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Abstract: In this study, we have investigated Cd, Cr, Cu, Ni, Pb and Zn in the biomonitors Mytilus chilensis and Nacella (P) magellanica sampled along seven selected sampling sites along 170 km of the coastal area of the Beagle Channel (Tierra del Fuego, Argentina) in four sampling campaigns: 2005, 2007, 2011 and 2012. The control charts were built by applying Johnson's (Biometrika 36: 149-175, 1949) probabilistic method for the first time in this marine area. We determined the metal concentration overlap ranges in the selected biomonitors (as well as medians and distribution), and the overlap bioaccumulation index (OBI) with respect to the lowest (OBI-L1) and the highest (OBI-L) extreme values of the overlap metal concentration ranges. The OBI can be used as an integrative tool in the management of prevailing unpolluted/polluted marine coastal ecosystems. It consents to identify the most suitable organisms for managing several environmental conditions where an ecosystem quality control is needed. The OBI-L1 index can be employed as a preventive signal of alarm when the contamination process is in its early stages. For Cd, Cr, Cu, and Ni, Nacella showed high OBI-L values that suggest its use as a biomonitor for mainly polluted marine ecosystems, in particular for Cd. Mytilus showed high Cd values for the OBI-L1 which means that this species is highly sensitive to a very low variation of the Cd levels in seawater. Good OBI-L1 values were instead obtained for Mytilus for Cr and Cu, showing the good aptitude of these organisms to detect minimum variations of trace metals concentrations in seawater. The OBI index and its related guidelines have both theoretical and practical implications in environmental management. They can be used, for instance, in environmental prevention from events such as oil spills or other marine disasters. Marine ecosystems are complex systems. According to the Ashby's Law (1957, 1958), the understanding of a complex system (requisite variety) depends on the information variety owned by the observer. The OBI index enhances the observer's information variety about the performance of the molluscs as metal biomonitors in marine ecosystems. Eventually, here we propose to conceptualize the wide set of

biomonitoring knowledge endowment as an open and evolutionary endowment of information variety supporting the environmental management.

Highlights:

- Six metals were determined (2005→2012) in two molluscs → Beagle Channel Patagonia
- Control charts were built, the metal overlap bioaccumulation index (OBI) is proposed
- OBI can be used as an integrative tool in the marine ecosystems management
- OBI enhances the observer's information variety of metal biomonitors performance
- Biomonitoring is an open information endowment for the environmental management

Performance of two Patagonian molluscs as trace metal biomonitors: the overlap bioaccumulation index (OBI) as an integrative tool for the management of marine ecosystems

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Abstract

In this study, we have investigated Cd, Cr, Cu, Ni, Pb and Zn in the biomonitors *Mytilus chilensis* and *Nacella* (P) *magellanica* sampled along seven selected sampling sites along 170 km of the coastal area of the Beagle Channel (Tierra del Fuego, Argentina) in four sampling campaigns: 2005, 2007, 2011 and 2012. The control charts were built by applying Johnson's (Biometrika 36: 149-175, 1949) probabilistic method for the first time in this marine area. We determined the metal concentration overlap ranges in the selected biomonitors (as well as medians and distribution), and the overlap bioaccumulation index (OBI) with respect to the lowest (OBI-L1) and the highest (OBI-L) extreme values of the overlap metal concentration ranges. The OBI can be used as an integrative tool in the management of prevailing unpolluted/polluted marine coastal ecosystems. It consents to identify the most suitable organisms for managing several environmental conditions where an ecosystem quality control is needed. The OBI-L1 index can be employed as a preventive signal of alarm when the contamination process is in its early stages. For Cd, Cr, Cu, and Ni, *Nacella* showed high OBI-L values that suggest its use as a biomonitor for mainly polluted marine ecosystems, in particular for Cd. *Mytilus* showed high Cd values for the OBI-L1 which means that this species is highly sensitive to a very low variation of the Cd levels in seawater. Good OBI-L1 values were instead obtained for *Mytilus* for Cr and Cu, showing the good aptitude of these organisms to detect minimum variations of trace metals concentrations in seawater. The OBI index and its related guidelines have both theoretical and practical implications in environmental management. They can be used, for instance, in environmental prevention from events such as oil spills or other marine disasters. Marine ecosystems are complex systems. According to the Ashby's Law (1957, 1958), the understanding of a complex system (requisite variety) depends on the information variety owned by the observer. The OBI index enhances the observer's information variety about the performance of the molluscs as metal biomonitors in marine ecosystems. Eventually, here we propose to conceptualize the wide set of biomonitoring knowledge endowment as an open and evolutionary endowment of information variety supporting the environmental management.

Keywords

Biological monitoring; Beagle Channel; *Mytilus chilensis*; *Nacella* (P) *magellanica*; baseline metal levels; Johnson's method; control charts; environmental performance; information variety.

1. Introduction

Moving from the seminal works of Goldberg (1975; 1986) and Phillips (1977), the use of marine organisms as biomonitors for metal pollution in seawater became a key-relevant monitoring method in environmental studies. Molluscs are among the most used organisms as biomonitors for trace metal pollution in biomonitoring surveys (Directive 2000/60/EC; Krishnakumar et al., 2018) as they observe all the requisites, e.g. they have high concentration factors (CFs), the species are sedentary, ubiquitous and easily identifiable (Krishnakumar et al., 2018; Reguera et al., 2018). Bivalves and gastropod molluscs have the ability to accumulate high concentrations of organic and inorganic pollutants, and the chemical analysis of tissues of organisms gives evidence of the trace metals bioavailability in seawater and sediments over time (Hervé-Fernández et al., 2010; Duarte et al., 2011; Gupta et al., 2014; Marques et al., 2018; Buzzi and Marcovecchio, 2018; Krupnova et al., 2018; Joksimovic et al., 2018). Filter feeders permanently accumulate metals in their tissues filtering the surrounding water (i.e. 3–9 l/h g/dry mass), they seem to be more appropriate for reflecting metal concentrations in marine waters presenting a clear ecotoxicological relevance (Rainbow and Phillips 1993; Conti and Cecchetti, 2003). In the last decades, studies on bivalves and gastropod mollusks from different marine geographical areas have been extensively examined in a number of field studies contributing to a better knowledge on metal bioaccumulation processes (Giarratano et al., 2010; Aydın-Önen and Öztürk, 2017; Joksimovic et al., 2016, 2018; see reviews Beyer et al., 2017; Reguera et al., 2018). These studies have also contributed to the evaluation of possible human health risks resulting from their consumption (Yüzereroğlu et al., 2010; Connan and Tack, 2010; Stankovic et al., 2012; Conti et al., 2012a; Jović and Stanković, 2014; Shefer et al. 2015; Primost et al., 2017). For instance, a recent interesting study, connect the Cd contamination with the use of Mytilus chilensis valves as byproducts in agricultural applications (Blanc et al., 2018). Although the amounts of accumulated metals showed themselves as harmless for humans to ingest, they can be considered an index of human exposure as bivalves and patellid limpets are a common indigenous food in the studied areas (Conti, 2002; Kelepertzis, 2013; Yusà & Pardo, 2015; Pèrez et al., 2011, 2017). Gastropods are herbivorous and represent the second link of the trophic chain. They live on rocky substrata of tidelands and can survive out of the water for several hours. However, recent studies report the relevance of patellid limpets as omnivorous grazers (Burgos-Rubio et al., 2015); they take metals mainly from the food making most likely their capacity for elemental accumulation (Ahn et al., 2002).

Usually, in order to obtain ample information about different possible bioaccumulation patterns, the simultaneous use of several biological indicators has been recommended. This is connected with the fact that each species responds to a particular fraction of the contaminants present in seawater and also to different metal concentrations (Ruiz-Fernàndez et al., 2018). Suitable biological indicators are needed, in particular, to detect even minimal variations in the marine ecosystem accurately (Lam and Gray, 2003, Conti et al., 2015), this is strongly connected with environmental prevention programmes that have the aim to take action as soon as the biomonitors show significant variations in their contaminant concentrations.

