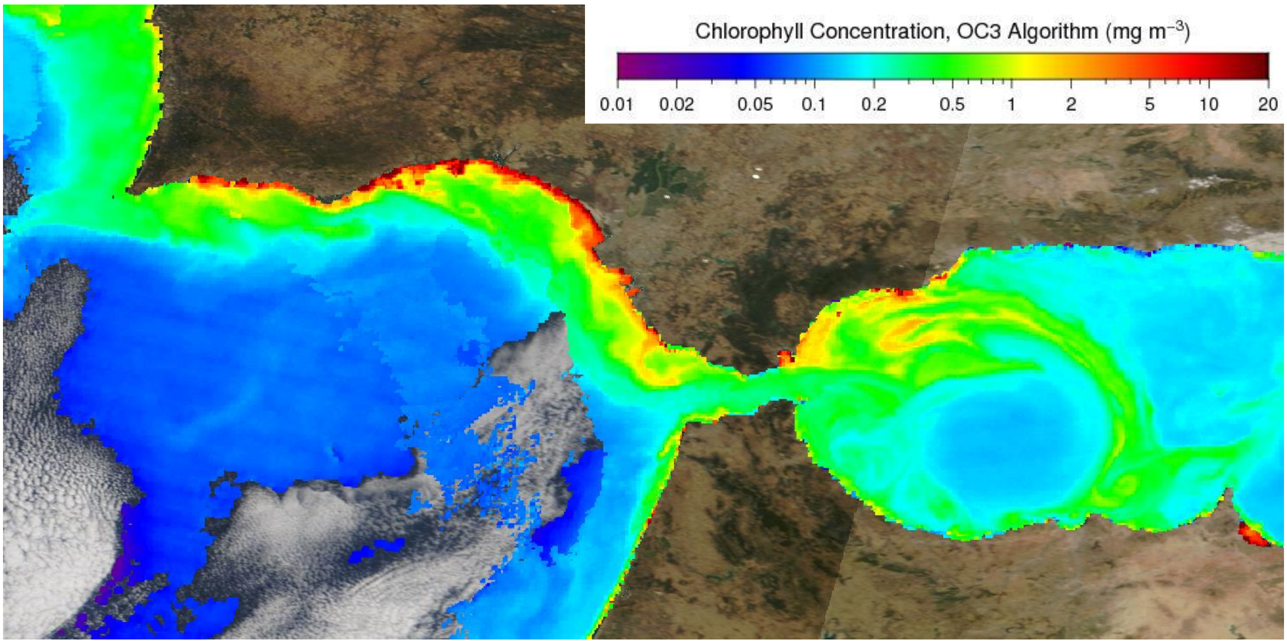

Figure 4.3. Satellite image of the chlorophyll concentration for the 8-day period (17th of July of 2019). **Source:** OESDIS, NASA.

4.3 Comparison with previous works in Ria Formosa

Due to the importance of Ria Formosa in Portugal in terms of ecological and socio-economic value a large number of research works have been conducted throughout the years, encompassing several and different areas along its extension. However, concerning the discharge effect of effluents from UWWTP on the water quality in Ria Formosa few studies can be pointed out. There was some research in 2001-2002 (Cravo et al., 2015) and 2006-2007 (Cravo et al., 2018), in areas affected by the main UWWTP discharges into the Ria Formosa including Tavira, on the low Gilão estuary, which allows a temporal comparison with the present work. Since the UWWTP in Tavira discharging its effluents on the Gilão estuary was decommissioned in 2007 and the new one close to Almargem channel it is only possible to compare data for the three sampling points in the Gilão low estuary including the area to the shellfish beds. There is also a study conducted in Ria Formosa considering the microbiological monitoring of bivalves from 1990 to 2009 (Almeida and Soares, 2012) that includes part of the area of this study, at the low estuary section that will be considered.

The range of temperature (T), salinity (Sal), pH, Oxygen saturation (O_2), ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}), silicate (SiO_4^{4-}) and chlorophyll *a* concentration for the Gilão river low estuary from the present study and for previous studies are represented in Table 8. It is noteworthy that the previous studies had more sampling points upstream the Gilão river than this study and so values such as salinity have a wider range. The TRIX index (Table 9), calculated for the sampling points at the low estuary section can be also compared with previous works.

From Table 8, it is observed that the *in situ* parameters have a similar range, except salinity, since previous works considered upstream stations, as mentioned before. The differences can derive from physical forcing such as the tidal range, seasonality and different meteorological conditions, like precipitation. The salinity is highly variable in estuaries, depending always on the local and proportion of the mixture between the two water masses endmembers (river and sea), and also on the tidal height and tidal phase (neap tide/spring tide/intermediate tide). Concerning the nutrient concentration, the values for this study are much lower, as reflected in the maximum and average values. This can be explained by the fact that when the previous studies were conducted the Tavira UWWTP was still active, discharging the effluents into the low estuary, what is not happening in the present work. As mirrored in the TRIX index (Table 9), the water quality improved to excellent in the period after the decommissioning of the Tavira UWWTP (1997) especially at the most upstream stations of the Gilão river low estuary (TV400N and TV900N), which quality is still maintained in the present study.

