Environmental Science and Pollution Research (2021) 28:1247–1253
https://doi.org/10.1007/s11356-020-11550-0

SHORT RESEARCH AND DISCUSSION ARTICLE

Use of survival rates of the barnacle *Chthamalus stellatus* as a bioindicator of pollution

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Received: 23 April 2020 / Accepted: 4 November 2020 / Published online: 10 November 2020

Abstract
Concentrations of heavy metals and trace elements in marine environments have increasingly become a problem for several ocean ecosystems, due to increments in pollution. Habitats daily exposed to extreme conditions, such as the intertidal rocky platforms and pools, are more vulnerable to pollution effects. In the coast of Punta del Hidalgo (Tenerife, Spain), we have located a water-treatment plant that could be pouring periodically pollutants to the near shore. We studied coverage and survival rates of the cirriped *Chthamalus stellatus* inhabiting the intertidal near the sewage pipe of the water plant of Punta del Hidalgo and in a control area in a proximate location. Concurrently, water samples from intertidal pools were obtained from both affected and control areas in order to corroborate the presence of pollutants, analyzing the concentrations of metals and trace elements. The results obtained clarified that the area near the underwater outfall presented higher percentage of coverage and mortality of *C. stellatus* than the control zone. The analysis of metal content in water samples also showed higher concentrations of metals for the affected area compared to the control one. We therefore propose the use of survival rates of populations of *C. stellatus* in the intertidal as bioindicators of metal pollution.

Keywords Sewage pipe · Barnacle · Bioindicator · Metal pollution

Introduction

Sewage pipes are regularly used for the evacuation of wastewater, purified or not, from water-treatment plants. The environment surrounding these emission systems requires a rigorous monitoring after being installed, which in many cases is not carried out. As a consequence, in case of a circuit collapse derived from heavy rain episodes, system failure, or overwork, there could be negative effects in the surrounding environment going unnoticed and, therefore, unreported. One of the polluting effects of mishandling sewage pipes is the increase of heavy metal concentrations in marine ecosystems (Costanzo et al. 2001; Dolenec et al. 2011; Herrera et al. 2020; Ramírez-Alvarez et al. 2007; Ruilian et al. 2008; Seubert et al. 2017).

Concentrations of metals in marine environments and their inhabiting organisms are recently becoming a global problem, given the increase input to these ecosystems of pollutants from human activities, which are having a considerable impact on marine communities and organisms in these environments. Industry, commerce, agriculture, tourism, and urbanization in coastal areas are considered direct and indirect continuous sources of pollutants (Fort et al. 2016; Halpern et al. 2008; Lozano-Bilbao et al. 2019, 2020a; Raimundo et al. 2013). Heavy metals are one of the main anthropogenic pollutants in coastal areas around the world. Metals constitute the main anthropogenic pollutants not only to marine organisms but also to the health of ecosystems, and even for humans.

Responsible Editor: Philippe Garrigues

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Hence, efficient techniques that assess this kind of pollution are needed. These techniques are typically expensive, so the presence and health of natural-distributed organisms is becoming increasingly used as bioindicators of pollution. Monitoring sessile bioindicator organisms would therefore allow for an immediate measurement of pollution levels in large areas and allow to take conservation measures. Since vital functions are potentially affected by the environment and its changes, both natural and anthropogenic, these bioindicators can be used to detect the presence of any of these effects. Even more, some authors consider the use of bioindicators more advantageous than the traditional physicochemical methods to assess ecosystem health (Atici et al. 2010; Dolenec et al. 2011, 2007; Verlecar et al. 2006). Within the group of crustaceans, barnacles are considered the best bioindicators of metal pollution (Amoozadeh et al. 2014) as they are capable of accumulating these elements, as has been seen in the species of giant barnacle in the Azores (Dionísio et al. 2013). These crustaceans have also been used as bioindicators of other stressors such as low salinity in estuaries, which seems to benefit the survival of barnacles (Poirrier and Partridge 1979), or to detect other types of contamination such as microplastics or organic matter (Xu et al. 2020; Vaezzadeh et al. 2021).

