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Response of a seagrass fish assemblage to improved wastewater treatment

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ABSTRACT

We compared the structure of a seagrass fish assemblage near a sewage outlet before and after improvements to wastewater treatment. To determine whether responses by the fish assemblage were due to changes in water quality or to other factors, comparisons were made with the structure of a fish assemblage from a nearby site unaffected by sewage effluent. Total species richness, density and biomass of fish, decreased at both sites over the 30-year period. An increase in mean trophic level near the sewage outlet following improvements in water quality indicated that wastewater treatment had another important effect. This result is consistent with the reductions in food webs supporting pelagic and benthic fishes that typically accompany decreases in nutrient inputs. Although improvements to wastewater treatment explained much of the variation in the structure of the fish assemblage at PC, our results also suggest that fishing and climate change, at both sites.

1. Introduction

Seagrass meadows are sensitive ecosystems under pressure from developments in the coastal zone (Duarte, 2002; Boudouresque et al., 2009; Waycott et al., 2009). Increased nutrients and particulate organic matter in sewage effluent from growing coastal populations is a problem of particular significance (Reish and Bellan, 1995; Airoldi and Beck, 2007; Stamou and Kamizoulis, 2009). The effects of such wastewater on soft-bottom and rocky reef ecosystems have been reported widely (Grigg, 1994; Otway et al., 1996; Hall et al., 1997; Smith et al., 1999; Reopanichkul et al., 2009), including from locations in the Mediterranean Sea (Guidetti et al., 2002, 2003; Azzurro et al., 2010). Typically, sewage pollution results in decreased biodiversity close to the point source (Reish and Bellan, 1995; Smith et al., 1999) and an increase in pelagic zooplanktivorous and omnivorous fishes as the effluent disperses (Hall et al., 1997; Guidetti et al., 2003).

Many countries have introduced improved treatment of wastewater to reduce the visual and ecological impacts of sewage effluent. However, relatively few studies have examined the changes in coastal fauna following installation of improved wastewater treatment systems (but see Bellan et al., 1999; Soltan et al., 2001; Perez et al., 2005; Ribeiro et al., 2008; Ourgaud et al., 2013). This study was conducted near Marseille, the second largest city in France. The population of Marseille and its urban conurbation, which now exceeds 1,800,000, produces 10,500 m^3 of sewage effluent per hour. This wastewater is discharged directly into Cortiou cove on the rocky coast to the east of the city (Fig. 1). The sewage outlet at Marseille is close to extensive areas of *Posidonia oceanica* seagrass habitat. This seagrass species grows to depths of 30–40 m in clear water and forms extensive meadows (Harmelin and True, 1964; Boudouresque et al., 1990, 2009, 2012; Pergent et al., 2012). These meadows support a high diversity of fish and invertebrates, and play important ecological, sedimentary and economic roles in the coastal zone of the Mediterranean Sea (Kikuchi and Pérès, 1973; Den Hartog, 1977; Kikuchi, 1980; Boudouresque and Meinesz, 1982; Harmelin-Vivien, 1983; Pollard, 1984; Pergent-Martini et al., 1994; Francour, 1997; Procaccini et al., 2003; Boudouresque, 2004; Personnic et al., 2014) and the species composition, trophic structure, diel migrations and seasonality of fish assemblages associated with *P. oceanica* are well known (Bell and Harmelin-Vivien, 1982, 1983; Deudero et al., 2008; Francour, 1997; Guidetti, 2000; Harmelin-Vivien, 1982, 1983; Harmelin-Vivien et al., 2000a; Kalogirou et al., 2010; Moranta et al., 2006; Seytre and Francour, 2013).

Until 1987, the wastewater from the sewage outlet at Marseille was untreated and included industrial and domestic effluents

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(Bellan et al., 1999). A primary treatment plant was then installed, which removed >70% of the organic matter (OM) and contaminants (Arfi et al., 2000). Since 2008, the wastewater from Marseille has received additional biological (tertiary) treatment which retains >90% of the dissolved and particulate organic matter. However, currently no fourth treatment, said "finishing" treatment is established to retain all metallic and organic contaminants. Here, we report changes to the fish assemblage associated with a *P. oceanica* seagrass meadow near the sewage outlet at Marseille following improvements to the wastewater treatment plant, and evaluate the extent to which these changes were due to better water quality.