Looking at the microbiological contamination, Almeida and Soares (2012) concluded that the *E. coli* concentration was below 1000 MPN/100 g in bivalves for the Tavira area (site 2). These authors, for this area from 1990 to 2009 found an average health status of the clam *Ruditapes decussatus* of class B, with about 10% of the time with class C and about 25% of class A. Comparing with the present study, the results are very similar, meaning that the source of *E. coli* concentration found presently are a not associated with wastewater discharge from the WWTP but rather an external source.

Table 8. Range and mean for low water (LW) and high water (HW) of temperature (T), salinity (Sal), pH, Oxygen saturation (O_2), ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}), silicate (SiO_4^{4-}) and chlorophyll *a* concentration for the Gilão river low estuary obtained from the present study and other referenced in the table.

		T (ºC)	Sal	pH	O_2 (%)	NH_4^+ (µM)	NO_3^- (µM)	PO_4^{3-} (µM)	SiO_4^{4-} (µM)	Chl*a* (µg/L)	Date	Ref.
Tavira	Min	13.20	2.30	7.25	56.00	n.d.	n.d.	n.d.	1.10	n.d.	May 2001 – December 2002	Cravo et al., 2015
	Max	36.40	37.70	8.19	140.00	44.00	39.60	14.20	108.70	16.70		
	LW	20.60	31.60	7.80	99.00	5.30	5.70	1.00	18.10	1.70		
	HW	20.40	34.30	7.96	108.00	2.15	2.50	0.50	5.00	1.00		
Faro-Northwest	Max	-	-	-	-	1801	~30	~160	-	-	July 2001 – May 2002	Cabaço et al., 2008
Tavira	Min	12.30	14.40	7.24	81.00	0.05	0.02	0.05	0.08	n.d.	May 2006 – December 2007	Cravo et al., 2018
	Max	24.80	37.80	8.82	142.00	92.30	25.30	7.51	55.6	6.60		
	LW	17.50	33.80	7.94	101.00	4.10	4.60	0.60	7.30	0.70		
Tavira	Min	12.37	30.20	7.76	81.40	0.10	0.02	0.02	0.20	0.18	June 2019 – February 2020	Present study
	Max	26.39	37.23	8.40	136.80	5.99	14.80	1.47	25.24	13.35		
	LW	19.96	35.90	8.07	102.50	1.92	2.26	0.22	5.98	1.41		
	HW	17.87	36.15	8.06	103.90	1.03	1.44	0.16	2.56	1.83		

Table 9. TRIX states with the respective corresponding stations for this study and other referenced in the table.

Date	Corresponding Station	TRIX State	Reference
May 2001 – December 2002	TV150N	Excellent	Cravo et al., 2015
	TV400N-TV900N	Good	
May 2006 – December 2007	TV150N – TV900N	Excellent	Cravo et al., 2018
June 2019 – February 2020	TV150N – TV900N	Excellent	Present study

This study was conducted within the CONPRAR project scope, which include more four UWWTPs discharging to the Ria Formosa coastal lagoon. Two of them suffered some alterations, the Olhão Poente and Faro Nascente were decommissioned in October 2018 and the new Faro-Olhão UWWTP join the influents from the previous decommissioned UWWTP (Jacob et al., 2020). Comparing the Almargem study area with the Faro-Olhão study area, the *E. coli* concentration was higher in Faro-Olhão and the water quality is worse. There, in the period between May and August of 2019, the higher values are not only associated with the highest flow discharged (~14000 m^3/day) but also because the UV disinfection was not working yet (Jacob et al., 2020). In past studies, a similar pattern was observed, where close to Faro Nascente UWWTP study area values were slightly higher for *E. coli* in comparison with Tavira, in the same period of time (Almeida et al., 2012).

The concentrations of nutrients, in Faro-Northwest UWWTP region, from July 2001 and May 2002, according to Cabaço, et al. (2008) were much higher (Table 9) than the ones found on this study, especially for ammonium and phosphate. By that time the volume discharge from the Faro Northwest UWWTP was similar to the volume discharging at the present moment in Tavira, meaning that the higher concentration in Faro Northwest did not result from the difference in volume discharged but with a more restricted circulation and exchanges along the tidal cycles. These exchanges are stronger in the Almargem channel than in Faro-Northwest, allowing the water in Almargem to have a high water quality.

4.4 Comparison with other coastal lagoons

In order to put in context the impact of wastewater discharge in the Almargem Channel with other lagoons, a comparison is conducted as shown in Table 10.

Table 10. Range and mean for low water (LW) and high water (HW) of, DIN (dissolved inorganic nitrogen), phosphate (PO_4^{3-}), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), silicate (SiO_4^{4-}) and chlorophyll *a* concentration for the different coastal lagoons.

