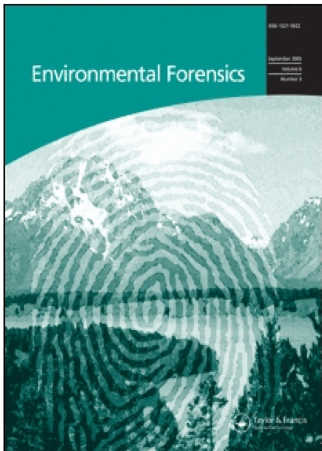

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Identifying the Source of Nutrient Contamination in a Lagoon System

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Nutrient concentrations within watercourses are often associated with the input of sewage or the runoff of fertilizers. Due to population increases, there has been a dramatic rise in the amount of fertilizer applied to land, as well as in the further development of sewage treatment plants (STPs), both of which can lead to significant discharges with associated eutrophication risks in coastal waters. The implementation of the European Union Water Framework Directive (EU-WFD) should improve the management and quality of European water bodies. The Ria Formosa Lagoon, Portugal, is one such water body monitored under the WFD where two inter-calibration sites have been developed, that of the Ancão Basin, which has a status of "high/good," and that of the Ramalhete Channel, with a status of "good/moderate." Spatial nutrient concentrations (NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}) and lipids were measured in two areas that were thought to contribute nutrients into the Ancão Basin; a river that flows through several golf courses and the Ramalhete Channel, which receives discharge from both Faro Airport and a STP.

Nutrient analyses showed that waters from the Ramalhete Channel had substantial concentrations, the highest of which was ammonium, which exceeded 180 μM near the sewage discharge site; however, concentrations diminished towards the sea, reaching 2.7 μM suggesting the utilization of NH_4^+ by seagrasses (*Zostera noltii*).The Atlantic Ocean was also a source of nitrate, contributing 1.8 μM, whereas sources of phosphate originated in the Ancão Basin catchment and included a commercial horticulture site, several golf courses, and the STP. Principal component analysis (PCA) and cross plots of the 5β-coprostanol/cholesterol and 5β-coprostanol/(5β-coprostanol + 5α-cholestanol) ratios identified areas of fecal contamination, highlighting several sites previously unknown to contain sewage matter. In general, nitrogen sources were associated with agriculture and phosphate originated from the golf complex and sewage discharges. These effects were partly mitigated, however, by eutrophic ponds between the river and the lagoon.

Keywords: sewage, nutrients, lagoon, golf courses, Portugal

Introduction

The use of parameters such as nutrient concentrations and lipid analyses within a body of water has proven to be a successful indicator of the quality of the water in relation to contaminant inputs (Desa et al., 2005; Kucuksezgin et al., 2006). High nutrient loadings can be attributed to the discharge of domestic, agricultural, and industrial waste (UNESCO, 1988), which are an increasing problem due to a global population increase. The deterioration of water quality due to nutrient enrichment has a detrimental effect to the biology within the water (Newton et al., 2003), and human health may be at risk. Issues of this magnitude have been addressed by the implementation of legislation and directives to protect waters that are both sensitive and of recreational, economic, or biological importance.

Reducing these detrimental consequences has led to regulatory pressure (National Research Council [NRC], 2000), which can be seen with the development of the WFD. This is a European Directive implemented to protect European rivers, coastal waters, and underground waters from deterioration. Every water body under investigation must achieve a status of "good" by 2015, with the status awarded in respect to the concentration of nutrients, oxygen, and biological elements. Loureiro et al. (2005) states that an interval of 3 months between measurement of physicochemical quality elements such as salinity, temperature, oxygen, and nutrients is needed, whereas the interval for biological assessments is longer, with a 6-month interval for phytoplankton and 3 years for macroalgae. Failure to meet deadlines or status may result in fines, making the monitoring, assessment of sources, and distribution processes within water bodies a priority.

The Ria Formosa Lagoon is a large lagoon in Europe (Mudge and Duce, 2005), covering 55 km of the Algarve coast. The widest part of the lagoon is approximately 6 km, with an average depth of 2 m (Ferreria et al., 2003). Freshwater input to

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the lagoon is small due to the nature of the dry climate; in the summer, water temperatures rise to an average of 24.5°C, which results in salinities of >35 PSU. In the winter months, temperatures within the lagoon average 18.25°C, with an average salinity of 35.5 PSU. Newton and Mudge (2003) suggest that 75% of the water may be exchanged daily; however, this may be the same 75% each day. The remaining 25% has a higher residence time and remains in the lagoon for longer, receiving more contaminants (Mudge et al., 2008). The Ria Formosa is of both local and regional importance due to recreation, tourism, aquaculture, and salt extraction (Newton, 1995). Problems that have arisen within the lagoon are due to rapidly increasing urbanization and intensified farming, which have resulted in a rapid population increase (Mudge and Duce, 2005). According to Mudge and Bebianno (1997), the population of the area increases from approximately 150,000 inhabitants to 450,000 during the summer months, with concomitant increases in the amount of sewage discharged. The main sewage discharge into the western area of the lagoon only undergoes primary treatment, which may lead to an increase in nutrient concentrations, causing a deterioration of the water quality.

