Original Articles

Managing complexity of marine ecosystems: From the monitoring breakdown structure (MBS) to the baseline assessment. Trace metal concentrations in biomonitors of the Beagle Channel, Patagonia (2005–2012)

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ABSTRACT

In this study we propose a conceptual framework, i.e. the Monitoring Breakdown Structure (MBS) as a tool for the management of marine ecosystems. The conceptual framework thinks through the complexity of marine ecosystems keeping into account the variety (space) and variability (time) dimensions. Consistently with the MBS we have built the control charts of trace metal concentrations of two selected biomonitors in the Beagle Channel (south Patagonia) (case study).

Thus, we have tested the aptitude of two species of mollusks as biomonitors of heavy metal (HMs) pollution. The selected species were the limpet *Nacella* (P) *magellanica* and the bivalve *Mytilus chilensis*. Seven hundred eighty-five samples were collected along 170 km of the coastal area of the Beagle Channel (BC), (Tierra del Fuego, Argentina) in seven selected georeferenced locations and four sampling campaigns (2005, 2007, 2011, 2012). Cd, Cr, Cu, Ni, Pb and Zn determinations in seawater and mollusks by atomic absorption spectrometry (AAS) were carried out. The calculation of the respective concentration factors (CFs), i.e. their capacity as strong bioaccumulators, was also conducted. This is of relevance because it aims to use these data as a baseline reference for other geographical areas. Second, we have compared metal bioaccumulation differences among sites and the contamination trend by building, for the first time, the control charts of the baseline metal concentrations in the biomonitors. For these purposes, we applied probabilistic Johnson's method.

Furthermore, the control charts (based on four years baseline data) allowed us to test the contamination trend by plotting data from 2012 vs 2011. Our results confirm N. magellanica as an extremely strong accumulator of Cd, and M chilensis strong bioaccumulator of Cd and Zn. Zn was the most abundant metal followed by Cu. Overall, regarding the contamination trend, based on thousands of determinations we observed that the six mean metal levels were quite constant over time. Moreover, metal distribution among sites turned out to be not univocal (no one site is more contaminated than the other sites). Thus, the expected hypothesis of Ushuaia Harbour as being the most contaminated site should be reconsidered. This reinforces the hypothesis of our data as baseline data (except for cadmium), that should be considered in management decisions about future environmental monitoring programs, i.e. preventing/managing marine accidents.

1. Introduction

Marine ecosystems are complex, and the protection of the biological complexity is one of the main drives of environmental management.

Biomonitoring studies, and in particular baseline studies involving heavy metals (HMs), have to be considered as an essential dimension of the overall environmental management.

HMs are natural trace constituents of the marine environment but,

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due to rapid urbanization and industrialization, their levels have hugely increased in the last decades. Pollution by HMs has been recognized as a global threat since the period of industrial revolution. HMs pollution can give severe health and environmental hazards due to their toxic nature (Jacob et al., 2018; Tornero & Hanke, 2016). This is strongly connected with their persistence and bioaccumulation capability (Rainbow & Phillips, 1993). Aquatic organisms accumulate HMs from water and sediments from low to higher trophic level (El Nemr et al., 2012; Krupnova et al., 2018; Reguera et al., 2018; Espejo et al., 2019). In particular, mollusks, being at the top of the food chain accumulate in soft tissues and shells a large amount of HMs through the skin and oral ingestion of food and water (Beyer et al., 2017; Blanc et al., 2018). Biomonitoring surveys are currently considered of high relevance in studies dealing with HMs contamination in marine ecosystems around the world (Conti et al., 2015). Mollusks are probably the most used organisms as biomonitors for trace metal pollution in biomonitoring studies (Pérez et al., 2011, 2017; Kim and Choi, 2017; Krishnakumar et al., 2018; Pérez et al., 2019; Bajt et al., 2019). In fact, they have all the characteristics needed for a biomonitor: e.g. the species are sedentary, ubiquitous and easily identifiable; and have high concentration factors (CFs); they should also have a simple mathematical function describing the accumulation process of HMs in the soft tissues of the biomonitor (Conti, 2002; Conti et al., 2007; Krishnakumar et al., 2018).

Bivalves and gastropod mollusks (i.e. patellid limpets) have the aptitude to accumulate an extensive range of organic and inorganic contaminants at elevated concentrations. The chemical analysis of soft tissues of mollusks can be correlated with the trace metals' bioavailability in seawater over time (Duarte et al., 2011; Joksimović et al., 2018; Marques et al., 2018; Conti et al., 2017; Pérez et al., 2019). Filter feeders permanently accumulate metals in their tissues filtering the surrounding water (i.e. 3–9 l/h g/dry mass) (Newell et al., 2005); they can reflect HMs concentrations in seawater as they respond only to the seawater fraction presenting a clear ecotoxicological relevance (Phillips, 1977; Rainbow and Phillips 1993; Stankovic et al., 2012). Numerous field studies have been conducted on bivalves and gastropod mollusks from different marine ecosystems aiming to give more in-depth knowledge on metal bioaccumulation processes (Conti and Cecchetti, 2003; Conti et al., 2008; Giarratano et al., 2010; Aydın-Önen and Öztürk, 2017; Joksimović et al. 2018; see reviews: Beyer et al., 2017; Reguera et al., 2018; Mearns et al., 2018). Moreover, several researches aimed to study the possible human health risks consequential from their consumption (Jović and Stanković, 2014; Ruiz-Fernández et al., 2018). The accumulated HMs can be considered an index of human exposure, as bivalves and, to a lesser extent, patellid limpets, are common indigenous food in south Patagonia (i.e. Tierra del Fuego province) mainly in coastal areas (Pèrez et al., 2017; Bigatti et al., 2018; Conti et al., 2012a). Gastropods are herbivorous and characterize the second link of the trophic chain. The selection of biomonitors depends on their proved ability in metal assumption and to detect even minimal differences in the marine ecosystem accurately. This is strongly related with environmental management which needs reliable information consistent with the complexity of the marine ecosystems (requisite variety) (Ashby, 1957, 1958; Conti et al., 2019) in order to take decisions at the early stage of the contamination processes (i.e. prevention programs).