2. Materials and methods

2.1. General approach

To assess the effects of improvements in water quality on the species richness, density, biomass and trophic structure of the fish assemblage associated with the *P. oceanica* seagrass habitat at Marseille, we used a 'before vs after, control vs impact' (BACI) sampling design. This design provided the opportunity to identify the relative importance of the effects due to improvements in wastewater treatment, and any other factors that may have altered over time, on the fish assemblage near the sewage outlet. The BACI sampling design involved comparing (1) the key features of the fish assemblage in the site near the sewage outlet ("Plateau des Chèvres") before wastewater was treated (1980) with key features of the assemblage four years after the commencement of tertiary treatment (2012); and (2) the key features of the fish assemblage near the sewage outlet with those of a 'control' assemblage from a seagrass meadow in an area unaffected by sewage effluent ("Côte Bleue") in 1980 and 2012. A better sampling design would have included comparisons with a seagrass meadow that remained under the influence of untreated sewage but no such site existed. The results of a fish survey at Plateau des Chèvres in 2000 have also been included to provide insight into the possible effects of primary wastewater treatment on the fish assemblage near the sewage outlet, albeit without the benefit of a comparison with a control site.

2.2. Study area

The two *P. oceanica* seagrass sites used for this study were located in the Bay of Marseille (Fig. 1). The site impacted by wastewater was at Plateau des Chèvres (PC) close to the sewage outlet at Cortiou in the south of the bay. The fish assemblage at PC was directly exposed to nutrient-rich wastewater (Arfi et al., 2000; Bellan, 1970; Bellan et al., 1980). The control seagrass meadow was at Côte Bleue (CB) in the north of the bay.

2.3. Sampling method

The fish assemblages were sampled with a small (1.5 × 0.5 m) beam trawl, with a stretched mesh size of 8 mm, purpose-built to catch fish from the *P. oceanica* seagrass ecosystem (Harmelin-Vivien, 1981). Fish were collected during the day and at night because of the diel differences that occur in activity rhythms, feeding behaviour and position in the water column for fishes associated with *P. oceanica* seagrass meadows (Harmelin-Vivien, 1982) (Fig. 2). At PC, sampling consisted of four trawls of 15 min duration during the day and the night in summer 1980 (Bell and Harmelin-Vivien, 1982) and five trawls of 10 min duration during the day and the night in winter. In 2000 and 2012, five trawls of 10 min duration were made during the day and the night in both summer and winter at PC. The trawls were made between depths of 8 and 18 m at a constant towing speed of 1.5 knots. The combined surface area covered by four 15-min trawls at PC in summer was ~4200 m^2 in 1980, whereas the combined surface area covered by five 10-min trawls at all other times was ~3500 m^2. The sampling at CB in summer and winter 1980 followed the same protocol used at PC. The sampling at CB in 2012 was also based on five 10-min trawls during the day and night in summer and winter. The catch from each trawl was separated into fish species, the number of each species was counted and the total length of each individual fish was

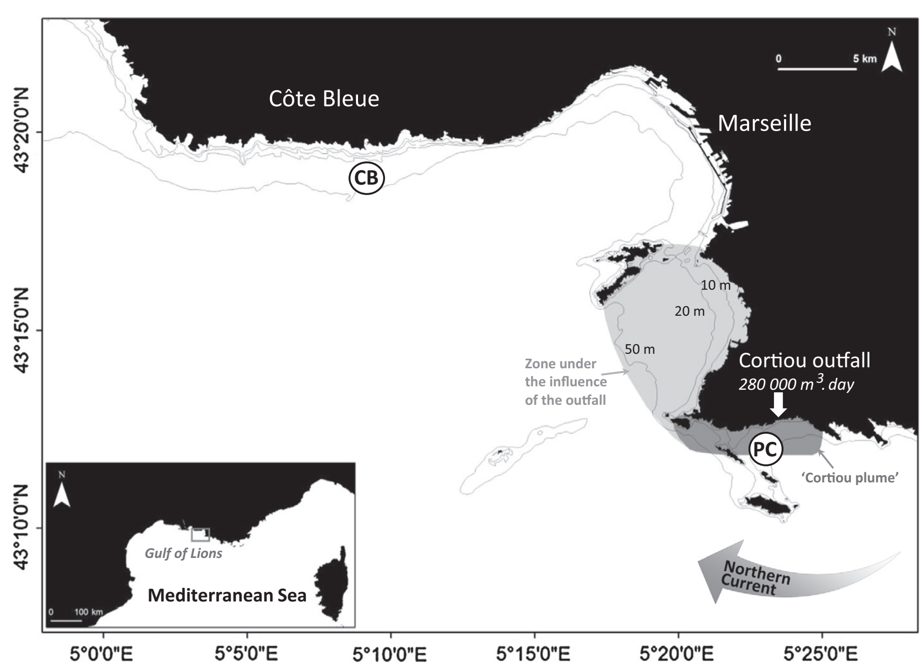

Fig. 1. Location of study sites and Cortiou area (in grey = zone under the influence of the sewage outfall (after Fraysse et al., 2013).

