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ORIGINAL ARTICLE

A case study of sewage discharge in the shallow coastal area of the Northern Adriatic Sea (Gulf of Trieste)

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Abstract

A case study was carried out in 2000 in the shallow coastal area of the Northern Adriatic Sea (Gulf of Trieste) where untreated domestic sewage and industrial wastes are discharged at rate of 5500 $m^3 \cdot day^{-1}$. The sewage plume above the outfall was followed using faecal coliforms (FC) and overturning length scale (l_T). The latter was rejected as a marker as the discharge conditions prohibit following the turbulence of sewage water. Intermittent sewage discharge is reflected in the minimal effect of eutrophication. Increase of phytoplankton biomass is thus only minor compared with the unpolluted area regardless of elevated concentrations of sewage-derived nutrients (confirmed by correlation coefficients between FC and NH_4^+, TP, PO_4^{3-}: 0.78, 0.71 and 0.67, respectively). Deteriorated trophic status, determined by the TRIX index, was observed only in the surface layer (average TRIX: 5.67). High FC content well above the regulation limit (up to 2.6×10^5 FC·100 ml^{-1}) represents, therefore, the major negative impact of the improperly treated waste for the risk to human health.

Problem

Coastal waters are highly variable ecosystems due to their natural characteristics, and may become even more vulnerable when exposed to negative consequences of human activities. In the shallow and semi-enclosed Gulf of Trieste (Northern Adriatic Sea) the variability of hydrological, physico-chemical and biological characteristics has been attributed mostly to its geo-morphological properties and the freshwater regimes of rivers that enter the Gulf (Olivotti *et al.* 1986; Mozetič *et al.* 1998; Malačič & Petelin 2001). The short southern coastline is also densely populated (232 individuals·km^{-2}) (Turk & Potočnik 2001) and concentrates several activities that exploit the economic value of the sea. It is therefore important that pollution pressure, which derives either from land-based sources or from different activities at sea, is kept under control.

Largely untreated domestic and industrial wastes are considered one of the main causes of the deterioration of the state of the Mediterranean Sea (UNEP/WHO 2007) as well as seawaters worldwide (*e.g.* Adingra & Arfi 1998; Braga *et al.* 2000; Aslan-Yilmaz *et al.* 2004). Besides faecal pollution and the deteriorative appearance of bathing waters, which are the immediate consequences of wastewater discharge, the other important aspect is eutrophication, which can be viewed as decreased biomass and number of species, proliferation of persistent species and community changes, mostly in benthic communities (Chisholm *et al.* 1997; Soltan *et al.* 2001; Savage *et al.* 2002), whereas the impact of sewage on pelagic communities is more difficult to detect. The dilute nature of the effluent obscures the impact of sewage discharge on the already complex coastal environment and thus makes distinguishing between the 'background' of receiving waters and wastewater an almost impossible task (Faganeli 1982; Mozetič *et al.* 1999), even in areas with high effluent flows and non-efficient wastewater treatments (Smith-Evans & Dawes 1996; da Silva *et al.* 2002). The greatest effects are usually observed in the immediate vicinity of the sewage discharge and rapidly decrease towards the open sea, where polluted and brackish waters are no longer detectable (Theodorou 1992; Torres & del Rio 1995). It is therefore crucial to describe the sewage plume

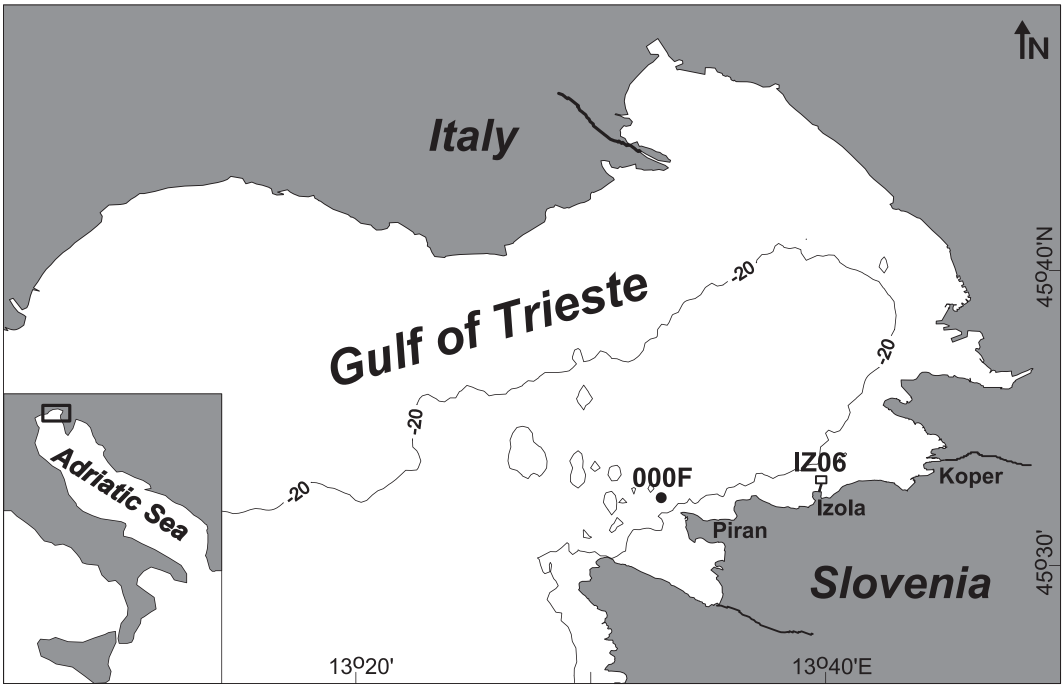

Fig. 1. Location of the sampling station IZ06 above the endpoint of the underwater pipe, which releases wastewaters from the municipality of Izola into the coastal waters. The rectangle denotes the grid of 11 stations, with station IZ06 being the central one. The position of the non-impacted, reference station 000F is also shown.

as precisely as possible and to measure the variations in parameters that most likely reflect the impact of sewage.

Such a study was performed in 2000 in the coastal area of the Gulf of Trieste, which receives largely untreated sewage from a small town on the Slovenian coast (Izola; Fig. 1). The sampling design followed that applied in the similar study of the outfalls of the neighbouring town Piran (Malačič 2005; Malačič & Mozetič 2005), where the overturning length scale was adopted. This is the measure of higher turbulence intensity and can serve as an indication of a formed patch of pollutant in the surrounding, *i.e.* marine environment.

