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Symptoms of Eutrophication in Intertidal Macroalgal Assemblages of Nuevo Gulf (Patagonia, Argentina)

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The city of Puerto Madryn, located within a section of Nuevo Gulf (Patagonia, Argentina) known as Nueva Bay, can be regarded as an important centre of anthropogenic impact. Intertidal assemblages of macroalgae were studied in order to test the hypothesis that the seaweed flora impacted by raw sewage would show significant differences in structure compared to that of control localities, mainly due to the increase in biomass of ephemeral and opportunist chlorophytes. Three rocky shores showing similar characteristics were selected. One of these was located near the sewage outfall of the city of Puerto Madryn. Two control shores were chosen approximately 30 km northward and southward of the latter. Four seasonal surveys were performed between June 1998 and April 1999. Biomass data of 35 macroalgal species were analysed by non-metric multidimensional scaling and the analysis of similarities permutation test. Species responsible for the differences between shores were identified by the similarity percentages routine. The macroalgal assemblage of the impacted site was significantly different from those of the two control shores throughout the year. *Ulva rigida*, *Corallina officinalis* and *Ralfsia* sp. were the species mostly responsible for these differences. Results indicate that the impacted shore showed signs consistent with an intermediate degree of eutrophication, particularly the increased abundance of the opportunist green alga *Ulva rigida*.

Introduction

Studies on benthic community structure may be an adequate tool to assess the impact caused by pollution (Borowitzka 1972, Littler and Murray 1975, 1978, Warwick 1986). Previous surveys of pollution impacts on coastal benthos in Argentina were focused on intertidal rocky shores dominated by mytilid bivalves (López Gappa *et al.* 1990, 1993) and estuarine sediments (Elías 1992).

In Nuevo Gulf (Patagonia, Argentina, Fig. 1), the only previous study on benthic communities including macroalgal assemblages was performed at a site located at a considerable distance from the zone of urban impact (Olivier *et al.* 1966). Therefore, background information previous to the present situation is lacking.

Part of Nuevo Gulf is included in the Valdés Peninsula system, recently declared a World Heritage Site by UNESCO. The city of Puerto Madryn (42° 45' S; 64° 55' W), located within a section of the Gulf known as Nueva Bay (Fig. 1), has 50,000 inhabitants and a constant harbour activity, and can be regarded as an important centre of anthropogenic impact.

Coastal water quality has been modified by sewage discharges from urban areas and the fishing industry. Freshwater entering Nueva Bay is characterised by high nutrient concentrations (25–37 mg L^{-1} of ammonium nitrogen and 3–4 mg L^{-1} of phosphate phosphorus, Estéves *et al.* 1997).

Among other benthic organisms, macroalgae, because of their sedentary nature, tend to integrate the effects of long-term exposure to adverse conditions (Borowitzka 1972). Therefore, the study of intertidal algal assemblages was considered useful in order to analyse whether eutrophication symptoms in Nueva Bay can be detected in the benthos, as well as a contribution to the knowledge of the benthic communities in this poorly known region.

The City of Puerto Madryn is currently developing a new system of urban wastewater treatment that will eliminate the discharge of sewage to the sea. Therefore, spatial surveys of the polluted site, as well as in one or more control localities, may serve as a future reference to detect changes indicating ecosystem recovery.

Based on previous studies on macroalgal assemblages impacted by organic pollution (Bokn *et al.* 1996, Borowitzka 1972, Littler and Murray 1975, Morand and Briand 1996, Munda 1993) we predicted that the seaweed flora in the vicinity of the Puerto Madryn sewage outfall would show significant differences in structure compared to that of control locali-