Numerous studies have been undertaken within the Ria Formosa investigating water quality with the aid of biological indicators (Hewitt and Mudge, 2004; Hopkins and Mudge, 2004; Chenery and, Mudge, 2005), lipid analysis (Mudge and Duce, 2005) and oxygen concentrations (Mudge et al. 2008); however, there is little information available regarding the biologically important nutrients (Newton and Mudge, 2005). These studies have determined the origin of contaminants and shown that both the Ramalhete Channel and the Esteiro de Maria Nova could be major contributors. The Ramalhete Channel contains primary treated effluent; conceptual box models of tidal flushing produced by Newton and Mudge (2003) and water masses identified by Mudge et al. (2008) show these waters to be carried into the Ancão basin. The Esteiro de Maria Nova, which flows into the Ancão Basin, receives inputs from a horticulture supplier, an STP, and several golf courses. The Ramalhete Channel and the Ancão Basin are both monitored for water quality under the WFD, with the Ramalhete Channel producing a score of "good/moderate" and the Ancão Basin "high/good" (Lourerio et al., 2005).

The objective of this investigation was to quantify the concentration of nutrients the Ancão Basin receives from the Ramalhete Channel and the Esteiro de Maria. Nutrients are present in high concentrations within sewage and through surface runoff on land such as golf courses. Therefore, the analysis of nutrients (NH_4^+, NO_2^-, NO_3^-, and PO_4^{3-}) will make it possible to determine sources of contamination and regions of contaminant accumulation. However, nutrient concentrations alone may not uniquely identify the source of contamination unless coupled to other measures.

One of the most useful groups of primary biomarkers are the sterols that have been used as tracers of inputs from various marine and terrestrial plants and animals and their degradation processes such as oxidation and reduction (Mudge and Duce, 2005). Sterols are amongst the most useful biomarkers because they are preserved well in sedimentary environments. Sterols can be used as tracers for sewage-derived material because cholesterol is biohydrogenated by higher animals to 5β-coprostanol. Green et al. (1992) identified that 5β-coprostanol can arise from other sources; however, it can still be used as a tracer for sewage because it arises in much higher concentrations in fecal matter. Using sterols together as a ratio can provide a wealth of information regarding environmental conditions. For instance, the cholestanol/cholesterol ratio may indicate in situ reduction, whereas a ratio >0.2 from 5β-coprostanol/cholesterol indicates fecal contamination (Grimalt et al., 1990). Epi-coprostanol/5β-coprostanol will indicate either age since discharge or anaerobic sludge digestion during the treatment process (McCalley et al., 1981).

Materials and Methods

Sampling

The region under investigation, the Ancão Basin, is situated at the western end of the Ria Formosa Lagoon (Figure 1a and b). Surface sediment samples and waters were collected from the two areas that were thought to contribute nutrients into the Ancão Basin: the Esteiro de Maria Nova, a river that flows past several golf courses, and the Ramalhete Channel, which receives discharge from the city sewage treatment plant. Twelve samples were collected within the Ramalhete Channel from the source beside Faro Airport down to the Ancão Basin and 12 samples were collected from the Esteiro de Maria Nova from the point where surface waters run. Figure 1 is a map of the Ria Formosa and the sample locations.

Water samples for nutrient analysis were collected in 330-mL polyethylene bottles by submerging them 0.5 cm under the water surface; bottles were rinsed three times with the sample water before collection. Bottles were returned to the laboratory in a cool box surrounded by ice packs where they were immediately frozen (−20°C) until analysis by the methods outlined in Grasshoff et al. (1983). Surface sediment scrapes of approximately 100 g were collected with a Teflon spatula and placed in a glass jar with an aluminum foil–lined lid. Samples were refrigerated (5°C) before lipid extraction.

Nutrient Analysis

NH_4^+

Water samples were defrosted, and immediately three 5-mL pseudo-replicates were extracted with 0.15 mL citrate buffer, 0.15 mL phenol reagent, and 0.15 mL trione. The samples were then refrigerated for at least 10 h before analyzing. The absorbance at 630 nm was then recorded in a spectrophotometer (Grasshoff et al., 1983).

NO_2^-

Aliquots (5 mL) were added to test tubes and 0.1 mL of sulphanilamide and 0.1 mL of N-(1-napthyl ethylenediamine