The selected species were the limpet *Nacella* (Patinigera) *magellanica* (Gmelin, 1971) and the bivalve *Mytilus chilensis* (Hupe' 1854). These species are ubiquitous and easy to collect and classify and are well distributed in South American seas (i.e. Beagle Channel, Magellan Strait, etc.). Biomonitors and seawater samples were collected in four sampling campaigns at the same time and the same seven geographically referenced sites in 2005, 2007, 2011 and 2012 (see details in Conti et al., 2019).

The first aim (Section 2) was to propose the Monitoring Breakdown Structure (MBS) conceptual framework that entails the complexity of

marine ecosystem keeping into account the variety (space) and variability (time) dimensions. Section 3 concerns the long-term experimental survey conducted in the Beagle Channel between 2005 and 2012 (case study, materials and method).

The second aim of this manuscript was to test (and confirm) Mytilus chilensis and Nacella magellanica as possible biomonitors of pollution of Cd, Cr, Cu, Ni, Pb and Zn in the Beagle Channel (BC) (Section 4). We also have conducted the analysis of metals in seawater (soluble fraction) in the seven sites of the BC in order to check their concentration factors (CFs), i.e. their capacity as strong bioaccumulators. The main goal of this step was to search for strategic (uncontaminated) areas able to provide background contamination levels in the BC. This is of relevance because it aims to use these data as a baseline reference for other geographical regions (Section 4.1).

The third aim (Section 4.2) was to compare metal bioaccumulation differences among sites in the BC. With this purpose, we have determined the baseline metal contamination among the four sampling campaigns (2005 → 2012) and its evolution by building, for the first time, the control charts of the selected metals measured in the biomonitors over time. Here we applied the probabilistic Johnson's method (Johnson, 1949). The study of the probabilistic distributions of trace metals concentrations in marine species can give useful information on their bioaccumulation patterns: a normal data distribution of metals indicates several independent and small additive factors affecting the measured quantity, and a log-normal distribution suggests multiplicative effects. The probabilistic approach here proposed consents to define metal concentration confidence intervals at 95% ranges of variability, through the normalization of any continuous probability distribution. Thus, we have built the quality control charts that can be inferred as a natural baseline HMs levels in the selected ecosystems by using the two selected biomonitors. The control charts built based on data of four years sampling with the limits at 95% (excluding possible outliers) consent to check, for instance, if the metal concentrations in the species analyzed in those years are, for instance, still included in the bounds of the control chart, or if they have changed with time (i.e. possible contamination event occurred). These aspects have been thoroughly debated elsewhere (Conti and Finoia, 2010: Conti et al., 2019). Furthermore (Section 4.3), we have checked the contamination trend by comparing/plotting data from 2012 vs 2011 by using the control charts built on the data basis of the four described sampling campaigns. The relevant findings connected with managerial issues as well as bioaccumulation patterns will be thoroughly debated.

2. Managing the complexity of marine ecosystem: A conceptual framework proposal

As pioneer enlightened by Leopold (1939), environmental management configure both a vision and a huge set of knowledge (scientific principles, methodologies, tools, standards, indicators, empirical data etc.) supporting the decisional processes (at macro, medium and micro level of the socio-economic system) of several and heterogeneous actors (institutions, policymakers, scientists, entrepreneurs, managers, activists etc.). One of the main purposes of environmental management is to protect biological complexity, which has become the keystone of the more recent theory on environmental management (Costanza et al., 1993: 26). According to this, biomonitoring has to be considered as a more and more crucial dimension of the overall environmental management. Moving from this conceptualization, and rooting in the managerial literature and the complexity perspective, this section aims to propose a conceptual framework of a specific biomonitoring process: the marine one. The proposed framework is consistent with the evolution of the ecological disciplines towards a complex based perspective. This perspective, on the one hand, considers the marine ecosystems as complex ones – i.e. systems that are featured by a high number of interdependent variables, with nonlinear relationships among them and uncertainty (Reed, 2008: 2418; Ciasullo et al., 2014; Broszeit et al.,

2019); on the other hand, it enlightens how the concepts of space and time ask to be connected and cannot be ignored using universal abstraction (Morin, 1986). The introduction of space and time make it possible to overcome the limits of that universalist abstraction which eliminated locality and temporality, and this represents one of the possible ways to face complexity in natural systems (Morin, 1986).

Space and time are fundamental dimensions in managing complexity in environmental systems as they put under attention two fundamental dimensions of the complexity itself: the variety (space dimension) and the variability (time dimension). The variety is a synchronous dimension: it refers to the differentiation of possible cases that can occur at the same time (e.g., different level of pollution at the same time in different areas). The variability is a diachronic dimension that refers to the possibility that a phenomenon presents later variants over time (e.g.: the change in the pollution level over time in the same area). The number of variations that the system presents at a given time and the degree of change over time is a measure of complexity.

According to this, the conceptual framework of our research is articulated along the two crucial dimensions of space and time. This forking articulation defines the basic Monitoring Breakdown Structure (MBS) of the research and configures a first milestone of the conceptualization. Along the space dimension, the monitoring process has been organised: a) by selecting seven strategic coastal sites along 170 km in the BC, Tierra del Fuego, south Patagonia (Argentina); b) by using for each coastal sites two biomonitors – i.e. *Mytilus chilensis* and *Nacella magellanica;* c) by measuring - in each coastal site - six metals (cadmium, chromium, copper, nickel, lead, and zinc) trough each biomonitor. In so doing, the proposed MBS captures three different levels of variety: that relating to the site; that related to the type of biomonitor used for the detection of metals in the marine ecosystem; that related to the type of metals.