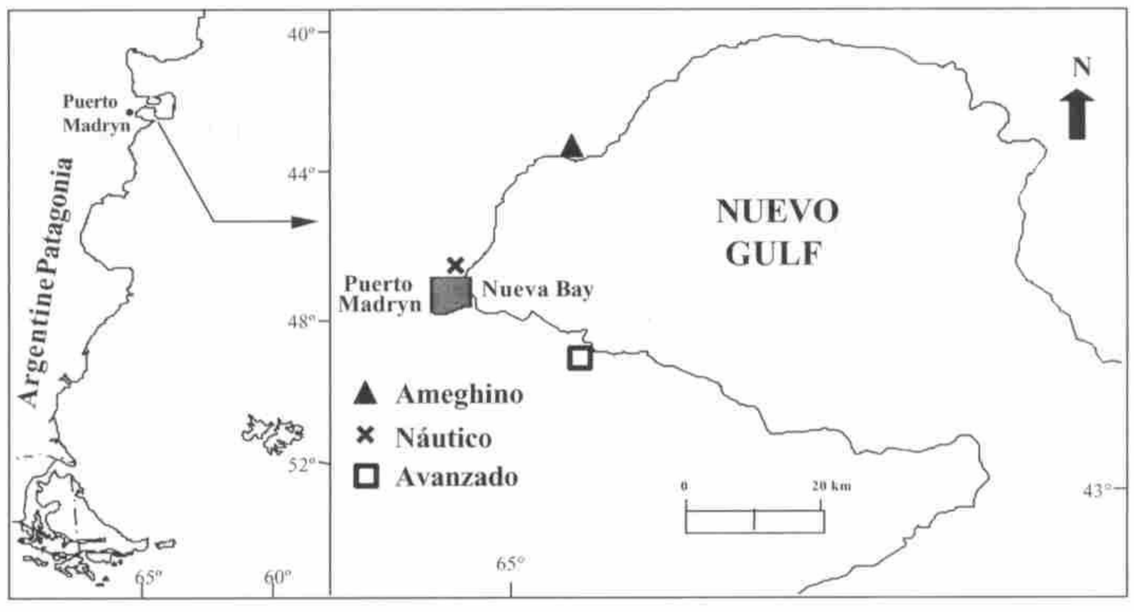

Fig. 1. Study area.

ties, mainly due to the increase in biomass of ephemeral and opportunist chlorophytes. The aim of this study is to test this hypothesis.

Study Site

Nuevo Gulf (Fig. 1) is a semi-enclosed basin of low hydrodynamics. As in many semi-desertic areas, natural freshwater inputs are lacking. Seawater temperature and salinity fluctuate yearly between 10 to 19.5 °C and 33.7 to 33.9‰, respectively (Solís 1998). Nuevo Gulf is located in the transition zone between the cold-temperate and warm-temperate biogeographic regions of the south-western Atlantic Ocean (Boschi 1979, Kühnemann 1972).

Nueva Bay, located in the western part of Nuevo Gulf, shows evidence of eutrophication, due to the input of urban and industrial effluents (Estéves *et al.* 1997), which are discharged in the intertidal zone. Urban outfall volume has been estimated as 6,000 to 8,000 m^3 d^{-1}. Sewage receives secondary treatment, but in recent years there has been a remarkable increase in volume due to human population growth. The treatment facility is no longer able to process all the wastewater properly, discharging it to the sea mostly as raw sewage. Therefore, the effluents represent an important input of nitrogen, phosphorus and organic matter to the Bay, which has a volume of 4×10^8 m^3 and a restricted water exchange with other areas of the Gulf (Estéves *et al.* 1997).

Material and Methods

Three rocky shores were selected within Nuevo Gulf. One of these was within Nueva Bay, near the Puerto Madryn sewage outfall (Náutico). The other two localities, Punta Ameghino and Punta Cerro Avanzado (hereafter referred to as Ameghino and Avanzado, respectively), were outside the Bay, approximately 30 km northward or southward of Puerto Madryn, respectively (Fig. 1).