Coastal Lagoon			DIN (µM)	PO_4^{3-} (µM)	NH_4+ (µM)	NO_2^- (µM)	NO_3^- (µM)	SiO_4^{4-} (µM)	Chl*a* (µg/L)	Date	Ref.
Ria Formosa (Almargem)		Min	0.12	0.01	0.08	n.d.	0.03	0.19	n.d.	June 2019 – February 2020	Present Study
		Max	141.08	38.02	106.6	11.94	22.51	49.56	141.07		
		LW	13.78	4.39	8.37	1.48	3.93	10.94	6.30		
		HW	13.48	4.23	8.23	1.42	3.84	10.59	6.25		
Ria de Aveiro	Winter	Min	n.d.	n.d.	-	-	-	n.d.	n.d.	February 2001	Lopes, et al., 2007
		Max	537.9	20.1	-	-	-	143.5	5.9		
		LW	220	3.66	-	-	-	86.65	1.9		
		HW	139	3.69	-	-	-	72.16	1.26		
	Summer	Min	6.5	0.5	-	-	-	10.0	1.2	September 2001	
		Max	504.8	33.9	-	-	-	72.3	11.3		
		LW	111	5.25	-	-	-	23.85	4.6		
		HW	56.5	2.56	-	-	-	22.04	1.98		
Ria de Vigo		Min	-	-	-	-	n.d.	n.d.	-	Maio 2001 – April 2002	Santiso, et al., 2008
		Max	-	-	-	-	12	14	-		
Mar Menor		Min	-	n.d.	-	-	-	-	490	September 2002 – October 2003	Velasco, et al., 2006
		Max	-	15000	-	-	-	-	12570		
		Mean	-	3000	-	-	-	-	2390		
Patos Lagoon	Winter	Max	-	170	500	-	-	-	-	February 2011	Marreto, et al., 2017
	Summer	Max	-	300	150	-	-	-	-	July 2011	

Ria de Aveiro (North of Portugal) is also a shallow coastal lagoon but suffers influence from an important river (Vouga). After amelioration on the wastewater treatment system, the domestic and industrial sewage is not discharged directly into the Ria de Aveiro, leading to a decrease of nutrient input (Figueiredo da Silva et al., 2002) and improved the water quality. In fact, in Ria de Aveiro less than 10% of the nutrient input comes from UWWTP (Lillebø et al., 2015). However, Ria de Aveiro watershed includes a large agricultural area, which input remain unchanged, so the nitrogen input was not affected by the implementation of the new wastewater treatment system, but the phosphorous loads were reduced, especially during the summer (Figueiredo da Silva et al., 2002). This was also recorded in the results for the Gilão river low estuary, where the nutrients concentrations decreased after the decommissioning of the UWWTP. However, there is still an important input of nutrients from the agriculture run-off in winter months after precipitation events. Comparing the nutrient concentrations, in general, the Ria de Aveiro during the summer and winter of 2001, reached concentrations twice as high as the ones found in this study (Table 10), especially concerning silica. However, it can be explained by the differences in the river flow (that in Gilão most of the time can be considered negligeable) and in the precipitation pattern. In Ria de Aveiro the precipitation reached at least 112 mm (in summer) and 624 mm (in winter) in 2001 (Lopes et al., 2007), while in Ria Formosa during these winter months in 2020 it was much lower (Table 6). Nevertheless, the concentrations have a similar temporal pattern of distribution and spatial trend, decreasing with the increase of salinity and mixture with seawater, and with distance from the nutrients source . The OHI (Overall Human Influence) index for Ria de Aveiro concerning the impact of the nutrient input has been considered “Moderate Low”, due to a “high” flushing potential while the input of nutrients from river and land run-off has been also considered “high” (Ferreira et al., 2003). In Ria Formosa the OHI index has been considered as “moderate”, with a “moderate” input of nutrients, which means that some eutrophication symptoms can be related to the nutrient input, while this system has a “high” dilution potential and a “low” freshwater inflow (Ferreira et al., 2003). However, Ria Formosa also can suffer from inputs of nutrients from the coast, especially during periods of upwelling events more frequent during April to October, under westerly winds (Relvas et al., 2007). Associated with those, algal blooms (toxic or not) can occur on the adjacent ocean (Barbosa, 2010) and entering this system increasing the biological activity of these waters and cause some impact if phytoplankton species are toxic, that can be accumulated by the bivalves, as mentioned before.

The hydrodynamics in Ria Formosa can be compared with Ria de Vigo (North of Spain), where there is a high rate of exchange of water between the coastal lagoon and the ocean. However, Ria de Vigo also receives a great riverine contribution. In this coastal lagoon, the nitrate concentration vary from not detected values to 12 µM and the silicate from not detected values to 14 µM, from May of

2001 and April of 2002 (Santiso et al., 2008), which values are similar to the ones found in this study. The water exchanges promoted by tidal influence allow the dilution of the nutrient input and microbiological contamination, not only from the UWWTPs but also from riverine and land run-off (Fernández et al., 2016). The water renewal every tidal cycle, prevents the decrease of the water quality, reason why regardless the inputs to the lagoon, it keeps oligotrophic and the bivalves maintain a health status of class A and B (Fernández et al., 2016), as those in the Tavira production area.

Mar Menor is a shallow coastal lagoon in the South of Spain that has a strong anthropogenic influence from the watershed that drains to it. This lagoon shows high concentrations of organic residues, fertilizers, pesticides and heavy metals, mainly driven from agricultural run-off, but it also receives the discharge of an urban wastewater (Lillebø et al., 2015). In Mar Menor, the UWWTP discharge increases during summer, corresponding to an increase of population (García-Pintado et al., 2007) that similarly to this study, led to an increase of the nutrient input into the water column. That study in Mar Menor also revealed that the principal source of nitrate is the agricultural run-off after rainfall, contrarily to the other nutrients which major source is the UWWTP, in accordance with that observed in the present study. The nutrient concentrations in Mar Menor are much higher (Table 10) than the ones found in this study. Despite the large amounts of nutrient inputs in Mar Menor, eutrophication events are not recorded since *Caulerpa prolifera* beds in the area, are able to consume part of these nutrients (Lillebø et al., 2015). TRIX index calculated for Mar Menor showed a TRIX similar over time, maintaining the oligotrophic conditions (Salas et al., 2008), similarly as found at the downstream points of the Almargem channel and the Gilão river low estuary. In the rest of the present sampling sites the water showed a worse water quality varying from mesotrophic to hypertrophic at low water. This shows the importance of autotrophic producers in controlling the nutrient contamination by their consumption that in Mar Menor contributed to improve the water quality. In Ria Formosa, the main driving mechanism controlling the water quality is the tidal exchange and water renewal each tidal cycle closely related with hydrodynamics.