The logical disarticulation of the spatial dimension - with reference to a specific time point (T_0, T_1, …T_n)- is represented graphically through a tree diagram: the Space Breakdown Structure (SBS) (see Supplementary Material Section Fig. S1, and the graphical abstract in the web site). This graphical tool subdivides the biomonitoring along the "space" dimension and allows to represent the elements object of observation and from which the data will be extracted; in particular the overall data obtained in S_n at a certain time (T_x) configures a specific data-package: it represents the basic set of information – a "modular" raw material – useful to develop the biomonitoring along the two complexity dimensions of space (variety) and time (variability).

Then, to represent the overall MBS, we combine the SBS with the time dimension by a further tool: the TimeSpace Monitoring Matrix

(Fig. 1). Given the selected sites and the time frame, this graphical tool represents the set of possible baselines obtainable from all available data. In particular, as each data is an *input* of information units, then the baseline is a deliberate *output* resultant from the combination of information units decided by a specific decision maker (researcher, policymaker, institution, etc.).

The way to represent the biomonitoring process by a TimeSpace Matrix is quite new and sheds new light on the biomonitoring process meant as a consistent set of *open* decisional processes. The adjective *open* aims to underline that the biomonitoring management (and more generally, environmental management) stems from "plastic" and subjective choices. "Plastic" because it deals with wicked problems (Rittel and Webber, 1973) and it is not possible to crystallize it in a given (i.e. unchangeable) set of knowledge; instead, it is strongly affected by and it co-evolves with the evolution of knowledge. "Subjective" because it is the expression, from time to time, of the quality and quantity of information that are identified, evaluated, selected and combined by the different decision makers (researchers, policy makers, government institutions, non-governmental organizations etc.). In turn, the aforementioned identification, selection and combination processes do not guarantee an optimal solution, but at best only a *satisfactory* solution because they depend strongly on subjective elements that characterize the specific decision maker such as: aims, cognitive frames, categorical values, biases, bounded rationality, heuristics etc. (Simon, 1955; Tversky and Kahneman 1981; Bandura, 1986; March, 1994; Kahneman, 2011). In this light, the decision maker is involved in the process of "becoming aware of": he selects in the potentially available information endowment those elements he considers relevant for the pursuit of his purpose and shapes the context (subjective) in which the decisions are then taken.

This is exactly the case concerning the build of the environmental managerial tools - such as the baselines and the control charts- accurately described and discussed in the following of this work: the building of these crucial biomonitoring tools really configures an open decision. Then, being aware of the open nature of the biomonitoring managerial decisions is relevant to understand the role that the subjective information endowment (Ashby, 1957, 1958; Barile, 2009; Faggioni and Simone, 2011) of the decision makers plays in terms of environmental management performances. As the famous French philosopher of science Gaston Bachelard (1929) remembers us "There are no simple things in nature, there are only simplified things". And the simplification – we add- is always influenced by less or more plasticity and subjectivity.

At the end of this section, it is interesting to observe that the
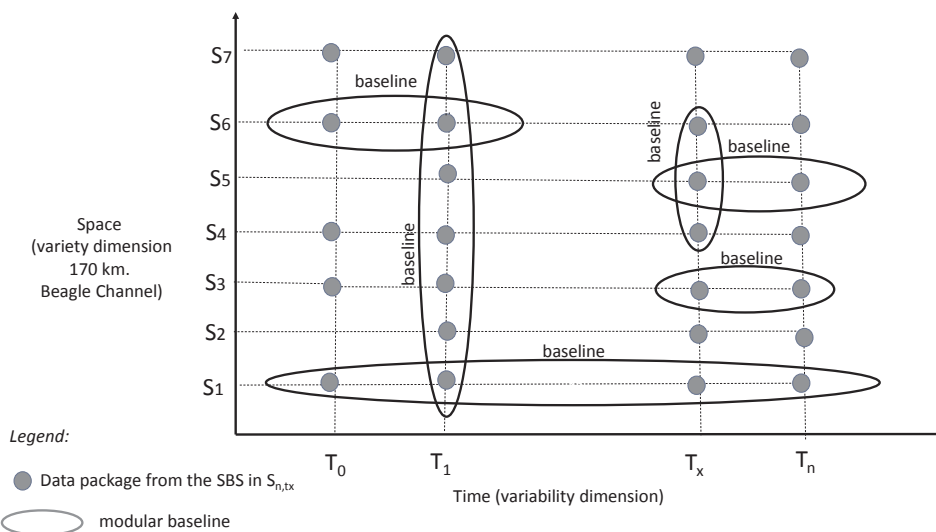

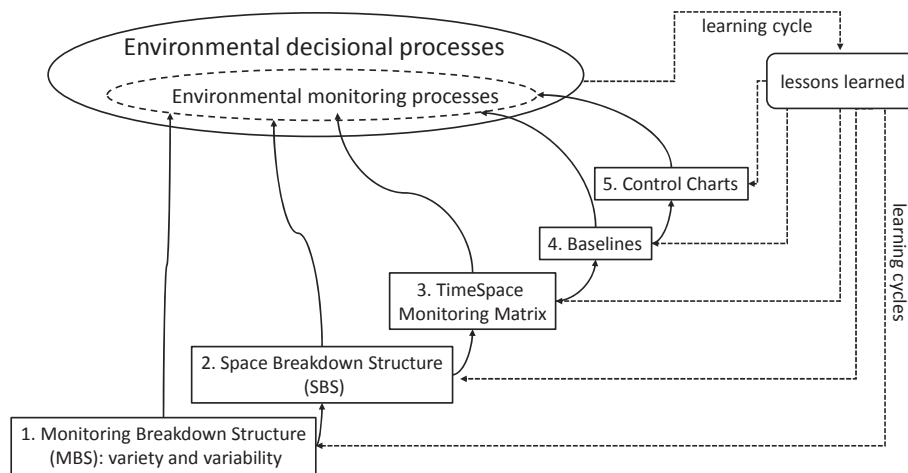**Fig. 1.** The Monitoring Breakdown Structure: The TimeSpace Monitoring Matrix.