Ammonium and phosphate values recorded by Estéves *et al.* (1997) varied from 13.4 µM L^{-1} and 1.55 µM L^{-1} off the city, to 0.9 µM L^{-1} and 1.12 µM L^{-1} at a distance of 15 km from the urban area, respectively. Ameghino and Avanzado, located approximately at 30 km from Puerto Madryn, are therefore not affected by the effluents, and can be regarded as adequate controls.

Intertidal rocky platforms showed a gradual profile, with a vertical differential of around 3 m in the three shores. They consisted of bare substratum, or were covered by sessile organisms (algae, barnacles, mussels). During the study period, the rocky platforms, as well as the intertidal community, were temporarily covered by sand deposits in all three localities.

Four seasonal surveys were performed between June 1998 and April 1999. Samples were collected along transects perpendicular to the shoreline. For each combination of locality and season, 10 sampling sites were established between the level of spring low tides and the nearest point to the cliff showing macroalgal coverage.

Sampling area and shape, a 25 × 50 cm rectangle, were determined by the minimum quadrat method (Alveal and Romo 1995). Each sample consisted of all macroalgae collected within the sampling quadrat. They were extracted using scrapers, bagged, labelled and stored at –13 °C. Macroalgae were identified in the laboratory. Their wet weights were measured to the nearest 0.01 g in a Sartorius analytical balance (Werke GmbH, Göttingen, Germany), after blotting the excess of water. The biomass of *Ralfsia* sp. was estimated from coverage data, after calculating wet weight of a known area (25 cm^2, n = 89, mean = 1.98 g, SD = 0.71 g).

The Plymouth Routines in Multivariate Ecological Research (PRIMER) statistical package (Clarke and Warwick 1994) was used to compare macroalgal assemblages among localities by means of non-metric multidimensional scaling (MDS, Kruskal and Wish 1978). A similarity matrix was obtained using the Bray-Curtis index (Bray and Curtis 1957). The 4th root transformation ($x' = x^{0.25}$, Clarke and Warwick

1994) was used to downweight the effect of dominant species. The hypothesis that macroalgal assemblages from the putatively impacted site (Náutico) significantly differed from the two control localities (Ameghino, Avanzado), was tested by means of the analysis of similarities (ANOSIM) permutation test (Clarke and Green 1988). The similarity percentages (SIMPER) routine (Clarke 1993) was then used to analyse which species were responsible for the differences between localities.

Results

Thirty-five macroalgal species were recorded during this study. Of these, 19 were common to all localities (Table I). *Corallina officinalis* and *Ralfsia* sp. may be regarded as dominant species both in the control sites and at Náutico, their average site biomasses frequently being 1 to 3 orders of magnitude higher than those of most other algae. *Ulva rigida* was dominant only at Náutico, where it reached very high average mass, particularly during spring. Other species were also abundant at certain combinations of locality and season, such as *Leathesia difformis* (Avanzado, summer), *Polysiphonia abcissa* (Avanzado, spring), *Chaetomorpha linum* (Náutico, spring), *Cladostephus* sp. (Avanzado, summer through autumn), *Antithamnion* sp. (Avanzado, spring through summer), and *Enteromorpha compressa* (Ameghino, winter; Náutico, spring through autumn) (Table I).

Stress values of the MDS plots varied between 0.11 and 0.15. Therefore, they can be regarded as potentially useful 2-dimensional representations of intersample distances in the similarity matrices (Clarke and Warwick 1994). Most Náutico samples tended to cluster apart from those of the two control localities in the MDS plots throughout the study period (Fig. 2). Accordingly, results of the ANOSIM permutation tests (Table II) showed that differences in structure between Náutico and Ameghino or Avanzado were always significant or highly significant. On the other hand, contrasts between both control localities reached significance just in winter.