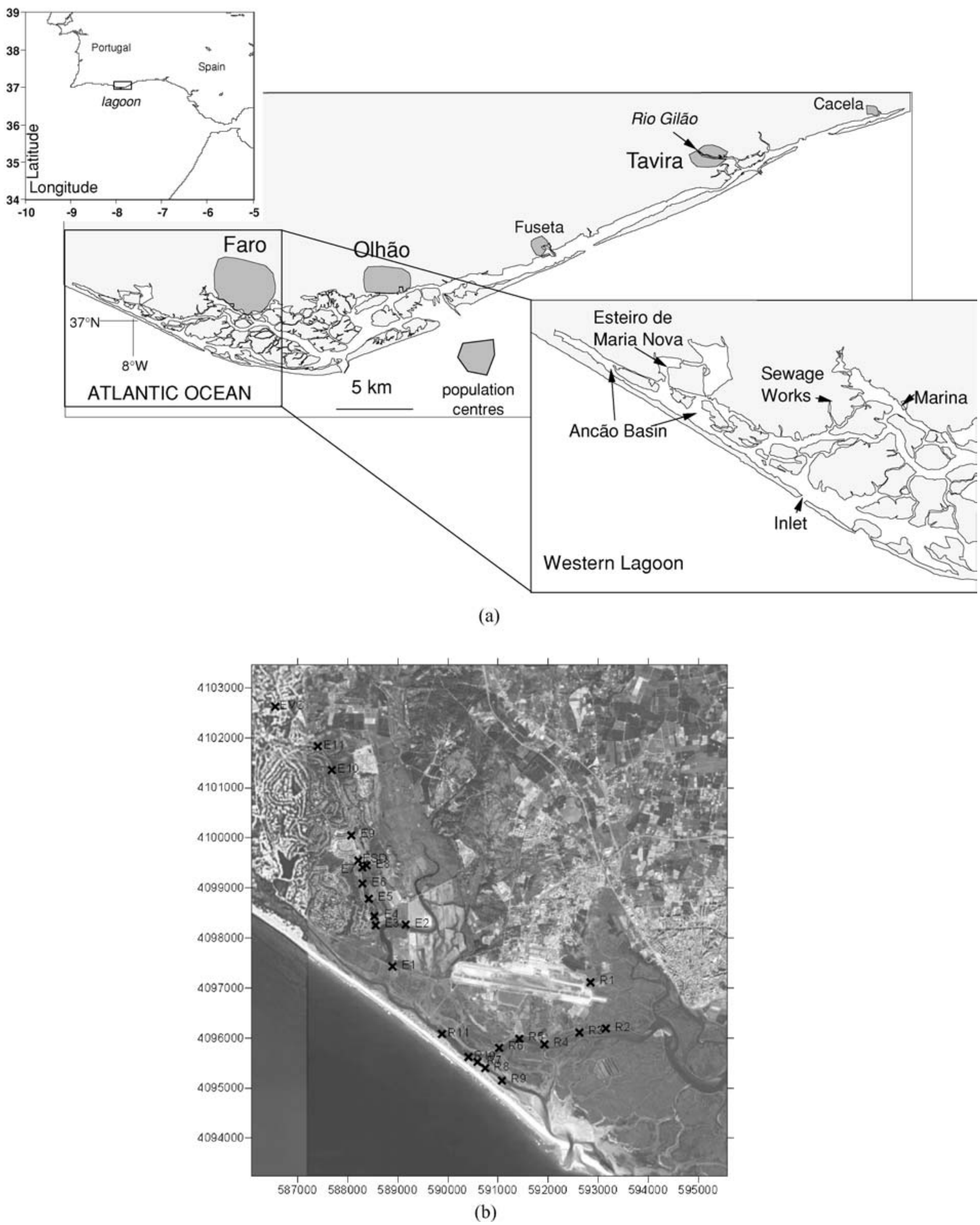

Figure 1. A) Map of the Ria Formosa, Portugal, and the western end of the lagoon under investigation (adapted from Mudge and Duce, 2005); and B) the sample locations in Ramalhete Channel and the Esteiro de Maria Nova.

dihydrochloride) were added. Absorbances were measured on a spectrophotometer at 543 nm.

NO_3^-

Nitrate concentrations were measured after reduction of NO_3^- into NO_2^- with a Cd column and NH_4Cl solution ($NO_3^- + Cd^0 + 2H^+ \rightarrow NO_2^- + Cd^{2+} + H_2O$). Pseudo-replicates of 5 mL were added to 5 mL of ammonium chloride buffer solution. The efficiency of the column was checked every 10 samples to ensure that the efficiency of reduction was >90%. If levels of reduction decreased, the column was reactivated by passing 150 mL of a 100-μm NO_2 standard and rinsed with NH_4Cl. The efficiency was then retested. If results showed reduction efficiency <90% then the procedure was repeated. Samples sufficiently reduced from NO_3^- to NO_2^- were analyzed as indicated previously.

PO_4^{3-}

Phosphate concentrations were measured by the addition of 0.15 mL mixed reagent (20 mL 9 M sulphuric acid, 4.5 mL ammonium molybdenum, and 0.5 mL of K(SbO) solution) and 0.15 mL ascorbic acid to a 5-mL sample. Absorbance at 880 nm was measured and related to a calibration curve.

Lipid Analysis

The method of Mudge and Norris (1997) was used for the extraction of lipids. Sediment samples (approximately 60 g) were weighed wet and refluxed for 4 h in 50 mL of 6% KOH in methanol. The liquid was decanted and centrifuged for 5 min at 600 $\times$ g. The supernatant was decanted and liquid-liquid extracted into 20 mL of hexane. A further 10 mL of hexane was added to ensure maximum sterol and fatty alcohol extraction. The hexane phase was then rotary evaporated to reduce the sample down to approximately 5 mL and 1 g of anhydrous sodium sulphate was added to remove any polar components. Samples were then reduced to dryness under nitrogen, derivatized with bis(trimethylsilyl) trifluoroacetamide (BSTFA), and analyzed on a gas chromatography/mass spectrometer.

Statistical Analysis

PCA was conducted to the lipid data set to determine internal relationships within the large data set. PCA determines the correlation between data by transforming an original set of potentially correlated variables, the raw data, into a new set of uncorrelated variables called *principal components* (PCs). The raw data were converted to proportions to remove the effect of concentration and were $\log_{10}$ transformed to normalize the values. PCA was performed using the statistical software package SIMCA-P by Umetrics (Version 10.0, Umed, Sweden, www.umetrics.com).