The choice of these biomonitors is subjected to their demonstrated aptitude in metal assumption. Metals present in marine waters may penetrate the cell membrane of molluscs after complexation due to the exopolysaccharide and protein contents present in their mucus secretions. After assumption, metals can be metabolized by metallothionein formation, the core detoxification mechanism present in invertebrate organisms (Amiard et al., 2006). These tolerance mechanisms were recognized as a strategy assumed by benthic organisms to survive with the stressors (Amiard-Triquet et al., 2011). On the other hand, metal absorption by molluscs can be associated with pH and ionic strength conditions and seasonality. These factors have to be considered in metal baseline level surveys (Duarte et al., 2011).

Several quality indexes have been proposed in the last decades with the aim to assess pollution in marine geographical areas both by using data analysis of the abiotic and biotic compartments. These indexes are often applied to screen the metal burden within sediment. The Biosediment Accumulation Factor (BSAF) was proposed by Szefer et al. (1999) and Lafabrie et al. (2007) with the aim to obtain the ratio between metal concentration in the organism and the sediment. The Geo-accumulation Index (Igeo) (Müller, 1979), and Pollution Load Index (PLI) are connected with the metal burden in sediments. Igeo gives useful information about the level of the metal burden in sediments for single elements while PLI gives a general symptom about the sediment pollution in the studied site. The definition is given by the formula (Tomlinson et al., 1980), $PLI = [CF_1 \times CF_2 \times CF_3 \cdots CF_n]^{1/n}$ where CF is the concentration factor of the metal *n* with respect to the background value in the sediment ($CF = C_{metal}/C_{background}$) (Tomlinson et al., 1980; Angulo, 1996). PLI denotes the number of times the obtained metal concentration surpasses the baseline concentration in the sediment (i.e. $PLI > 1$ means polluted, while < 1 indicates no pollution).

The Enrichment Factor (EF) was proposed by Ergin et al. (1991). Basically, its consents to detect anomalous metal concentrations in sediments by making geochemical normalization of heavy metal data to a conservative element, i.e. Al, Fe and Si. Usually, Fe is selected to normalize metal contaminants. It is given by: EF = $(M/Fe)_{sample}$[The ratio of metal and Fe concentration of the sample] / $(M/Fe)_{background}$ [the ratio of metal and Fe concentration of the background]. EF values lower than 1.5 indicate that heavy metals arise mainly from natural sources, whereas values higher than 1.5 can be connected with anthropogenic sources (Ergin et al., 1991; Barakat et al., 2012). USEPA Sediment Quality Guidelines (SQGs) and TEL/PEL [Threshold Effect Level/ Probable Effect Level] indexes have been proposed (Perin et al., 1997; Long et al., 1995). These indexes give a threshold concentration values (i.e. guidelines) for trace elements in sediments.

Other indexes, such as the condition index (CI) give information about the health of the bivalve, including several factors such as salinity, water temperature, food availability and the reproductive phase of bivalves (Okumus and Stirling, 1998), while the well-known Metal Pollution Index (MPI) is applied to make comparisons of the total metal content in mussels among different sites (Usero et al., 1997). The equation is $MPI= [Cf_1 \times Cf_2 \times \ldots Cf_n]^{1/n}$, Cf_n stands for the concentration of the metal n in the mussel sample.

In this work, the selected species were the bivalve *Mytilus chilensis* (Hupe` 1854) and the limpet *Nacella* (Patinigera) *magellanica* (Gmelin 1971). These species are well distributed in South American seas (i.e. Beagle Channel, Magellan Strait, etc.), are ubiquitous and easy to collect and classify; they were selected to fulfil the objectives of this study.

Going beyond classical biomonitoring studies (Conti et al., 2011; 2012b) in this work we have built, for the first time, the control charts for the metals bioaccumulation in the selected biomonitors in the Beagle Channel. The first aim is to determine the range of overlaps of metal concentrations and the overlap bioaccumulation index (OBI) with respect to the upper (OBI-L) and lower (OBI-L1) bound of the overlap range (Conti et al., 2015).

For this purpose, we apply the probabilistic Johnson’s method (Johnson, 1949). The study of the probabilistic distributions of trace metals concentrations in marine species can give reliable information on their bioaccumulation mechanisms. In fact, it is well known that a Gaussian (i.e. normal) distribution of metals suggests several independent and small additive factors affecting the measured quantity, and a log-normal distribution suggests multiplicative effects; these issues were discussed elsewhere (Conti and Finoia, 2010). The probabilistic approach here applied consents easily, by means of the normalization of any continuous probability distribution, to define metal concentration confidence intervals at 95% ranges of variability (Johnson, 1949; Miller and Miller, 2005). The use of OBI as an integrated tool in marine environmental management consents to identify the specific biomonitor (or biomonitors) needed for a particular condition of contamination that can arise from natural or anthropogenic activities (i.e. marine accidents).

The second aim is to analyze the theoretical and practical implications of the OBI index and its relative guidelines for the environmental management. Marine ecosystems are complex systems. According to the Ashby's Law (1957, 1958), the understanding of a complex system (requisite variety) depends on the information variety owned by the observer. In view of this, here we propose to conceptualize the wide set of biomonitoring knowledge capacity as an open and evolutionary endowment of information variety supporting the environmental management. These theoretical and practical implications will be fully debated.

2. Case study

2.1 Materials and method

2.1.1 Study area

Our study concerns seven sites in the Beagle Channel, Tierra del Fuego, south Patagonia (Argentina) (Figure 1). Tierra del Fuego has a unique ecosystem, and it is characterized by a wide range of wildlife and biodiversity (Pino et al. 2010). The Beagle Channel has high ecological relevance and is about 240 km long and 5 and 14 km wide. It separates Isla Grande de Tierra del Fuego from several smaller islands in the south. It owes its name to the British ship Beagle, employed by Charles Darwin to explore the area between 1833 and 1834. The main urban settlement in Tierra del Fuego is the city of Ushuaia that is the southernmost city of the world with ca. 60,000 inhabitants. Ushuaia is the most important port for the Antartic tourism and maritime traffic. Except for Ushuaia, the selected experimental sites lack any industrial site and can be considered not affected by anthropogenic activities.

Mytilus chilensis and *Nacella magellanica* samples were collected in seven selected stations situated along 170 km of the Beagle Channel (Fig. 1) in four sampling campaigns at the same time and in the same geographically referenced sites in 2005, 2007, 2011 and 2012. The six metals are cadmium, chromium, copper, nickel, lead, and zinc. Sampling, mineralization procedures, chemical protocols and results for the sampling campaigns 2005-2007 have been reported elsewhere (Pino et al., 2007; Conti et al. 2011, 2012b).

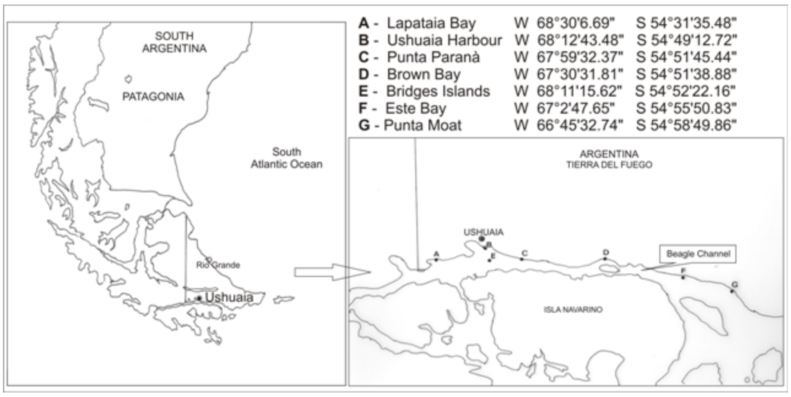

Figure 1. The study area

2.1.2. Metal overlap ranges and the overlap bioaccumulation index (OBI)

Johnson's method (1949) was applied to heavy metal concentrations to generate frequency curve systems by translation. This method allowed 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).