Results of SIMPER analyses (Table III) indicate that species mostly responsible for differences in structure between Náutico and both control localities were invariably *Corallina officinalis*, *Ralfsia* sp. and *Ulva rigida*. The latter constantly had higher biomass values in the vicinity of the sewage outfall (Náutico) than in both control localities. The red alga *Corallina officinalis* was always more abundant at Ameghino than at Náutico, and also showed this pattern from winter through summer at Avanzado. Although always highly ranked in the results of the SIMPER tests, the crustose brown alga *Ralfsia* sp. showed no consistent seasonal preferences, either for Náutico or for the control localities (Table III).

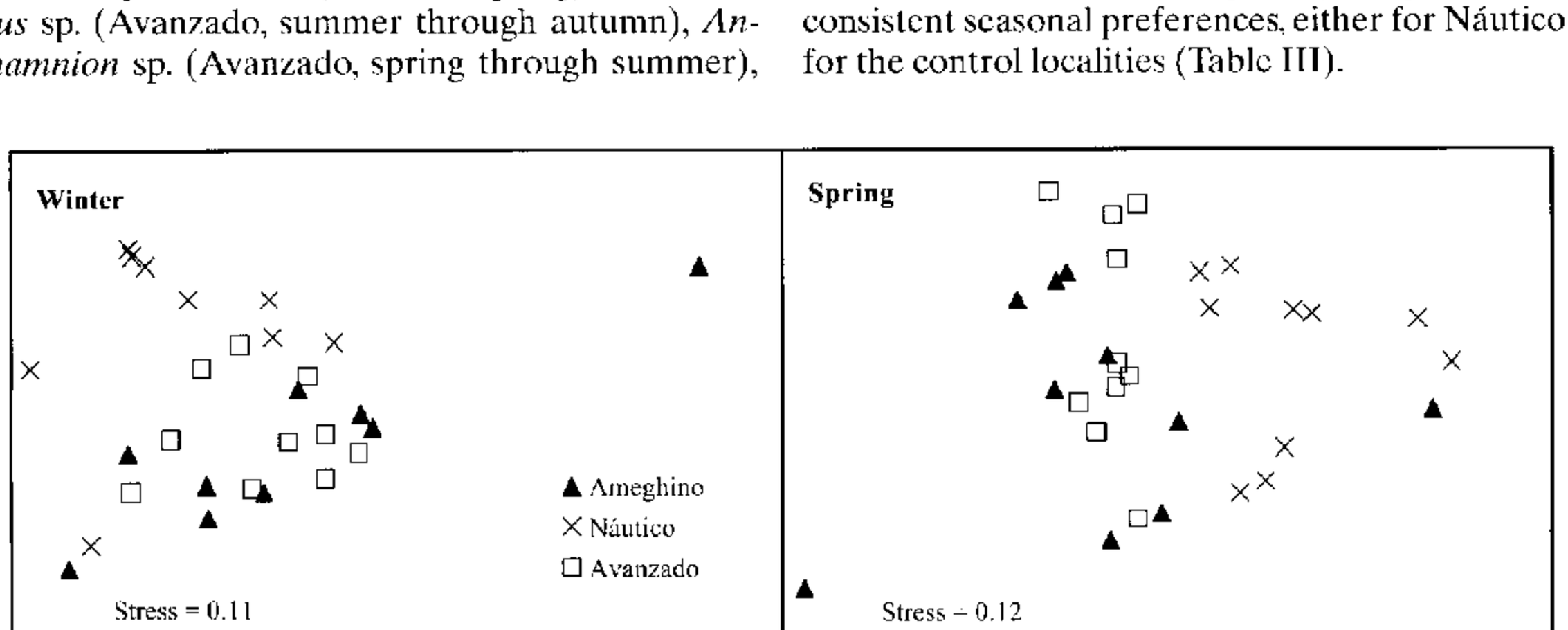

Fig. 2. MDS plots for each season. Two winter samples (one from Náutico and another from Ameghino) were omitted. Since they contained just *Enteromorpha compressa*, a species absent in all other winter samples, its presence caused the collapse of the plot.