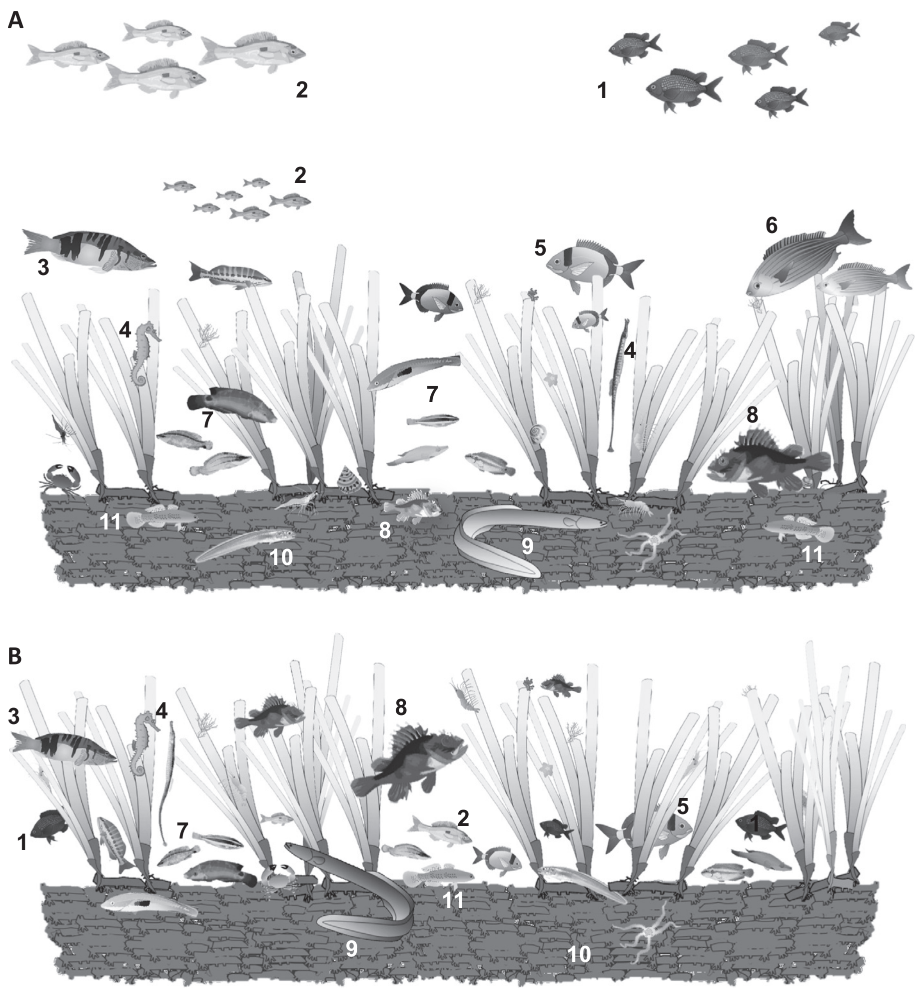

Fig. 2. Diel vertical distributions of fishes in Posidonia oceanica meadows; A = day and B = night (redrawn from Harmelin-Vivien, 1982). 1: Pomacentridae (*Chromis chromis*), 2: Centracanthidae (*Spicara maena, S. smaris*), 3: Serranidae (*Serranus cabrilla, S. scriba*), 4: Syngnathidae (*Hippocampus guttulatus, Syngnathus typhle*), 5 & 6: Sparidae (*Diplodus vulgaris, Sarpa salpa*), 7: Labridae (*Coris julis, Symphodus* spp., *Labrus* spp.), 8: Scorpaenidae (*Scorpaena notata, S. porcus*), 9: Congridae (*Conger conger*), 10: Ophidiidae (*Ophidium barbatum, Parophidion vassali*), 11: Gobiidae (*Gobius cruentatus, G. geniporus*).

measured to the nearest mm and weighed to the nearest gram wet weight.

2.4. Assemblage characteristics and trophic structure

The species richness (number of fish species), and density (total number of individuals) and biomass (total weight of fish) per 100 m^2, were calculated for PC and CB in 1980 and 2012, and for PC in 2000. Fish species were also attributed to one of five trophic categories based on their feeding habits and spatial distribution, following Bell and Harmelin-Vivien (1983): zooplanktivores, herbivores, mesocarnivores-1, mesocarnivores-2 and macrocarnivores (Table 1).

2.5. Data analyses

The effect of season on total density and biomass of fish at each site during each year was tested using the non-parametric Man–Whitney *U* test because the data were not normalised. To analyse differences in the relative density and biomass of species between sites, seasons and years, the data for density and biomass of each species per 100 m^2 were square-root transformed to reduce the

Table 1
The five trophic categories of fish associated with *Posidonia oceanica* seagrass meadows (after Bell and Harmelin-Vivien, 1983).