Knowing that in coastal studies sessile organisms are ideal for monitoring, we considered the survival rates in the barnacle *Chthamalus stellatus* as a possible bioindicator of high concentrations of heavy metals. This barnacle forms a typical band limiting the upper intertidal zone of the Canary Islands archipelago, which is why it may be highly influenced by anthropic impacts on the coasts (González et al. 2012; Navarro et al. 2005; Lozano-Bilbao et al. 2020b).

Our aim is to assess if intertidal pools near the sewage pipe are polluted by heavy metals and trace elements. Concurrently, we want to explore the survival rate in the population of *Chthamalus stellatus* in the intertidal surrounding the sewage pipe and if it could be considered a bioindicator of coastal pollution.

Materials and methods

Samples were taken in the intertidal of Punta del Hidalgo in low tide (28° 34′ 5.17″ N 16° 19′ 35.45″ W) during September 2018 (Fig. 1). For the experimental design, the sampling area under the same condition was divided into two zones. The area of influence of the submarine emissary and a control area where there is little anthropic activity. A photograph of three 50 × 50 cm quadrants was obtained for each area to determine the coverage and percentage of alive and dead specimens of *Chthamalus stellatus* using ImageJ 1.45V software. The dead specimens were determined by observing the external structure of the barnacle: if it had an internal structure, it was classified as “alive,” but if not, as “dead” (Clavier et al. 2009).

To determine heavy metal and trace element concentrations in intertidal pools, 10 water samples were taken from each zone. The salinity and temperature of each pool was measured in situ using handheld conductivity meter WTW COND315i, to extrapolate the results of the metal concentration to its corresponding unit (Herrera et al. 2020).

Water sample

For the study, 20 samples of seawater were taken: 10 in the area of the submarine sewage pipe and 10 in the control zone; these data have been published in (Herrera et al. 2020), of which we have taken only the data from the control zone and the sewage pipe zone (see the preparations of the water samples in Herrera et al. 2020).

Statistical analysis

For the statistical analysis, univariate ANOVAs executed by permutations (Anderson 2001) with Euclidean distances (Anderson and Ter Braak 2003) were performed for both datasets, *C. stellatus* quadrants and metal content in water samples.

In order to investigate the survival rates of *C. stellatus* among the considered locations, one-way design was used with the fixed factor “Zone” with 2 levels of variation (“Sewage pipe” and “Control zone”). The variables included in the analysis of *C. stellatus* were the Alives (count of alive *C. stellatus* individuals), Alives per m^2, Dead (count of dead *C. stellatus*), Dead per m^2, % Dead, and % of substrate coverage of the species.

For the analysis of metal content in the water samples, the variables included were the concentration in mg/kg of the following metals: Al, Cd, Pb, B, Cr, Cu, Fe, Li, Ni, V, Zn. Relative dissimilarities among locations were determined using multidimensional scaling (MDS) in which the metals that best explained data variability were represented as vectors.

In all the analyses, there are 4999 permutations were used in all analyzes units and the significant factors (p value < 0.01) (Anderson 2004). Additionally, all the results were represented by using boxplot graphs. The statistical packages PRIMER 7 & PERMANOVA+v.1.0.1 were used for the statistical analyses.

Results

The area near the sewage pipe presented the highest counts of *C. stellatus* (Fig. 2). Also, this area had greater number of alive specimens and 10% higher mortalities than the Control Zone (Fig. 2, Table S1).