Results

Ammonium

The highest concentrations of ammonium were found at the point of discharge from the STP in the Ramalhete Channel with a concentration of 181 μM $\pm$ 1.4. The concentration decreased from this point of discharge through the Ramalhete Channel to 4.8 μM $\pm$ 0 .99 at a station located where the Ramalhete joins the inlet channel from the ocean. Concentrations within this channel were noticeably lower, with an average concentration of 2.1 μM $\pm$ 0.6. The Esteiro de Maria Nova had higher concentrations with a maximum of 146 μM $\pm$ 5.84 measured at a sampling station located downstream of a STP. Ammonium concentrations had greater spatial variance in the Esteiro de Maria Nova than those within the Ramalhete Channel; however, the majority of elevated ammonium values were measured where the river meandered past the golf courses, leading to an average concentration of 90 μM $\pm$ 4.2. The spatial distribution of ammonium can be seen in Figure 2A.

Nitrite

Only five stations within the Ramalhete Channel had measureable nitrite concentrations (Figure 2B) above the limit of detection (<0.01 μM). The highest concentration was at the STP discharge pipe, with a concentration of 0.46 μM $\pm$ 0.01, and the remaining sites within the Ramalhete Channel had lower concentrations between 0.3 μM $\pm$ 0.02 and 0.4 μM $\pm$ 0.02; all values fell within the status of "good" under the European Environment Agency (EEA) criteria for the assessment of nutrient levels in transitional, coastal, and marine waters (EEA, 2001). Nitrite within the Esteiro de Maria Nova was substantially higher with a maximum concentration of 89 μM at two consecutive sites down stream of a horticultural business. Concentrations exceeded the status of "bad" at four sites within the Esteiro de Maria Nova (Table 1). Within the golf course complex, nitrite concentrations ranged between 3.55 μM $\pm$ 0.02 and 18.61 μM $\pm$ 0.51 varying the status from "good" to "bad".

Nitrate

Nitrate within the Ramalhete Channel was generally low, with the highest concentration at the point of sewage discharge (4.7 μM $\pm$ 1.48), while an adjacent site had a concentration of only 0.6 μM $\pm$ 0.1. A small peak in nitrate (1.84 μM $\pm$ 0.74) was measured at the inlet, an opening to the Atlantic Ocean. All nitrate results within this area can be classified as good when based on the EEA guidelines for nutrient levels in transitional, coastal, and marine waters (Table 1). Nitrate concentrations within the Esteiro de Maria Nova were considerably higher (Figure 2C), with a maximum concentration of 93.3 μM $\pm$ 6.11 at the source of the river. Concentrations declined away from this source to a minimum of 3.6 μM $\pm$ 0.2, with the status improving from bad to good as the river enters the Ria Formosa.