Category	Family/*species*	Comments
Zooplanktivores	*Chromis chromis, Spicara maena, S. smaris, Boops boops.*, Atherinidae	These species lived in the water column during the day, feeding mainly on pelagic zooplankton, and rested inactive close to the substrate at night (except the Atherinidae)
Herbivores	*Sarpa salpa*	
Mesocarnivores-1	Labridae	This abundant fish family consumed mainly molluscs but copepods, amphipods, echinoderms and polychaetes were also significant food items for some species
Mesocarnivores-2	*Diplodus* spp., *Spondyliosoma cantharus*, Mullidae, Syngnathidae, Gobiidae, Blenniidae, Bothidae	Several species showed a strong preference for polychaetes, amphipods, small crustaceans, echinoderms and molluscs
Macrocarnivores	Congridae, Ophidiidae, Gadidae, Scorpaenidae, Serranidae, *Labrus viridis*	The main food types were decapods (carid shrimps and brachyurans), large amphipods and teleosts

influence of rare or abundant species. The Bray–Curtis similarity index was then used to generate a rank similarity matrix, which was converted into a non-metric Multi-Dimensional Scaling ordination (nMDS) (Clarke, 1993; Clarke and Gorley, 2006). Variations between density and biomass of species at each site and year were described by nMDS, using the PRIMER software package (Clarke and Warwick, 1994). Analyses were retained if the stress levels from the 2-dimensional plots were lower than 0.2 (Clarke, 1993). Correlations of taxa-specific density and biomass with the 2-dimensional ordination plots of samples were displayed with correlation vectors, calculated with the Spearman rank correlation index. A cluster routine was used to explore potential grouping factors, before proceeding with nMDS analysis (Anderson et al., 2008). This procedure showed that season was the less important factor and so it was not considered during analysis of the composition of the fish assemblage. A Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson, 2001; McArdle and Anderson, 2001; Anderson et al., 2008) was performed on the model, including all terms and interactions, to compare the effects of site, year and diel variation on the composition of the fish assemblages based on density and biomass data. This method is equivalent to a parametric MANOVA but free from the assumptions of normality and homoscedasticity of residuals (Anderson, 2001). *P*-values were obtained using a permutation procedure, with 999 permutations of residuals under a reduced model.

To analyse changes in the trophic structure of fish assemblages between 1980 and 2012, species within each of the five predefined feeding categories (Table 1) were assigned to a trophic level (TLevel) (Appendix A. Supplementary material), according to the literature (Stergiou and Karpouzi, 2002; Karachle and Stergiou, 2008; Froese and Pauly, 2013). The species richness, size-structure of the main fish species (total length), density and biomass of each trophic category were calculated for 1980 and 2012. To compare each of these variables between sites and years, we performed a PERMutational univariate ANalysis Of Variance (PERMANOVA univariate) based on the Euclidean distance (Legendre and Legendre, 2012). In addition, density and biomass percentages of each species were multiplied by its specific trophic level, and a mean TLevel was then calculated for the whole fish assemblage at each site in 1980 and 2012 for both density and biomass.

3. Results

3.1. Temporal variation in fish assemblages

A total of 76 species and 24 families of fish was recorded from the two seagrass meadow sites (Table 2). The Labridae (13 spp.) was the most diverse fish family, followed by Gobiidae (12 spp.), Sparidae (8 spp.), Syngnathidae (5 spp.), Gobiesocidae (4 spp.) and Ophidiidae, Scorpaenidae, Serranidae, Callionymidae and Soleidae (3 spp.) (Appendix A. Supplementary material). The remaining families were each represented by only one or two species. Labridae, Sparidae, Scorpaenidae, Centracanthidae and Pomacentridae contributed 93% of total fish density at PC and 84–86% at CB, and 70–93% of the total biomass at PC and 87–90% at CB (Table 3).

Although the same families dominated at both sites over time (Table 3), the fish assemblages differed significantly between 1980 and 2012 (PERMANOVA univariate, $F = 8.194$, $p < 0.01$) in term of species richness (Table 2). Species richness decreased at both sites, but the difference was greater at PC (−11% species, −33%) than at CB (-5 species, −10%) (Table 2). The mean total density and biomass of fish per 100 m^2 also decreased substantially between 1980 and 2012 at both sites (Table 2). These decreases were due mainly to reductions in the abundance of the sparid *Boops boops*, the centracanthid *Spicara maena* and the labrids *Coris julis*, *Symphodus cinereus* and *S. rostratus* (Appendix A. Supplementary material). The decreases occurred principally in summer and were more pronounced at PC than CB (Fig. 3). However, the seasonal differences in fish density and biomass were significant only at PC in 1980 (Man–Whitney *U* test, $p < 0.001$, in both cases).