Table I. Mean algal biomass (g) per locality and season.

	Ameghino				Náutico				Avanzado			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Bryopsis sp.	–	–	–	–	< 0.01	–	0.01	–	–	–	–	–
Chaetomorpha aerea (Dillwyn) Kützing	–	< 0.01	–	–	< 0.01	–	–	–	0.04	< 0.01	0.01	< 0.01
Chaetomorpha linum (Müller) Kützing	–	–	–	–	0.03	3.08	0.52	1.09	–	–	–	–
Cladophora sp.	–	–	< 0.01	0.02	–	< 0.01	0.03	–	< 0.01	0.01	–	0.02
Codium vermilara (Olivi) Delle Chiaje	< 0.01	–	–	–	0.27	–	–	–	–	0.03	–	–
Enteromorpha compressa (Linnaeus) Greville	1.48	0.04	–	0.14	0.14	3.40	3.92	2.46	–	0.06	–	–
Enteromorpha sp.	< 0.01	–	–	< 0.01	–	–	0.02	–	–	< 0.01	–	–
Ulva rigida (C. Agardh) Thuret	0.03	0.28	–	0.01	1.04	27.50	7.39	17.31	< 0.01	0.33	0.29	0.07
Anotrichium furcellatum (J. Agardh) Baldock	–	–	–	< 0.01	–	–	–	0.01	< 0.01	< 0.01	< 0.01	0.08
Antithamnion sp.	0.64	0.01	0.13	0.46	–	–	–	< 0.01	0.48	1.05	2.04	0.63
Aphanocladia spp.	< 0.01	–	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	–	0.38	< 0.01	< 0.01
Callithamnion gaudichaudii C. Agardh	–	0.29	0.09	< 0.01	–	–	–	–	–	–	0.02	0.32
Ceramium rubrum (Hudson) C. Agardh	0.47	0.02	0.05	0.37	< 0.01	0.05	0.04	0.81	0.01	0.30	< 0.01	0.24
Ceramium strictum Greville *ex* Harvey	< 0.01	–	< 0.01	0.04	–	–	0.01	0.10	< 0.01	< 0.01	0.01	0.04
Corallina officinalis Linnaeus	39.78	59.00	49.60	33.82	8.91	9.98	6.22	25.79	68.56	47.92	16.07	17.59
Gelidiella sp.	–	–	0.03	< 0.01	–	–	–	–	0.28	0.01	< 0.01	0.23
Gracilaria gracilis (Stackhouse) Steentoft, Irvine *et* Farnham	–	–	–	–	–	–	–	0.05	–	–	–	–
Heterosiphonia sp.	–	–	–	–	–	< 0.01	–	–	–	< 0.01	< 0.01	–
Lomentaria clavellosa (Turner) Gaillon	< 0.01	–	–	0.17	–	–	–	–	–	0.43	< 0.01	< 0.01
Nothogenia fastigiata (Bory) Parkinson	–	–	–	–	–	–	–	–	0.10	–	–	–
Polysiphonia abcissa Hooker *et* Harvey	0.48	–	–	–	–	< 0.01	–	–	0.28	3.37	0.55	< 0.01
Polysiphonia argentinica Taylor	0.07	–	0.08	0.45	–	–	–	0.01	0.01	< 0.01	0.07	0.05
Polysiphonia brodiaei (Dillwyn) Greville	0.50	0.01	0.06	0.11	–	< 0.01	0.05	–	0.05	0.78	0.23	0.43
Polysiphonia sp.	–	–	–	–	–	–	–	–	–	–	–	0.02
Streblocladia camptoclada (Montagne) Falkenberg	< 0.01	–	< 0.01	0.26	0.38	0.46	0.59	1.25	0.01	0.09	0.43	0.03
Cladostephus sp.	–	–	< 0.01	0.01	0.04	0.01	< 0.01	–	0.45	0.15	6.02	2.15
Cutleria multifida (Smith) Greville	–	–	–	–	–	–	–	–	0.01	–	–	–
Dictyota aff. *dichotoma* (Hudson) Lamouroux	–	0.06	–	< 0.01	–	–	–	–	–	–	–	–
Halopteris sp.	–	–	–	–	–	–	–	< 0.01	< 0.01	–	–	–
Leathesia difformis (Linnaeus) Areschough	–	0.17	0.73	0.69	–	–	–	–	–	0.03	2.49	–
Ralfsia sp.	25.80	18.20	10.08	19.74	5.06	6.20	18.65	12.40	2.31	8.68	10.39	12.60
Sphacelaria sp.	0.14	< 0.01	< 0.01	0.55	0.02	< 0.01	0.07	< 0.01	0.08	0.02	0.03	0.01
Undaria pinnatifida (Harvey) Suringar	–	–	–	–	–	0.05	–	–	–	–	–	–
Cyanobacteria	–	< 0.01	< 0.01	< 0.01	–	–		–	–	–	0.20	< 0.01
Tube-dwelling diatoms	0.10	–	< 0.01	< 0.01	–	–	–	–	–	–	–	< 0.01