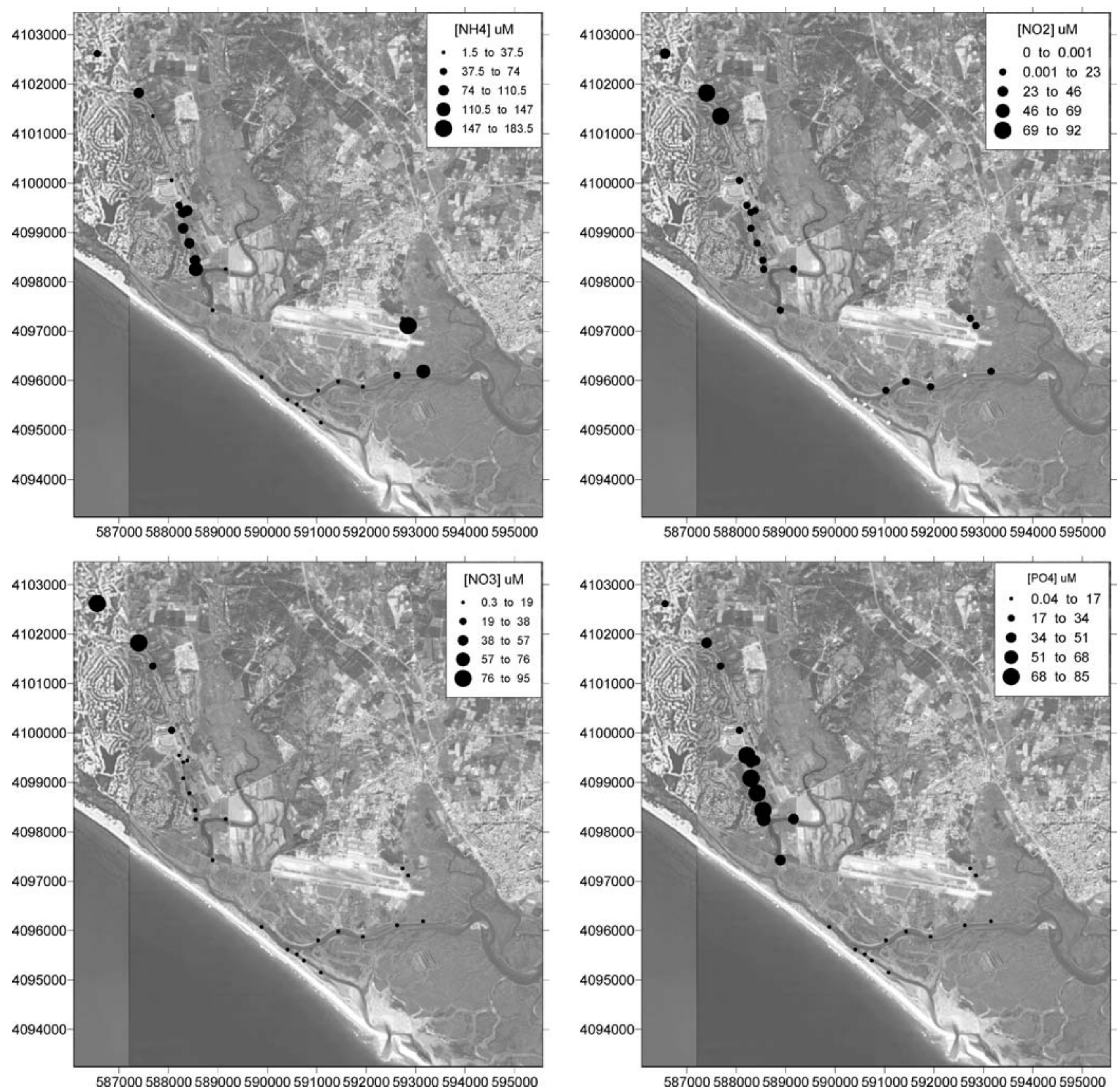

Figure 2. A) Ammonium, B) nitrite, C) nitrate, and D) phosphate concentrations in μM.

Phosphate

Concentrations of phosphate (Figure 2D) follow the same trend of the other nutrients within the Ramalhete Channel with the maximum concentration at the STP (2.4 μM, $\pm$ 0.3), which is deemed as bad according to the EEA criteria (Table 1). Phosphate concentrations decreased down the Ramalhete Channel to a minimum of 0.04 μM $\pm$ 0.03 in the inlet channel; the status improves to good by the EEA criteria. A small increase to 0.16 μM $\pm$ 0.04 was measured where the Ancão Basin meets this channel. Phosphate within the Esteiro de Maria Nova is greatest adjacent to the STP with a concentration of 83.7 μM $\pm$ 4.83 considerably above the concentration needed to be classed as bad. The concentrations decrease to 44.0 μM $\pm$ 19.2 at the point where the river enters the lagoon. Lower concentrations (22.9 μM $\pm$ 0.12) were measured at a site upstream from the STP, although this is still classified as bad.

Table 1. *European Environment Agency (EEA) criteria for the assessment of nutrient levels in transitional, coastal, and marine waters (EEA, 2001)*

Quality status	Nitrate & nitrite (μM)	Phosphate (μM)
Good	<6.5	<0.5
Fair	6.5–9.0	0.5–0.7
Poor	9.0–16.0	0.7–1.1
Bad	>16.0	>1.1

Lipids

The distribution of sewage-derived compounds has previously been determined in this area; Mudge and Duce (2005) identified sources, sinks, and pathways of contaminants with lipid biomarkers whilst Hewitt and Mudge (2004), Hopkins and Mudge (2004), and Chenery and Mudge (2005) identified the presence of sewage contaminants with biological indicators. It is possible to separate sewage, marine, and terrestrial inputs with biomarkers, and to determine their relative influence at each site. Long-chain fatty alcohols (C_{22}-C_{30}) are indicative of a terrestrial input due to the composition of leaf waxes, while short-chain fatty alcohol (C_{11}-C_{21}) suggests a marine source (Tulloch, 1976). The most common sterol biomarkers used for establishing organic matter sources are marine markers (cholesterol), phytoplankton (cholest-5,22-dien-ol, brassicasterol, dinosterol), terrestrial (β-sitosterol and ergosterol), and sewage (5β-coprostanol; Mudge and Bebianno, 1997).

A total of nine sterols and 19 fatty alcohols in the range C_{11}-C_{30} were identified and used in the PCA. Compounds that originate from similar sources can be seen to cluster in the loadings plot (Figure 3A). Three source vectors can be identified in relation to the fatty alcohol carbon length and the sterols. In