Fig. 2. The environmental monitoring processes: the recursive learning cycles.

proposed "open view" leads to see the environmental monitoring processes – and more generally the environmental management – as a set of decisions giving rise to multiple and recursive learning cycles over time. If we take the case study of Patagonia presented in this paper, we firstly observe that each step in which biomonitoring process was articulated (in sequence: definition of the MBS, SBS, TimeSpace Monitoring Matrix, the building of the baselines and the control charts) constitutes a phase of elaboration and provides information for the successive phases. This flow of information not only is the input for the decision-making, but stimulates feedback loops in term of lessons learned (Fig. 2): decision makers learn from their past experiences, from their success and failures, by observing the best (or worst) practices, by rejecting extant solutions and by exploring new ones (Watzlawick et al., 1974; Senge, 1997; Argyris and Schön, 1996). The learning cycle could involve a change in the specificity of the response by correcting errors in a given (i.e. unchangeable) set of alternatives (type 1 learning or single-loop learning); or it could involve a change in the type 1 learning (double-loop learning): for example a corrective change of the set of alternatives within which the choice is made (Bateson, 1979). Beyond the type of learning, what is important to stress is that each lesson learned involves a change in the information endowment available for the decision makers. In environmental management, the overall information endowment available for the decision makers constitutes the main element influencing the quality of the decisions. The enrichment of the information endowment (both in terms of breadth and depth) emerging from the learning cycles is therefore crucial for the improvement of the performances of the environmental management itself (for example in terms of effectiveness and efficiency of the biomonitoring processes, increasing of the sustainability of the industrial processes, pollution decreasing, etc.). Through time, learning cycles make the information endowment co-evolutive according to the changes of the context: again an aspect of the open nature of the environmental monitoring processes we described in this section.

3. Experimental: Biomonitoring the Beagle Channel (2005–2012) with MBS conceptual framework

3.1. Material and methods

3.1.1. Study area and analytical protocols

Seven strategic coastal sites were selected along 170 km in the BC, Tierra del Fuego, south Patagonia (Argentina) (Fig. 3). Tierra del Fuego is characterized by a wide range of wildlife and biodiversity. The main urban city in Tierra del Fuego is Ushuaia that is the southernmost city of the world with ca. 60,000 inhabitants; there are a few manufacturing

industries, especially the electronic ones, and Ushuaia is the most important harbor for Antarctic tourism and maritime traffic (Pino et al., 2010).

Except for Ushuaia, the selected experimental sites lack any industrial location and can be considered uncontaminated by anthropogenic activities (see for instance, Gil et al., 2019). *Mytilus chilensis* and *Nacella magellanica* samples were collected in four sampling campaigns 2005–2007 and 2011–2012. The six metals are cadmium, chromium, copper, nickel, lead, and zinc. Sampling and chemical protocols, analytical quality control of methods, i.e. mineralization procedures (accuracy control), precision, seawater analysis and the concentration factors (CFs) calculus, graphite furnace atomic absorption spectrometry (GFAAS) and Flame atomic absorption spectrometry (FAAS) conditions, have been previously reported (Conti et al. 2011, 2012a).

3.1.2. Statistical analysis

Johnson's method (1949) was applied to HMs concentrations to generate frequency curve systems by translation. This method consents the normalization of data, an important condition for the determination of confidence intervals. The application of the normalization procedure has been carried out through the use of the SuppDists package of R (Wheeler 2013). In our previous study (Conti et al., 2019) we used the control charts to build the overlap bioaccumulation index (OBI). In this study, the control charts for the selected biomonitors were built for the first time to determine differences among sites, i.e. to define baseline metals levels, and to study the evolution of contamination in this supposedly uncontaminated area.

It is worth noting that this study concerns about 13,000 thousands analytical metal determinations in samples collected in the considered years. For comparison between/among medians, a non-parametric test with Chi-square distribution was applied.

4. Results and discussion

4.1. Mytilus chilensis and Nacella magellanica as biomonitors of metal pollution

Tables 1 and 2 show mean ± SD metal concentrations in the selected biomonitors, and mean ± SD metal concentrations in seawater samples for the four sampling campaigns in the seven selected sites of the Beagle Channel; and the concentration factors (CFs).

Seawater analysis of the six metals (soluble fraction) for all samples was carried out to determine the CFs and, subsequently, to test the aptitude of biomonitors as a strong metal bioaccumulators. As previously described, metals speciation in seawater and thus their
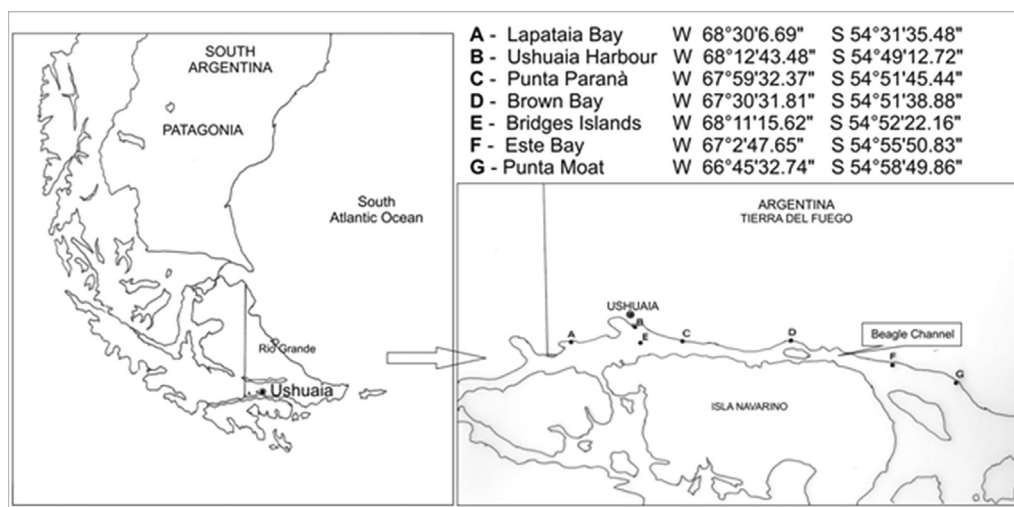
A - Lapataia Bay	W 68°30'6.69"	S 54°31'35.48"
B - Ushuaia Harbour	W 68°12'43.48"	S 54°49'12.72"
C - Punta Paranà	W 67°59'32.37"	S 54°51'45.44"
D - Brown Bay	W 67°30'31.81"	S 54°51'38.88"
E - Bridges Islands	W 68°11'15.62"	S 54°52'22.16"
F - Este Bay	W 67°2'47.65"	S 54°55'50.83"
G - Punta Moat	W 66°45'32.74"	S 54°58'49.86"