The additional sampling done at PC in 2000 showed that although the total number of fish species decreased only slightly after the introduction of primary wastewater treatment, the abundance of individuals was drastically reduced (Table 2). This was mainly due to the disappearance of the zooplanktivore *B. boops* (Appendix A. Supplementary material). By 2012, the other species that had disappeared at PC in addition to *B. boops* belonged to the Gobiidae, Syngnathidae, Mullidae and Bothidae.

Multivariate analyses, based on both density and biomass, indicated that fish assemblages varied significantly among years and between day and night, but not between sites (see Fig. 4A for the results of the nMDS based on fish density). There were no significant interactions between factors, indicating that the fish assemblages varied in similar ways at both sites (Table 4).

The pattern in the nMDS ordination based on density was influenced mainly by 12 species (Fig. 4B). In 1980, high densities of *S. maena*, *S. cinereus*, *Syngnathus tiphle*, *B. boops* and *Mullus surmuletus* were associated strongly with samples taken at night, whereas *Gobius geniporus* was prominent in the trawls made during the day. In contrast, *Scorpaena porcus* and *Chromis chromis* were most common in the samples taken in 2012 at night, while *Diplodus sargus* and *Serranus scriba* occurred mainly during the day in 2012.

An increase in the proportion of thermophilic (warmer water) species and a decrease in northern (more tolerant to cooler water) species were observed over the 30-year period. For example, the relative proportions of *Symphodus ocellatus* (southern) *and S. cinereus* (northern) changed from 14%:86% in 1980 to 25%:75% in 2012; and *S. scriba* (southern) *and S. cabrilla* (northern) changed from 0%:100% in 1980 to 28%:72% in 2012 (Appendix A. Supplementary material).

3.2. Changes in trophic structure

The trophic structure of the fish community changed significantly at PC between 1980 and 2012 (Fig. 5). At PC, mesocarnivores-1 (labrids) comprised 64% of the total density in 1980, but represented only 49% in 2012 ($F = 6.08$, $p < 0.05$). Zooplanktivores

Table 2
Total species richness, mean density (individuals 100 m^{-2}) and mean biomass (g 100 m^{-2}) at the two study sites between 1980 and 2012 *(± standard error)*.

	Plateau des Chèvres			Côte Bleue	
Year	1980	2000	2012	1980	2012
Number of trawls	18	20	20	18	20
Total species richness	44	41	33	53	48
Number of individuals	3071	1732	814	1869	820
Total biomass (g)	32828.26	36446.04	15379.3	30039.87	12392.97
Mean density (individuals 100 m^{-2})	17.40 *(3.00)*	12.68 *(1.63)*	5.86 *(0.81)*	10.12 *(1.28)*	5.91 *(0.62)*
Mean biomass (g 100 m^{-2})	187.54 *(37.74)*	271.90 *(36.62)*	110.80 *(21.97)*	149.13 *(32.56)*	89.29 *(11.04)*

Table 3
Mean density (individuals 100 m^{-2}) and biomass (g 100 m^{-2}) of fish families caught at Plateau des Chèvres and Côte Bleue in 1980 and 2012. Families in bold represent the five dominant families, *se* = *standard error,* – = no data.