Areas in the Slovenian coastal sea influenced by wastewater discharges have been surveyed for years within the framework of monitoring activities of the Barcelona convention. This study represents, therefore, an upgrade of these activities and it also meets some objectives of the initiative INTERREG III CBC Phare (Italy-Slovenia), project OBAS (Biological Oceanography of the Northern Adriatic Sea), carried out during 2000–2006.

Material and Methods

Wastewater disposal and sampling location

The pollution load of the Izola municipality is estimated to be up to 23,000 population equivalent (PE) for domestic sewage and 10,000 PE for industrial waste (Turk & Potočnik 2001). Waste is collected in a treatment basin where only rough mechanical purification (screening by 1-cm rake) is carried out. The effluent is pumped from the sedimentation basin and discharged into the 12-m-deep seawater column through an approximately 200-m-long pipe. In 2000 the average daily flow rate of effluent was estimated to be around 5300 $m^3 \cdot day^{-1}$ considering the total yearly amount of working hours (7739 h), capacity (70 $l \cdot s^{-1}$) and average daily operation (21.2 h) of the pump (E. Požar, personal communication). It must be stressed that the pump is switched on when the basin is filled. Just before the pipe sweeps into the sea, a discharge from the fish-cannery pre-treatment plant (flow rate 225 $m^3 \cdot day^{-1}$) joins the last ground collector of the sewage system, thus influencing the nature of the effluent.

Fieldwork

Four samplings were performed in the year 2000 to define the impact of Izola's sewage discharge on seawater properties (ecological survey) and to detect its spread across the sea surface and along the vertical (CTD survey). To accomplish the latter a detailed oceanographic survey, including distribution of the overturning length scale, was carried out on 10 May 2000 on a grid of 11 stations distributed above the outlet (Fig. 1). Within the grid, the stations were separated by 0.1′ (185 m) in S–N direction and 0.1′ (130 m) in E–W direction.

Surveys of the chemical and biological properties of the sea water were carried out at the central station of the grid – IZ06 (45°32.64′ N, 13°39.75′ E; Fig. 1), at a depth of about 12 m on 6 March (A), 10 May (B), 29 August (C), and 23 October 2000 (D). The chosen dates reflect mixed and stratified water columns and different growing seasons of the phytoplankton.

Samples were taken at six depths (0.3, 1, 3, 5, 7 m and above the bottom) during three surveys, whereas on 10 May a different sampling scheme was applied. On that date the sea water was sampled at 10 depths where small-scale wiggles were detected on salinity profiles (see Fig. 2), and where turbulent effluent was expected. Seawater samples were collected using 5-l Niskin bottles and were kept in a dark and cold place prior to analysis in the laboratory.

Vertical profiles of temperature, salinity, density and oxygen were obtained using a fine-scale multiparameter CTD (conductivity, temperature, depth) probe with vertical resolution of about 2.5 cm for a conventional drop speed of about 1 m·s^{-1} (instrumental details in Malačič 2005). The overturning length scale (l_T) was calculated as the root-mean square (rms) of vertical displacement over layers of thickness of 0.5 m.

Information about the input load was gained from chemical and microbiological analysis of sewage samples that were collected at the outflow of the treatment basin and at the fish-cannery pre-treatment plant in the morning hours before each survey.

Analyses

Concentrations of inorganic nutrients (nitrite, nitrate, ammonia, phosphate, silicate) from unfiltered seawater and filtered sewage samples were measured using standard colorimetric procedures (Grasshoff *et al.* 1999). Total nitrogen (TN) and total phosphorus (TP) were analysed in unfiltered samples (Grasshoff *et al.* 1999). Sewage samples were filtered through glass-fibre filters (Whatman GF/F).

Dissolved oxygen (O_2) was determined following the modified standard Winkler method (Grasshoff *et al.*

