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Shark parasites as bio-indicators of metals in two South African embayments

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ABSTRACT

Concentrations of metals in the tissues of the sharks *Callorhinchus capensis*, *Rhinobatos annulatus* and *Rhinobatos blochii* collected in False Bay and Saldanha Bay, South Africa, in 2013 were investigated. Metal concentrations in the tissue of the parasites *Gyrocotyle plana* infecting the spiral intestine of *C. capensis* and *Proleptus obtusus* infecting the stomach of *R. annulatus* and *R. blochii* were also analysed. *G. plana* showed accumulation of arsenic (4073.52 ± 5561.54 μg/g), manganese (522.16 ± 578.21 μg/g), lead (64.87 ± 101.7 μg/g), titanium (1821.42 ± 1348.16 μg/g) and zinc (12439.57 ± 9743.60 μg/g). These results when compared to baseline values, showed that accumulation of the metals in *G. plana* are orders of magnitude higher than those in the surrounding environment and 2 to 6 times the concentration of the surrounding host's tissues. These results show the usefulness of marine endoparasites as early warning indicators of heavy metal pollution.

1. Introduction

Trace metal concentrations stemming from land-based activities are an ever increasing threat to marine environments (Robinson and Avenant-Oldewage, 1997). These metals have long residence times and concentrate in sediments and within marine organisms (Islam and Tanaka, 2004). Marine organisms are particularly susceptible to these pollutants and there is support for their bio-accumulation through the food web (Sadiq, 1992).

The use of biological indicator organisms to define areas of trace metal pollution is most attractive, as these organisms not only concentrate metals from water and sediment to concentrations higher than their surrounding environment, but they may also represent a time-averaged value for the relative biological availability of metals at each site studied (Rainbow and Phillips, 1993). The effects of metals on organisms is associated with their inhibition of enzymatic systems, and in high concentrations, they act on the surface tissue of organs as protein precipitants (Sures, 2001). Once absorbed, they concentrate in protein rich organs (e.g., lymphocytes, liver, muscle etc.), and at high concentrations cause a variety of morphological, inhibitory and behavioural responses (Bryan et al., 1979 provides a comprehensive review on the impact of metals on marine organisms). Biological indicator organisms provide valuable information about the chemical state of their environment.

Metal pollution studies in South African waters that utilized bio-indicator species mainly focussed on metal concentrations in mussels *Mytilus galloprovincialis* and *Perna perna* and abalone *Haliotis midae*. Most of these studies were performed in the 1970's and early 1980's, as part of the National Marine Pollution Monitoring Programme (Cloete and Oliff, 1976; Cloete and Watling, 1981; Gardner et al., 1983). In 1985, the South African National Committee for Oceanographic Research (SANCOR) developed the Marine Pollution Research Programme (MPRP) as a framework for pollutant research (Hennig, 1985). As part of the MPRP the Mussel Watch Programme was established, but these data were only made available to the public recently (Atkinson et al., 2006; Sparks et al., 2014). Sparks et al. (2014) evaluated the data collected from 1985 till 2008 and concluded that metal concentrations along the west coast of the Cape Peninsula (from Noordhoek to Milnerton) were highly variable (e.g. not detected to 1625.6 μg/g for zinc) with the highest average concentrations for zinc (186.2 ± 125.6) and iron (129.3 ± 163.3 μg/g).

Apart from the Mussel Watch Programme, there has been a sharp decline in marine metal pollution research since the 1990's. The majority of research currently being undertaken in South Africa are extremely diverse in their objectives and practice (O'Donoghue and Marshall, 2003). These studies are linked to impact assessment studies by local authorities (e.g. municipalities) and exposure studies on local fauna, sediment and water quality (Wepener and Degger, 2012). The majority of these studies are conducted by university research groups to establish baselines for a variety of marine organisms, often with the intent of measuring the potential health risks to the general public (Bosch et al., 2013, 2015, 2016a, 2016b; Erasmus, 2004; Reinecke et al., 2012).

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As top predators, sharks have been shown to accumulate higher concentrations of metals in their tissues than organisms occupying lower trophic levels in the food web. This is attributed to biomagnification (accumulating metals from food) due to their hierarchy in the food web (Al-Reasi et al., 2007; Marcovecchio et al., 1991). It has also been demonstrated that sharks harbour a variety of parasitic organisms that are highly effective accumulators of metals (Malek et al., 2007). The usefulness of parasites in environmental monitoring has long been established, with three publications summarizing results and identifying trends within the literature by analysing more than 150 publications (Blanar et al., 2009; Lafferty, 1997; Poulin, 1992). The reviews identified that parasites are effective metal accumulators, concentrating metals to orders of magnitude above their host.