The control charts for the selected biomonitors were built to determine the overlap range between the two biomonitors and for the definition of the OBI (Conti et al., 2015). Briefly, it consists in:

Definition of the overlap range for the studied metals

Given the $Q_{i,2.5}$ and $Q_{i,97.5}$ values, corresponding to the minimum and the maximum metal concentration levels respectively for the range determined according to Johnson's method, we build the control chart for the i_{th} species. Analogously, $Q_{j,2.5}$ and $Q_{j,97.5}$ are determined for the j_{th} species. Then, the overlap range for the i_{th} and j_{th} species is defined according to the following extreme values:

$$I_{min}=\max(Q_{i,2.5}, Q_{j,2.5}) \text{ with } i=1, 2,..,k \text{ and } i \neq j \qquad \text{[Eq. 1]}$$

$$I_{max}=\min(Q_{i,97.5}, Q_{j,97.5}) \text{ with } i=1, 2,..,k \text{ and } i \neq j \qquad \text{[Eq. 2]}$$

We have considered the percentiles Q2.5 and Q97.5 instead of the extreme values (minimum and maximum) in order to exclude from the definition of the overlap ranges anomalous values and outliers.

Definition of bioaccumulation index with respect to the maximum and minimum overlap range

The indexes of bioaccumulation (OBI-L_i) for the i_{th} species with respect to $Q_{i,97.5}$ is defined as:

$$OBI\text{-}L_i = \frac{Q_{i,97.5}}{\mathrm{I}_{\max}} \qquad \text{with i=1, 2,..,k} \qquad \text{[Eq. 3]}$$

OBI-L is generally ≥ 1 and becomes 1 when $Q_{i,97.5} = I_{max}$

The OBI-$L1_i$ for the i_{th} species with respect to $Q_{i,2.5}$ is defined as:

$$OBI\text{-}L1_i = \frac{\mathrm{I}_{\min}}{Q_{i,2.5}} \qquad \text{[Eq. 4]}$$

For comparison between medians, a no parametric test with Chi-square distribution was applied.

3. Results and Discussion

Tables 1-2 show mean ± sd metal concentrations in the selected biomonitors for the four sampling campaigns in the seven selected sites of the Beagle Channel.

Table 1: Metal concentrations in the selected biomonitors for the 2011-2012 sampling campaigns in seven sites of the Beagle Channel (mean ± sd, µg g^{-1} d.w.)[a]

	Cd	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
Mytilus c. (n=140)	0.86±0.42	0.62±0.35	5.96±1.92	1.14±0.40	0.49±0.41	81.7±31.9
Nacella m. (n=140)	2.64±1.03	1.69±1.20	8.65±9.49	2.13±1.44	0.35±0.54	51.9±10.7

[a] Data for *N. magellanica* in muscle and viscera were standardized by using the method reported in Conti et al. (2012a).

Table 2. Metal concentrations in the selected biomonitors for the 2005-2007 sampling campaigns in seven sites of the Beagle Channel (mean ± sd, µg g^{-1} d.w.)[a] (Conti et al., 2011; 2012b)

	Cd	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
Mytilus c. (n=278)	0.75 ±0.48	0.45±0.29	6.14±2.04	0.92±035	0.42±0.36	83.2±50.8
Nacella m. (n=171)	5.42±2.51	1.21±0.84	7.79±2.87	2.79±1.38	0.50±0.57	53.0±9.5

[a] Data for *N. magellanica* in muscle and viscera were standardized by using the method reported in Conti et al. (2012a).

3.1. Overlap metal concentration ranges and overlap bioaccumulation index (OBI)

Table 3 shows Johnson’s classification of probability distributions, control chart limits, the extremity values of the overlap range, and the OBI-L and (OBI-L1) (see section 2.1.2 for definitions) for each metal in *Mytilus chilensis* and *Nacella magellanica* (µg/g d.w.).

[table 3 next page]

Table 3. Johnson's classification of probability distributions, control chart limits, overlap ranges (µg/g d.w.) and the OBI calculated for each metal in *Mytilus chilensis* (n=418) and *Nacella magellanica* (n=311). SU – unbounded, SB – bounded, and SL – log-normal distribution.

Cd	**Type of distribution**		**Range ($Q_{2.5}$, $Q_{97.5}$)**	**L**	**L1**
Mytilus c.	SL		0.19-2.00	1.00	5.16
Nacella m.	SU		0.98-9.96	4.98	1.00
Total	SB	Overlap	0.98-2.00		
Cr					
Mytilus c.	SB		0.13-1.33	1.00	1.62
Nacella m.	SB		0.21-3.39	2.54	1.00
Total	SB	Overlap	0.21-1.33		
Cu					
Mytilus c.	SU		1.99-10.98	1.00	1.95
Nacella m.	SU		3.88-34.93	3.18	1.00
Total	SB	Overlap	3.88-10.98		
Ni					
Mytilus c.	SL		0.45-1.84	1.00	1.51
Nacella m.	SU		0.68-6.69	3.64	1.00
Total	SL	Overlap	0.68-1.84		
Pb					
Mytilus c.	SB		0.13-1.22	1.23	1.00
Nacella m.	SB		0.089-0.99	1.00	1.46
Total	SB	Overlap	0.13-0.99		
Zn					
Mytilus c.	SU		30.7-205.9	3.61	1.46
Nacella m.	SU		44.9-57.1	1.00	1.00
Total	SU	Overlap	44.9-57.1		

Figures 2-7 show the control charts for each metal built for the two selected biomonitors with their obtained overlap metal concentrations (color figures are reported in the online version of the manuscript). Observed values are on x-axes, and values calculated by Johnson's method are on y-axes. Inside the plot are reported: the medians ± m.a.d. (median absolute deviation) with the OBI-L and OBI-L1 (in brackets), the first and third quartile, the lower and upper bounds of baseline range (Q2.5 and Q97.5), the range of overlap (i.e. the common metal concentration range for both species, see the arrow) and the percentile position of the upper and lower bound of the range of overlap. The histograms of values are shown outside of the plot. This study was conducted from data collected in four sampling campaigns (2005, 2007, 2011, 2012) in the Beagle Channel as above mentioned.

Figure 2 shows that the concentrations for *Mytilus* are lower than the overall median and with very low variability (i.e. m.a.d.) than those found for *Nacella* specimens. The medians for the two species were significantly different ($\chi 2$ (1) = 450.5, $p < 0.001$). The limits of the overlap range were 39.1 and 60.5 percentiles, and they constitute 20% of the total data. The obtained OBI show that *Mytilus* is highly sensitive to low seawater Cd concentrations (OBI-L1 = 5.16, see Table 3), which means it detects fivefold lower Cd levels in seawater with respect to the minimum overlap range. On the contrary, *Nacella* has higher bioaccumulation Cd surplus, that is OBI-L=4,98 which means it detects five times higher Cd levels with respect to the upper extreme bioaccumulation overlap range (see the arrow in Figure 2). For instance, the OBI-L for Cd in *Nacella* was obtained after dividing the $Qi,_{97.5}$ value (i.e. 9.96 µg/g, see Table 3) by the extreme upper value of the overlap range (i.e. 2.00 µg/g) according to Eq. 3 (see section 2 and Table 3). These results agree with our previous studies in other distant geographical areas (i.e. Tyrrhenian sea) for another patellid limpet (i.e. *Patella caerulea*) that showed high bioaccumulation Cd surplus (OBI-L) (Conti et al., 2010).