Fig. 3. The study area.

bioaccumulation, depend on several factors such as sampling protocol, environmental and physicochemical conditions, salinity, humic substances, trophic chain, water hardness, etc.; and they have to be understood with some caution. On the other hand, the bioaccumulation phenomenon does not immediately reflect acute contamination in the studied areas; it mainly reflects the history of contamination. These aspects have been thoroughly debated elsewhere (Turner et al., 2008; Conti et al., 2012a).

Mean dissolved metal levels determined in the BC seawaters were low (see Tables 1 and 2), in particular < 18 and < 10 ng/L for Cd in the four sampling campaigns. Ni showed < 100 ng/L in all the sampling campaigns; and Pb decreased from 1176 ng/L in the first and second sampling campaigns (2005–2007) to 744 ng/L in the third and fourth sampling campaigns (2011–2012).

For the metals of major concern in surface waters (i.e. Cd, Pb, Ni and Hg) the Water Framework Directive (WFD-2013/39/EU) reports Environmental Quality Standards (EQS) expressed as annual average values (AA-EQS). The mean seawater concentrations of Cd, Ni, Pb determined in this study (expressed as ng/L, Tables 1 and 2) are well

below the WFD-EQS that are: 0.2 μg/L for Cd; 8.6 μg/L for Ni, and 1.3 μg/L for Pb.

Comparing Cd, Cu and Zn concentrations in the BC seawater (soluble fraction) with previous studies, they resulted to be clearly lower than those of medium-low contaminated and uncontaminated sites in Tyrrhenian areas (Campanella et al., 2001; Conti and Cecchetti, 2003; Conti et al., 2010).

CFs were calculated as described in Tables 1 and 2. Our results confirm Nacella as extremely strong accumulator of Cd (CF = 301 × 10³ in 2005–2007; and 272 × 10³ in 2011–2012 sampling campaigns); and a good accumulator of Cu, Ni, Pb, and Zn (Tables 1 and 2) (see for discussion, Conti et al., 2011, 2017; Shefer et al., 2015). This is also consistent with the herbivorous diet of the limpet, taking into account that CFs can be connected with the passage of contaminant through the trophic chain (Ahn et al., 2002; Wang and Ke, 2002).

Likewise, very high CFs were obtained for all the analyzed metals in M. chilensis in the 2011–2012 sampling campaigns. In particular 88.8 × 10³ and 53.3 × 10³ for Cd and Zn respectively (see Table 1). For

Table 1
Metal concentrations in the selected biomonitors for the 2011–2012 sampling campaigns in the Beagle Channel (mean ± SD, μg/g dry weight)[a] (Conti et al., 2019); metal concentrations in coastal seawater samples, (ng/L) (mean ± SD) (n = 7 stations) and CFs[b] × 10³.

	Cd	Cr	Cu	Ni	Pb	Zn
Mytilus c. (n=140)	0.9±0.4	0.6±0.3	6.0±1.9	1.1±0.4	0.5±0.4	82±32
Nacella m. (n=140)	2.6±1.0	1.7±1.2	8.7±9.5	2.1±1.4	0.4±0.5	52±11
Seawater (n = 28)	<10	-	584±225	< 100	744±782	1584±634
CFs x 10³ (Mytilus c.)	88.8[c]	-	10.5	11.8[c]	0.68	53.3
CFs x 10³ (Nacella m.)	272.7[c]		15.3	22.0[c]	0.48	33.8

[a]Data for *N. magellanica* in muscle and viscera were standardized by using the method reported in Conti et al. (2012b). [b]CF = Co/Csw, where Co = mean concentration in the organism (μg/g d.w.) and Csw = mean concentration in seawater (ng/L). CFs are referred to the soluble fraction of seawater. Mean salinity recorded during sampling: 33 ± 1 NaCl/litre. [c]CFs are here intended as a minimum possible CF value obtained for mollusks samples.
Data shown in shaded boxes are highlighted because of their similarity with those of Table 2.

Table 2
Metal concentrations in the selected biomonitors for the 2005–2007 sampling campaigns in the Beagle Channel (mean ± SD, µg/g d.w.)[a], metal concentrations in coastal seawater samples, (ng/L) (mean ± SD) (n = 7 stations) (Conti et al., 2011, 2012a) and CFs[b] × 10^3.

