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Baseline metals concentration in the sea star *Anasterias minuta* in San Matías Gulf, Atlantic Ocean

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Abstract: Environments contaminated with metals can induce accumulation of trace metals in soft tissues of marine fauna resulting in negative effects. The oral brooding sea star *Anasterias minuta* is one of the most conspicuous macro-invertebrate top-predator species in the Atlantic Patagonian rocky shores that could accumulate trace elements and be used as biomonitor of coastal metal pollution. The aim of this study was to quantify metals concentrations on gonads of *A. minuta* populations in an anthropogenic impacted and in a natural protected area. Sea stars *A. minuta* were collected from two rocky intertidal populations in San Matías Gulf, near an iron-ore loading wharf at Punta Colorada (PC), and in the Natural Protected Area Puerto Lobos (PL), located 35 km south to PC. The essential metals Fe, Mn, Zn, Cu, Cr and Ni, and the non-essential metal Cd and As were present in gonads of *A. minuta* from both populations. However, the essential metal Co and the non-essential metal Pb were only detected in the Natural Protected Area PL, with values below to quantification limit at PC. As high metal concentrations can impact negatively on sea star populations and shore habitats, this metal survey obtained from gonads of *A. minuta* populations could be useful as a reference to start a monitoring program in urbanized and marine protected areas.

Keywords: Metals concentration, Asteroidea, Atlantic Patagonia, Anthropogenic impact.

Resumen: Concentración de metales de referencia en la estrella de mar *Anasterias minuta* en el Golfo San Matías, Océano Atlántico. Los ambientes contaminados con metales pueden inducir a la acumulación de metales trazas en los tejidos blandos de la fauna marina, resultando en efectos negativos. La estrella de mar incubadora *Anasterias minuta* es una de las especies de depredadores superiores de macroinvertebrados más conspicuas en las costas rocosas de la Patagonia Atlántica que podría acumular elementos traza y usarse como biomonitor de la contaminación costera por metales. El objetivo de este estudio exploratorio fue cuantificar las concentraciones de metales en las gónadas de poblaciones de *A. minuta* en un área con impacto antrópico y en un Área Natural Protegida. Las estrellas de mar *A. minuta* fueron recolectadas de dos poblaciones intermareales rocosas del Golfo San Matías, cerca de un muelle de carga de mineral de hierro en Punta Colorada (PC), y en el Área Natural Protegida Puerto Lobos (PL), ubicada 35 km al sur de PC. Los metales esenciales Fe, Mn, Zn, Cu, Cr y Ni, y los metales no esenciales Cd y As estuvieron presentes en las gónadas de ambas poblaciones de *A. minuta*. Sin embargo, el metal esencial Co y el metal no esencial Pb fueron solo detectados en el Área Natural Protegida PL, con valores inferiores al límite de cuantificación en PC. Dado que las altas concentraciones de metales pueden tener un impacto negativo en las poblaciones de estrellas de mar y los hábitats costeros, estos datos de metales obtenidos de las gónadas de las poblaciones de *A. minuta* podrían ser útiles como referencia para comenzar un programa de monitoreo en áreas urbanizadas y marinas protegidas.

Palabras claves: Concentración de metales, Asteroidea, Patagonia Atlántica, Impacto antropogénico.

INTRODUCTION

Metals are natural components of the environment, frequently present as trace elements of continental rocks, water column, in soil, or available through food chains (Elberling *et al.*, 2003). Some metals are essential elements in living organisms, acting as cofactors for many enzymes and for stabilizing structures of proteins. Nevertheless, metal concentrations and their potential toxic effects may be anthropogenically enriched, mainly if industrial and urban wastes are discharged directly on the seabed (Loring & Asmund, 1989; Amin, 1995; Elberling *et al.*,

2003; Prashanth *et al.*, 2015). Metal mining activities generate serious environmental problems due to the generally low solubility of contaminants in sea water and their accumulation, mainly in sediments with negative effects over benthic organisms living there (Elberling *et al.*, 2003; Danis *et al.*, 2004; Benedicto *et al.*, 2008). Increased non-essential metals concentrations seem to reduce reproduction, behavior (Furness & Rainbow, 1990; Rhora, 2005), and have effects in skeletal morphogenesis (Temara *et al.*, 1997) in some marine species. Consequently, marine fauna accumulate trace metals in soft body tissues reaching higher concentrations than environmental levels (De Moreno *et al.*, 1997; Vázquez *et al.*, 2007; Idaszkin *et al.*, 2017), while toxicity could occur when the rate of metal uptake exceeds the combined rates of detoxification and excretion (Rainbow, 2007). Coastal species are more vulnerable to anthropogenic disturbances than those from offshore (Thompson *et al.*, 2002). This is because intertidal ecosystems are more prone to direct interaction with physical and chemical alterations of the habitat, such as city waste discharges.