[Figure 2 next page]

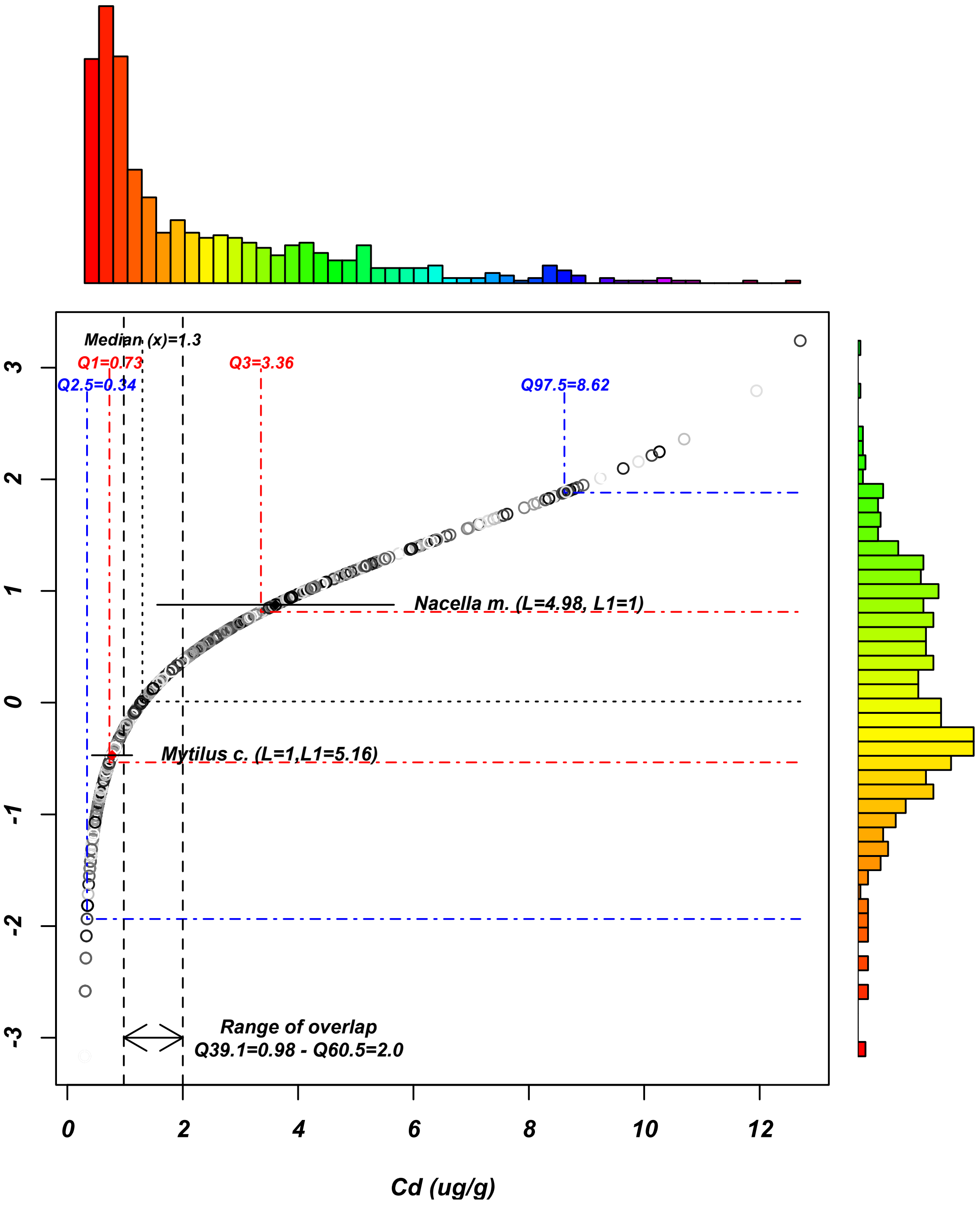

Figure 2. Control chart for Cd built for the two selected biomonitors with their obtained overlap metal concentrations (µg/g d.w.).

Figure 3 shows that the Cr concentrations detected for *Mytilus* are lower than the overall median and lower than *Nacella* which bioaccumulates at the Q3 level of the distribution of the two selected biomonitors. On the other hand, *Mytilus* bioaccumulates Cr in a narrow range showing low variability with respect to *Nacella* specimens. The medians for the two species were significantly different ($\chi2$ (1) = 109.5, p <0.001). The limits of the overlap range were 8.5 and 79.4 percentiles, and they constitute approximately 70% of the total data. The obtained OBI (Table 3) show that *Nacella* has high bioaccumulation Cr surplus (OBI-L= 2.54).

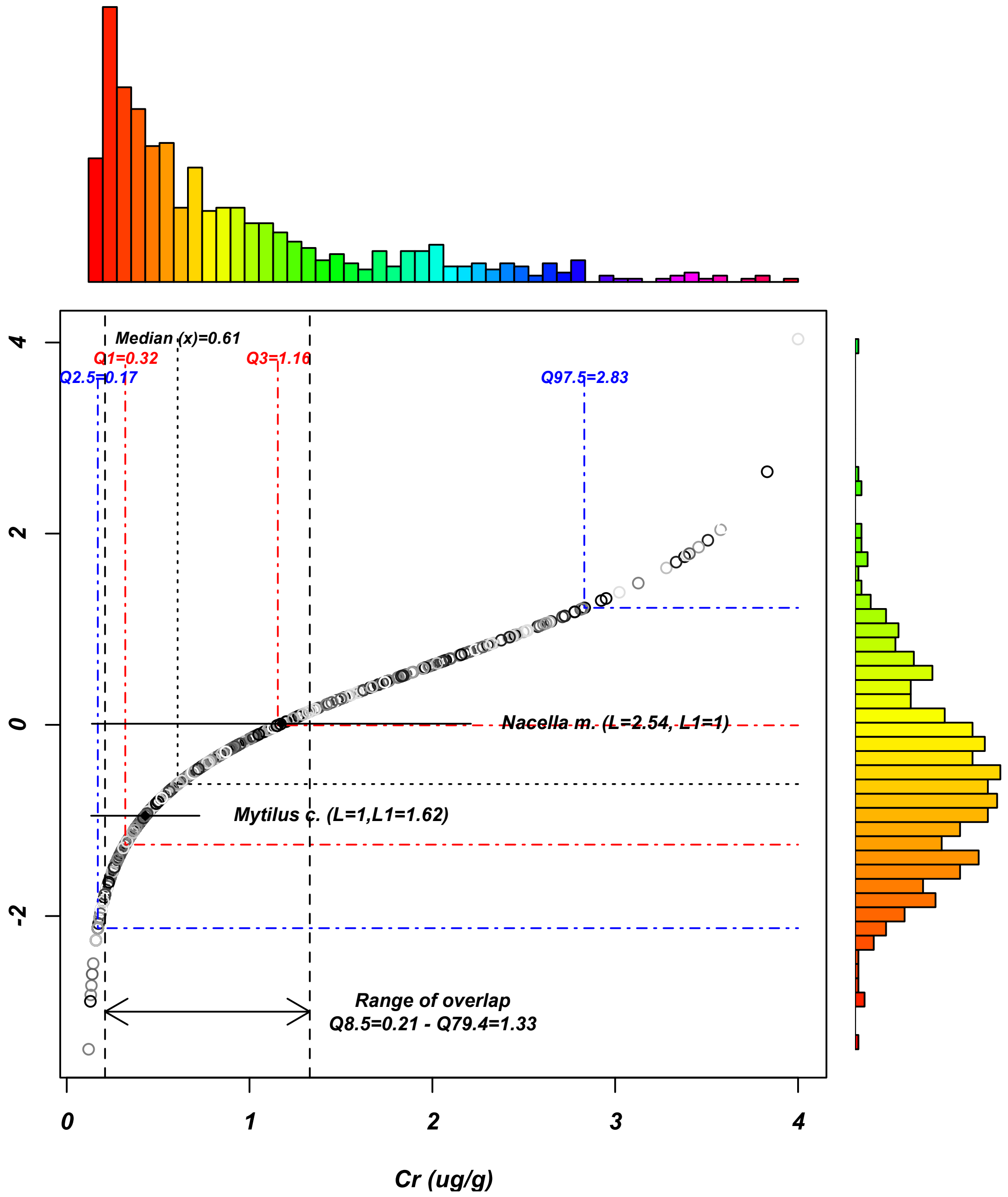

Figure 3. Control chart for Cr built for the two selected biomonitors with their obtained overlap metal concentrations (µg/g d.w.).

Figure 4 shows that the Cu concentrations detected for *Mytilus* are lower than the overall median and bioaccumulate in a similar range of Cu concentrations with respect to *Nacella*. The species showed low range variability for Cu bioaccumulation. The medians for the two species were significantly different ($\chi^2(1)=26.83$, $p<0.001$). The limits of the overlap range were 6.8 and 93.5 percentiles, and they constitute approximately 87% of the total data. The OBI (Table 3) show that *Nacella* has high bioaccumulation Cu surplus (OBI-L= 3.18) better responding to higher Cu concentrations in seawater.