The Patos Lagoon estuary in South Brazil, is surrounded by a big city (Rio Grande), with high anthropogenic influence in the lagoon, especially due to poorly treated effluents discharges (industrial and domestic). There, the circulation of the water bodies is influenced by the wind stress that affects the patterns dispersion/distribution of the anthropogenic inputs (Seiler et al., 2020), rather than the tides as observed in Ria Formosa. In Patos Lagoon, also the input of nutrients is increased during precipitation periods (end of winter and spring), leading to of eutrophication processes (Marreto et al., 2017). In the Patos lagoon, the concentration of ammonium is higher in winter and higher concentration of phosphate in summer (Table 10) (Marreto et al., 2017). Despite the nutrient input by the UWWTP discharge in the Almargem channel, the general trophic condition was mesotrophic, by the high water exchanges and dilution while in the Patos lagoon the excessive inputs of phosphorus and nitrogen generate algal blooms causing a global hypereutrophic condition of the system (Marreto et al., 2017).

5. Conclusion

This work was aiming at a better understanding of the impact the UWWTP on the Almargem channel and of the low estuary on the nearby shellfish production areas in terms water quality including microbiological contamination. The main conclusions of this study are:

- The Almargem UWWTP effluent impacts the water quality in the Almargem channel till about 750 m - 1000 m downstream from the discharge point. This impact is mainly reflected in the high nutrients and dissolved oxygen concentrations, and microbiological contamination (especially *E. coli*) . However, the values of microbiological contamination, in general, comply the limit imposed for the discharge license, not affecting the bivalve production zone. In the shellfish beds there were values indicative of some microbiological contamination, from external sources, leading the production area to have a general classification of Class B, meaning that bivalves must be depurated before commercialization.
- The water quality assessed by the TRIX index, was mostly oligotrophic (downstream 1500 m to the Tavira channel and at the Gilão low estuary) becoming progressively to eutrophic (at the upstream stations closer to the effluents discharge point on the Almargem channel until 1000).
- Seasonally, the nutrient concentration at the Almargem Channel was higher in summer (dry season), which can be associated with the increase of the population, when the volume of the input can be expectedly higher. However, under periods of rainfall, land runoff provides an increase of nitrate and silicate at the Almargem stream and Gilão low estuary.
- Along the last 20 years, at the Gilão river low estuary, the water quality and microbiological contamination improved since the decommissioning of the Tavira UWWTP in 2007 and present a water quality significantly higher than at Almargem channel upstream the 750 m, as expressed by TRIX.
- The most important factor for the decrease of the contamination in the study area is the tidal regime and water renewal each tidal cycle, that during flood is able to cause a significant dilution effect that improve substantially the water quality.
- Despite the amplitude of variability of water quality was attained during spring tides, the highest impact of effluents on the water quality was recorded during the neap tides due to increased residence time and decreased water renewal, when the biological processes of photosynthesis and respiration can be intensified.
- Comparing with the other places in Ria Formosa especially with both Faro UWWTPs, the Almargem channel showed to be less impacted, with better water quality highly responsive to high renewal rate each tidal cycle and strong hydrodynamics.

- The water quality in Tavira study areas is relatively similar to other costal lagoons worldwide (Ria de Aveiro, Ria de Vigo and Mar Menor) despite responding to lower nutrient loads. However, when compared with other lagoons (ex: Patos Lagoon – Brazil) receiving high nutrient loads and suffering lower water renewal through exchanges with the sea Tavira study area has a better water quality.

References

Águas do Algarve (2019) *ETAR de Almargem*. Available at: https://www.aguasdoalgarve.pt/content/etar-de-almargem-0 (Accessed: 2 January 2020).

Almeida, C., Soares, F. (2012) 'Microbiological monitoring of bivalves from the Ria Formosa Lagoon (south coast of Portugal): A 20 years of sanitary survey', *Marine Pollution Bulletin*. Elsevier Ltd, 64(2), pp. 252–262. doi: 10.1016/j.marpolbul.2011.11.025.

Andrade, J. P. (1990) *A importância da Ria Formosa no ciclo biológico de Solea senegalensis Kaup 1858, Solea vulgaris Quencel 1806, Solea lascaris (Risso, 1868) e Microchirus azevia (Capello, 1868)*. Faro: UCTRA, Universidade do Algarve. pp. 410

Barbosa, A. (2010) 'Seasonal and interannual variability of planktonic microbes in a mesotidal coastal lagoon (Ria Formosa, SE Portugal): impact of climatic changes and local human influences', in *Coastal Lagoons: critical habitats of environmental change*, pp. 335–366.