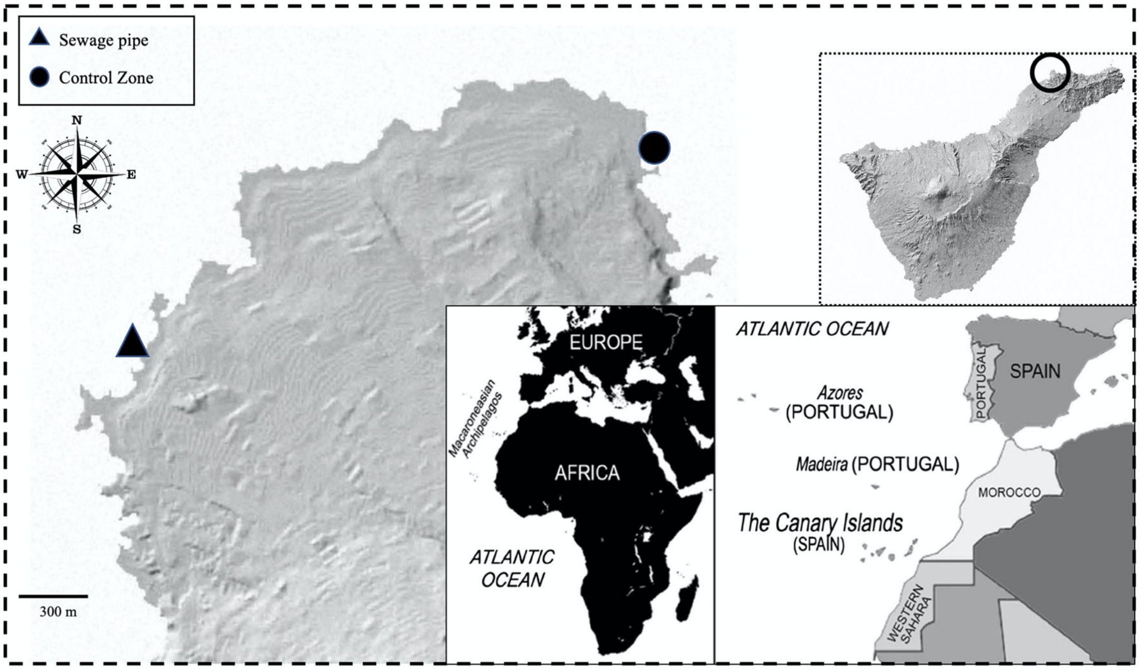

Fig. 1 Map of the study area. Sewage pipe (28° 34′ 20.59″ N; 16° 19′ 56.59″ W), Control zone (28° 34′ 38.68″ N; 16° 19′ 05.54″ W)

For the metal content analysis in intertidal pools, the samples nearest the sewage pipe presented the highest concentrations for all trace elements and metals except for Cr and Fe which have a higher concentration in the Control Zone (Fig. 3, Table S2).

According to the factor "Zones," the results of univariate permutational ANOVAs showed a significant effect between zones with the highest abundance in the sewage pipe zone for the factors Alives m^2 ($F = 10.609$, p value = 0.0082), Dead m^2