Sea stars are widely distributed around the world with ca. 1900 species grouped into 36 families (Pawson, 2007; Mah & Blake, 2012). Sea stars occupy meaningful ecological roles, such as the North Pacific *Pisaster*, whose influence in the structure of benthic communities on rocky shores is well known (Paine *et al.*, 1985; Mah & Blake, 2012). In the last two decades, sea stars have been used as bioindicators in the evaluation of anthropogenic waste fluxes, such as heavy metals (Temara *et al.*, 1998; Den Besten *et al.*, 2001; Danis *et al.*, 2004, 2006). High contaminant levels by industries emissions, such as persistent organic pollutants, nutrients, oils and heavy metals (Islam & Tanaka, 2004), can lead to DNA damage, abnormal embryonic development, reproductive inhibition, and impaired offspring quality (Den Besten *et al.*, 1989; Trieff *et al.*, 1995; Au *et al.*, 2001a, b; Yang & Xiong, 2015), which can, in turn, affect sea stars populations (Temara *et al.*, 1998; Danis *et al.*, 2006). Abnormalities in sea stars populations, such as deviations from pentamerism, suggest environmental perturbations on the metamorphosis of larvae or abnormal regeneration of arms (Hotchkiss, 2000; Kolandhasamy & Subramanian, 2012; Maheswaran *et al.*, 2015; Arribas *et al.*, 2017).

The oral brooding sea star *Anasterias minuta* Perrier, 1875 is one of the most conspicuous macro-invertebrate top-predator species in the Atlantic Patagonian rocky shores, which preys upon a wide range of organisms (Gil & Zaixso, 2008; Brogger *et al.*, 2013; Arribas *et al.*, 2017). In the last fifty years, Patagonian coasts suffered an increase in anthropogenic population, tourism, industries, and maritime traffic (Yorio *et al.*, 2001; Commendatore & Esteves, 2007; Chomnalez, 2011; Márquez *et al.*, 2017; Primost *et al.*, 2017). Playas Doradas is a recreational area in Río Negro Province (Atlantic Patagonia, Argentina) located in the San Matías Gulf. The area has a mineral port, located south to the town, which used to distribute iron by ships and throw away metal wastes, as iron pellets to the marine environment (*pers. obs.*). Although the iron mine "Hierro Patagónico Rionegrino S.A." (HIPARSA) is inactive since November 2016, an open-pit iron ore deposit of ca. 5 thousand tons remains near the pier of Punta Colorada (Zanettini, 2008). Therefore, the volatility of the continental dust (Paparazzo *et al.*, 2018), such as iron deposit, added to the strong winds in the area (Genchi, 2012) could affect coast populations and ecosystem, representing a serious risk to the flora and fauna (Gurzau *et al.*, 2003). Puerto Lobos was declared Natural Protected Area in 1998. It used to be an old wool jetty now used as touristic and recreational activities related to whale, bird and sea lion watching, and artisanal fishing (Zanettini, 2008; Morsán & Ciocco, 2011).

The aim of this exploratory study was to determine for the first time the essential and non-essential metals concentrations in gonads of the rocky intertidal sea star *A. minuta* from two populations of different anthropogenic impact, near the iron-ore loading wharf Punta Colorada (PC) and in the Natural Protected Area Puerto Lobos (PL), located 35 km south to PC.

MATERIAL AND METHODS

Study site and sampling populations

The survey was conducted in two rocky intertidal shores ca. 35 km separated from each other in the south of the San Matías Gulf, Atlantic Patagonia: Punta Colorada (41°42' S – 65°1' W, hereafter PC, Fig. 1A) near Playas Doradas town, and the Natural Protected Area Puerto Lobos (41°57' S – 65°4' W, hereafter PL; Fig. 1B). Both rocky outcrops belong to a group of volcanic and marine sediments with large pyroclastic contribution (Kokot *et al.*, 2004). At PC shore, there is a 1,000 meters length ore wharf that was used to distribute iron from Sierra Grande Mine (MCC S.A.) to the dock (Zanettini, 2008), where