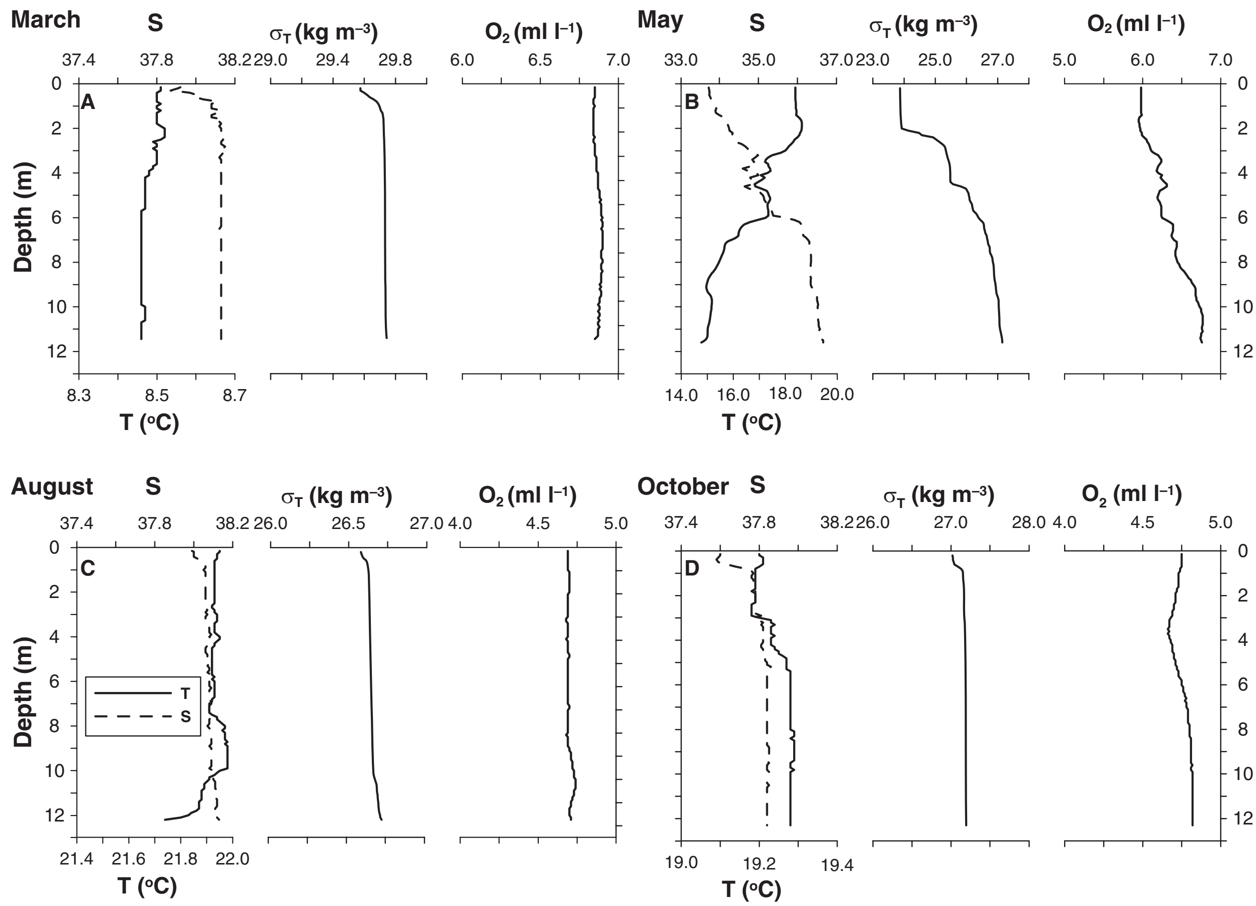

Fig. 2. Vertical profiles of temperature, salinity, density (σ_T) and dissolved oxygen recorded at station IZ06 during four surveys in the year 2000.

1999). The same method was applied for determining the biochemical oxygen demand (BOD_5) in water samples after they were incubated for 5 days in the dark at room temperature.

Concentrations of total suspended matter (TSM), particulate organic carbon (POC) and particulate nitrogen (PN) in sewage and seawater samples were analysed on Whatman GF/F glass-fibre filters, which were pre-combusted for 3 h at 480 °C to eliminate organic contaminants. Samples of sewage liquid (up to 100 ml) and seawater (1 l) were filtered and the material collected on the filters was rinsed several times with distilled water to remove salts. All samples were freeze-dried for 24 h. TSM was determined gravimetrically. The POC and PN content of the freeze-dried and acid-washed sample was determined using a Carlo Erba model 1108 elemental analyzer (Hedges & Stern 1984).

Chlorophyll *a* concentrations (Chl *a*), corrected for phaeopigments, were determined fluorometrically on a Turner 112 fluorimeter (Holm-Hansen *et al.* 1965). Subsamples of 20 ml were filtered onto 0.22-μm Millipore filters, extracted in 90% acetone and measured for fluorescence.

Samples for phytoplankton analysis were preserved with neutralized formaldehyde (2% final concentration). Phytoplankton cells were identified and counted in subsamples of 50 ml on an inverted microscope where 100 fields of the bottom sedimentation chamber were examined at 400× magnification (Utermöhl 1958).

The number of faecal coliform bacteria (FC) was determined following the recommendations of UNEP/WHO (1994). Water samples were filtered through the 0.45-μm pore-size sterile Millipore filters and incubated on FC-agar medium at 44.5 ± 0.2 °C for 24 h. Results are expressed as number of FC·100 ml^{-1}.

The trophic status of seawater was estimated by applying the trophic index TRIX (Vollenweider *et al.* 1998), taking into account concentrations of Chl *a*, dissolved inorganic nitrogen (DIN) and TP, and the absolute deviation (%) from oxygen saturation. The index is scaled from 0 to 10.

Relationships between different parameters, measured at station IZ06 during four surveys, were tested using linear correlation analysis and the significance was calculated with the Student's *t*-test. Data, except for salinity, temperature, density, l_T and TRIX index, were log transformed to achieve normal distribution. Statistical analysis MANOVA was used to compare the influenced station IZ06 and the reference station 000F (45°32.42′ N, 13°33.05′ E; Fig. 1) using nutrients, chlorophyll *a* and trophic index. To obtain a more reliable comparison with a larger number for statistical analysis (n = 68 for both stations), data from 1999 to 2001 were also considered; these data were acquired during regular monitoring programs of pollution hot spots along the coast and of the quality of coastal sea at stations IZ06 and 000F, respectively. Overlapping sampling dates in the 3-year period were picked out for the comparison.

Results

Sewage composition

During the first survey in March, samples from the fish-cannery treatment basin were not taken. Concentrations of all parameters were higher in the industrial wastes of the fish-cannery, except for nitrate and nitrite (Table 1). Ammonia filled up almost the entire pool of DIN. More than 50% of total nitrogen was organic in both types of wastes. The predominant form of phosphorus was inorganic phosphate (PO_4^{3-}) in domestic wastes and organic P compounds in the fish-cannery wastes. Higher C/N ratios in the effluent of the fish-cannery (on average 37) reflect a composition richer in fats compared with the domestic sewage (mean C/N = 14). FC were counted only in the domestic sewage and on average they were 4.3×10^6 FC·100 ml^{-1}.

Daily loads of pollutants, calculated from their concentrations and daily flow rates of both effluents (in Turk & Potočnik 2001), were roughly estimated to vary from 15.8 to 166 kg DIN, 0.7 to 24 kg PO_4^{3-}, 2.2 to 32 kg SiO_4^{4-}, 26 to 1340 kg TN, 4.5 to 33 kg TP, and 256 to 1853 kg TSM.

Physical properties of the water column and the overturning length scale

Results of four vertical CTD casts above station IZ06 show the presence of a surface lens of less-saline water at each survey (Fig. 2). These surface lenses were 1–1.5-m-thick during the surveys A, C and D and the salinity oscillations within the lenses were in the range 0.05–0.21. These small vertical gradients of salinity induced a weak pycnocline that was always present near the surface (depth of approximately 1 m), whereas the rest of the water column was generally homogenized. Vertical profiles of dissolved oxygen (Fig. 2) showed no peculiarities; the highest concentrations (up to 6.9 ml·l^{-1}) were measured during the spring period.

Stratified conditions were observed only on 10 May. During this survey not only we measured the lowest salinity of all campaigns (33.73) but the surface layer was more perturbed and much thicker. Salinity oscillations were especially pronounced in the layer between 3 and 6 m (maximal difference 1.32). The upper mixed surface layer of fresher water with an average temperature of 18.47 °C was separated from deeper layers by a sharp pycnocline at a depth of 2 m. Another, but

Table 1. Composition of the effluent of the Izola sewage system and of the fish cannery, collected before each survey in the year 2000.