Table II. Results of ANOSIM tests.

	Global probability	Paired Contrasts		
		Náutico vs. Ameghino	Náutico vs. Avanzado	Ameghino vs. Avanzado
Winter	0.000	0.011	0.001	0.020
Spring	0.001	0.007	0.001	0.116 NS
Summer	0.001	0.001	0.016	0.168 NS
Autumn	0.039	0.028	0.033	0.399 NS

NS: non-significant

Table III. Results of SIMPER tests for significant ANOSIM contrasts.

Species	Mean abundance	Mean abundance	Percentage	Cumulative (%)
Winter	Náutico	Avanzado		
Corallina officinalis	8.91	68.56	29.35	29.35
Ralfsia sp.	5.06	2.31	14.23	43.58
Ulva rigida	1.04	0.00	13.54	57.12
Winter	Náutico	Ameghino		
Corallina officinalis	8.91	39.78	21.16	21.16
Ralfsia sp.	5.06	25.80	20.72	41.87
Ulva rigida	1.04	0.03	11.34	53.21
Winter	Avanzado	Ameghino		
Corallina officinalis	68.56	39.78	24.93	24.93
Ralfsia sp.	2.31	25.80	19.52	44.45
Polysiphonia abcissa	0.28	0.48	6.43	50.88
Spring	Náutico	Avanzado		
Corallina officinalis	9.98	47.92	18.68	18.68
Ulva rigida	27.50	0.33	18.64	37.31
Ralfsia sp.	6.20	8.68	14.46	51.77
Spring	Náutico	Ameghino		
Ulva rigida	27.50	0.28	22.88	22.88
Corallina officinalis	9.98	59.00	22.39	45.26
Ralfsia sp.	6.20	18.20	16.74	62.00
Summer	Náutico	Avanzado		
Ralfsia sp.	18.65	10.39	17.85	17.85
Corallina officinalis	6.22	16.07	14.79	32.65
Ulva rigida	7.39	0.29	12.48	45.13
Antithamnion sp.	0.00	2.04	7.19	52.32
Summer	Náutico	Ameghino		
Corallina officinalis	6.22	49.60	23.21	23.21
Ralfsia sp.	18.65	10.08	18.97	42.18
Ulva rigida	7.39	0.00	15.27	57.45
Autumn	Náutico	Avanzado		
Corallina officinalis	25.80	17.59	17.34	17.34
Ulva rigida	17.30	0.07	16.08	33.43
Ralfsia sp.	12.40	12.60	15.95	49.38
Enteromorpha compressa	2.46	0.00	6.59	55.96
Autumn	Náutico	Ameghino		
Ralfsia sp.	12.40	19.74	19.01	19.01
Corallina officinalis	25.80	33.82	17.78	36.79
Ulva rigida	17.30	0.01	17.17	53.96

Lists of species have been truncated after cumulative percentage exceeded 50 %.