	Cd	Cr	Cu	Ni	Pb	Zn
Mytilus c. (n=278)	0.8 ±0.5	0.5±0.3	6.1±2.0	0.9±0.3	0.4±0.4	83±51
Nacella m. (n=171)	5.4±2.5	1.2±0.8	7.8±2.9	2.8±1.4	0.5±0.6	53±9
Seawater (n = 28)	<18	-	311±233	< 100	1176±1243	768±369
CFs[b] x 10^3 (Mytilus c.)	43.0[c]	-	20.4	9.5[c]	0.37	112
CFs[b] x 10^3 (Nacella m.)	301.1[c]	-	25.9	28.2[c]	0.44	71.3

[a]Data for *N. magellanica* in muscle and viscera were standardized by using the method reported in Conti et al. (2012b). [b]CF = Co/Csw, where Co = mean concentration in the organism (µg/g d.w.) and Csw = mean concentration in seawater (ng/L). CFs are referred to the soluble fraction of seawater. Mean salinity recorded during sampling: 33 ± 1 NaCl/litre. [c]CFs are here intended as a minimum possible CF value obtained for mollusks samples.
Data shown in shaded boxes are highlighted because of their similarity with those of Table 1.

the two biomonitors, in all the four years' sampling, Zn was the most abundant metal followed by Cu. The high Zn levels determined in this study could be attributed to the aptitude of bivalves to regulate Zn which may not be moved along the food chain, thus limiting its concentration levels in the food chain (Cheung and Wang, 2008). However, the capability of mollusks to regulate essential metals (i.e. Cu and Zn) concerns a limited range of environmental concentrations of these metals (Phillips, 1995).

Even though HMs accumulation is dependent on their speciation –which is severely influenced by the physicochemical environment-(Muse et al., 2006) a comparison between other results recently obtained for Patagonian seas is still relevant.

Recently, Primost et al. (2017) reported data for mollusks in other Patagonian sites (Nuevo Gulf) with different levels of anthropogenic activities. As expected, Pb, Cd, Cu and Zn concentrations in our study were lower than those of gastropods from Nuevo Gulf site. Similarly, for bivalves, our mean levels for Cd and Cu were lower than those reported for the Nuevo Gulf site (i.e.1.16 µg/g d.w., and 9.5 µg/g d.w. respectively). On the contrary, we have determined higher mean levels of Zn in Mytilus (i.e. 82.5 µg/g d.w) in the BC (four years sampling) than those reported by Primost et al. (2017) for bivalves (i.e. 46.0 µg/g d.w.).

Overall, regarding the contamination trend conducted on the two biomonitors collected over four years, we clearly observe that the six mean metal levels were quite constant (see grey highlighted data Tables 1 and 2); excepting Cd in Nacella which decreased from 5.4 µg/g (2005–2007) to 2.6 µg/g (2011–2012) (see Tables 1 and 2 and also Section 4.3).

4.2. Comparing metal bioaccumulation differences among sites in the Beagle Channel (baseline levels)

The control charts (reported in the Fig. 4, S2–S12) describe the observed concentrations of metals, these are shown on the x-axis, while on the ordinates the concentrations are standardized and normalized according to the Johnson method. In the charts are reported: the general median of data calculated on the basis of data collected during the four sampling campaigns (2005, 2007, 2011, 2012); the medians calculated for each site ± the median absolute deviation (m.a.d.); the first

and the third quartile and the limits of the 95% control chart.

Due to its major concern here we discuss cadmium [Cd and its components are identified as priority hazardous substances by the WFD]. Figs. 4 and S2 show the cadmium control charts for the two selected biomonitors.

Fig. 4 shows that the median Cd concentrations in *Mytilus* are at the lowest levels in Ushuaia Harbor (all multiple median comparison tests [hereafter MMCT], between the median of this site and the other sites, were significant at p < 0.05) while the highest levels were observed at Punta Moat (the test was not significant (n.s.) only with the median Cd concentration of Este Bay). The medians calculated at Este Bay and Lapataia Bay are between the 25th and 50th percentile, while for the other sites the medians are distributed between the 50th and 75th percentiles. The overall median is 0.72 µg/g while the confidence interval includes values in the range 0.19–2.00 µg/g. Considering the cadmium risk limit for bivalves established by Mercado Comùn del Sur (MERCOSUR/GMC, 2011), we found that 65% of bivalves exceeded this risk limit (see Fig. 4, green line - online version of the MS). Even if consumption levels are low in the BC, this aspect deserves attention.

From Fig. S2 we observe that the median Cd concentrations in Nacella are at the lowest levels in Ushuaia Harbor; the highest levels were found at Punta Moat (all MMCT were significant at p < 0.05). This median exceeds the 75th percentile. The medians calculated in all the other examined sites are between the 25th and 75th percentiles. The overall median is 3.71 µg/g while the confidence interval is in the range 0.98–9.96 µg/g.

This is also consistent with the overlap bioaccumulation indexes (OBI) previously obtained for these biomonitors (Conti et al., 2019). Mytilus resulted highly sensitive to low seawater Cd concentrations (OBI-L1 = 5.16), which means it detects fivefold lower Cd levels in seawater with respect to the minimum overlap range according to Johnson's method. Conversely, Nacella showed higher bioaccumulation Cd surplus, e.g. OBI-L = 4.98 which means it detects about five times higher Cd levels with respect to the upper extreme overlap range (see for details Conti et al., 2019).