Family	Plateau des Chèvres								Côte Bleue							
	1980				2012				1980				2012			
	Density	se	Biomass	se	Density	se	Biomass	se	Density	se	Biomass	se	Density	se	Biomass	se
Ophidiidae	0.05	*(0.01)*	0.82	*(0.15)*	0.16	–	3.53	–	0.02	(0.002)	0.22	*(0.03)*	0.04	*(0.01)*	0.74	*(0.12)*
Syngnathidae	0.40	*(0.02)*	2.43	*(0.10)*	0.13	(0.003)	0.68	*(0.02)*	0.38	*(0.01)*	1.45	*(0.04)*	0.33	*(0.01)*	2.36	*(0.09)*
Scorpaenidae	**0.58**	***(0.02)***	**30.10**	***(1.08)***	**1.65**	***(0.10)***	**42.91**	***(2.82)***	**0.92**	***(0.03)***	**50.27**	***(1.24)***	**0.66**	***(0.03)***	**17.81**	*(0.86)*
Serranidae	0.10	*(0.01)*	0.96	*(0.09)*	0.12	(0.004)	3.10	*(0.15)*	0.18	*(0.01)*	1.05	*(0.07)*	0.55	*(0.01)*	9.66	*(0.29)*
Sparidae	**2.10**	***(0.09)***	**11.76**	***(0.28)***	**0.43**	***(0.01)***	**17.06**	***(0.47)***	**0.66**	***(0.02)***	**5.10**	***(0.09)***	**0.20**	***(0.01)***	**6.05**	*(0.17)*
Centracanthidae	**3.51**	***(0.22)***	**42.33**	***(2.90)***	**0.53**	***(0.05)***	**10.31**	***(0.80)***	**1.76**	***(0.12)***	**31.81**	***(3.36)***	**0.20**	***(0.03)***	**0.97**	*(0.10)*
Mullidae	0.11	*(0.02)*	0.29	*(0.06)*	–	–	–	–	0.21	*(0.02)*	1.23	*(0.06)*	0.01	–	0.04	–
Pomacentridae	**0.39**	***(0.11)***	**6.83**	***(1.89)***	**0.23**	**–**	**5.45**	**–**	**0.27**	***(0.09)***	**5.00**	***(1.67)***	**0.60**	***(0.15)***	**6.86**	*(1.72)*
Labridae	**9.50**	***(0.06)***	**81.73**	***(0.48)***	**2.49**	***(0.02)***	**25.54**	***(0.16)***	**4.93**	***(0.03)***	**36.76**	***(0.18)***	**2.84**	***(0.01)***	**39.24**	*(0.20)*
Gobiidae	0.44	*(0.01)*	3.45	*(0.06)*	0.02	(0.001)	0.59	*(0.03)*	0.48	*(0.01)*	6.07	*(0.15)*	0.12	*(0.002)*	0.96	*(0.03)*
Rare species	0.22	*(0.02)*	6.84	*(0.14)*	0.12	(0.001)	1.65	*(0.02)*	0.30	(0.002)	10.18	*(0.09)*	0.35	*(0.003)*	4.60	*(0.04)*

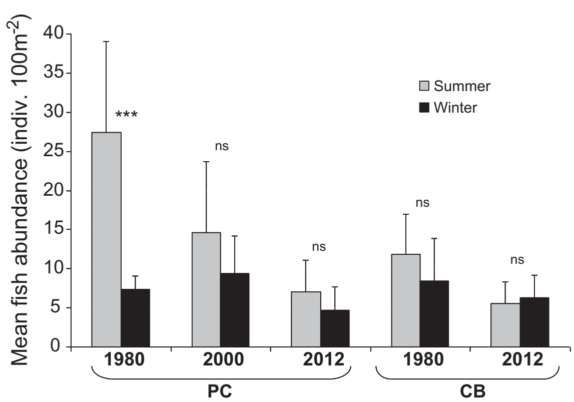

Fig. 3. Mean seasonal density of fish in seagrass meadows at PC (Plateau des Chèvres) and CB (Côte Blelue) from 1980 to 2012. Results of Man–Whitney *U* tests between summer and winter values are indicated (*** = significant at $p < 0.001$, ns = not significant, $p > 0.05$).

also decreased significantly in density over the 30-year period (from 22% to 10%). Conversely, macrocarnivores and mesocarnivores-2 increased significantly from 6% to 30%, and 9% to 11%, respectively at this site. At CB, no significant difference between 1980 and 2012 was observed. However, zooplanktivores also decreased (from 24% to 12%). The herbivore *Sarpa salpa* was caught only at CB in 2012 and represented only 1% of fish density. Similar patterns were observed for biomass. These changes resulted in an increase in the trophic level of the whole fish assemblage at PC of +0.18 (3.40–3.58), but little change at CB, i.e. +0.02 (3.50–3.52) (Fig. 5).

4. Discussion

This study demonstrates that seagrass fish assemblages can be used as indicators for improvements in wastewater treatment and, more generally, for changes in environmental conditions. Our results revealed conspicuous changes in species richness, density, biomass and trophic structure of fish assemblages associated with *P. oceanica* seagrass meadows between 1980 and 2012, concomitant with changes in water quality and consistent with observations of the effects of sewage outfalls on coastal fish communities elsewhere. The greater abundance of zooplanktivores fishes associated with the seagrass meadow under the influence of untreated wastewater at Marseille (PC) than at the control site (CB) in 1980 mirrors the observations that sewage effluent enhances planktonic food webs (Grigg, 1994; Hall et al., 1997; Menge, 2000; Guidetti et al., 2002, 2003). The greater numbers of benthic fishes (Bothidae, Mullidae and Gobiidae) at PC than at CB in 1980 are also consistent with observations of relatively high abundances of benthic fish species near sewage outlets (Spies, 1984; Ribeiro et al., 2008; Azzurro et al., 2010) due to the elevated densities of polychaete worms and other macrobenthos in such areas (Spies, 1984; Reish and Bellan, 1995; Bellan et al., 1999; Arfi et al., 2000 and references therein).