Discussion

This study shows that the macroalgal assemblage at Náutico, a site located in the vicinity of the Puerto Madryn sewage outfall, was significantly different from those of two control localities of similar physical characteristics, but situated at considerable distances from any source of urban and industrial pollution. *Ulva rigida*, *Corallina officinalis* and *Ralfsia* sp.

were the species mostly responsible for this difference. These algae were present at all localities, but their abundance varied in space and time.

Although the green alga *Ulva rigida* was found both in the impacted and control sites, its average biomass was always highest at Náutico, where it was dominant in spring and was one of the most abundant species during the remaining seasons. This is one of several species of the genus *Ulva* that have been characterised in the literature as opportunist algae, proliferating in eutrophicated shores (Morand and Briand 1996, Menesguen 1992, Pugnetti *et al.* 1992).

Other opportunist algae, reportedly favoured by organic enrichment are *Chaetomorpha linum*, *Gracilaria gracilis* and *Enteromorpha compressa* (Morand and Briand 1996). The first two species were found only at Náutico. *Enteromorpha compressa* occurred in significant amounts at Náutico throughout the study period, a fact that could be associated with the influence of organic enrichment, although lower amounts of this green alga were also present on other shores. *Enteromorpha compressa* forms a conspicuous belt at high intertidal levels at San José Gulf, a neighbouring area far away from any source of anthropogenic disturbance (Boraso de Zaixso 1996, Sánchez and Zaixso 1995). This and other species of *Enteromorpha* were also recorded in other localities of the Argentinean coast (Boraso 1975, Hall and Boraso 1975, Zaixso *et al* 1978), not always affected by human activities. Therefore, the presence of *Enteromorpha per se* should not necessarily be regarded as a symptom of eutrophication.

The occurrence of dense stands of green algae, particularly *Ulva rigida*, has been associated with the increase of nitrogen concentrations in water (Morand and Briand 1996, Menesguen 1992, Pugneti *et al.* 1992, Viaroli *et al.* 1992). Chemical analyses of water performed recently in Nuevo Gulf (Estéves *et al.* 1997) showed that high nutrient concentrations (ammonium, nitrate, etc.) in coastal areas were associated with the input of urban and industrial effluents from the city of Puerto Madryn.

A historical study on the composition of beached seaweeds deposited on Puerto Madryn shores during the last 7 decades reports the replacement of species dominance from *Codium* spp. to *Ulva* spp. (and more recently to *Undaria pinnatifida*) as a symptom of environmental changes (Piriz *et al.*, unpublished results).

Present results indicate that the algal assemblage of Náutico shows signs consistent with an intermediate degree of organic enrichment. Borowitzka (1972), Littler and Murray (1975) and López Gappa *et al.* (1990), found that intertidal sites heavily impacted by the discharge of effluents are characterised by relatively low values of algal coverage (mainly *Enteromorpha* sp., Cyanobacteria and tube-dwelling diatoms) and extensive areas of bare substratum. On the other hand, intermediate zones located between coarsely polluted and relatively clean areas show high abundance of green algae (*Ulva* and/or *Enteromorpha* spp.).

Since spring (October 1998), sand deposits have covered mid-intertidal areas of Náutico almost completely. During this season, Náutico was colonised by thalli of *Ulva rigida* attached to sand grains. The belt of *Ulva rigida* persisted during the following summer and autumn, together with their sand substratum. Algae were absent, however, on sand beds deposited at Ameghino and Avanzado.

Although background data of pre-disturbance conditions were lacking, comparison of impacted and control localities, together with information gathered from different shores throughout the world, strongly suggest that the macroalgal assemblage of Náutico shows some signs related to organic enrichment, particularly the increased abundance of the opportunist green alga *Ulva rigida*.

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