date	6 March	10 May	29 August	23 October	Mean	SD
Izola sewage system						
NO_2^- (mg·l^{-1})	0.13	0.01	0.01	0.01	0.04	0.06
NO_3^- (mg·l^{-1})	1.28	0.04	0.01	0.07	0.35	0.62
NH_4^+ (mg·l^{-1})	20.14	19.50	30.10	31.22	25.24	6.28
DIN (mg·l^{-1})	21.55	19.54	30.10	31.29	25.62	5.94
PO_4^{3-} (mg·l^{-1})	4.33	4.53	2.21	3.38	3.61	1.06
SiO_4^{4-} (mg·l^{-1})	4.53	5.49	6.01	4.19	5.06	0.84
TN (mg·l^{-1})	252.61	38.42	73.23	80.66	111.23	96.03
TP (mg·l^{-1})	6.19	5.00	5.14	5.37	5.43	0.53
BOD_5 (mg·l^{-1})	267.21	226.34	168.50	234.55	224.15	41.08
TSM (mg·l^{-1})	200.56	92.27	162.08	294.44	187.34	84.30
POC (mg·l^{-1})	71.22	56.10	69.33	130.52	81.79	33.17
PN (mg·l^{-1})	4.19	4.43	6.01	10.07	6.18	2.72
C/N	17	13	12	13	14	2
FC·100 ml^{-1}	2.2E+06		3.9E+06	9.9E+06	5.3E+06	4.0E+06
Fish cannery						
NO_2^- (mg·l^{-1})		0.01	0.01	0.01	0.01	0.00
NO_3^- (mg·l^{-1})		0.03	0.01	0.02	0.02	0.01
NH_4^+ (mg·l^{-1})		69.99	120.70	70.75	87.15	29.06
DIN (mg·l^{-1})		70.03	120.71	70.77	87.17	29.05
PO_4^{3-} (mg·l^{-1})		18.30	2.87	27.83	16.33	12.60
SiO_4^{4-} (mg·l^{-1})		9.90	15.42	11.08	12.13	2.91
TN (mg·l^{-1})		114.12	334.92	254.86	234.63	111.78
TP (mg·l^{-1})		20.23	63.05	66.51	49.93	25.78
BOD_5 (mg·l^{-1})		1460.13	3658.54	2884.56	2667.74	1115.13
TSM (mg·l^{-1})		1138.00	8235.00	6677.14	5350.05	3729.98
POC (mg·l^{-1})		402.84	4325.76	3805.77	2844.79	2130.71
PN (mg·l^{-1})		17.92	80.37	107.44	68.58	45.91
C/N		22	54	35	37	16

On the morning of 6 March, effluent from the fish cannery was not taken. Mean values of the parameters and standard deviations (SD) are shown.

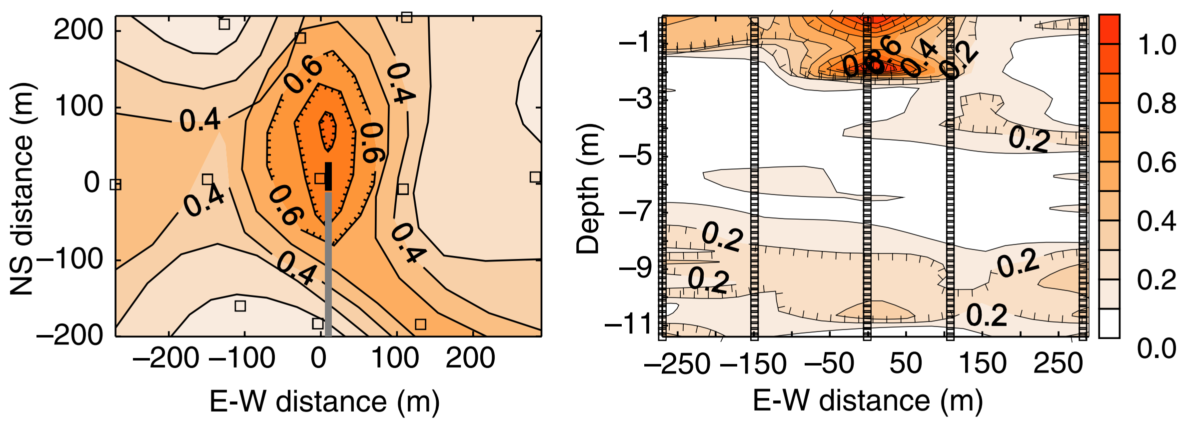

Fig. 3. Left: horizontal distribution of I_T at a depth of 0.2 m on 10 May 2000 above the outfall off Izola. Right: vertical distributions of I_T in E–W direction that cuts through the central station of the near-field for the respective cruises. Small bars mark the positions where CTD casts were made; dark straight line marks the pipe in a simplified way. [From Malačič & Mozetič (2005), their Fig. 3 (bottom part); with the permission of ACS journals.]

weaker, pycnocline was detected at a depth of 4 m. On May 10 we also performed a CTD survey across the grid of 11 stations to estimate the extent of the near-field of the sewage plume. From the distribution of the overturning length scale it is clear that the sewage was rising to the surface, where, in a layer of thickness of about 2.5 m, it was spreading horizontally, reaching an extent of several 100 m (Fig. 3).

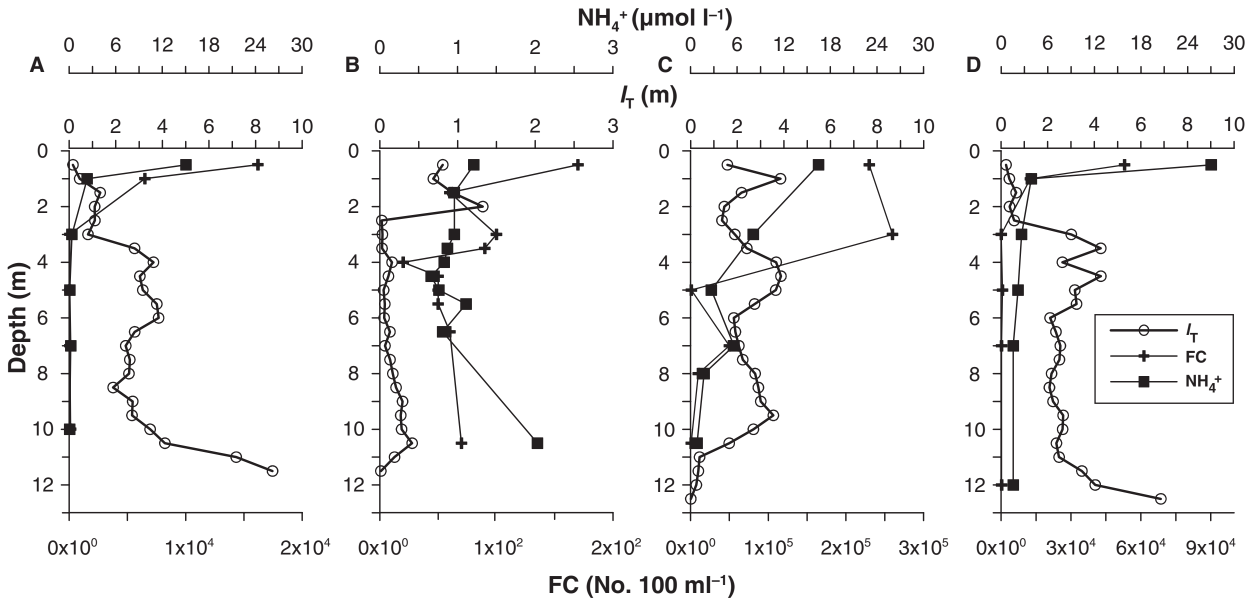

Fig. 4. Vertical distribution of ammonia, faecal coliform bacteria (FC) and overturning length scale (l_T) at station IZ06 during four surveys in the year 2000. A, March; B, May; C, August; D, October.