All results and sites' classification are briefly commented in the Supplementary Material (SM) section; i.e. Figs. S2–S12 show the control charts for Cd in Nacella, and Cr, Cu, Ni, Pb and Zn, respectively, for the two selected biomonitors.
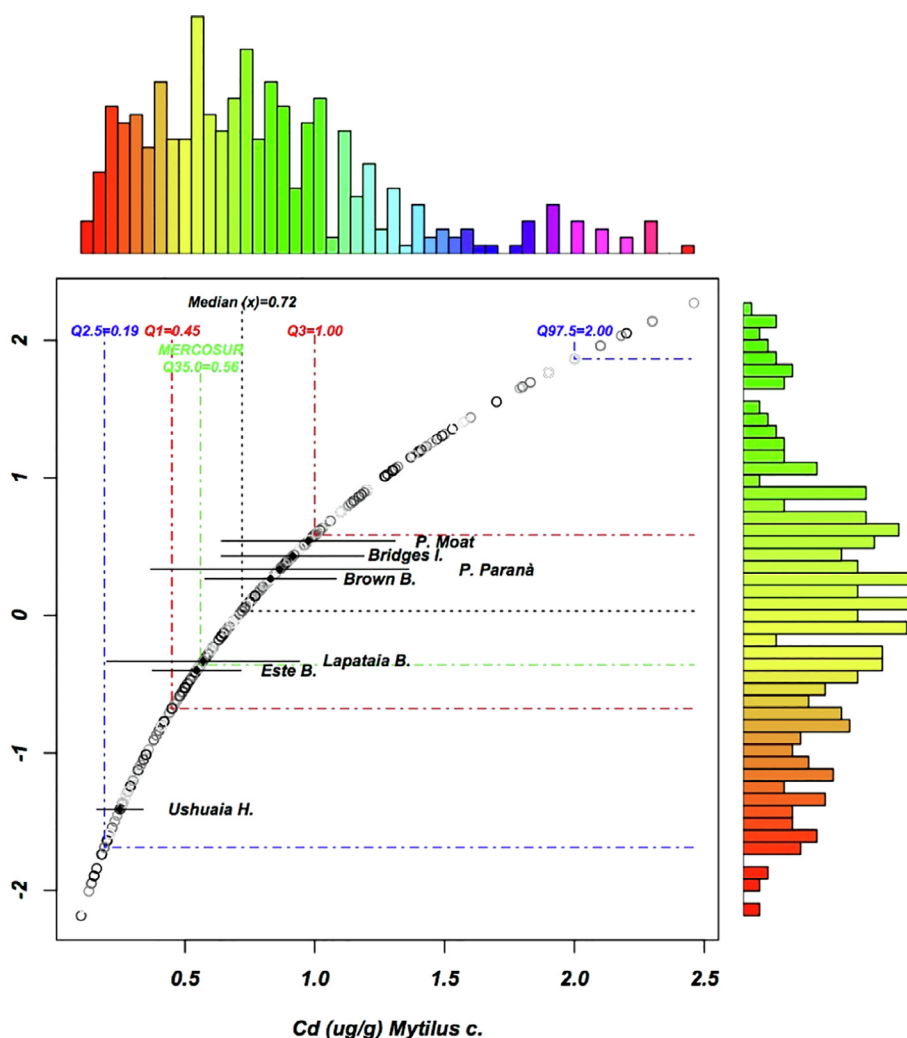
Fig. 4. Control chart for the Cd concentrations (μg/g d.w.) in *Mytilus chilensis* (n = 418) in the four sampling campaigns (2005, 2007, 2011, 2012). OBI-L1 = 5.16 (see text). The risk limit for Cd in bivalves established by MERCOSUR/GMC (2011) is reported (see text).

From these results (Figs. 4, S2–S12) we can infer some relevant findings:

-similar Cd bioaccumulation patterns are observed for the two biomonitors for Ushuaia Harbour and Punta Moat sites (at about 150 km of distance, see map Fig. 3) (Figs. 4 and S2). Surprisingly, Ushuaia harbour showed the lowest levels of Cd and Punta Moat the highest ones;

- on the contrary, as expected, the highest levels of Pb and Zn were detected contemporary by the two biomonitors at Ushuaia harbour;
- as to Cr, Mytilus detected the lowest levels at Ushuaia harbour (Fig. S3), while the highest ones were detected at P. Moat. Conversely, Nacella showed (as expected) their highest Cr and Cu concentrations levels in the Ushuaia Harbour (Figs. S4 and S6);
- on the basis of these results (Figs. 4, S2–S12), that involved years of sampling , we observe that the metal distribution among sites is not univocal and it is clear that none of these sites is more contaminated than the others.

However, this apparently anomalous and complex metal behavior above described, in particular for Cd, is difficult to explain. The geographic position of Punta Moat site (Fig. 3) is exposed to open sea and can be more influenced with upwelling coastal currents that occur periodically in this area of the BC. Metal biogeochemistry in coastal waters is a matter of debate in the scientific context (Price and Morel,

1990; Hervé-Fernández et al., 2010). On the other hand, water chemistry and organism's physiology can have a relevant role in metal toxicity (for instance, see review for Ni, Blewett and Leonard, 2017) and their bioaccumulation capabilities. Valdés et al. (2006) proposed that Cd is removed from the superficial layer in the ocean by the bioaccumulation process in the organisms and, subsequently throughout the biota sedimentation process.

Consequently, we assume that the organic matter oxidation process yields metals (Cd in particular) and nutrients to the water body. The upwelling coastal currents have been proposed as a process responsible for the regulation of Cd in the coastal environment (Van Geen and Husby, 1996). Moreover, this mechanism is applied as a marker of water circulation (Takesue et al., 2008).

As reported above there are several electronic factories in Tierra del Fuego (about 30 in the area of Ushuaia). The Río Grande city has emerged as a manufacturing and assembly hub for laptops, high-definition televisions, and cell phones in the last decades. The currently functioning sewage treatment plant was built recently (2018). We assume that the problem is the treatment of industrial wastes which, although regulations have been implemented in this regard (i.e. Environmental law n. 55 of Tierra del Fuego province), they have yielded negative results with judicial aftermath. Being a very important tourist area with a high level of ship traffic, the problem of washing liquid waste from ships is another relevant problem (i.e. bilge water).