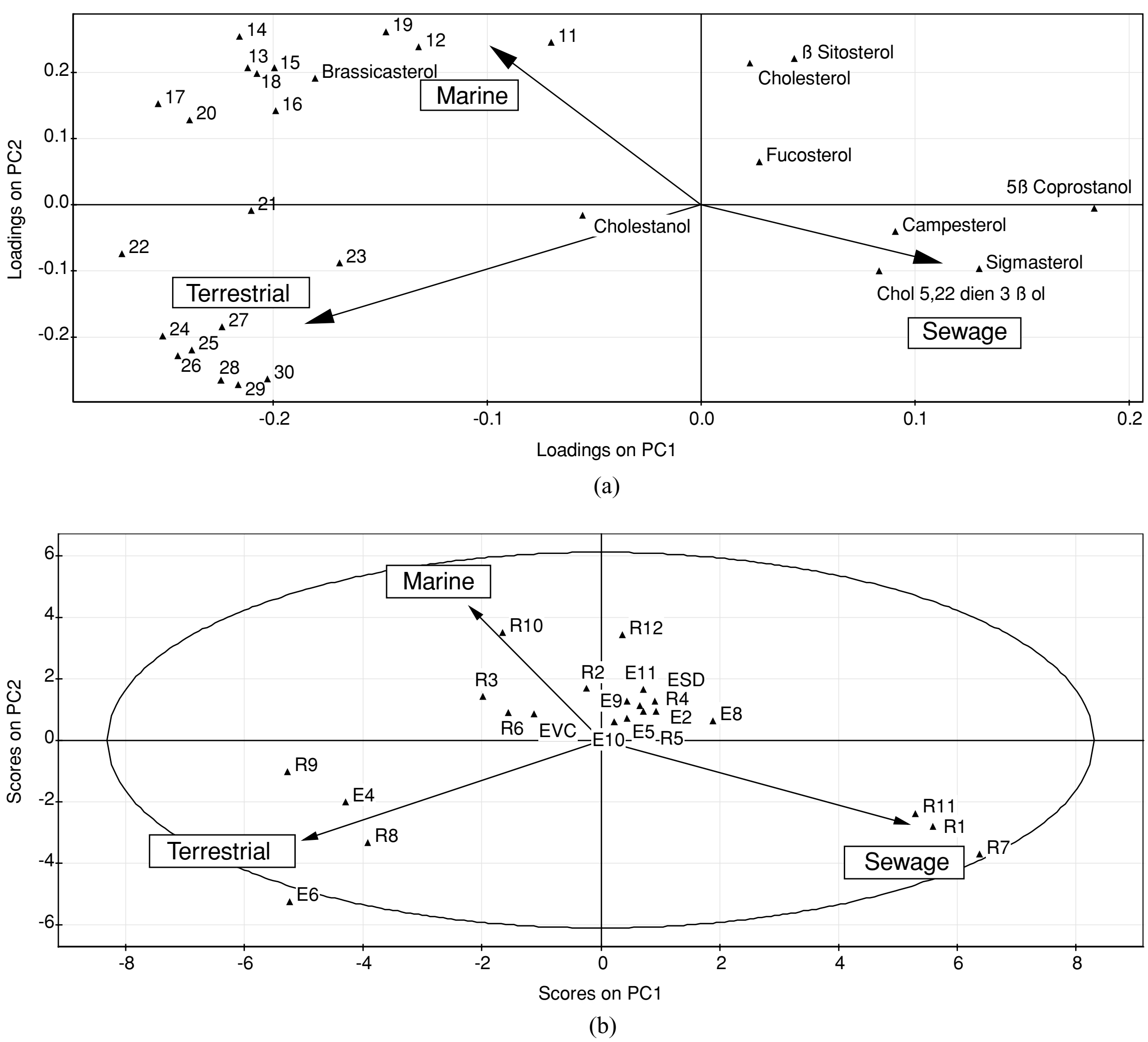

Figure 3. A) Loadings and B) scores on the first two principal components for all the fatty alcohol and sterols with imputed source vectors. In the loadings plot, the numbers refer to the carbon chain length of the fatty alcohols; in the scores plot, sites labeled "R" correspond to the Ramalhete Channel and "E" to the Esteiro de Maria Nova.

order to relate these compounds to sites, the loadings plot is used in conjunction with the score plot (Figure 3B). The majority of sites exhibiting a marine signature are from the Ramalhete Channel: terrestrial signatures exist in both the Esteiro de Maria Nova and Ramalhete Channel. Three sites with a characteristic sewage signature were all within the Ramalhete Channel (R1, 7 and 11).

Ratios of Sewage Biomarkers

The classic ratio between the fecal stanol and parent sterol (5β-coprostanol/cholesterol) indicates fecal contamination, with raw sewage having a value of $\sim$10, but all values greater than 0.2 may be considered contaminated (Grimalt et al. 1990). These authors suggest a further ratio used to measure human sewage, 5β-coprostanol/(5β-coprostanol + 5α-cholestanol), where a value <0.3 signifies an uncontaminated area and a ratio >0.7 suggests sewage contamination (Grimalt et al. 1990). In environmental forensic investigations, it is always useful to have at least two different measures telling the same story and in this case these ratios can be used together in a single plot (Figure 4).

The sterols suggest that several sites contain fecal matter, four of which show contamination in both the sterol ratios. The highest ratio produced using the 5β-coprostanol/(5β-coprostanol + 5α-cholestanol) was site R7, which is located at a sewage discharge site, a result that corresponds to the findings of Mudge and Duce (2005). The maximum 5β-coprostanol/cholesterol ratio was 2.8, measured at a site adjacent to an STP where primary treatment occurs before discharge. This site, R1, is adjacent to an STP discharge pipe within the Ramalhete Channel, whereas ESD is a STP discharge pipe within the Esteiro de Maria Nova. Two sites (E5 and R2) show a fecal fingerprint with the 5β-coprostanol/cholesterol ratio; however, the 5β-coprostanol/(5β-coprostanol + 5α-cholestanol) produces a more uncertain result.