From the vertical profiles of l_T which are plotted together with FC and ammonia (Fig. 4), commonly considered indicative parameters of sewage pollution, it is difficult to distinguish a general pattern of l_T distribution. Profiles of surveys A and D were quite similar, both having a distinctive maximum (8.7 and 6.8 m) at the bottom and the lowest value (around 0.2 m) in the upper layer. Vertical distribution of l_T during survey B was just the opposite: a maximum (1.3 m) was found at 2 m depth and was the lowest of all maxima recorded. The most variable distribution of l_T was that of survey C, with several local maxima along the vertical and the minimum at the bottom.

Faecal pollution and nutrients

The highest concentrations of ammonia (Fig. 4) were generally measured in the surface layer (15.04–27.03 μmol·l^{-1}) and decreased sharply towards the bottom to reach values (<0.1–1.55 μmol·l^{-1}) typical for the undisturbed marine environment. The exception was the sampling on 10 May, when the peak concentration of ammonia (2.03 μmol·l^{-1}) was measured in the layer above the bottom; this sampling, however, differed from the others by being the one with the lowest values of both ammonia and FC. The distribution of FC along the water column (Fig. 4) almost followed that of ammonia. Peaks of FC were recorded in the surface layer and, similarly to ammonia, bacteria rapidly decreased in the layers below the surface. Occasionally the surface sewage plume expanded down to 3 m depth (29 August). On 10 May vertical oscillations of FC were more pronounced; however, their highest concentration (170 FC·100 ml^{-1}) was much lower than the highest ever measured (2.6×10^5 FC·100 ml^{-1}).

The highest concentrations of phosphate (Fig. 5) were always measured in the surface layer (0.91–1.44 μmol·l^{-1}) except on 10 May, when the maximum was observed at the bottom (1.19 μmol·l^{-1}). An increase of bottom concentration was also observed on 6 March. Peaks of TP (data not shown) coincided with peaks of phosphate, which indicates that at the highest concentrations the majority of phosphorus compounds were in the inorganic form. The vast majority of total nitrogen was, on the contrary, in the organic form (on average 80%; data not shown), whereas nitrate was on average the main inorganic form (4.05 ± 1.77 μmol·l^{-1}), though it never reached instantaneous concentrations as high as ammonia (Fig. 5). Concentrations of nitrate varied from 1.25 to 7.85 μmol·l^{-1}.

Concentrations of silicate (data not shown) were in the range of those regularly measured in the adjacent coastal waters (0.86–11.12 μmol·l^{-1}).

The cross-correlation analysis revealed several correlations at a level of statistical significance of 5% (Table 2). One of the highest and most statistically significant correlation was found between FC and ammonia (r = 0.78), followed by correlation coefficients between FC and TP (r = 0.71), PO_4^{3-} (r = 0.67), DIN (r = 0.66) and NO_2^- (r = 0.52). In 17 pairs of quantities with r ≥ 0.65, TP and

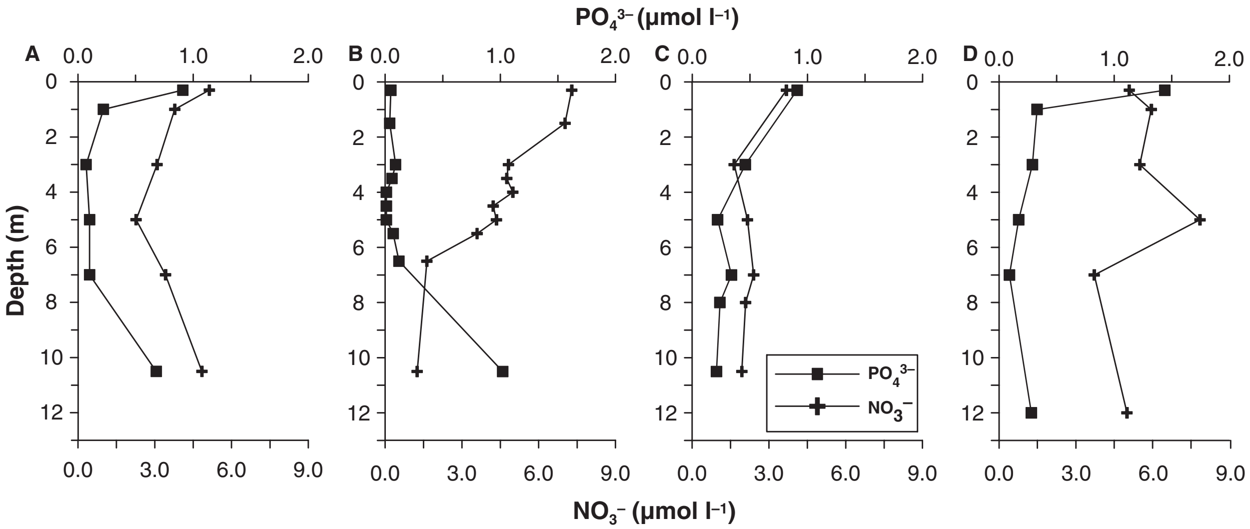

Fig. 5. Vertical distribution of phosphate and nitrate at station IZ06 during four surveys in the year 2000. A, March; B, May; C, August; D, October.