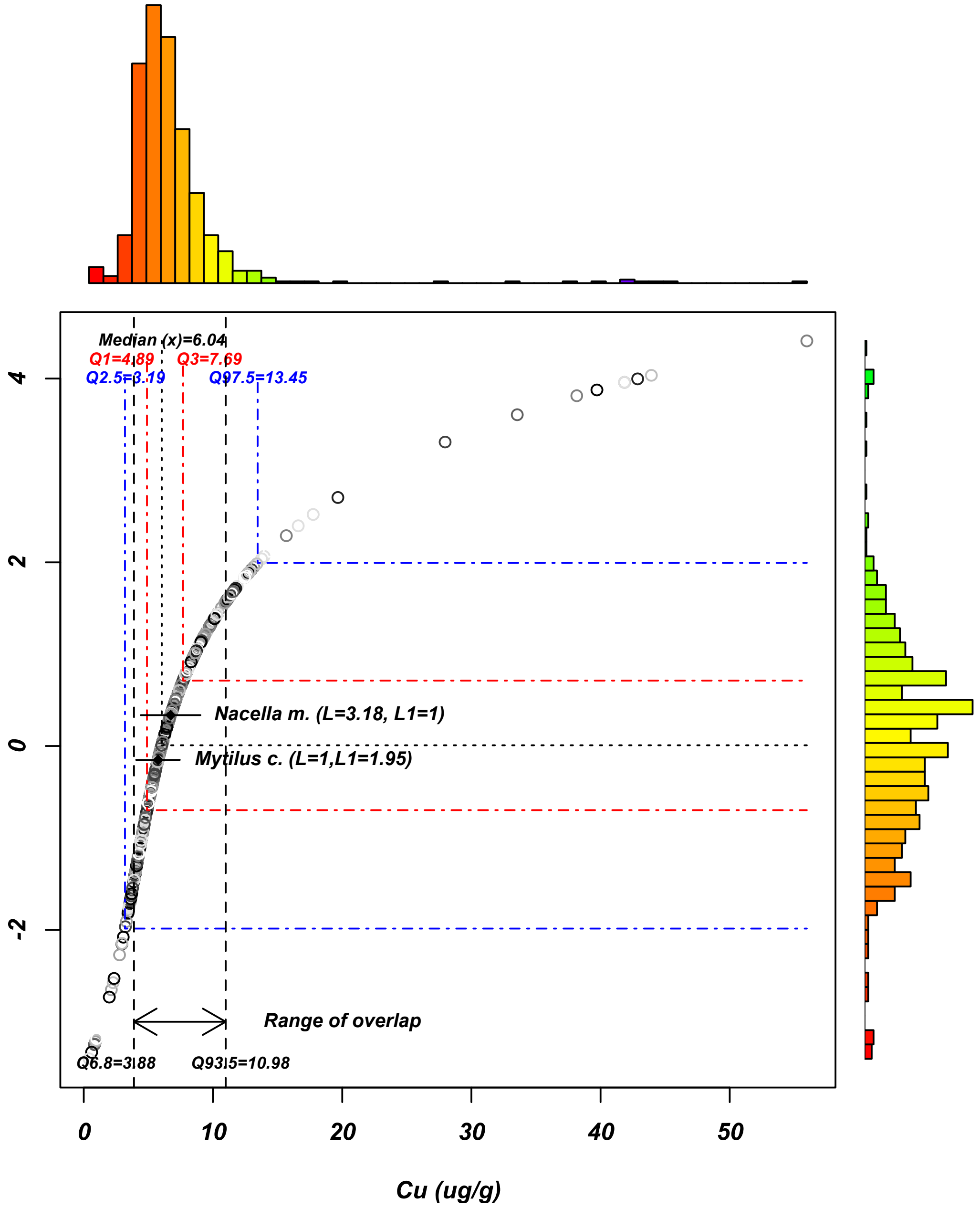

Figure 4. Control chart for Cu built for the two selected biomonitors with their obtained overlap metal concentrations (µg/g d.w.).

Figure 5 shows that the median Ni concentrations detected for *Mytilus* are lower than the overall median and lower than *Nacella* which is higher than the Q3 quartile which considers the distribution of the two biomonitors. Similarly to the other metals, *Mytilus* bioaccumulates in a narrow range of concentrations. The medians for the two species were significantly different ($\chi^2(1)=321.5$, $p<0.001$). The limits of the overlap range were 11 and 93.5 percentiles, and they constitute approximately 52% of the total data. The bioaccumulation indexes (Table 3) show that *Nacella* has high Ni bioaccumulation surplus (OBI-L= 3.64).

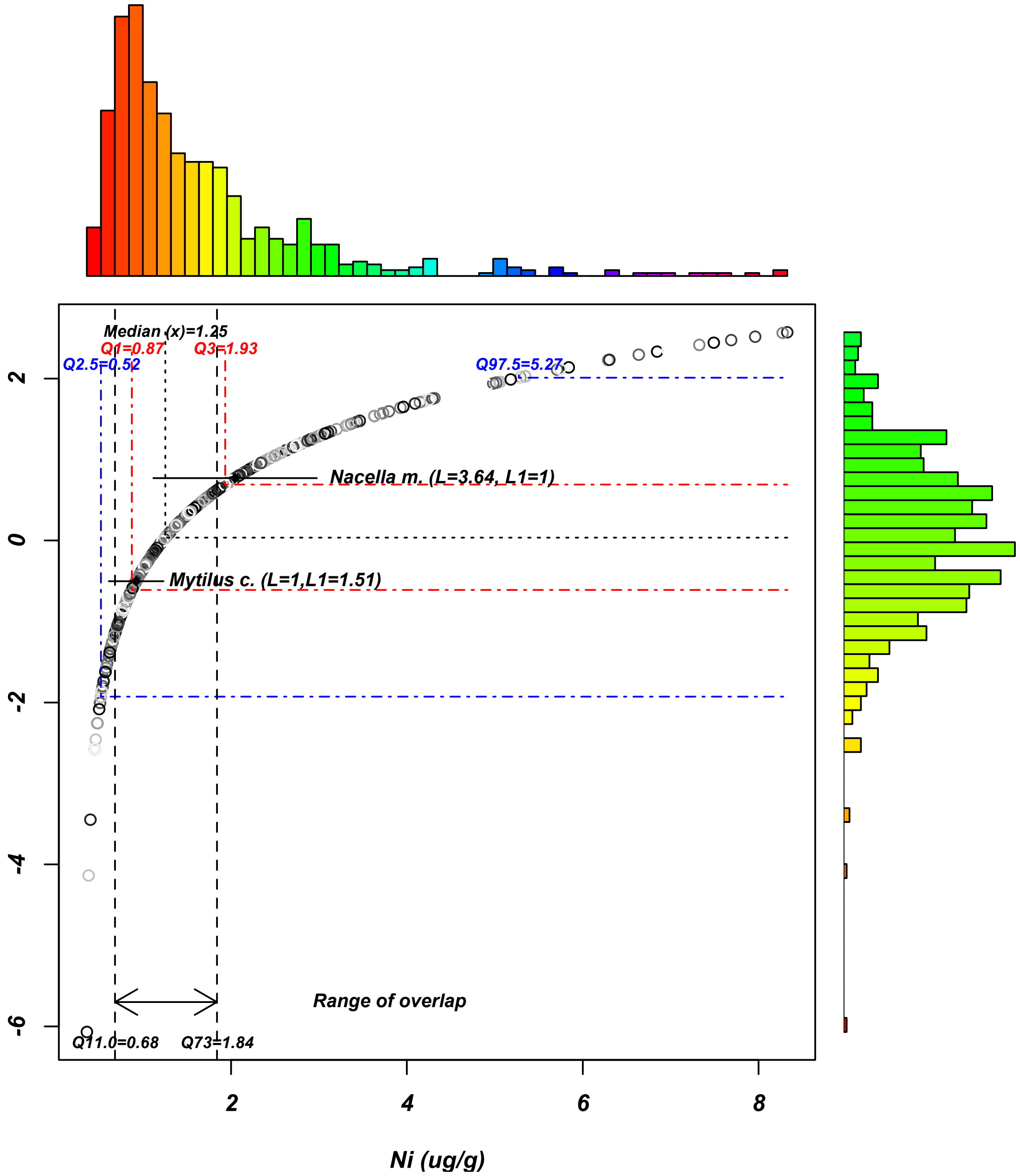

Figure 5. Control chart for Ni built for the two selected biomonitors with their obtained overlap metal concentrations (µg/g d.w.).

