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# Detecting human mitigation intervention: Effects of sewage treatment upgrade on rocky macrofaunal assemblages

María Bustamante <sup>a,\*</sup>, Stanislao Bevilacqua <sup>b</sup>, Javier Tajadura <sup>a</sup>, Antonio Terlizzi <sup>b</sup>, Iosé Ignacio Saiz-Salinas <sup>a</sup>

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#### ABSTRACT

The effectiveness of secondary *vs* primary treatments of wastewaters in mitigating the effects of sewage discharge on the multivariate structure and diversity of rocky invertebrate assemblages was assessed over a nine-year period through a beyond-BACI experimental design. Assemblages from different tidal levels (i.e. mid-shore, low-shore, 3 m and 8 m subtidal) were sampled at the impact location (*I*) and three control locations (*Cs*) at a hierarchy of spatial scales. The improvement in water treatment significantly changed the structure, diversity and cover of low intertidal assemblages. Faunal cover at 8 m subtidal increased significantly after the treatment upgrade at *I*. The secondary treatment also affected patterns of spatial heterogeneity between *I* and *Cs* for mid-shore and 3 m subtidal assemblages. This study demonstrates that powerful experimental designs combined with univariate and multivariate analytical approaches are fundamental in distinguishing the subtle effects of human impact from those of natural processes.

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#### 1. Introduction

Sewage discharge has long been recognised as one of the most widespread threats affecting coastal ecosystems (Pearson and Rosenberg, 1978). Population increases along coastlines have multiplied inputs of freshwater, nutrients and pollutants, mainly through sewage outfalls, which could have drastic effects on the structure and functioning of the marine ecosystem (Halpern et al., 2007). As a consequence, monitoring the effects of sewage discharge on marine biota has become a major environmental concern (Nixon, 1995).

Eutrophication, toxicity, turbidity, sedimentation and decreases in salinity are the major potential effects of sewage effluents producing chronic stress in marine assemblages (Cromey et al., 1998; Gray, 1992; Pastorock and Bylard, 1985; Saiz-Salinas, 1997). These detrimental effects may reduce species richness (Wear and Tanner, 2007), causing assemblages to be dominated by just a few species (Roberts et al., 1998) and altering their overall structure (Terlizzi et al., 2002). However, stress level from sewage discharge can vary considerably depending on effluent treatments and flow rates (Del-Pilar-Ruso et al., 2010), as well as on the physical

environmental conditions in which the effluent is discharged (Pastorock and Bylard, 1985).

Environmental investigations on the effects of sewage discharge have been carried out both on soft sediments (e.g. Gee and Warwick, 1985: Lindegarth and Hoskin, 2001: Saiz-Salinas, 1997: Stark et al., 2003) and on hard substrates (e.g. Garaffo et al., 2012; Hindell and Quinn, 2000; Reopanichkul et al., 2009; Smith, 2000). The information available on the impact of sewage effluents on assemblages inhabiting rocky habitats includes seaweeds and sessile invertebrates (e.g. Smith, 2000; Terlizzi et al., 2005a), meiofauna (Fraschetti et al., 2006), epiphytes (Piazzi et al., 2004) and fish (Guidetti et al., 2003; Smith et al., 1999). However, several studies examined shallow subtidal habitats (e.g. Terlizzi et al., 2005a; Underwood and Chapman, 1996) as sewage disposal is often released at very few metres in depth, whereas deeper subtidal rocky substrates have been less investigated. Moreover, sewage wastes are often released in the intertidal zone (e.g. Ashton and Richardson, 1995; Garaffo et al., 2012; Smith, 2000), and the effect of sewage discharge in a given habitat could be not necessarily localized, possibly affecting also adjacent habitats (Elías et al., 2005). Therefore, cross-habitat assessments are likely to provide a more comprehensive view of direct and indirect effects of sewage discharge on marine ecosystems.

The Urban Waste Waters Treatment Directive (91/271/EEC), established to prevent the environment from the adverse effects of

<sup>&</sup>lt;sup>a</sup> Department of Zoology and Cell Biology, Faculty of Science and Technology, University of the Basque Country, Box 644, E-48080 Bilbao, Spain

b Lab. Zoology and Marine Biology, Department of Biological and Environmental Science and Technologies, University of Salento, I-73100 Lecce, Italy

Corresponding author. *E-mail address:* maria.bustamante@ehu.es (M. Bustamante).

sewage discharges, and its achievements need to be integrated into the objectives of the Water Framework Directive (WFD, 2000/60/EC) to ensure good ecological status for all waters by 2015. However, most studies assessing the effects of sewage effluents on rocky habitats have focused on the potential impact of the sewage discharged and few attempts have been made to evaluate the effectiveness of wastewater treatments (Bokn et al., 1996; Díez et al., 2009; Pagola-Carte and Saiz-Salinas, 2001; Soltan et al., 2001), which, in turn, is essential for the management of treatment plants, upgrading sewage treatments, and mitigating sewage impacts on marine ecosystems.