Table 2. Top part: Coefficients of cross-correlation analysis performed on a dataset of physical, chemical, microbiological and biological parameters (n = 530), which were measured in the seawater at station IZ06 during four surveys in the year 2000. Bottom part: pairs of quantities with the correlation coefficient ≥ 0.65, in decreasing order.

	temperature	salinity	σ_T	l_T	FC	PO_4^{3-}	TP	NO_2^-	NO_3^-	NH_4^+	DIN	TN	SiO_4^{4-}	Chl *a*	TSM	Phyto	TRIX
temperature																	
salinity	−0.13																
σ_T	**−0.73**	**0.76**															
l_T	0.10	**0.61**	0.32														
FC	0.35	**0.52**	0.10	0.08													
PO_4^{3-}	0.03	**0.68**	**0.41**	0.30	**0.67**												
TP	0.14	**0.44**	0.19	0.07	**0.71**	**0.87**											
NO_2^-	**0.48**	**0.53**	0.04	**0.53**	**0.52**	**0.65**	**0.65**										
NO_3^-	−0.06	−0.34	−0.20	−0.09	−0.13	−0.25	−0.08	0.09									
NH_4^+	**0.51**	0.18	−0.21	−0.19	**0.78**	**0.51**	**0.66**	**0.45**	0.04								
DIN	0.26	0.08	−0.12	−0.12	**0.66**	**0.43**	**0.65**	**0.49**	**0.52**	**0.79**							
TN	**−0.57**	**0.61**	**0.76**	0.20	**0.40**	**0.55**	**0.47**	0.23	0.25	0.16	**0.46**						
SiO_4^{4-}	**0.85**	−0.32	**−0.76**	0.04	0.15	−0.02	0.20	**0.48**	0.13	0.35	0.25	**−0.57**					
Chl *a*	0.05	−0.14	−0.15	0.06	−0.17	0.17	0.15	0.37	**0.53**	0.12	0.27	0.13	0.27				
TSM	0.19	**0.43**	0.14	0.35	**0.38**	**0.66**	**0.54**	**0.49**	**−0.58**	0.17	−0.05	−0.01	0.12	−0.06			
Phyto	−0.18	**−0.41**	−0.14	**−0.45**	**−0.40**	−0.07	−0.05	−0.21	0.17	0.06	0.00	−0.10	0.00	**0.49**	−0.18		
TRIX	**0.41**	−0.17	−0.38	−0.21	0.34	**0.41**	**0.60**	**0.55**	**0.39**	**0.65**	**0.74**	0.09	**0.57**	**0.64**	0.09	0.37	

order	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
parameter 1	TP	SiO_4^{4-}	DIN	NH_4^+	σ_T	TN	TRIX	TP	PO_4^{3-}	PO_4^{3-}	DIN	TSM	NH_4^+	NO_2^-	NO_2^-	DIN	TRIX
parameter 2	PO_4^{3-}	Temp.	NH_4^+	FC	Sal.	σ_T	DIN	FC	Sal.	FC	FC	PO_4^{3-}	TP	PO_4^{3-}	TP	TP	NH_4^+
r	0.87	0.85	0.79	0.78	0.76	0.76	0.74	0.71	0.68	0.67	0.66	0.66	0.66	0.65	0.65	0.65	0.65

All data, except for temperature, salinity, σ_T, l_T and the TRIX index were log-transformed. Values in bold are significant at P < 0.05 level.

PO_4^{3-} are five times involved and FC, DIN and NH_4^+ four times. Conversely, silicate and nitrate did not correlate with FC. Nitrate was rather highly correlated with Chl *a* (r = 0.53) and inversely correlated with suspended matter (r = −0.58).

Phytoplankton assemblage

Phytoplankton biomass varied from 0.62 to 2.96 μg Chl $a \cdot l^{-1}$ and it was more or less uniformly distributed throughout the water column (Fig. 6). Nevertheless, the

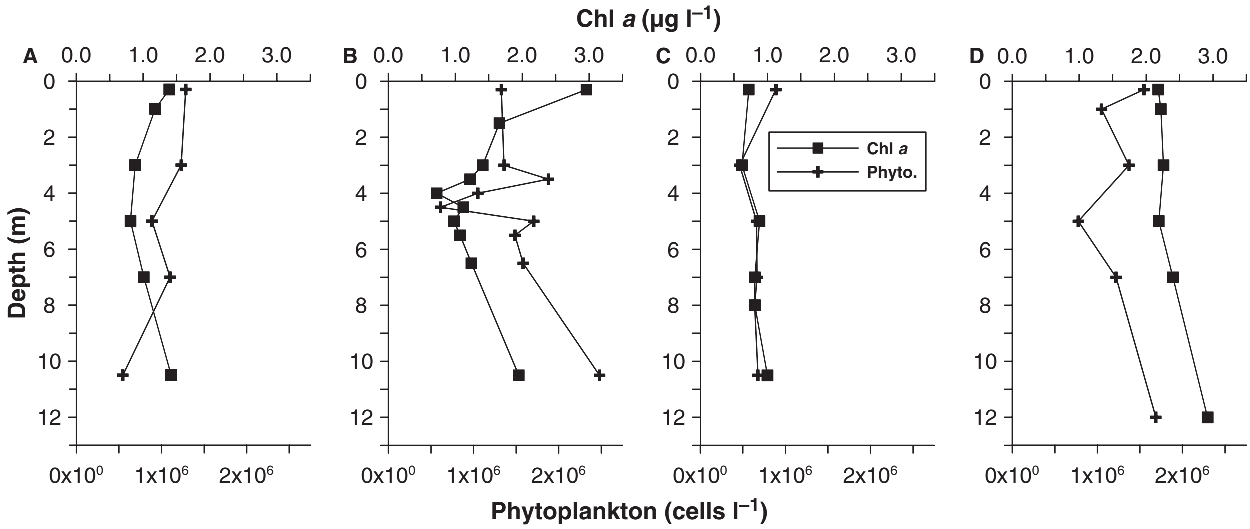

Fig. 6. Vertical distribution of phytoplankton biomass using chlorophyll *a*, and of phytoplankton abundance at station IZ06 during four surveys in the year 2000. A, March; B, May; C, August; D, October.

highest concentrations of Chl *a* were always measured in the bottom layer (1.00–2.92 $\mu g \cdot l^{-1}$) except on 10 May. During the sampling the pronounced vertical oscillations of Chl *a* concentrations and a surface maximum (2.96 $\mu g \cdot l^{-1}$) were observed.

Phytoplankton abundance displayed a similar pattern when compared to phytoplankton biomass (Fig. 6), which is also indicated by the statistically significant correlation between the two parameters (r = 0.49 between 'Phyto' and 'Chl *a*' in Table 2). Total abundance varied from 4.6×10^5 cells$\cdot l^{-1}$ (survey C) to 2.5×10^6 cells$\cdot l^{-1}$ (survey B) and the largest variations along the vertical were again observed during the May survey (B), when high values > 10^6 cells$\cdot l^{-1}$ were regularly counted.