Figure 6 shows that the median Pb concentrations detected for *Mytilus* are slightly higher than the overall median and also higher than *Nacella*. However, the Pb medians were not significantly different (χ^2(1)=0.93, n.s.) The limits of the overlap range were 17 and 92.5 percentiles, and they constitute approximately 75% of the total data. The Pb OBI shows that the two species have a similar bioaccumulation Pb pattern and it is strictly connected with the wide overlap range obtained (Table 3, Figure 6).

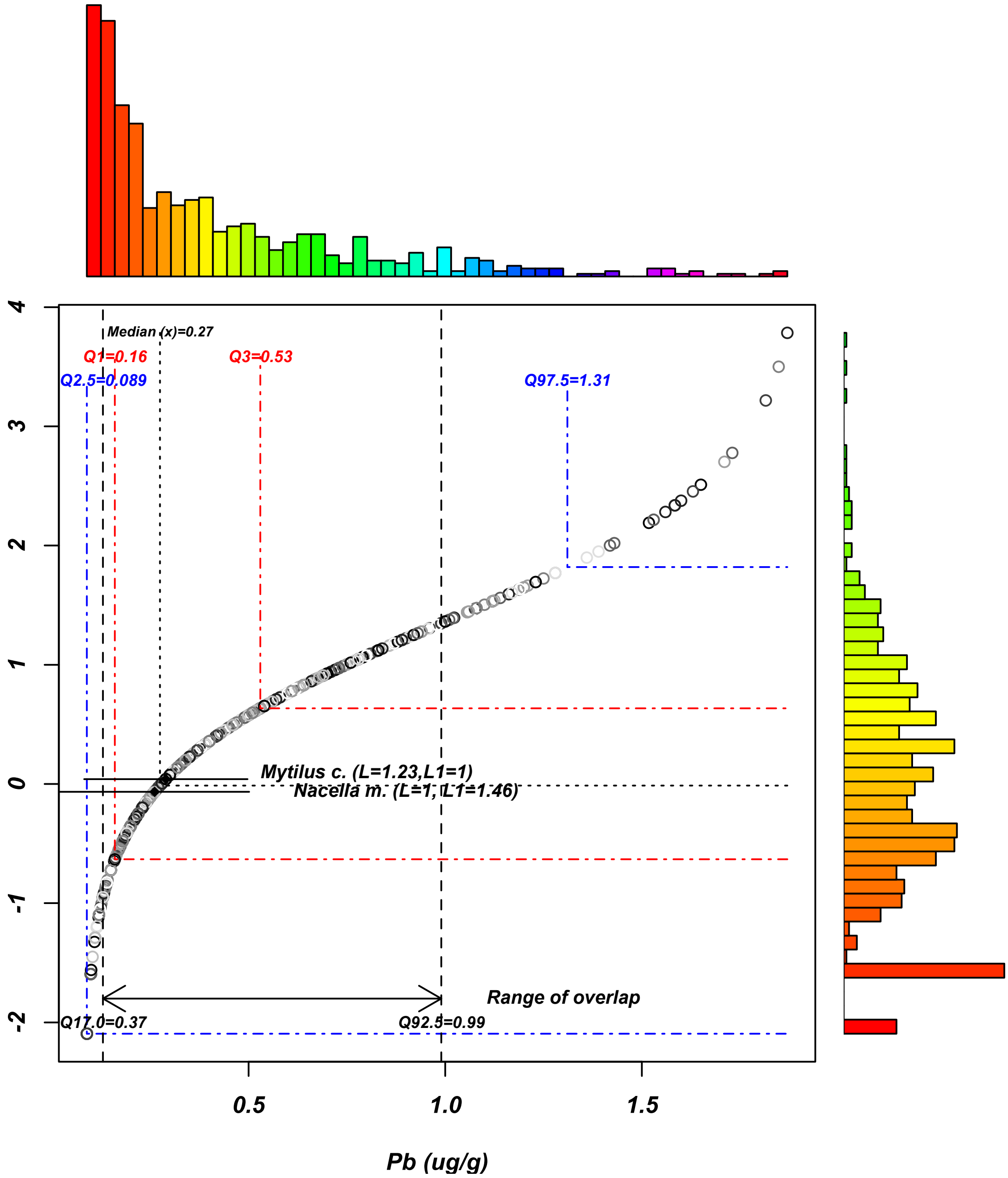

Figure 6. Control chart for Pb built for the two selected biomonitors with their obtained overlap metal concentrations (µg/g d.w.).

Figure 7 shows that the median Zn concentrations detected for *Mytilus* are higher than the overall median and also higher than *Nacella*; the Zn medians were significantly different (χ^2(1)=171.3, p<0.001). The limits of the overlap range were 17 and 48 percentiles, and they constitute approximately 31 % of the total data. The Zn OBI-L (Table 3, Figure 7) show that *Mytilus* has high bioaccumulation Zn surplus (OBI-L= 3.61).

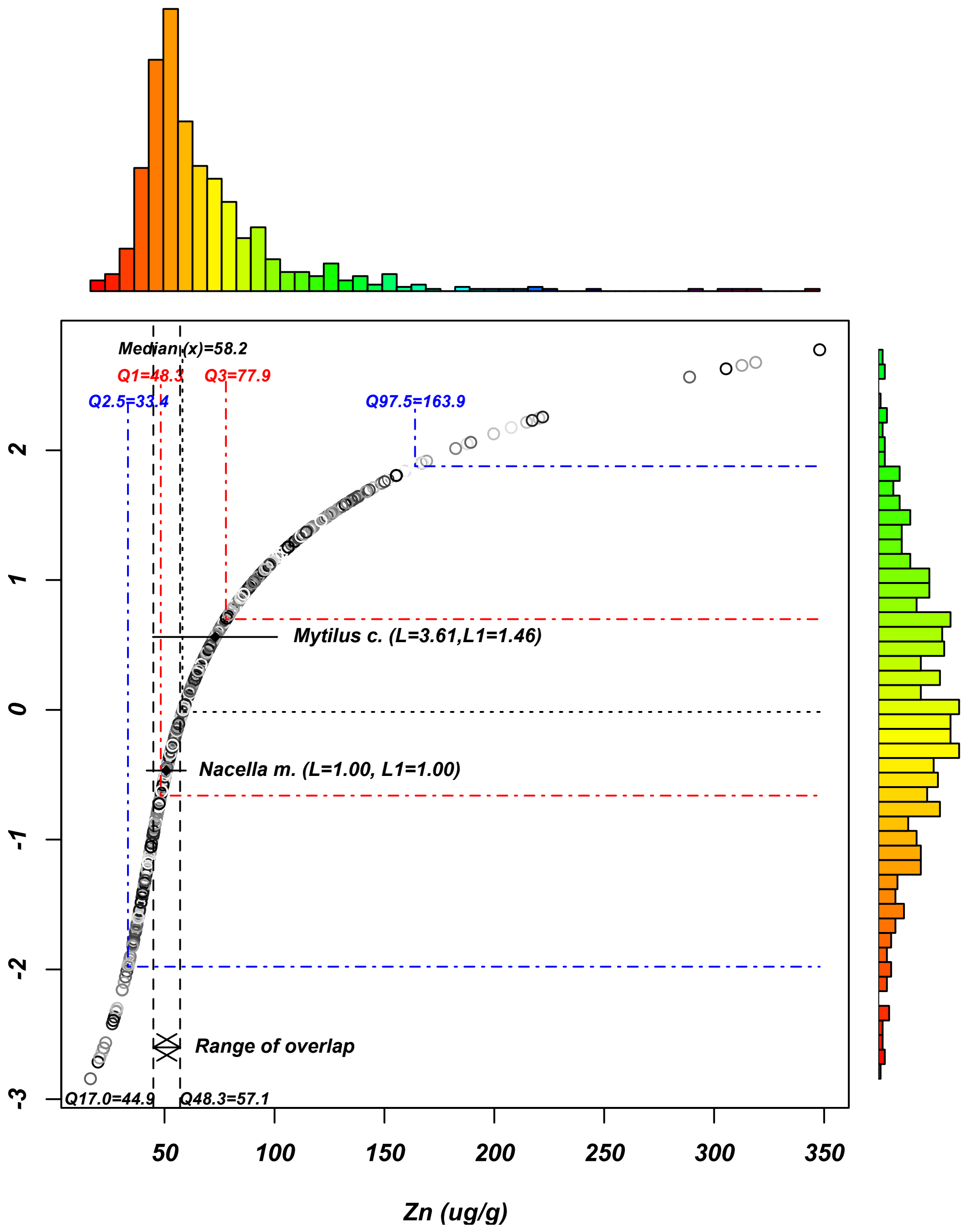

Figure 7. Control chart for Zn built for the two selected biomonitors with their obtained overlap metal concentrations (µg/g d.w.).