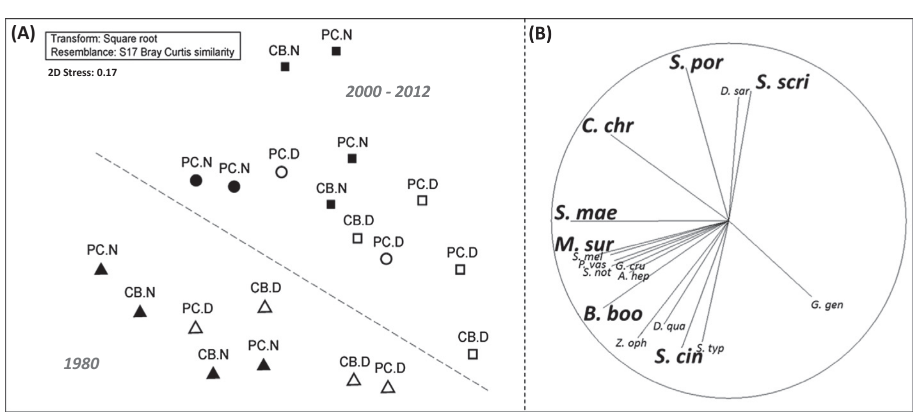

Fig. 4. (A) Two-dimensional nMDS ordination plot of mean density according to year (1980 = △, 2000 = ○ and 2012 = □), site (PC = Plateau des Chèvres and CB = Côte Bleue) and diel period (D = day, white and N = night, black), (B) correlation vectors of taxa densities (Spearman), note that only species >0.60 are plotted and that species with absolute contributions >0.70 are in bold italics; 2D stress: 0.17.

Table 4
Permutation multivariate analysis of variance (PERMANOVA) based on square root transformation and Bray–Curtis dissimilarities for fish density (A) and biomass (B). The sources of variance are year (1980–2000–2012), site (Plateau des Chèvres and Côte Bleue), and diel period (day and night). ns = not significant, $**P < 0.01$. P-values were obtained by 999 permutations of residuals under a reduced model.

Source of variation	*df*	MS	*F*	*p*		
(A)						
Year	2	3402.10	4.07	0.002	**	1980 > 2012
Site	1	1560.50	1.87	0.070	ns	PC ⩾ CB
Day – night	1	3103.80	3.71	0.007	**	Day < night
Year × site	1	1105.70	1.32	0.235	ns	
Year × day – night	2	416.09	0.50	0.941	ns	
Site × day – night	1	192.45	0.23	0.982	ns	
Year × site × day – night	1	408.34	0.49	0.889	ns	
Residuals	10	836.55				
Total	19					
(B)						
Year	2	2774.90	2.76	0.005	**	1980 > 2012
Site	1	1850.90	1.84	0.090	ns	PC ⩾ CB
Day – night	1	3409.50	3.39	0.013	**	Day < night
Year × site	1	1197.40	1.19	0.296	ns	
Year × day – night	2	640.40	0.64	0.836	ns	
Site × day – night	1	274.75	0.27	0.963	ns	
Year × site × day – night	1	579.01	0.58	0.767	ns	
Residuals	10	1005.80				
Total	19					

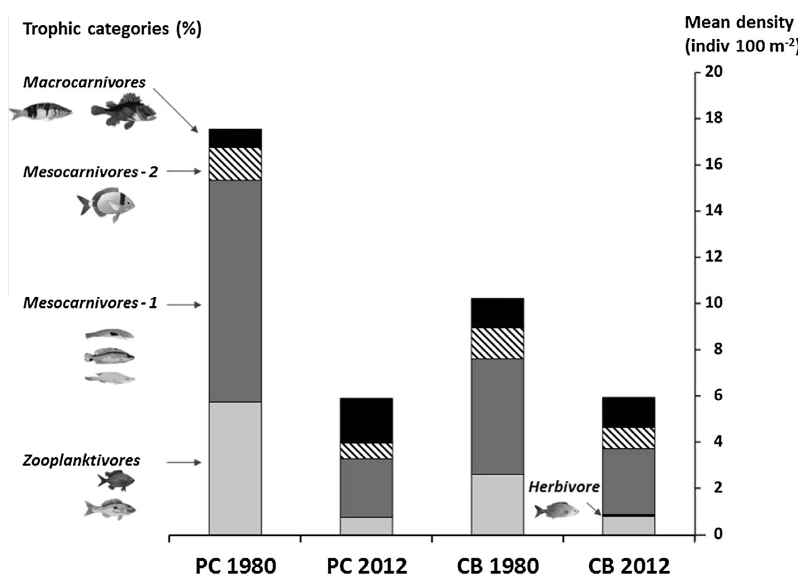

Fig. 5. Changes of the trophic structure of fish assemblages at PC (Plateau des Chèvres) and CB (Côte Bleue) between 1980 and 2012, based on density (individuals per 100 m^2).