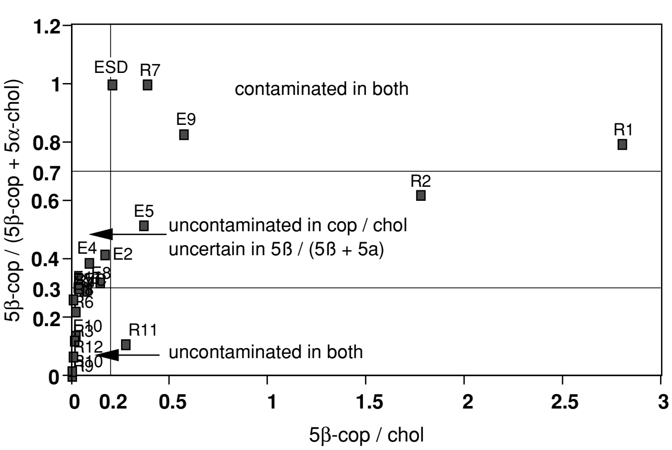

Figure 4. 5β-coprostanol/cholesterol and 5β-coprostanol/(5β-coprostanol + 5α-cholestanol) ratios highlighting sites in which fecal contamination is greatest.

Discussion

Concentration of nutrients within the Ramalhete Channel follow a consistent pattern, with the greatest concentration for all nutrients found at the stations located within close proximity of the STP. The STP receives waste from Montenegro and Faro, the latter being of a considerable size. Sewage undergoes primary treatment within the installation, which comprises the screening of large material; large settling ponds act as a natural tertiary treatment as ultraviolet radiation from the sun may reduce the number of harmful pathogens before discharge. However, due to the lack of secondary treatment or nitrate and phosphate stripping phase, the discharge is nutrient rich. Ammonium concentrations decrease away from the source, suggesting nutrient assimilation or nitrification. Concentrations of both nitrite and nitrate do not increase away from the source, suggesting nitrification is not occurring to such a large extent to reduce the ammonium concentrations. Dense seagrass beds of *Zosetra noltii* occupy the banks of the Ramalhete Channel, suggesting nutrient assimilation: ammonium is the preferential state for nitrogen uptake as it is in a reduced state and so energetically beneficial to organisms (Andrews et al., 2004). Concentrations of ammonium within the Esteiro de Maria Nova were greatest at the point of sewage discharge where primary, secondary, and (natural ultraviolet) tertiary treatment occurred. High concentrations may be due to two factors: firstly, the hydrolysis of urea shown by Sanchez-Monedero et al. (2001) and the degradation of organic compounds found in sewage. A rapid reduction in ammonium concentrations was observed where the river exits the golf course complex and enters several large lakes covered in algal mats. Such algal mats are indicative of eutrophication (Paerl, 1997) and suggest that the algae are stripping the water of ammonium, leading to this eutrophic state. These blooms will also lower the concentration of ammonium in waters entering the Ria Formosa Lagoon.

Nitrite and nitrate follow similar spatial trends with highest concentrations near the source of the Esteiro de Maria Nova, a horticultural operation. Watercress and other green salad vegetables are grown in large channels filled with nutrient-rich water; surplus water enters a drainage system that flows offsite and forms the source of the Esteiro de Maria Nova during the summer months. A decrease in nitrite concentrations occurs after the sewage treatment plant; this may be due to the oxidation state of the water in the discharge (Figure 5). Nitrate and nitrite concentrations within the Ramalhete Channel were negligible in comparison to those found within the Maria Nova; however, a small rise in nitrite was determined at two sampling sites. This rise coincided with the results from the lipid analysis and the findings of Mudge and Duce (2005) in which the sewage tracer 5β-coprostanol was found (Figures 3B and 4); this suggests the input of sewage contributes to the nitrite loading. An increase in ammonium and nitrate was not measured at this site (R7), which may be due to the rapid utilization by the seagrass beds; however, utilization of nitrite was not so great as it is not the preferential state of nitrogen for organisms.

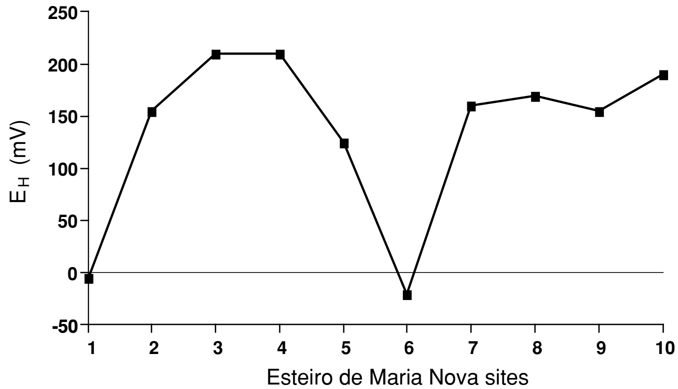

Figure 5. Graph of the redox potential (E_H) measurements measured at sampling stations along the Esteiro de Maria Nova. The minimum at station 6 may be due to deposition of organic matter from the sewage discharge near station 7.

Nitrate concentrations again are small in comparison to those of the Maria Nova, whereas a second source of nitrate, the Atlantic Ocean, was in agreement with the findings of Newton (1995).