In this context, a matter of debate is the explanation of the atypical behavior of the bioaccumulation patterns of some metals, such as Cd, which showed high median levels of bioaccumulation. These can be linked with metal biogeochemistry in coastal waters (Price and Morel, 1990). The upwelling coastal currents push Cd and nutrients arising from the oxidation process of the organic matter to the superficial layer of the aquatic medium. Then, Cd is accumulated by organisms from the superficial layer in the ocean. The upwelling coastal currents were proposed as a mechanism responsible for the regulation of Cd in coastal environment (Geen and Husby, 1996).

From these results we can draw some relevant findings:

i. *Nacella* showed high OBI-L values for Cd (4.98), Cr (2.54), Cu (3.18) and Ni (3.64) (Table 3 and Figures 2, 3, 4, 5 respectively), demonstrating its strong ability to accumulate these metals from seawater. In particular, for Cd, this study confirms that patellid limpets (i.e. *Nacella* and *Patella caerulea*) are excellent biomonitors for Cd in seawater, as also confirmed by the high Concentration Factors (CFs) levels obtained in our previous studies in Mediterranean areas (Conti et al., 2010);

ii. these results confirm the high aptitude of *Mytilus* as a good biomonitor for Zn (OBI-L = 3.61) as also reported for other coastal areas from Korea (Kim and Choi, 2017), and from Mediterranean areas where high Zn CFs with respect to soluble metal concentrations in seawater have been reported (i.e. CFs = 27,000, Conti and Cecchetti, 2003);

iii. *Mytilus* showed high Cd values for the OBI-L1 (i.e. 5.16, Table 3, Figure 2) which means that this species is highly sensitive to a very low variation of the Cd levels in seawater (i.e. about five times with respect to the lower bound of the overlap range). Good OBI-L1 values were instead obtained for *Mytilus* for Cr and Cu (Table 3, Figures 3 and 4 respectively);

iv. moreover, the Pb OBI indexes showed that the two species have similar bioaccumulation Pb patterns, i.e. the two species can be used indifferently for Pb bioaccumulation assessment.

3.2. Supporting the management of the marine ecosystems: the OBI index and the perspective of complexity.

Environmental management typically faces complex problems (i.e. problems featured by a high number of interdependent variables, with nonlinear relationships among them and uncertainty) (Reed, 2008: 2418; Ciasullo et al., 2014).

Going deeper, environmental management issues are featured by the following critical dimensions. Firstly, they do not have a univocal formulation: the information selected, organized and exploited to understand an environmental problem depends upon one’s set of values (culture) and upon one’s knowledge capacity for solving it (Barile, 2009). Problem setting, problem understanding and problem-solving are strictly intertwined: e.g. the pollution problems according to the neoclassical vs the ecological perspective. Neoclassicals support the efficiency standard (connected with profit), while ecological economists support the safety and sustainability standards claiming for more stricter rules for protecting the environment and people's health.

Secondly, the environmental management issues usually do not allow a best (i.e. optimal) solution, as they allow just a "satisficing" solution. That is to say: to describe an environmental problem adequately, one has to develop a comprehensive inventory of all conceivable solutions ahead of time, but this inventory is simply not available because of the complexity of the environmental system itself.

Thirdly, with environmental management, any decision, after being implemented, will cause several unpredictable feedbacks over time and space. The full feedbacks cannot be foreseen at all until the effects have entirely run out.

According to the previous description, -and compared to the antithetical "tame" problems -, environmental management issues configure typical "wicked" problems (Rittel and Webber, 1973).

And for all the above reasons, environmental management is difficult to define (Burrow, 2005: 4).

Moving from the seminal work by Leopold (1939), environmental management could be defined at the same time a vision and a wide endowment of knowledge, scientific principle and tools supporting the related decisional processes. One of the main purposes of environmental management is to provide proactive or preventive decisions and measures that contribute to maintain the sustainability of the relationship between human being activities and natural environment for a long range. In particular, crucial is the goal of protecting biological complexity, which has become the keystone of the more recent theory on environmental management (Costanza et al., 1993: 26).

According to this, biomonitoring is an essential critical dimension of the environmental management meant as decisional processes, frameworks, methodologies and tools focused on the complex environmental issues. In fact, biomonitoring is a wide consistent set of knowledge endowment (theoretical principles, methodologies, tools, standard, empirical data etc.) able to provide relevant information supporting the heterogeneous decisional processes (at macro, medium and micro level of the socio-economic system) related to the environment. Moving from this view/conceptualization/approach, and rooting in the complexity perspective, this section discusses the role of the OBI index as a tool to integrate the extant set of the biomonitoring knowledge endowment.

The use of molluscs as biological indicators of trace metal in marine geographical areas is well-known. However, their intrinsically different suitability to play their role as bioindicators, according to the different level of metal concentrations in seawater, should be better stressed. In fact, from the environmental management point of view -and biomonitoring is, of course, an essential, critical dimension of the environmental management-, this could lead to a necessary further level of knowledge in this field. As we described in the previous sections by the OBI index, the two selected biomonitors (*Nacella* and *Mytilus*) show a different level of capabilities in giving a feedback according to the metal, its speciation, the presence of concomitants (i.e. other metallic species), and its concentration in seawater (Muse et al., 2006). In particular, the OBI index enlightens the following useful rule for an effectively (i.e. successfully) management of the molluscs as metal biomonitors in marine ecosystems. As described above, the two species have a similar performance inside the overlap range of metal concentrations. On the contrary, outside of the overlap range they provide different responses, i.e. OBI-L1 values higher than 1.0 suggest high sensitivity to low seawater metal concentrations (unpolluted sites). Likewise, values of OBI-L higher than 1.0 suggest high sensitivity to high concentrations (polluted sites). The OBI index and its relative guidelines have both theoretical and practical implications as follows.

Firstly, marine ecosystems are complex systems. Roughly, by a complex system, we mean "one made up of a large number of parts that interact in a nonlinear relationship. In such systems (biological and physical systems, social systems, symbolic systems etc.), the whole is more than the sum of the parts, not in an ultimate, metaphysical sense, but in the important pragmatic sense that, given the properties of the parts and the laws of their interaction, it is not a trivial matter to infer the properties of the whole. In the face of complexity, as in-principle reductionist may be at the same time pragmatic holistic" (Simon, 1962: 468). A complex system shows a surprising and hard to predict (i.e. uncertain) behavior because it is nonlinear (Casti, 1994; Anderson, 1999: 217; Faggioni and Simone, 2011). Its components interact with one another via web of feedback loops and the change of one or two parameters a small amount can severely change the behavior of the whole system, and the whole can be very different from the sum of the elements. For these reasons, in a complex system, it is often very difficult to predict what will be the final output because the causal links are ambiguous (causal ambiguity). The uncertainty is connected with the fact that in complex systems cause and effect are not closely related in time and space; the output loses the direct causal relationship with the input, and the effects of an input may occur on very different time horizons. Because of bounded rationality (Simon, 1947; 1978) often these feedbacks are distant in time and space and cannot be predicted and calculated (unexpected results). There is frequently a lag time between a short-term advantage and a long-term disadvantage and because of bounded rationality it has not been possible to predict that long-term disadvantage. So unexpected and unwanted outputs occur, and they usually take too long time to observe and to react to.