The most abundant groups were autotrophic nanoflagellates and diatoms, which on average represented 52% and 45% of the total abundance, respectively. In the March and October surveys, diatoms dominated the community (55–80% of total abundance) reaching densities up to 1.3×10^6 cells$\cdot l^{-1}$ on 23 October. The most abundant diatoms in the March survey were *Skeletonema costatum s. l.*, *Pseudo-nitzschia calliantha* and *Chaetoceros* spp., whereas in October various species of *Chaetoceros* dominated the community, followed by *Leptocylindrus danicus*, *Cerataulina pelagica* and *Cylindrotheca closterium*. In May there was clear evidence of a nanoflagellate bloom that developed throughout the water column with maximal abundances in the bottom layer (2.2×10^6 cells$\cdot l^{-1}$). On 29 August, nanoflagellates and diatoms (dominated by *C. closterium*, *P. calliantha* and *Lauderia annulata*) reached similar densities. Dinoflagellates, coccolithophorids and silicoflagellates were poorly represented (on average around 1% of total abundance). In the taxonomically heterogeneous group of nanoflagellates we were able to identify organisms belonging to cryptophytes, chrysophytes, euglenophytes and green algae (chlorophytes and prasinophytes), which at times became important. On 10 May, cryptophytes were more abundant than diatoms (up to 5×10^5 cells$\cdot l^{-1}$), representing on average 24% of total abundance. During other surveys their contribution was much lower. Another important group during the May survey was that of green algae.

Trophic status

The trophic status of the coastal area was determined by applying the trophic index TRIX (Table 3). Its values varied from 3.48 (survey B, 5 m depth) to 6.16 (survey D, surface layer), thus encompassing very different trophic conditions. High values of TRIX, which indicate less favourable trophic conditions, were generally found in the surface layer and only once at the bottom (survey B). TRIX values were also correlated to the measured parameters and, as expected, significant correlations were found with those parameters (*e.g.* TP, DIN, Chl *a*) which define this index. However, the correlation cannot be very high due to the non-linear dependence of TRIX on these quantities.

Discussion

Detecting the sewage plume above the discharge

In our study we combined both faecal bacterial counts and *in situ* measurements of the physical properties of the water (temperature, salinity, density and its derived

Table 3. Trophic status of seawater at station IZ06 during four surveys in the year 2000, described by values of the TRIX index.

	TRIX			
depth (m)	6 March	10 May	29 August	23 October
0.3	4.82	5.13	4.90	6.16
1.0	4.07			5.36
1.5		4.97		
3.0	3.62	4.81	4.83	5.15
3.5		4.63		
4.0		4.28		
4.5		4.24		
5.0	3.48	4.35	4.12	5.05
5.5		4.75		
6.5		4.42		
7.0	4.00		4.63	4.76
8.0			4.00	
10.5	4.38	5.25	3.92	
12.0				4.82
(±SD)	4.06 (0.49)	4.68 (0.36)	4.40 (0.44)	5.22 (0.51)

Mean values of the parameter and standard deviations (SD) are shown.

parameter l_T). Previous studies of the spatial distribution of l_T in the Gulf of Trieste (Malačič 2005; Malačič & Mozetič 2005) showed that l_T could serve as a quick indicator of turbulent alien water, although several constraints to this approach have been pointed out. The turbulence of alien sewage water is far from being the only one detected by l_T as the ambient fluid already has a layered structure of turbulence generated by physical phenomena (*e.g.* internal waves, their breaking, wind-induced turbulence).

It is likely that these natural 'disturbances' masked the turbulence of the sewage water during our surveys as the l_T approach did not produce satisfactory results. There was no meaningful visual correlation between l_T and FC (Table 2) which could confirm that the presence of turbulent alien water was due to sewage discharge. Variations of l_T, even in the range of a few metres, were not detected on the profiles of FC. Yet in the situation of the most pronounced stratification, during survey B, when it is less likely that the ambient turbulence could mask the turbulence of the sewage water, vertical profiles of l_T and FC only roughly matched.

Other constraints in using l_T above the Izola submarine outfall are related to the steadiness of the discharge and to the sloping bed. The discharge at Izola emerges from a single orifice and is driven by pumps, intermittently putting bursts of sewage into the sea and, secondly, the outfall is on a seabed with a slope (around 10/200 = 0.05), which can create trapped and reflected internal waves in stratified fluids.

It is likely that in such complex near-coast areas other methods for tracking the sewage would be more appropriate (*e.g.* fluorescent dyes, radioactive isotopes or sewage-derived water-soluble markers) (Smith-Evans & Dawes 1996; Managaki *et al.* 2006).

Water quality and eutrophication effect

Variable hydrological conditions, mostly an unstable water column and irregular emptying of the sewage-collection basin, also blur another effect of sewage discharge, *i.e.* the extent of eutrophication, in the surrounding seawater.

As the concentrations of BOD_5, TSM, TP and NH_4^+ in the effluent (Table 1) were always above national regulation limits (25 mg $BOD_5 \cdot l^{-1}$, 80 mg $TSM \cdot l^{-1}$, 2.0 mg $TP \cdot l^{-1}$ and 10 mg $NH_4^+ \cdot l^{-1}$) (Official Gazette of Republic of Slovenia 1996) we expected that the poor quality of the effluent would also be reflected in the deteriorated water quality of the coastal sea. Abundance of FC was, indeed, elevated especially in the surface layer (up to 2.6×10^5 FC·100 ml^{-1}) and 60% of all measurements of FC have exceeded the national regulation limit for bathing waters (100 FC·100 ml^{-1}) (Official Gazette of Republic of Slovenia 1988). Although this regulation cannot be applied to water bodies which are not designed for recreational activity, it nevertheless gives an accurate estimate of water quality. However, the nearest beach is less than 300 m southwest of the outfall; therefore there is a risk that the sewage plume could occasionally reach it (see Fig. 3).