Mainstone and Parr (2001) state that urban wastewater, particularly from sewage treatment effluent sources, can provide a major source of phosphorus to river systems, and whilst it is the major source of phosphate into the Ramalhete Channel, concentrations fall within the lowest class spatially. A decrease in concentrations away from the source may be due to the accumulation in sediments (Gomez et al., 1999) or because of the dilution away from the source. Concentrations within the Maria Nova are very high when the river enters the golf course complex and are three orders of magnitude greater than most of the levels recorded by Clark (2001) in the Ria Formosa, and more than 10 times higher than the phosphate sewage discharges of 7 μM documented by Mudge and Ball (2006). High concentrations of phosphates from detergents (UNESCO, 1988) and foams were seen at the STP discharge site during collection. Concentrations decreased as the river exited the golf courses and entered the large eutrophic lakes, dramatically lowering the concentration before it enters the lagoon.

Sterol and fatty alcohol analysis highlighted three sites within the Ramalhete Channel that exhibited a characteristic sewage signature. Sampling station R7 had high concentrations of 5β-coprostanol, confirming the results of Mudge and Duce (2005). There were concomitant increases in the nitrite concentration. A surprising result was that sampling site R2, which is located at the point of sewage discharge, did not produce a sewage signature within the PCA plot (Figure 3B). When compared with the sterol ratios in Figure 4, site R2 shows strong contamination in the ratio 5β-coprostanol/cholesterol but does not unambiguously cross the 0.7 threshold in the 5β-coprostanol/(5β-coprostanol + 5α-cholestanol) ratio. Bacteria preferentially produce 5α-cholestanol from cholesterol in anaerobic reducing sediments and reducing environments are often associated with areas that receive a high organic input such as sewage discharge sites. This may lead to the production of significant post-discharge production of 5α-cholestanol thereby lowering the proportion of 5β-coprostanol in the sedimentary ratio.

Two sites within the Esteiro de Maria Nova had a sewage signature (Figure 4); one of these (E5) was the sewage discharge point, but another station, E9, was also contaminated with sewage. This suggests that there is a previously unreported point source of sewage to the Esteiro de Maria Nova or that the horticulture supplier is applying sewage sludge as a natural fertilizer through what is known as the *safe sludge matrix* (Agricultural Development and Advisory Service [ADAS], 2001). This permits the application of enhanced treated sludge to salad vegetables, and it is possible that components of the treated sludge are accumulating at E9, leading to the sterol results shown in Figure 4. Grain size analysis shows that sediment at E9 comprises 80% clay (Figure 6) and implies that a lower current velocity would allow the settlement of fine-grained particulate matter and, therefore, the accumulation of contaminants.

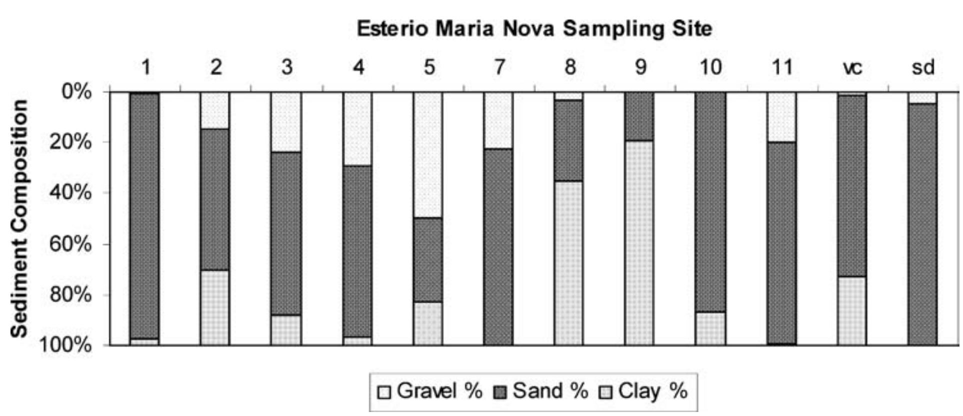

Figure 6. Graph of the sediment composition shown as a percentage of gravel, sand, and clay fractions; vc, horticultural company; sd, sewage discharge point.

Conclusion

The combined use of nutrients and lipids has allowed the determination of both sewage and surface runoff into the Ancão Basin. Analyses show that the Esteiro de Maria Nova had the highest concentrations of nutrients, dwarfing those measured within the Ramalhete Channel. Several significant inputs of nutrients in the Esteiro de Maria Nova were discovered; nitrate, nitrite, and ammonium originated from the horticultural company, while phosphate was associated with the golf complex. All sites fell into the status of bad when judged by the EEA criteria for the assessment of nutrients in transitional, coastal, and marine waters, which potentially would influence the status of the Ancão Basin under the WFD. However, the reported status under the WFD is good and this may be due to the large ponds through which the Esteiro de Maria Nova passes before discharging into the lagoon. These lakes become eutrophic due to the high nutrient loading received from the Esteiro de Maria Nova and as a result the large algal mats strip nutrients from the river, reducing the nutrient concentration that enters the lagoon. While eutrophication is seen as an environmental problem, in this case it is acting as a protection mechanism to maintain the health of the western end of the lagoon. Nutrient inputs to the Ramalhete Channel are principally from the STP, and while the nutrient concentration within the discharge is high, the dense seagrass beds assimilate much of the nutrients reducing the water concentrations. Several sites within the Ramalhete Channel had sewage signatures.

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