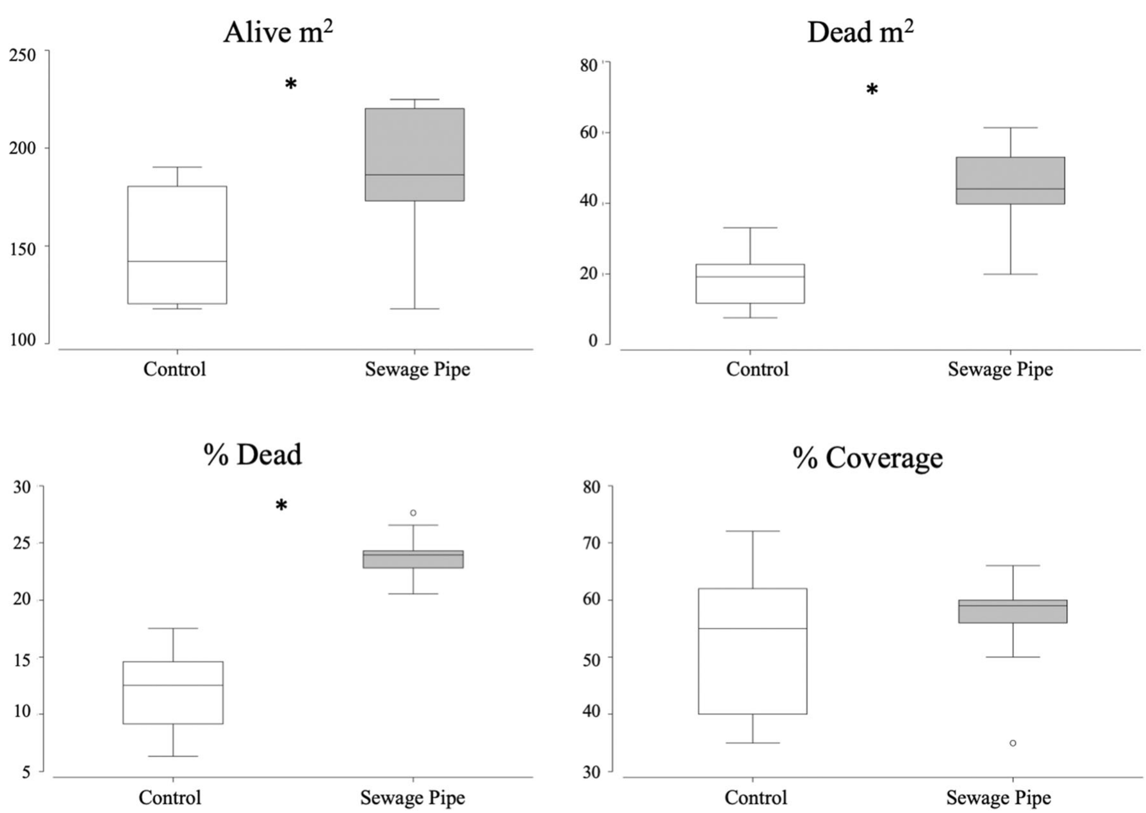

Fig. 2 Boxplots comparing Alive m^2, Dead m^2, % Dead, and % Coverage of *C. stellatus* in each of the studied zones. Significant differences have been marked with an asterisk (*)