A comparison made between station IZ06 and the reference station 000F (see Fig. 1, Table 4) showed that both stations are statistically significantly different over the 3-year period at the significance level of 5% (Table 5). All parameters were on average higher at station IZ06 but those that contributed the most to this difference were TP, PO_4^{3-}, NH_4^+ and DIN. Nitrate was on average 1.2 times higher at station IZ06, but the maximal value was rather lower compared with the reference station (Table 4). This indicates that augmented nitrate concentrations often occur at station IZ06 but are not related to sewage discharge, as suggested by the correlation coefficient in Table 2. The average values of the TRIX index show a slight difference in trophic status, being high (3.59) at the reference station and good (4.71) at station IZ06 (Table 4). The persistently poorest conditions indicated by the average moderate trophic status (5.67 ± 0.89) are found only in the surface layer of station IZ06 where positively buoyant sewage plume usually emerges. Below this layer, nutrient concentrations are similar to 'unpolluted' values and follow the seasonal distribution. Only on one occasion, 10 May, did the back-

Table 4. Comparison of the mean concentrations of nutrients and Chl *a* measured at station IZ06 and at reference station 000F in the period 1999–2001.

	NO_3^- (μmol·l^{-1})	NO_2^- (μmol·l^{-1})	NH_4^+ (μmol·l^{-1})	PO_4^{3-} (μmol·l^{-1})	SiO_4^{4-} (μmol·l^{-1})	DIN (μmol·l^{-1})	TN (μmol·l^{-1})	TP (μmol·l^{-1})	Chl *a* (μg·l^{-1})	TRIX
station IZ06										
Mean	3.85	0.14	2.06	0.15	7.24	7.72	45.18	0.45	0.98	4.71
Mean+1*SD	9.83	0.40	7.80	0.62	12.57	16.27	88.47	1.07	1.81	5.45
Mean–1*SD	1.51	0.05	0.54	0.04	4.17	3.67	23.07	0.19	0.54	3.97
Min	0.01	0.02	0.07	0.01	0.86	2.02	3.06	0.03	0.22	2.97
Max	12.77	0.84	70.37	11.45	17.96	70.58	287.28	12.68	2.96	7.02
station 000F										
Mean	3.18	0.13	0.93	0.04	7.10	5.07	41.22	0.18	0.75	3.59
Mean+1*SD	8.69	0.46	2.00	0.09	11.44	10.19	62.22	0.38	1.40	4.48
Mean–1*SD	1.16	0.04	0.43	0.02	4.40	2.52	27.30	0.09	0.40	2.71
Min	0.35	0.01	0.09	0.01	2.13	1.70	12.35	0.01	0.11	1.01
Max	20.18	1.21	3.70	0.27	20.01	21.10	79.48	0.48	2.83	5.38

Mean values of nutrients and Chl *a* were calculated as geometric means, whereas those of TRIX as arithmetic means. Minimal (Min) and maximal (Max) values and 1 standard deviation (1*SD) are shown.

Table 5. Results of the multivariate analysis of variance (MANOVA) of nutrients, Chl *a* and TRIX measured in the seawater at station IZ06 and reference station 000F in the period 1999–2001.

parameter	F	P
NO_3^-	1.236	0.2685
NO_2^-	0.309	0.5792
NH_4^+	16.599	**0.0001**
PO_4^{3-}	39.362	**0.0000**
SiO_4^{4-}	0.044	0.8335
DIN	10.823	**0.0013**
TN	0.841	0.3608
TP	40.636	**0.0000**
Chl *a*	6.052	**0.0152**
TRIX	52.672	**0.0000**

Values in bold are significant at $P < 0.05$ level (Wilks' $\lambda = 0.422$, df1 = 10, df2 = 117).

ground conditions prevail throughout the whole water column (thermo-haline stratification; see Fig. 2).

The observed discrepancy between high nutrient concentrations and the minor biological response is even more accentuated if we consider that ammonia is, on average, the preferred nitrogen form for phytoplankton (Dortch 1990; Dortch *et al.* 1991; Watson & McCauley 2005) and that nitrate uptake is often inhibited in the presence of ammonia at concentrations $> 1\ \mu$mol·l^{-1} (Dortch *et al.* 1991). This preference for ammonia was recently linked to some species of the genus *Alexandrium* (Cannon 1990; Sorokin *et al.* 1996; Takeuchi & Yoshida 1999; Maguer *et al.* 2007), which might have important ecological and human health-related implications, these taxa being Harmful Algal Bloom (HAB) forming organisms. Cannon (1990) also observed *Alexandrium minutum* blooms in a strongly nutrient-enriched area close to sewage discharge in an Australian estuary. No occurrences of this sort were ever observed in our case.

In addition to nitrogen, whatever its form, phosphorus is also one of the major nutrients for phytoplankton growth. In many coastal waters more than half of the phosphate uptake can be due to bacteria (Turk *et al.* 1992; Turk & Hagström 1997). In our study the majority of phosphorus was in the inorganic form, especially at high concentrations of TP, and due to the P-depleted organic substrate, bacteria might have prevailed over phytoplankton for phosphate. This was previously observed in several enrichment experiments carried out in the Gulf of Trieste (Malej *et al.* 2003) when the maximal rate of bacterial production was measured on the second or third day of experiments in enclosures with P addition. The same was also recorded in an enclosure enriched with sewage from the Izola treatment basin (Authors' unpublished data).

Little biological response was observed in other confined areas such as estuaries which receive high concentrations of sewage-originated phosphate and ammonia (Aslan-Yilmaz *et al.* 2004; Pavlidou *et al.* 2004; Mallin *et al.* 2005; O'Higgins & Wilson 2005; Garcia-Barcina *et al.* 2006). O'Higgins & Wilson (2005) suggested that due to physical and chemical processes (accumulation and remineralization) organic matter remains entrapped in the estuary. In the case of short water retention time, phytoplankton growth is not sustained in spite of eutrophic levels of nutrients (Demir & Kirkagac 2005).

Conclusions

The intermittent discharge of effluent and the discharge of sewage onto a sloping bed near the shoreline do not allow an accurate tracking of the sewage plume with the otherwise successfully applied approach of the overturning length scale. The effect of intermittency is also reflected in the minimal effect of eutrophication in the case of phytoplankton. High amounts of nutrients probably dilute rapidly into the surrounding media under unsteady conditions, not allowing a sustainable growth of phytoplankton. Deteriorated average conditions as indicated by moderate trophic status are observed only in the surface layer.

The major negative influence of improperly treated wastewaters is demonstrated mostly by the microbiological quality of the coastal sea. High concentrations of pollution-related parameters in the effluent, in many cases well above national regulation limits, are evidence of that. There is a constant threat to human health during the bathing season and to the integrity of seafood (*i.e.* angling) due to the spreading of the sewage plume towards the beach and its presence in the surface layer. To solve the problem of poor sewage treatment, the municipality of Izola will, in the near future, send their sewage through a recently built pipeline system to a new sewage treatment plant, which is designed for tertiary wastewater treatment.

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