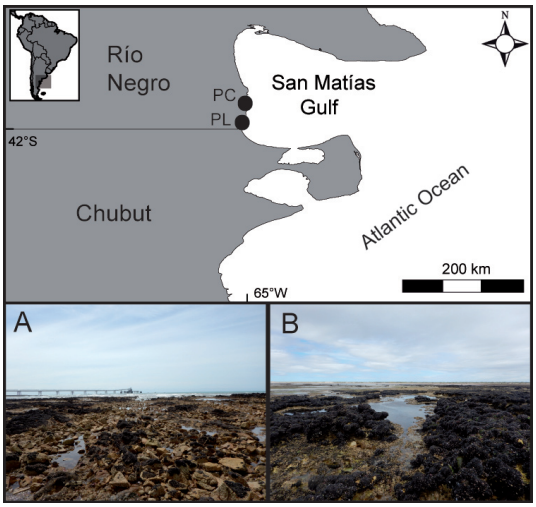

Fig. 1. Sampling sites (A) Punta Colorada iron-ore loading wharf (PC) and (B) the Natural Protected Area Puerto Lobos (PL) on the SW Atlantic coast (Argentina).

a 1,500 meters conveyor belt can load ships with a maximum of 2,000 tons per hour. This pier was active until a few months before our survey.

Sea stars of similar size were collected from low intertidal level at PC (ca. 400 m south of pier, n = 21) and PL (ca. 35 km south of pier, n = 18) in the austral summer of 2017, when gonads reach maturity in both sexes before the brooding season (Gil *et al.*, 2011; Pérez *et al.*, 2015). Sea stars size (as the longest arm length R) was measured with Vernier calipers (± 0.01 mm) and weighted to calculate wet biomass (± 0.1, g m^{-2}). Individuals were stored in refrigerated plastic containers and transported to the laboratory. Metals concentrations were measured in *Anasterias minuta* dry gonads at both localities. Gonads of *A. minuta* were dissected and lyophilized to be digested in a Novawave microwave Digestor to quantify metal levels (μg g^{-1} ± SD). The measurements were made on an inductively coupled plasma optical emission spectrometer (ICP-OES) Agilent 720 (Marinho *et al.*, 2018). To achieve the minimum recommended sample in the microwave digestor (dry weight ca. 0.5 g), a random pooled of among two and four individuals were carried out (eight and six replicates were analyzed at PC and PL, respectively).

RESULTS

Sea stars mean R size (± SD) and wet biomass (± SD) from the population at PC was 26.1 ± 3.1 mm and 5.7 ± 1.4 g, respectively. At PL, sea stars population presented a mean R size of 24.8 ± 1.9 mm and a wet biomass of 5.3 ± 1.4 g. Sea stars collected did not show significant differences between R sizes (t-test, t= 1.89, p = 0.065) or wet biomass (t-test t = 0.76, p = 0.45) between populations at both localities.

Similar metals were found in gonads of populations of the sea star *Anasterias minuta* at both localities (Table 1). However, the essential metal Co and the non-essential metal Pb were only detected in the gonads of the *A. minuta* population of the Natural Protected Area PL, and were below to the quantification limit in all gonads sampled at the iron-ore loading wharf PC. Otherwise, the essential metals Fe, Mn, Zn, Cu, Cr and Ni, and the non-essential metal Cd and As were present in gonads of both sea star populations (Table 1).

DISCUSSION

In our study, essential and non-essential metals were quantified in gonads of both sea star populations separated ca. 35 km away from each other. While similar metal types were found in gonads of the brooding sea star *Anasterias minuta* in both populations, some metals as Co and Pb were only detected in the Natural Protected Area PL, being under the quantification limit in the iron-ore loading wharf PC. Previous studies have shown similar metal compositions with variable values of essential and non-essential metals in tissues of sea stars and their effects over echinoderm populations (Den Besten *et al.*, 1989, 2001; Flammang *et al.*, 1997; Temara *et al.*, 1997, 1998, 2002; Danis *et al.*, 2004, 2006). Negative effects derived from high metal concentrations have been found in marine populations at contaminated areas, such as decrease in abundance and biomass (Menge *et al.*, 2016), retarded embryo development (Trieff *et al.*, 1995; Kobayashi & Okamura, 2004, 2005), and effects in DNA and enzymatic processes (Jakimska *et al.*, 2011a). Experimental studies in the sea star *Asterias rubens* have shown that exposures to high concentrations of essential metals reduced righting time (Sköld *et al.*, 2015), increased numbers of coelomocytes and proliferation of epithelial cells (Oweson *et al.*, 2010), while increased levels of HSC70 in immune cells (Matranga *et al.*, 2012). On the other hand, the increase of non-essential metal concentrations seem to reduce reproduction, behavior (Furness & Rainbow, 1990; Rhora, 2005), and have effects in skeletal morphogenesis (Temara *et al.*, 1997) in some marine species. For example, skeletal material stiffness and tough-