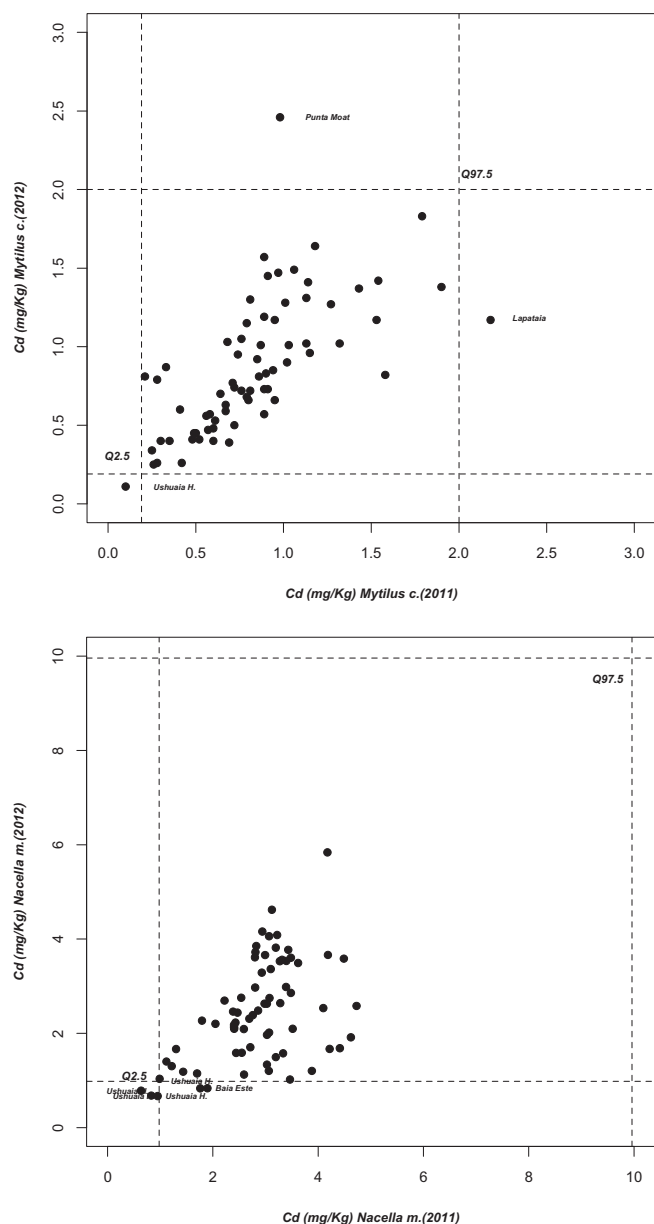However, it is hard to found the direct source of these contaminants

Fig. 5. Cd control charts built based on data from the 2011–2012 period.

4.3. The contamination trend in the Beagle Channel

Figs. 5 and S13–S17 show the metals contamination trend checked by plotting data from 2011 vs 2012 based on the control charts built with the limits at 95% (i.e. based on data of four years sampling). The aim was to verify, for each species, if the concentrations detected in those years were included in the bounds mentioned above of the control chart, or if they have changed with time.

Fig. 5 shows that the Cd concentrations for the two biomonitors in 2011–2012 are almost all within the limits of the control charts defined through the application of Johnson's method. Only one sample of Mytilus from Lapataia showed higher concentration in 2011 (outside of the limits) while all samples were within the chart limits in 2012. Conversely, only one bivalve sample from Punta Moat increased its Cd concentration in 2012 (outside of the control chart limits). Concentrations below the Q2.5 limit are not discussed here because, in any case, they are extremely low (i.e. see the low Cd values obtained for Ushuaia Harbor detected by Nacella, Fig. S2).

Fig. S13 shows the concentrations of Pb for the two biomonitors in 2011–2012, that are mainly within the limits of the control charts (i.e. Nacella samples in particular), with the exception of some samples that were beyond the upper limit of the control chart (i.e. some few Mytilus samples from different sites (i.e. Este Bay, Lapataia, Ushuaia Harbor, etc.). Similar evolution trend is observed for the other metals (i.e. Cr, Cu, Ni and Zn, Figs. S14–S17) where their concentrations in the majority of samples resulted within the control charts in the 2011–2012 period, with some few exceptions.

This study further confirms (see also Section 4.1) that the contamination trend of the metal concentrations in the biomonitors is quite homogeneous/constant in the studied years (see shaded boxes Tables 1 and 2).

5. Conclusions

A complexity-based conceptual framework to manage marine ecosystems is proposed. This study strongly confirms the aptitude of the selected mollusks as biomonitors of HMs pollution in remote areas. We have built the control charts by means of Johnson's statistics and metal baselines ranges have been defined, based on thousands of determinations from 2005 to 2012. Our results confirm N. magellanica as an extremely strong accumulator of Cd, and M. chilensis strong bioaccumulator of Cd and Zn and very good accumulator of Cu, Ni and Pb.

Overall, regarding the contamination trend, we clearly observe that the six mean metal levels were quite constant over time, and the metal distribution among sites is not univocal. Thus, the expected hypothesis of Ushuaia Harbour as being the most contaminated site should be reconsidered. These results reinforce the hypothesis of our data as baseline data (except for cadmium) that can be considered in management decisions about future environmental protection programs (i.e. preventing/managing marine accidents).

However and as told under discussion, further studies involving challenging analytical developments as functional speciation at ultratrace levels are needed in order to establish geochemical mechanisms for a bunch of metals in different marine coastal ecosystems. A study of possible concomitants able to trigger accumulation or depress it should be useful for a more integral understanding.

along 170 km of the BC coast, and it seems there is no particular industrial fingerprint from the other sites, excepting Ushuaia Harbor. The determined levels of metals in the BC are at the lowest levels than those found for other areas in the world. Recently, a summary of trace metal concentrations in mussels worldwide has been reported (Lu et al., 2019). The authors reported mean levels of 2.93, 4.04, 34.7, 5.82, 7.98 and 255 µg/g d.w. for Cd, Cr, Cu, Ni, Pb and Zn respectively. Our results (Tables 1 and 2) were clearly lower (and with lower variability) than those reported for mussels worldwide (which showed, as expected, high SD values). However, the study by Schøyen et al. (2017) reported lower levels than those of the BC for the six metals in Mytilus edulis from background and slightly impacted stations in Norwegian coastal sites (i.e.: 0.35–8.7 µg/g. w.w. for Cd in the BC versus 0.13–0.34 µg/g w.w., expressed as 10 and 90 percentile respectively, in a six-months sampling campaign).

Thus, the expected hypothesis of Ushuaia Harbour as being the most contaminated site should be reconsidered.

manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2019.05.013.

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