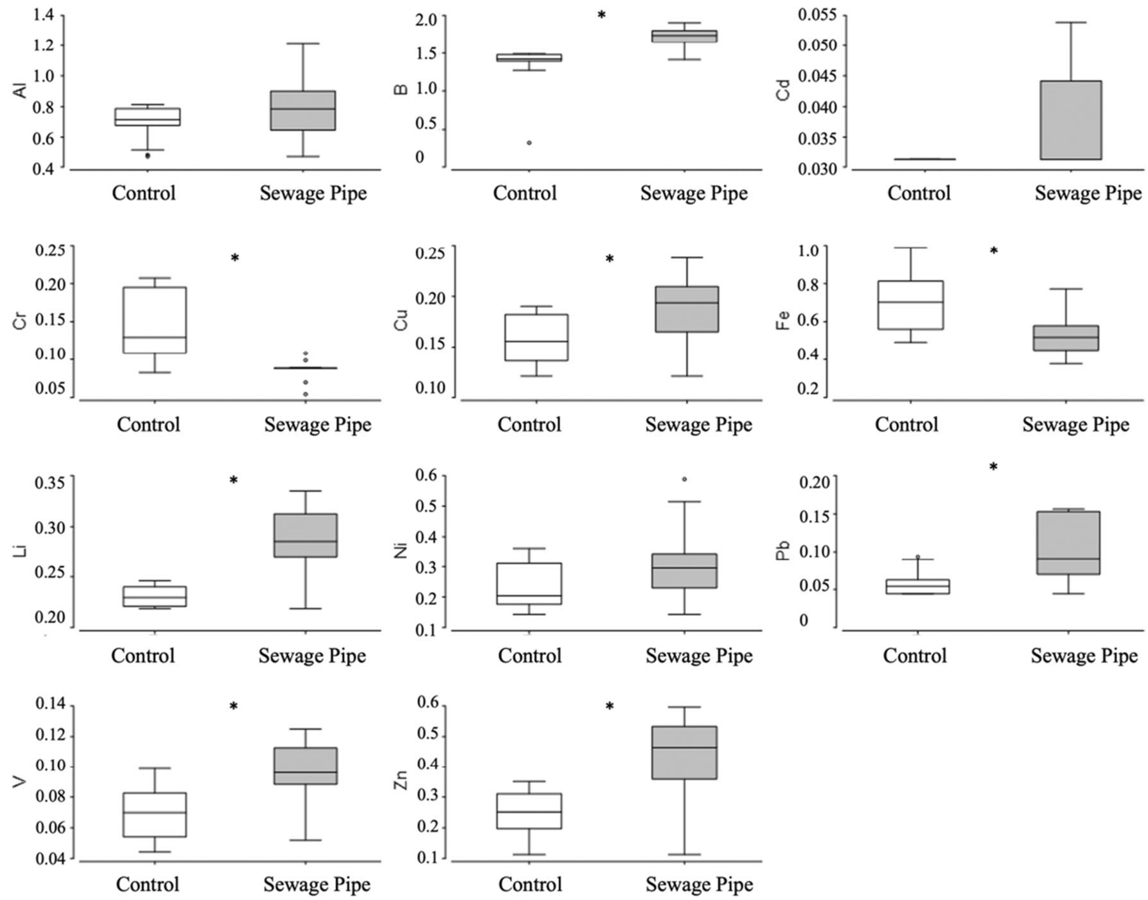

Fig. 3 Boxplots comparing metal content (Al, B, Cd, Cr, Cu, Fe, Li, Ni, Pb, V, and Zn) in each of the studied zones. Significant differences have been marked with an asterisk (*)

($F = 37.155$, p value = 0.0004), and % Dead ($F = 49.039$, p value = 0.0002) (Fig. 2). Only % Coverage presented no significant differentiation ($F = 0.34661$, p value = 0.5411).

The results of univariate permutational ANOVAs on metal content also showed a significant difference between zones ($F = 7.3745$, p value = 0.0002). Concentrations of heavy metals and trace elements were higher in the water samples collected near the sewage pipe for all metals except Cr, which was higher in the control intertidal pools (Fig. 3, Table S2). These results are visible in the non-metric MDS analysis with the metal contents that best explain the data variability as a vector representation (Fig. 4).

Discussion and conclusion

The present study proposes that heavy metal pollutants from a sewage pipe can cause unnatural mortalities in populations of *C. stellatus*. The wastewater discharges from the sewage pipe would raise heavy metal and trace element concentrations in seawater near this emissary, as Fig. 3 shows for intertidal pools in the area surrounding the sewage pipe. These inorganic compounds are very toxic for marine organisms (Widdows et al. 2017). Consequently, sessile and filter-feeding species inhabiting the area surrounding the sewage pipe, like *C. stellatus*, would be the most negatively affected by emissions of this submarine outfall, as high mortality rates show (Table S1, Fig. 2).

There are several studies revealing the influence that sewage pipes have on marine ecosystems and how they alter the chemical composition of the sea and their organisms, as well as how these organisms can be used as bioindicators of pollution (Lozano-Bilbao et al. 2018; Verlecar et al. 2006; Žvab Rožič et al. 2015). *Chthamalus stellatus* is a barnacle that lives attached to rocky coastal substrates, in the mid to low eulittoral zone on the Canary archipelago and in other areas of the Atlantic and Mediterranean Sea. This species withstands aerial exposure during low tides thanks to two mobile opercular plates that close to avoid drying effects inside the carapace of the animal. As a filtering organism, it uses cirrus to retain particles present in the water to consume as food, which makes it susceptible to contamination as it would be unable to avoid the ingestion of pollutants along with its filtered food (Cabral-Oliveira and Pardal 2016; Hawkins et al. 2000; O'Riordan et al. 2004). For that reason, it could be considered the best bioindicator of metal contamination