Barners, R. S. K. (1980) *Coastal Lagoons*. Cambridge, UK: Cambridge University Press. pp. 106

Bettencourt, a M., Bricker, S. B., Ferreira, J. G., Franco, A., Marques, J. C., Melo, J. J., Nobre, A., Ramos, L., Reis, C. S., Salas, F., Silva, M. c., Simas, T., Wolff, W. J. (2004) 'Typology and reference conditions for Portuguese transitional and coastal waters', pp. 98.

Bettencourt, F., Almeida, C., Santos, M.I., Pedroso, L., Soares, F. (2013). 'Microbiological monitoring of Ruditapes decussatus from Ria Formosa Lagoon (South of Portugal)'. *Journal of Coastal Conservation* . 17 (3), 653–661. doi: 10.1007/s11852-013-0264-1

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*. Elsevier Ltd, 96, pp. 188–196. doi: http://dx.doi.org/10.1016/j.marpolbul.2015.05.030

Cabaço, S., Machás, R., Vieira, V., Santos, R. (2008) 'Impacts of urban wastewater discharge on seagrass meadows (*Zostera noltii*)', *Estuarine, Coastal and Shelf Science*. Elsevier Ltd, 78(1), pp. 1-13. doi: https://doi.org/10.1016/j.ecss.2007.11.005

Clesceri, L.S., Greenberg, A.E., Eaton, A. D. (1998) *Standard Methods for the Examination of Water and Wastewater*. 23th edn, *American Public Health Association*. 23th edn. Washington DC. pp. 1546

Cloern, J. E. (2001) 'Our evolving conceptual model of the coastal eutrophication problem', *Mar Ecol Prog Ser National Research Council*, 210, pp. 223–253.

Correira, C., Torres, A.F., Rosa, A., Cravo, A., Jacob, J., de Oliveira Júnior, L., Garel, E. (2020) 'Export of dissolved and suspended matter form the main estuaries in South Portugal duing winter conditions' *Marine Chemistry*, 224. doi: 10.1016/j.marchem.2020.103827

Cravo, A. *et al.* (2015) ‘Determining the footprint of sewage discharges in a coastal lagoon in South-Western Europe’, *Marine Pollution Bulletin*. Elsevier Ltd, 96(1–2), pp. 197–209. doi: 10.1016/j.marpolbul.2015.05.029.

Cravo, A., Ferreira, C., Jacob, J., (2019) ‘Water quality improvement in Ria Formosa since the early 2000?’, *Sanitation aproaches and solutions and the sustainabl development goals*. 307-322

Davidson, K., Gowen, R. J., Tett, P., Bresnan, E., Harrison, P. J., McKinney, A., Milligan, S., Mills, D. K., Silke, J., Crooks, A.-M. (2012) ‘Harmful algal blooms: How strong is the evidence that nutrient ratios and forms influence their occurrence?’, *Estuarine, Coastal and Shelf Science*, 115, pp. 399–413. doi: 10.1016/j.ecss.2012.09.019.

Diário da República (1987). DL (Decreto-Lei) 373/87, série I, nº282, 1987-12-09, pp. 4257-4263.

Diário da República (1998). DL (Decreto-Lei) 236/98, série I-A, nº176, 1998-08-01, pp. 3676-3721.

Diário da República (2004). DL (Decreto-Lei) 149/2004, série I-A, nº 145, 2004-06-22, pp. 3805-3809.

Dionísio, L. C., Rheinheimer, G., Borrego, Juan J. (2000) ‘ Microbiological Pollution of Ria Formosa (South of Portugal)’, *Marine Pollution Bulletin* 40(2):186-193 doi: https://doi.org/10.1016/S0025-326X(99)00206-4

EU, 2000. Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for Community action in the field of water policy. Official Journal of the European Communities L 327, 1e72.

FAO (2004). *Marine Biotoxins* in FAO food and Nutrition Paper, Vol: 80. Rome, Italy: FAO of the United Nations

Fernández, E., Álvarez-Salgado, X. A., Beiras, R., Ovejero, A. (2016) 'Coexistence of urban uses and shellfish production in an upwelling-driven, highly productive marine environment: The case of the Ría de Vigo (Galicia, Spain)', *Regional Stuudies in Mairne Science*, 8, pp. 362-370 doi: http://dx.doi.org/10.1016/j.rsma.2016.04.002

Ferreira, J. G., Simas, T., Nobre, A., Silva, M. C., Shifferegger, K., Lencart-Silva, J. (2003)

Identification of sensitive areas and vulnerable zones in transitional and coastal portuguese systems. Applications of the United States National Estuarine Eutrophication Assessment to the Minho, Lima, Douro, Ria de Aveiro, Mondego, Tagus, Sado, Mira, Ria Formosa and Guadiana systems.

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, W., Verner-Jeffreys, D., Baas, J., Petersen, J. K., Wright, J., Calixto, V., & Rocha, M. (2013). FORWARD - Framework for Ria Formosa Water Quality, Aquaculture, and Resource Development.

Figueiredo da Silva, J., Duck, R. W., Hopkins, T. S., Rodrigues, M. (2002) 'Evolution of the nutrient inputs to a coastal lagoon: the case of the Ria de Aveiro, Portugal', *Hydrologia, 475/476s*, pp. 379-385 doi: 10.1023/A:1020347610968

Frigstad, H. *et al.* (2011) ‘Seasonal variation in marine C:N:P stoichiometry: Can the composition of seston explain stable Redfield ratios?’, *Biogeosciences,* 8(10), pp. 2917–2933. doi: 10.5194/bg-8-2917-2011.