This paper presents the results of metal concentrations in three species of shark (*Callorhinchus capensis* (Dumeril, 1985), *Rhinobatos annulatus* (Muller and Henle, 1841) and *R. blochii* (Muller and Henle, 1841)) and selected endoparasites (*Gyrocotyle plana* (Linton, 1924) and *Proleptus obtusus* (Dujardin, 1845)), and discusses the potential use of this data as early warning systems for metal accumulation in two South African marine embayments.

2. Methods

Fifty specimens of three shark species were collected between March 2013 and March 2014 at two localities (Fig. 1) in the Western Cape Province of South Africa. Nineteen individuals of *Callorhincus capensis* and 15 of *R. annulatus* were collected from commercial beach seine net fishermen at the coast between Sunrise Point and Strandfontein Point in False Bay, while 4 individuals of *R. annulatus* and 13 of *R. blochii* were collected in Saldanha Bay with the aid of a beach seine net (50 m long and 1.5 m deep) with a mesh size of 1 cm (Table 1). The sharks were transported to the Department of Biological Sciences, University of Cape Town and frozen individually in plastic bags at –20 °C until processing.

Prior to dissection the sharks were thawed individually at room temperature, their sex determined, weighed to the nearest gram (g), and measured for total length and standard length (base of tail) measured to the nearest millimetre (mm). A survey of parasitic fauna was conducted as outlined by MacKenzie and Abaunza (2005). After an external examination for macroparasites, gills were removed, separated into petri dishes and examined with a dissecting microscope at 10× magnifimagnification (Leica EZ4). Sharks were eviscerated and organs separated. The alimentary canal was cut open and the contents examined with a dissecting microscope for parasites. Kidney, liver, muscle, gall bladder, and gonad samples were smeared and examined at 40× magnification (Leica ICC50, DM750) for microscopic parasites. Any parasites found during these processes were kept in 10% formalin and the count was recorded. Parasites were identified as far as possible using the literature (Beverley-Burton et al., 1993; Bih Awa, 2012; Freer and Griffiths, 1993; Linton, 1924; McLachlan, 2011; Rohde, 2005; Yeld, 2009).

Table 1
Collection details of samples of *Callorhincus capensis* (CC), *Rhinobatos annulatus* (RA) and *Rhinobatos blochii* (RB).

Year	Date of capture	Species	Location	Sample size (n)	Size range (g)
2013	March	RA	Saldanha Bay	3	1436–1988
2013	May	CC	False Bay	9	443–2885
2013	June	CC	False Bay	2	1148–2263
2013	June	RB	Saldanha Bay	13	807–3900
2013	November	CC	False Bay	8	132–373
2013	November	RA	False Bay	15	161–1520
2014	March	RA	Saldanha Bay	1	745

Samples of gonad, kidney, intestine (with bolus removed), liver and muscle were kept and re-frozen at −20 °C for metal analysis (see Table 2 for list of metals). All macroscopic parasites were also kept and frozen. Frozen fish tissue samples were allowed to thaw at room temperature and 4 g of sample was weighed into acid washed glass Petri dishes, to the closest 0.001 gram (g). All glassware was acid washed (2% hydrochloric acid bath) prior to weighing and use in ovens. The tissue samples were then dried for 48 h in a Memmert TV30 oven at 70 °C. At 24 h the dried tissue samples were weighed and placed back into the ovens. Once 48 h was completed, the samples were re-weighed and these weights were compared to the 24 h measurement to determine if all moisture was removed. If the values were not consistent then samples were returned to the oven for a further 24 h, or until the weight did not change. Once dried, samples were placed in plastic cryo.s™ (Greiner bio-one) vials and sealed with Parafilm M (plastic paraffin film) prior to digestion and metal analysis. 0.5 g of sample was weighed with a Sartorius CP225D scale and placed into Teflon microwave digestion flasks (also referred to as “bombs”), along with 10 ml of 65% Suprapur™ nitric acid (Merck, South Africa) and 1 ml of 30% hydrogen peroxide. The digestion of the tissue samples was done using a CEM Mars 6 Microwave Reaction System for approximately two hours at 200 °C. The samples were diluted with Milli-Q water to 50 ml and decanted into 50 ml falcon tubes (Cellstar™ Tubes, greiner bio-one). The falcon tubes were stored in a fridge at 4 °C until analysis using an Inductively Coupled Plasma-Mass Spectrometer (Perkin Elmer, NexION 300 ICP-MS). Dog fish liver certified reference

Fig. 1. Map of Southern Africa indicating study locations (False Bay and Saldanha Bay), with nearby city of Cape Town and town St. Helena Bay for reference.