All of these qualities of the complex system—heterogeneity in the parts, richness of interaction between them and uncertainty—have the same implication: the quantities of information that flow, either from system to observer or from part to part, are very high and the observer risks to not possess all the information to understand the complex system and its dynamic. And it is because the quantities are large that the limitation is likely to become dominant in the selection of the appropriate scientific strategy to study those complex system. According to the Ashby's Law (1957, 1958), the understanding of a complex system (requisite variety) depends on the information variety endowment owned by the observer. In other words, in order to understand and effectively manage a complex system, the more the complexity of the system under focus (expressed in terms of its variety) increases, the more the level of the information variety (i.e. richness, diversity of the information endowment) possessed by the observer/decision maker must increase. The recognition of the limitation implied by the law of requisite variety "may, in time, also prove useful, by ensuring that our scientific strategies for the complex system shall be new strategies, genuinely adapted to the special peculiarities of the complex system" (Ashby, 1958: 13). According to this, modeling the method is a problem itself, because formulating a method in a certain way leads to different results than formulating it in another. Using certain lenses and not others, making use of certain cognitive frame and not others leads to different results and generates different inputs for decision makers.

Again, modeling the method is a wicked problem: its setting and usefulness depend upon the self-confidence (information endowment) of the observer.

Thus, a primary dimension of complexity of the marine ecosystems management (applying, calibrating and calculating, controlling, tuning and interpreting) is closely associated with the employed environmental models.

The OBI index enhances the observer's information variety about the performance of the molluscs as metal biomonitors in marine ecosystems: it gives further helpful feedback (i.e. information) about the specific attitude of each species of molluscs to detect specific metals in marine waters. In so doing, it enriches the information endowment about the potential performances of molluscs as biomonitors. The OBI index alerts that the choice of mollusc species is not neutral to the aim to effectively biomonitoring (i.e. managing) marine ecosystems. In other words, the selection of the mollusc species is a critical decisional process that in turns involves management of information (searching, collecting, organizing, interpreting data, etc.) and that asks for a problem solving (i.e.

finding a solution that is- even not the best- at least satisficing). In fact, the OBI index aims to support those decisional processes with the purpose to have more reliable results about the marine metal pollution. This conceptualization leads to focus the attention on the level of fit between the exploitable information variety provided by the OBI index and the specific requisite variety needed at a local level (i.e. in a particular context) to successfully manage the environment. In so doing, the proposed conceptualization also promotes helpful reflections on the potential gap between the information variety provided by the OBI index and the requisite variety asked at a local level. In particular, we propose to conceptualize the wide set of biomonitoring knowledge endowment as an open and evolutionary endowment of information variety supporting the management of the environment (Figure 8). The more this endowment becomes rich in variety, the more the observer/decision maker is provided by the requisite variety to face the complex challenges related to the management of the environment.

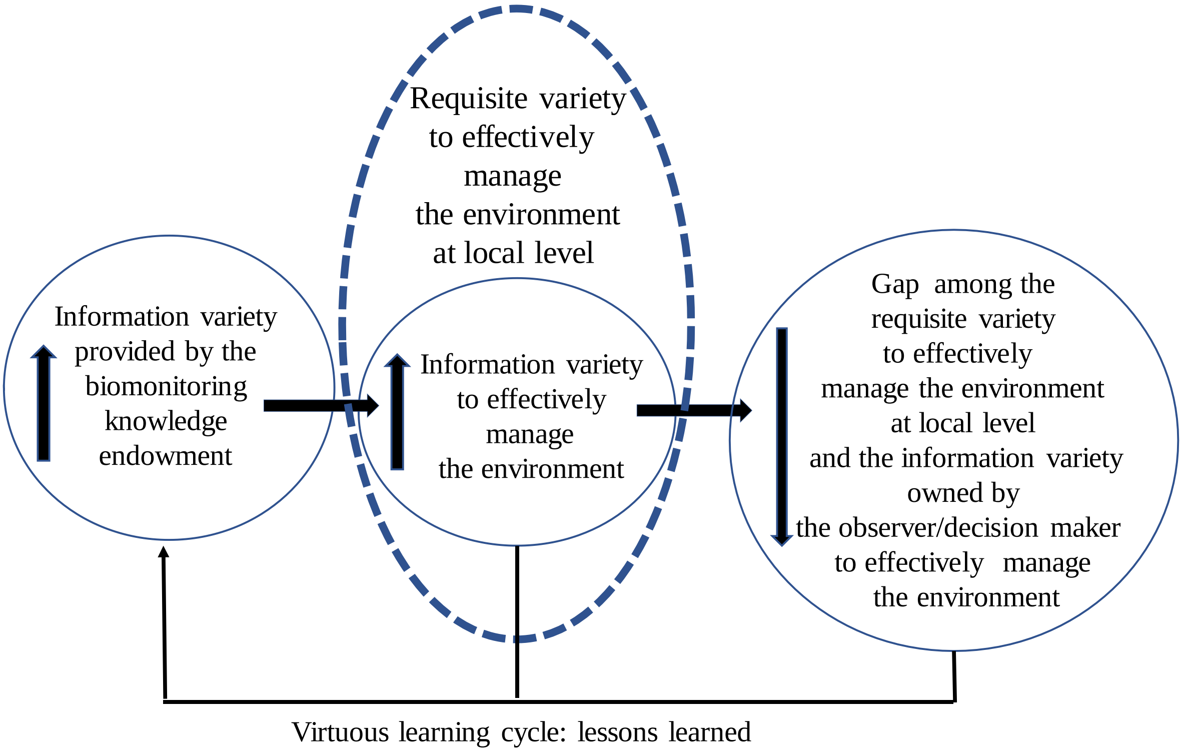

Figure 8: the virtuous cause-effect relationship among the increase of the information variety and the decrease of the gap between the requisite variety at a local level and the information variety owned by the observer to successfully manage the environment at local level. *Source*: our elaboration.

Conclusions

From this study, we can make several inferences and conclusions. By means of Johnson's probability method, we built the quality control charts for Cd, Cr, Cu, Ni, Pb and Zn in *Mytilus chilensis* and *Nacella* (P) *magellanica* in order to define the range of overlaps of metal concentrations between the two selected species. By means of this approach, we can find the overlap bioaccumulation index (OBI) with respect to the lowest (OBI-L1) and the highest (OBI-L) extreme values of the overlap metal concentrations range. The OBI is here, for the first time, applied to a baseline data collected in four sampling campaigns (i.e. 2005, 2007, 2011, 2012) in the same referenced sites in Tierra del Fuego, Beagle Channel (south Patagonia, Argentina). The OBI can be used as an integrative tool in the management of unpolluted or polluted marine ecosystems; it consents to identify the most suitable organisms for managing several environmental conditions where an ecosystem quality control is needed.

For Cd, Cr, Cu, and Ni, *Nacella* showed high OBI-L values that suggest its use as a biomonitor for prevalently polluted marine ecosystems. In particular, Cd for *Nacella* showed the highest OBI-L values suggesting that *Nacella* can be used as biomonitor in contaminated marine ecosystems were high Cd levels in seawater are suspected.

On the contrary, *Mytilus* showed high Cd values for the OBI-L1 which means that this species is highly sensitive to a very low variation of the Cd levels in seawater. Good OBI-L1 values were obtained for *Mytilus* for Cr and Cu showing the good aptitude of these organisms to detect minimum variations of trace metals concentrations in seawater. The OBI-L1 index can be employed prevalently in unpolluted marine ecosystems as a preventive signal of alarm when the contamination process is in its early stages.

The OBI index has both theoretical and practical implications in environmental management. It can be exploited, for instance, in environmental prevention from events such as oil spills or other marine disasters.

Marine ecosystems are complex systems showing heterogeneity in the parts, richness of interaction among them and uncertainty, and have the same implication: according to the Ashby’s Law (1957, 1958), the understanding of a complex system (requisite variety) depends on the information variety owned by the observer. The OBI index increases the observer’s information variety about the performance of the molluscs as metal biomonitors in marine ecosystems. In this study, we propose to conceptualize the wide set of biomonitoring knowledge endowment as an open and evolutionary endowment of information variety supporting the management of the environment.

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