Froelich, P. N. (1988) 'Kinctic control of dissolved phosphate in natural rivers and estuaries: A primer on the phosphate buffer mechanism' *Limnology and oceanography*. 33(4) pp. 649-668

García-Pintado, J., Martínez-Mena, M., Barberá, G. G., Albaladejo, J., Castillo, V. M. (2007) 'Anthropogenic nutrient sources and loads from a Mediterranean catchament into a coastal lagoon: Mar Menor, Spain' *Science of the Total Environment*, 373. pp. 220-239 doi: 10.1016/j.scitotenv.2006.10.046

Gay, K. (1990) *Water Pollution*. London, UK: Franklin Watts. pp. 144

Grasshoff, K, Erkhardt, M, Kremling, K. (1983) *Determination of nutrients*, *Methods of Seawater Analysis*. New York: Verlag Chemie. doi: 10.1002/9783527613984.ch10.

Jacob, J., Correia, C., Torres, A. F., Xufre, G., Matos, A., Ferreira, C., Reis, M. P., Caetano, S., Freitas, C. S., Barbosa, A. B., Cravo, A. (2020) 'Impacts of Decommissioning and Upgrading Urban Wastewater Treatment Plants on the Water Quality in a Shellfish Farming Coastal Lagoon (Ria Formosa, South Portugal)', *Journal of Coastal Research* 95, pp. 45-50. doi: 10.2112/SI95-009.1

Johnson, K. S. (2010) ‘Simultaneous measurements of nitrate, oxygen, and carbon dioxide on oceanographic moorings: Observing the Redfield ratio in real time’, *Limnology and Oceanography*, 55(2), pp. 615–627. doi: 10.4319/lo.2009.55.2.0615.

Kjerfve, B. (1994) *Coastal Lagoons*. Elsevier Oceanography Series. Amesterdam, The Netherlands: Elsevier Science B.V. pp. 576

Lees, D. (2000) ‘Viruses and bivalve shellfish’, *International Journal of Food Microbiology*, 59(1-2), pp. 81-116. doi:10.1016/S0168-1605(00)00248-8

Leote, C. and Epping, E. (2015). Sediment–water exchange of nutrients in the Marsdiep basin, western Wadden Sea: Phosphorus limitation induced by a controlled release? *Continental Shelf Research*, 92, 44–58. doi: 10.1016/j.csr.2014.11.007

Lillebø, A. I., Stålnacke, P., Gooch, G. D. (2015) *Costal Lagoons in Europe: Integrated Water Resources Strategies* IWA Publishing. doi: 10.2166/9781780406299

Lloret, J., Marín, A. and Marín-Guirao, L. (2008) 'Is coastal lagoon eutrophication likely to be aggravated by global climate change?', *Estuarine, Coastal and Shelf Science*, 78(2), pp. 403–412. doi: 10.1016/j.ecss.2008.01.003.

Lopes, C. B., Lillebø, A. I., Dias, J. M., Pereira, E., Vale, C., Duarte, A. C. (2007) 'Nutrient dynamics and seasonal succession of phytoplankton assemblages in a Southern European Estuary: Ria de Aveiro, Portugal', *Esturaine, Coastal and Shelf Science*, 71, pp. 480-490, doi: doi:10.1016/j.ecss.2006.09.015

Lorenzen, C. J. (1967) 'Determination of Chlorophyll and Pheo-Pigments: Spectrophotometric Equations', *Limnology and Oceanography*, 12(2), pp. 343–346. doi: 10.4319/lo.1967.12.2.0343.

Marreto, R.N., Baumgarten, M.G.Z. and Wallner-Kersanach, M. Trophic quality of waters in the Patos Lagoon estuary: a comparison between its margins and the port channel located in Rio Grande, RS, Brazil. (2017) *Acta Limnologica Brasiliensia*, 29(11) doi: 10.1590/s2179-975x10716

Mcnulty, J. K. (1977) 'Discharge of Sewage', in *Coastal Ecosystem Menagement*. John Wiley and Sons, Inc., pp. 604–610.

Metcalf & Eddy (1995) *Wastewater Engineering - Treatment, Disposal and Reuse*. 3rd edn. Tata McGraw-Hill.

Mudge, S. M., Bebianno, M. J. (1997) 'Sewage contamination following an accidental spillage in the Ria Formosa, Portugal', *Marine Pollution Bulletin*, 34(3), pp. 163–170. doi: 10.1016/S0025-326X(96)00082-3.

Mudge, S. M., Icely, J. D., Newton, A. (2008) 'Residence times in a hypersaline lagoon: Using salinity as a tracer', *Estuarine, Coastal and Shelf Science*, 77(2), pp. 278–284. doi: 10.1016/j.ecss.2007.09.032.

Newton, A., Icely, J. D., Flacao. 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), pp. 1945–1961. doi: 10.1016/j.csr.2003.06.008.

Newton, A., Mudge, S. M. (2003) 'Temperature and salinity regimes in a shallow, mesotidal lagoon, the Ria Formosa, Portugal', *Estuarine, Coastal and Shelf Science*, 57(1–2), pp. 73–85. doi: 10.1016/S0272-7714(02)00332-3.