A major issue in environmental impact assessment concerns the confounding effects of natural variability, especially in rocky habitats (Fraschetti et al., 2005). Patterns of assemblage composition and abundance typically change at a range of spatial and temporal scales, making it essential to distinguish natural ecological variations from those caused by anthropogenic disturbance (Benedetti-Cecchi, 2001; Fraschetti et al., 2005; Hewitt et al., 2001; Underwood, 1994). Elucidating the effects of human disturbance could be further complicated, as impacts may cause changes in the multivariate structure of assemblages affecting not only mean, but also variance, or both (Terlizzi et al., 2005a). As a consequence, human impacts may induce differences in the average location of sampling units in the multivariate space defined by species variables and/or variation in the dispersion of sampling units around their average location (Anderson et al., 2006). Thus, quantifying human-driven changes in the spatial heterogeneity of assemblages may be of great help in detecting those effects of human disturbance that may remain unnoticed focusing exclusively on the average structure of impacted assemblages.

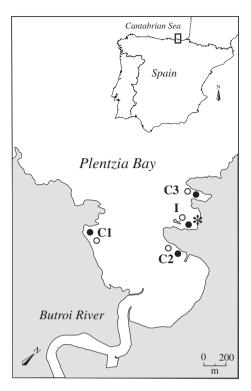
Experimental designs and analytical procedures capable of separating natural variability from human-induced changes have been developed (Anderson et al., 2008; Underwood, 1997). In this context, beyond-BACI designs are of great help, as they are able to detect community changes in a potentially impacted location compared to multiple reference locations at different spatial and temporal scales, from before to after the occurrence of a given disturbance (Underwood, 1994).

Here, we examine the effectiveness of secondary vs primary (i.e. biological vs settlement) urban wastewater treatment over nine years using a beyond-BACI design, investigating the potential effects of improved sewage treatment on rocky intertidal and subtidal benthic assemblages at a hierarchy of spatial scales. Our null hypothesis is that the average structure and spatial heterogeneity of assemblages were invariant to treatment upgrading, whereas we expect differences from before to after the treatment implementation in the contrast between the impacted and control locations. Responses of treatment effect also include variations from before to after occurring just in the impacted location and only in the event that such changes resemble more the impact location assemblages to the control locations ones.

#### 2. Materials and methods

#### 2.1. Study locations and sampling design

The study area (43° 24′ 56.09″ N, 2° 57′ 10.67″ O) is located in Gorliz, a town on the northern coast of Spain (Fig. 1). The outfall serves up to 20,000 inhabitants and has an average treated flow rate of  $4.3^{-2}$  m/s. Raw sewage was released into an intertidal area for 40 years before physical—chemical treatment was introduced in 1998. A further improvement of wastewaters in the form of biological treatment was implemented in 2006. This biological treatment incorporates a BIOFOR wastewater nitrification/denitrification system prior to discharging.



**Fig. 1.** Study area showing the positions of the disturbed location (I), control locations (C1, C2 and C3) and the sewage outfall (\*) in northern Spain. Black and white circles: intertidal and subtidal locations, respectively.

Sampling was carried at four locations: the outfall (hereafter indicated as *I*) and 3 control locations (C1, C2 and C3, hereafter indicated as *Cs*). As the effects of variations in environmental stress levels may depend on the physical and biological features of the system, four different habitats were selected and sampled independently in order to investigate the effects of sewage treatment under different environmental settings. The selected habitats were: mid shore (tidal height: 1.5–2.5 m), low shore (tidal height: 0.7–1.2 m), 3 m deep subtidal and 8 m deep subtidal.

The intertidal levels of location I were within a 25 m radius of the point of discharge, and the subtidal levels of I were within a 150 m radius of the discharge, and were situated in order to find suitable habitats at the chosen depths. Cs were located within a radius of 400-1000 m of the discharge. All locations were separated by 100s of metres. At all tidal levels, the assemblages investigated were those inhabiting substrates (either continuous bedrock platform or large blocks) with gentle to moderate slopes.

At each location (50 m in length) a set of three sites (ten metres apart, 5 m in length) was selected. At each site a set of three replicates (1 m apart,  $40 \times 40$  cm quadrats) was selected. The faunal assemblages at each location were sampled biannually between 2001 and 2009, making a total of 3 times before the implementation of biological treatment (2001, 2003 and 2005) and twice afterwards (2007 and 2009). Sampling always took place in summer (from June to August).

A non-destructive sampling which consisted of visually assessed estimates of invertebrate species cover per quadrat was used following the scale proposed by Pèrés and Picard (1964) as a reference: + (<1%), 1 (1-5%), 2 (>5-25%), 3 (>25-50%), 4 (>50-75%) and 5 (>75-100%). Mean cover of species was calculated for each quadrat using the median of each range. A total of 180 observations were obtained for each of the four habitats. As a result of the sampling a total of 167 species were recorded, comprising 27 species of Porifera, 31 Cnidaria, 18 Annelida, 32 Arthropoda, 30

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