Table 1. Metal concentrations ($\mu g\ g^{-1}$ ± SD) in gonads of the sea star *Anasterias minuta* from two populations, the pier area Punta Colorada (PC, n = 21) and the marine natural protected area Puerto Lobos (PL, n = 18). NA not available, ND below the quantification limit. * Value detected in only one sample

Metals	**PC ($\mu g\ g^{-1}$ ± SD)**	**PL ($\mu g\ g^{-1}$ ± SD)**
Essential		
Fe	21.0 ± 11.7	64.2 ± 47.1
Mn	1.6 ± 0.3	4.9 ± 3.7
Ca	3670.8 ± 1906.0	NA
Zn	65.8 ± 8.7	142.4 ± 172.5
Cu	13.9 ± 1.9	17.2 ± 2.0
Co	ND	0.6*
Cr	1.8 ± 1.0	5.3 ± 7.2
Ni	0.7 ± 0.5	1.9 ± 1.1
Non-essentials		
Cd	3.9 ± 6.5	2.9 ± 0.7
Pb	ND	4.8*
As	27.1 ± 8.9	26.0 ± 2.7
Ba	NA	30.5 ± 48.0

ness of *Asterina rubens* decreased in Pb contaminated areas (Temara *et al.*, 1997; Moureaux *et al.*, 2011). Arribas *et al*. (2017) registered sea stars with abnormal characteristics at the PC locality, whereby deeper experimental and field studies in *A. minuta* are necessary to elucidate the effect of differentially increased environmental metals alongshore in sea star populations using the baseline data obtained at this work, as well as the potential use of this species as bioindicator of contamination.

Mollusks, crustaceans, and echinoderms can act as bioindicators to evaluate the availability of environmental metals. The accumulation degree in tissues depends on the properties of the species, the environmental conditions, and the level in the trophic position (Temara *et al.*, 2002; Jakimska *et al*., 2011b). The accumulation of metals in the animal body is highly dependent on diet, where animals at the top of the trophic pyramid present higher metal levels in their tissues (Jakimska *et al.*, 2011b). For example, ecotoxicologists often use mussels as bioindicator, although they do not always indicate the relationship between tissue and environmental conditions, as is the case of the asteroid *Asterias rubens* in Norway (Temara *et al.*, 2002). In species such as *A. minuta*, which preys upon a wide range of species and the main preys depend on the environmental availability (Gil & Zaixso, 2008; Arribas *et al.*, 2017), future research on metals accumulation should contemplate concentrations focus on several species from the trophic chains, as recommended by Temara *et al.* (2002) and Jakimska *et al.* (2011b), and assess the availability of the metals in the environment where individuals develop (seawater and benthos).

Although environmental data is not available and only gonads were analyzed in the present work, this baseline information of metals in *A. minuta* from two localities (an anthropically impact area and a Natural Protected Area) showed the importance of metals quantification and monitoring of different populations alongshore in a generalist or opportunistic predator species, such as *A. minuta*. In populations that present a brood protection reproductive strategy with low dispersal ability, as *A. minuta* (Salvat, 1985; Gil *et al*., 2011), marine contamination such as uncontrolled industries waste (Pearse *et al.*, 2009) can generate aberrations during the embryonic and larval development (Den Besten *et al.*, 1989; Trieff *et al.*, 1995; Kobayashi & Okamura, 2004, 2005; Glynn & Colley, 2008; Pearse *et al.*, 2009) leading to a decrease in local sea star populations, and a cascade effect in the benthic communities. More studies may strengthen the biological implications of metal contamination on *A. minuta* and their ecological impact on rocky intertidal assemblages in Atlantic Patagonia.

CONCLUSIONS

Even though sea stars could be used as biomonitors of early warning signals of metal pollution and the quality of coastal waters (Temara *et al.*, 1998, 2002), an exhaustive ecological impact and monitoring program should be implemented in the urbanized and natural areas of Patagonian coasts to evaluate the health of marine organisms and their populations, which provide ecosystem services. In addition, it should be evaluated other *A. minuta* organs, such as pyloric caeca (detoxified storage) or the whole sea star, as well as the anatomy of the brooders, seawater and local sediments to explore relationships between concentration detect in the biota and those in the environment.

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