Nixon, S. W. (1995) 'Coastal marine eutrophication: A definition, social causes, and future concerns', *Ophelia*, 41(1), pp. 199-219. doi: 10.1080/00785236.1995.10422044 ISSN: 00785326.

Oliveira, J., Cunha, A., Castilho, F., Romalde, J.L., Pereira, M.J. (2011) 'Microbial contamination and purification of bivalve shellfish: Crucial aspects in monitoring and future perspectives - A mini-review', *Food Control*. Elsevier Ltd, 22(6), pp. 805–816. doi: 10.1016/j.foodcont.2010.11.032.

O'Neill, K., Schreider, M., McArthur, E., Schreider, S. (2015) 'Changes in the water quality characteristics during a macroalgal bloom in a coastal lagoon', *Ocean & Coastal Management*. Elsevier Ltd, 118, pp. 32-36. doi: 10.1016/j.ocecoaman.2015.04.020

Pacheco, A., Ferreira, Ó., Williams, J. J., Garel, E., Vila-Concejo, A., Dias, J. A. (2010) 'Hydrodynamics and equilibrium of a multiple-inlet system', *Marine Geology*. Elsevier B.V., 274(1–4), pp. 32–42. doi: 10.1016/j.margeo.2010.03.003.

Penna, N., Capellacci, S. and Ricci, F. (2004) 'The influence of the Po River discharge on phytoplankton bloom dynamics along the coastline of Pesaro (Italy) in the Adriatic Sea', *Marine Pollution Bulletin*, 48(3–4), pp. 321–326. doi: 10.1016/j.marpolbul.2003.08.007.

Portuguese Institute for Sea and Atmosphere (2020) *Bivalves*. Available at: https://www.ipma.pt/en/bivalves/ (Accessed: 8 January 2020).

Razinkovas, A., Gasiünaitè, Z., Viaroli, P., Zaldívar, J. M. (2008) 'Preface: European lagoons - Need for further comparison across spatial and temporal scales', *Hydrobiologia*, 611(1), pp. 1–4. doi: 10.1007/s10750-008-9463-4.

Redfield, A.C., Ketchum, B.H., Richards, F. (1963) 'The influence of organisms on the composition of sea water', in Hill, M. N. (ed.) *The Sea*. New York: John Wiley, pp. 26–77.

Relvas, P., Barton, E.D., Dubert, J., Oliveira, P.B., Peliz, Á., Silva, J.C.B., Santos, A.M.P. (2007) 'Physical oceanography of the western Iberia ecosystem: Latest views and challenges', *Progress in Oceanography*, 74(2-3) pp. 149-173. doi: 10.1016/j.pocean.2007.04.021

Rodrigues, M., Cravo, A., Friere, P., Rosa, A., Santos, D. (2020) 'Temporal assessment of the water quality along an urban estuary (Tagus estuary, Portugal)' *Marine Chemistry*. 223. doi: 10.1016/j.marchem.2020.103824

Salas, F., Teixeira, H., Marcos, C., Marques, J. C. Pérez-Ruzafa, A. (2008) Applicability of the trophic index TRIX in two transitional ecosystems: the Mar Menor lagoon (Spain) and the Mondego estuary (Portugal) *ICES Journal of Marine Science*, 65, pp. 1442–1448.

Seiler, L. M. N., Fernandes, E. H. L., Siegle, E. (2020) 'Effect of wind and river discharge on water quality indicators of a coastal lagoon' *Regional Studies in Marine Science*, doi: https://doi.org/10.1016/j.rsma.2020.101513

Serpa, D., Jesus, D., Falcão M., Fonseca, L. (2005) 'Ria Formosa ecosystem: socioeconomic approach'. Relat. Cient. Téc. IPIMAR n.º 28, 50.

Statham, P. J. (2012) 'Nutrients in estuaries - An overview and the potential impacts of climate change', *Science of the Total Environment*. Elsevier B.V., 434, pp. 213–227. doi: 10.1016/j.scitotenv.2011.09.088.

Tett, P., Gilpin, L., Svendesen, 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, pp 1635-1671. doi: 10.1016/j.csr.2003.06.013

The European Parliament and the Council of the European Union (1979) *Directive 79/409/EEC, of 2 April 1979 concerning the conservation of wild birds'. Official Journal of the European Communities*. Brussels

The European Parliament and the Council of the European Union (1991) *Directive 91/271/EEC, of 21 May 1991 concerning urban waste-water treatment'. Official Journal of the European Communities*. Brussels.

The European Parliament and the Council of the European Union (2004) *Regulation (EC) No. 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption, Official Journal of the European Union*. Brussels.

The European Parliament and the Council of the European Union (2006) *Directive 2006/113/EC of the European Parliament and of the Council of 12 December 2006 on the quality required of shellfish waters, Official Journal of the European Union*. Brussels.

Turner, R. E. (2002) 'Element ratios and aquatic food webs', *Estuaries*, 25(4 B), pp. 694–703. doi: 10.1007/BF02804900.

United Nations (2019) *Sustainable Development Goals*. Available at: https://www.un.org/sustainabledevelopment/ (Accessed: 2 January 2020).

Utermöhl, H. (1958) ‘Zur Vervollkommnung der quantitativen Phytoplankton-Methodik’, *SIL Communications, 1953-1996*, 9(1), pp. 1–38. doi: 10.1080/05384680.1958.11904091.