After improvements to treatment of wastewater from the city of Marseille, the quantity of nutrients, organic matter and contaminants at PC decreased considerably. The primary treatment filtered out 70% of the suspended material (>2 μm) and was also effective for some trace metals (from 40% for copper to 75% for lead) (Perez et al., 2005). The tertiary treatment retains >90% of the dissolved and particulate organic matter, and most trace metal (sewage plant monitoring unit, pers. comm.). This resulted in a general recovery of most benthic communities in the Cortiou area: soft-bottom macrobenthos (Bellan et al., 1999 and references therein), macroalgal communities on rocky reefs (Soltan et al., 2001) and the *P. oceanica* seagrass meadow itself (Pergent-Martini et al., 2002). Benthic communities are the most relevant and permanent integrators of water quality and are used as bioindicators of the impact of wastewater inputs (Bellan et al., 1999; Arfi et al., 2000).

Our analyses also show that the seagrass fish assemblage at PC responded to the change in water quality. From 1980 to 2012, the species richness, density and biomass of the fish assemblage decreased, especially during summer, whereas the mean trophic level increased. Similar trends in species richness, density and biomass also occurred at CB, however, the changes were greater at PC. These results suggest that key features of fish assemblages associated with *P. oceanica* seagrass meadows changed at two spatial scales, across the Bay of Marseille and more locally near the sewage outlet at Cortiou (Fraysse et al., 2013). These trends were more pronounced in summer (Fig. 3), the time of year when recruitment occurs, and when fish feed and grow more actively (García-Rubies and Macpherson, 1995; Planes et al., 1999).

The change in trophic structure between 1980 and 2012 is also a strong indicator of the response of the fish assemblage at PC to the improvement in wastewater treatment. In parallel with the reduction of nutrients and organic matter from the sewage outlet at Cortiou (Fraysse et al., 2013; Perez et al., 2005), there was a decrease of zooplanktivores and mesocarnivores-1, and an increase of macrocarnivores. The corresponding increase in trophic level of the fish assemblage at PC from 3.40 to 3.58 between 1980 and 2012 (Fig. 5), made it comparable to the TLevel recorded for seagrass meadow fish assemblages in Mediterranean marine protected areas (3.57) (Harmelin-Vivien, 2000b). The increase in TLevel made the fish assemblage at PC similar to the one at CB, strongly suggesting that tertiary treatment of wastewater allowed the ecosystem at PC to return to normal.

Although this study focuses on the effects of improved water quality on a seagrass fish assemblage, the data suggest that fishing pressure and climate change may also have played some role in the observed changes between 1980 and 2012. The general decrease in fish density and biomass observed at PC and CB were presumably related to the high fishing pressure throughout the Bay of Marseille because most fish species living in *P. oceanica* beds are targeted by local fisheries (Leleu et al., 2014). Between 1980 and 2012, we also detected a significant decrease in the total length of *C. julis*, *S. rostratus*, *S. tinca* and *S. porcus*, species targeted by artisanal and recreational fisheries. This contention is supported by the lower density and biomass of fishes in seagrass and rocky reef habitats in the Northwestern Mediterranean open to fishing compared to locations where these habitats are within marine protected areas (Harmelin-Vivien et al., 1995; Harmelin-Vivien, 2000b; Guidetti and Claudet, 2010).

The most parsimonious explanation for the increase in the relative proportions of warm-water species like *Symphodus ocellatus* and *S. scriba* is the documented increase in the temperature of the Mediterranean Sea over the past few decades (Lejeusne et al., 2010 and references therein). Continuous records from sites in the north-western Mediterranean Sea show that water temperature at the surface and to a depth of 80 m has increased by ~1 °C since 1975 (Prieur, 2002; Vargas-Yáñez et al., 2008). The warming of sea surface temperature would have enabled the northward migration and population increase of warm-water species from the southern Mediterranean (Francour et al., 1994; Sabates et al., 2006).

We conclude that monitoring fish assemblages is a practical way of assessing the efficiency of wastewater treatment plants, provided that the sampling is designed to (1) test for variation in the entire fish community and its trophic structure, not just changes in selected 'indicator' species; and (2) separate the effects of other factors that can influence the abundance and species composition of fish assemblages, particularly fishing pressure and climate change.

We also conclude that although our analysis was based on sampling at an impacted site and a control site before and after the introduction of tertiary wastewater treatment in 2008, future investments to test the effects of improvements in wastewater treatment on fish assemblages should be based on more comprehensive sampling to incorporate temporal and spatial variation in fish assemblages. Ideally, there should be multiple samples at the impacted site, and at more than one control site, before and after the introduction of improvements to wastewater treatment (Underwood, 1992, 1993).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2014.11.038.

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