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Osmoregulation of the resident estuarine fish *Atherinella brasiliensis* was still affected by an oil spill (Vicuña tanker, Paranaguá Bay, Brazil), 7 months after the accident

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ABSTRACT

An oil tanker loaded with methanol and bunker oil has exploded in November 2004 in Paranaguá Bay, in front of Paranaguá Harbor, southern Brazil. In order to investigate the chronic effects of an oil spill on a resident estuarine fish, the Brazilian silverside *Atherinella brasiliensis* was sampled 1, 4, and 7 months after the spill, from 2 sites inside Paranaguá Bay, and also from a reference site inside nearby Guaratuba Bay, non-affected by the spill. Increases in plasma osmolality (reaching ~525 mOsm/kg H_2O, or ~70% above values in reference fish) and chloride (reaching 214 mM in site C, or ~51% above values in reference fish) were detected 4 months after the spill, in parallel with branchial carbonic anhydrase inhibition (to 56% of the activity measured in reference fish) in silversides obtained from the contaminated sites. Plasma cortisol concentration increased progressively in samples from fish obtained 4 (462 ng/mL) and 7 (564–650 ng/mL) months after the spill, when compared to values in reference fish (192 ng/mL). Osmoregulation of a resident estuarine fish is still affected by an oil spill, months after the accident. It is, thus, a sensitive tool for the evaluation of the chronic effects of oil spills inside tropical estuarine systems, and *A. brasiliensis* is proposed as an adequate sentinel species for monitoring protocols.

1. Introduction

Teleost fish are excellent osmoregulators, attaining a fair degree of extracellular fluid (ECF) homeostasis, even when challenged with a fluctuating saline regime, such as necessarily happens in estuaries. Estuarine fish are thus especially euryhaline, hyper-regulating at low salinities and hypo-regulating at high salinities, through the transepithelial ion transport function by their gills, gastrointestinal tract, and kidneys (Evans et al., 2005; Marshall and Grosell, 2006). As a result, these organs are especially susceptible to pollutant action (e.g., Skaggs and Henry, 2002; Evans et al., 2005; Monserrat et al., 2007).

The effect of pollutants on the function of osmoregulation in aquatic animals in general has been sparsely investigated (Depledge et al., 1995; Monserrat et al., 2007). When osmoregulatory impairment has been studied, the focus was on the osmoregulation-related enzymes Na^+, K^+-ATPase and carbonic anhydrase (reviewed in Monserrat et al., 2007). Given that osmoregulation is essential for estuarine animals, further studies on the extracellular osmotic homeostasis of estuarine fish are required, especially in relation to oil spills (Katsumiti et al., 2009). Most studies on the effect of pollutants on fish osmoregulation dealt with either freshwater (e.g., ArasHisar et al., 2004), or transient estuarine species (e.g., Pacheco and Santos, 2001; Katsumiti et al., 2009). As estuaries are frequently the site of oil spills, the study of its effects on a resident estuarine fish is relevant.

The Harbor of Paranaguá is located inside the estuarine complex of Paranaguá Bay (Fig. 1). On November 15th, 2004 the Chilean oil tanker Vicuña exploded in front of the harbor, releasing a total of 1416 tons of oil, mostly (87%) of the bunker type. Despite the placement of containment buoys around the shipwrecked vessel, intense contamination of the Bay has ensued, as approximately 285 tons of oil were not recovered and spread through the extension of the Bay (IAP, 2005). Environmental effects of oil spills on coastal areas have been previously evaluated, for example after the Exxon Valdez accident in Alaska in 1989, the Sea Empress accident in Southwest Wales in 1996, or the Prestige oil spill in the Iberian Peninsula in 2002. Investigations on these previous accidents have been carried out within a time window ranging from 2 weeks to 6 years after the spills, using several tissues of both invertebrates and fish. Organisms have been investigated for changes in histopathology, activities of cholinesterase, glutathione-S-transferase (GST), catalase, glutathione reductase (GR), 7-ethoxyresorufin-O-deethylase (EROD), or either bile hydrocarbons, DNA damage, or peroxisomal proteomics, as biomarkers (e.g., Harvey et al., 1999; Martínez-Gómez et al., 2006, 2009, 2010; Fernandes et al., 2008; Tim-Tim et al., 2009). To the best of our knowledge, the only other study focusing on osmoregulation as a biomarker of effect for monitoring an oil spill is that of our group, on the acute effects of this same oil spill on the transient estuarine catfish *Cathorops spixii* (Katsumiti et al., 2009).

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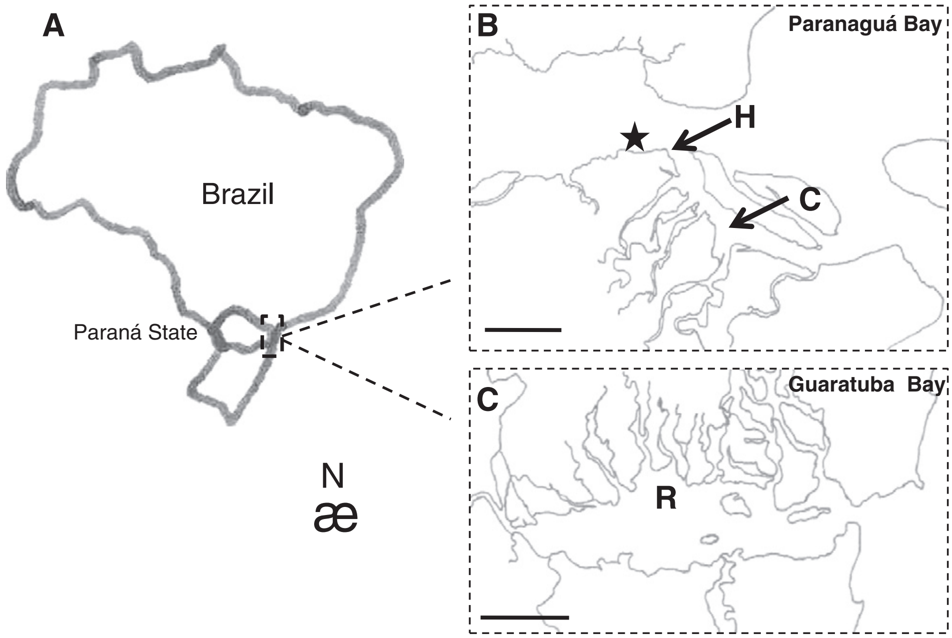