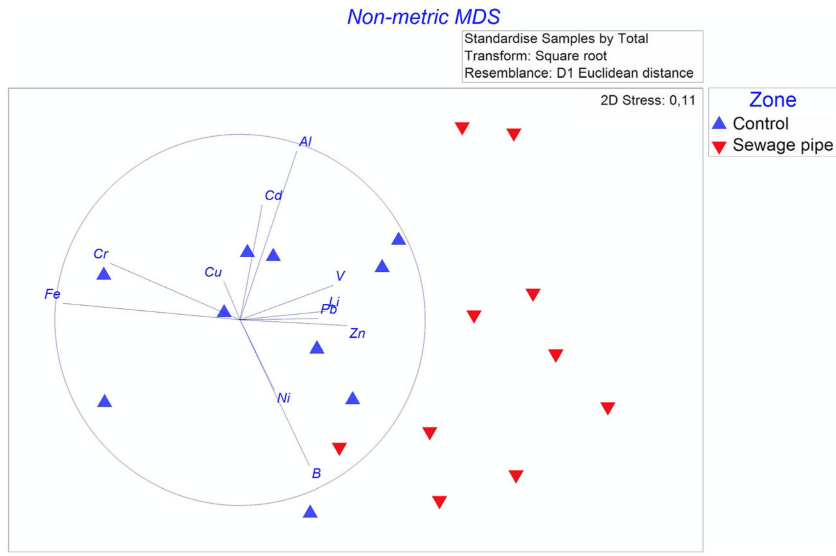

Fig. 4 Non-mMDS, based on Euclidean distances of square-root-transformed data of the heavy metal and trace element content in intertidal pool water samples compared between zones. The metals that better explain the differences are represented as vectors

within crustaceans, as seen in other barnacles (Amoozadeh et al. 2014).

There are numerous studies on organisms that can be used as bioindicators of different forms of marine pollution. Not only have barnacles been seen as good bioindicators of metal, microplastic, and organic matter pollution (Amoozadeh et al. 2014; Xu et al. 2020; Vaezzadeh et al. 2021), but there are also many other animals; for example, mussels and fish such as *Boops boops* are bioindicators of microplastic pollution (Bonanno and Orlando-Bonaca 2018; Garcia-Garin et al. 2019; Li et al. 2019); sea urchins, squid, and fish are bioindicators of the presence of organochlorine (Juma et al. 2018; Parra-Luna et al. 2020; Ueno et al. 2003); fish and sea urchins can also be bioindicators for heavy metal and trace element pollution (Chiarelli et al. 2019; Plessl et al. 2017); even blue sharks have been studied as bioindicators of marine pollution, measuring their stress levels (Alves et al. 2016). Additionally, Lozano-Bilbao et al. (2018) proposed the concentrations of δ15N in the anemone *Anemonia sulcata* as bioindicator of pollution in the same area.

In all of the previously cited works regarding heavy metal bioindicators, removal and sacrifice of the animals is needed in order to determine pollution levels. In contrast, in the present work, we attempt to establish a non-invasive methodology that would allow scientist to monitor coastal pollution without extracting any organism.

In conclusion, we propose the barnacle *Chthamallus stellatus* to be considered a bioindicator of heavy metal and trace element pollution in intertidal zones, since our results show that the higher concentrations of heavy metals and trace elements present in seawater are related to the higher mortality rate of *C. stellatus*. Specifically, monitoring of *C. stellatus* mortality rates could serve as a natural indicator of the presence of anthropic pollutant outfalls in the surrounding area.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-020-11550-0.

Acknowledgments We would like to thank two anonymous reviewers for their helpful comment s on this manuscript.

Authors' contributions Enrique Lozano-Bilbao: Introduction, Materials and methods, Results, Discussion and conclusion

Sara Gonzáalez-Delgado: Introduction, Materials and methods, Results, Discussion and conclusion

Jesús Alcázar-Treviño: Introduction, Materials and methods, Results, Discussion and conclusion

Data availability Data from this work is available upon request.

Compliance with ethical standards

Competing interests The authors declare that they have no conflict of interest.

Ethical approval For the study, no animals had to be killed, so it is not applicable.

Consent to participate The authors consent.

Consent to publish The authors consent.

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