Vollenweider, R. A., Giovanardi, F., Montanari, G., Rinaldi, A. (1998) ‘Characterization of the trophic conditions of marine coastal waters with special reference to the NW Adriatic Sea: proposal for a trophic scale, turbidity and generalized water quality index’, *Environmetrics*, 9(3), pp. 329–357. doi: 10.1002/(sici)1099-095x(199805/06)9:3<329::aid-env308>3.3.co,2-0.

Whitfield, A. K. (2011) *Coastal Lagoons – Critical Habitats of Environmental Change*, *Marine Biology Research*. CRC Press, Boca Raton, FL, pp. 417. doi: 10.1080/17451000.2010.538064.

Annex

Table 1. Lipophilic toxins concentration present in shellfish in Tavira from June 2019 to January 2020, no data was available for February 2020. Highlighted in red are the values above the legal limit (160 µg of okadaic acid/ kg) (data from IPMA).

Date	**Specie**	**Lipophilic Toxins (µg of okadaic acid/ kg)**
5/June/2019	*Mytilus edulis*	299
13/June/2019	*Mytilus edulis*	127
19/June/2019	*Mytilus edulis*	89
26/June/2019	*Mytilus edulis*	144
8/July/2019	*Mytilus edulis*	148
17/July/2019	*Mytilus edulis*	142
22/July/2019	*Mytilus edulis*	178
29/July/2019	*Ruditapes decussatus*	59
5/August/2019	*Mytilus edulis*	136
14/August/2019	*Mytilus edulis*	82
22/August/2019	*Mytilus edulis*	85
28/August/2019	*Ruditapes decussatus*	223
	Crassostrea gigas	200
	Mytilus edulis	523
2/September/2019	*Crassostrea gigas*	118
4/September/2019	*Mytilus edulis*	527
11/September/2019	*Mytilus edulis*	251
	Ruditapes decussatus	89
	Crassostrea gigas	57
16/September/2019	*Mytilus edulis*	103
23/September/2019	*Mytilus edulis*	83
2/October/2019	*Mytilus edulis*	132
30/October/2019	*Mytilus edulis*	122
6/November/2019	*Mytilus edulis*	139
13/January/2020	*Mytilus edulis*	76

Table 2. *E. coli* concentration present in shellfish in Tavira from June 2019 to February 2020. Highlighted in red are the values within the health classification of C, in yellow for the class B and in green for the class A (data from IPMA).

Date	Specie	*E. coli* (NMP/100 g)
4/ June/2019	*Ruditapes decussatus*	230
	Crassostrea gigas	130
	Mytilus edulis	< 18
17/July/2019	*Ruditapes decussatus*	3500
	Crassostrea gigas	< 18
	Mytilus edulis	790
5/August/2019	*Crassostrea gigas*	< 18
	Mytilus edulis	230
6/August/2019	*Ruditapes decussatus*	110
2/September/2019	*Ruditapes decussatus*	490
	Crassostrea gigas	110
4/September/2019	*Mytilus edulis*	1300
2/October/2019	*Ruditapes decussatus*	1100
	Crassostrea gigas	78
	Mytilus edulis	330
25/November/2019	*Ruditapes decussatus*	1400
	Crassostrea gigas	78
26/November/2019	*Mytilus edulis*	330
9/December/2019	*Ruditapes decussatus*	1100
	Crassostrea gigas	790
	Mytilus edulis	110
27/January/2020	*Ruditapes decussatus*	790
	Crassostrea gigas	< 18
	Mytilus edulis	2400
26/February	*Ruditapes decussatus*	9200
	Crassostrea gigas	<18
	Mytilus edulis	230

Table 3. Harmful phytoplankton present in the water column in Tavira from June 2019 to February 2020. Highlighted in red are the values above the legal limit for the harvesting of shellfish (data from IPMA).

Date	Bacillariophyceae ASP producer	Dinophyceae DSP producer	Dinophyceae yiessotoxins producer	Dinophyceae AZP producer
5/June/2019	3280	-	200	-
17/June/2019	-	-	80	-
26/June/2019	-	40	15720	-
10/July/2019	-	120	13840	-
17/July/2019	-	280	2174620	-
25/July/2019	-	40	-	-
12/August/2019	13940	-	-	-
21/August/2019	9840	1760	720	-
28/August/2019	-	37840	-	-
4/September/2019	6560	80	40	3280
9/September/2019	1640	80	-	-
18/September/2019	-	40	-	3280
23/September/2019	15580	-	-	-
2/October/2019	105780	-	160	-
7/October/2019	200080	40	40	-
16/October/2019	7380	320	-	9840
26/October/2019	102500	400	40	-
30/October/2019	88560	120	-	-
7/November/2019	7380	-	-	-
13/November/2019	246820	-	-	-
21/November/2019	168920	-	-	-
25/November/2019	247640	-	-	-
5/December/2019	51660	-	-	-
9/December/2019	4100	-	-	3280
6/January/2020	8200	40	-	-
15/January/2020	87760	280	40	-
22/January/2020	154980	80	-	-
29/January/2020	-	320	-	-
5/February/2020	-	-	-	-
13/February/2020	-	1480	120	4920
19/February/2020	1960	-	-	-
27/February/2020	15040	120	80	1640