Fig. 1. Map of the study sites located along the southern coast of Brazil (A), in the State of Paraná. The study sites H and C are shown, in Paranaguá Bay (B), and the Reference site (R) is shown in Guaratuba Bay (C). The black star in B indicates the site of the explosion of the Vicuña tanker, and the arrows point to the sampling sites H and C (for Cotinga Island, the island above the site). Scale bars: B and C: 5 km.

This study was conducted under the working hypothesis that the function of osmoregulation of a resident estuarine species would still be affected by a severe oil spill, months after the accident. This has been examined through the analysis of 1) its extracellular osmotic (and chloride) homeostasis, 2) levels of the stress- and osmoregulation-related hormone cortisol, and 3) carbonic anhydrase activity in gills and kidneys. In order to be able to associate possible disturbances with the oil spill, the short-term osmoregulatory capacity of the species was also evaluated.

2. Materials and methods

2.1. Fish species

The Brazilian silverside *Atherinella brasiliensis*, Quoy and Gaimard, 1824, occurs in the Atlantic coast of South America, from Venezuela to Rio Grande do Sul, the southernmost Brazilian State (Nelson, 1994). It is a resident estuarine species, and is very abundant in the Bays of Paranaguá and Guaratuba. The species completes its whole life cycle (from egg to adult) inside the same estuary (Fávaro et al., 2003), and is abundant in shallow areas of Bays, and coastal waters, in brackish waters, especially along the Southeastern-Southern coast of Brazil (e.g., Figueiredo and Menezes, 2000; Pessanha and Araujo, 2001).

2.2. Fish sampling

2.2.1. Paranaguá Bay

The Brazilian silversides *A. brasiliensis* from Paranaguá Bay were sampled in December 16–17, 2004, March 16–17 and June 16–17, 2005, following the effects of the oil spill from the Vicuña explosion for a period of 7 months. Fish were always sampled from 2 sites inside the Bay. Site H (UTM 22J 753669/7177045) was located next to where the explosion took place, in front of the Harbor, while site C (UTM 22J 759406/7172786) was located closer to the exit of the Bay, next to Cotinga Island (Fig. 1). Fish were obtained using picaré nets (seine beach, 30.0 m long × 3.0 m height, 2.0 m mouth diameter, mesh of 0.5 cm between adjacent knots) dragged by 2 men standing at ~1–1.5 m deep water.

Petroleum products have been measured in the sediment and in the water of Paranaguá Bay, at several locations inside the Bay (IAP, 2005), but only until Jan 04, 2005 for the sediment analyses, and until March 1st, 2005 for the water analyses. Eight days after the accident, petroleum hydrocarbons (total of 16 compounds: naphtalene, fluorene, anthracene, acenaphtilene, phenantrene, pyrene, benzo (a) pyrene, among others) measured in the sediment ranged between 33 and 220 ng/g sediment in the area next to the explosion of the tanker (site H), and between 319 and 480 ng/g sediment in the more distant site, site C (IAP, 2005). On Dec 20th, 2004, three days after our first sampling date, values were between 234 and 330 ng/g sediment, in the area covering our two sampling sites (IAP, 2005). In the water, oils and greases, polycyclic aromatic hydrocarbons, and benzene, toluene, xilene, and ethyl benzene were detected from November 16th, 2004 until January 8th, 2005, in the whole area covering both sites. Unfortunately, no follow up of environmental levels of oil derivatives has been conducted, so there are no data available for our second and third sampling dates (in March and June 2005). However, on all sampling dates, oil was still observed along the margins of the Bay, on the vegetation, and on the rocks (Souza-Bastos, personal observation). Salinity in Paranaguá Estuarine Complex ranges between 1 and 33, measured respectively in Antonina Bay and at the entrance of the Complex (Siqueira and Kolm, 2005).

2.2.2. Reference site: Guaratuba Bay

Reference fish were obtained from Guaratuba Bay (site R, UTM 22J 735488/7136974) on April 23–24, 2006. Cast nets (4.0 m mouth diameter, 1.0 cm mesh between adjacent knots) were thrown from a boat, next to a shellfish culture located inside the Bay. Water temperature, salinity, pH, and transparency were always measured, at all fish sampling sites, and were very similar among the dates and sites, in both Bays. Furthermore, fish were always caught between 6:00 and 8:00 am (Table 1).

Guaratuba Bay is a large estuarine system, located to the south of Paranaguá Bay (Vendel and Chaves, 1998; Chaves and Bouchereau, 1999) (Fig. 1), with a general salinity range of 3–37 (Chaves and Bouchereau, 1999). The main sources of contamination in Guaratuba Bay are sparce and sporadic, mainly from domestic sewage and boat traffic, especially during summer months, thus resulting in a degree of