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Comparing the metallic elemental compositions of Kelp Gull *Larus dominicanus* eggs and eggshells from the Swartkops Estuary, Port Elizabeth, South Africa

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ABSTRACT

Metals attributed to pollution may increase their concentrations above the geological background and pose toxic challenges towards humans and biota. We analysed sixteen Kelp Gull eggs and eggshells for 30 metallic elements from the Swartkops Estuary (SE), an important recreational, industrial, and ecological asset for Port Elizabeth, the region, and South Africa. Mean concentrations for eggshell and egg content for Hg was 0.02 and 0.4 mg/kg dm, Cr was 4 and 18 mg/kg dm (the highest yet recorded for any gull or tern egg), for Zn 2.1 and 62 mg/kg dm, for Sr 880 and 12 mg/kg dm, for V 170 and 1.3 mg/kg dm, and for Co 1.7 and 0.002 mg/kg dm, respectively. Zink, Se, and Hg, increased on a dry-mass basis from sediment via small fish to gull egg content, indicating bioaccumulation. No effect on eggshell thickness was seen. We also determined that eggshell concentrations cannot be used as a proxy for egg content concentrations. Mercury, Cr, V, Co, and Zn were elements we identified as potentially problematic that require source identification and mitigation. Further research into other high-trophic animals such as herons, egrets, cormorants, and otters in the SE system is proposed.

Keywords: Laridae, mercury, chromium, copper, zinc, vanadium, cobalt, Indian Ocean

1. Introduction

The addition of toxic compounds to the environment from increasing recreational, residential, and industrial activities places more stress on receiving ecosystems (Martin & Randal, 1987). Once pollutants such as metals and persistent organic pollutants enter aquatic ecosystems, they are difficult, if not impossible, to remove (UN Environment, 2018) and can subsequently contaminate food webs. When pollution-associated metallic elements increase in sediment or water, their toxicity can also increase, possibly affecting humans and organisms (Burger & Gochfeld, 2003; Burger & Gochfeld, 2007; Pizzol et al., 2011; Suiçmez et al., 2006). The concentrations of metallic elements in sediment can act as an indicator of exposure and possible accumulation by sediment-associated organisms. The accumulation in organisms at lower trophic levels may subsequently be biomagnified by and affect higher trophic-level organisms (Burger & Gochfeld, 2000; Dauwe et al., 1999; Nel et al., 2015).

Metallic element concentrations elevated above their natural background within the ocean have become a global problem because organisms such as fish, marine mammals, and birds can be negatively affected (Klaassen & Watkins, 2015; Suiçmez et al., 2006). Because authorities, researchers, and the public are concerned about the health of polluted ecosystems as well as food safety, bioindicators such as animals can be used for more than just scientific importance as it can inform policy and mitigation measures required (Burger et al., 2009).

Birds are good bioindicators because different species and guilds occupy different trophic levels, proving a measure of trophic bioaccumulation of pollutants (Burger & Elbin, 2015a, Burger & Elbin, 2015b; Mansouri et al., 2012a). The concentrations of pollutants within a bird can be externalised via, amongst others; feathers, eggs, glandular oil, and excreta (Burger & Elbin, 2015a; Burger & Elbin, 2015b; Burger & Gochfeld, 2003; Dauwe et al., 1999; Mansouri et al., 2012a). Eggs, oil, and feathers can therefore be a non-lethal (to adults) method of assessing pollutants such as metallic elements within the environment and the body of the bird (Bond & Lavers, 2011; Dauwe et al., 1999; Peck et al., 2016). Literature also suggests that eggshells of certain species can be used to infer those of egg contents for certain nonessential elements (cadmium, lead, arsenic and mercury), providing the opportunity to use discarded eggshells, thereby reducing the effects on bird populations (Ayaş, 2007; Dauwe et al., 1999; Hashmi et al., 2015).

The Swartkops River (South Africa) flows through an urbanised area where it forms part of the Swartkops Estuary (SE; Fig. 1). The SE is situated approximately 15 km north of Port Elizabeth on the coast of the Indian Ocean (Lord & Thompson, 1988; Martin & Baird, 1987). The SE is a recreational asset to people, and an ecological asset to many species of birds and fish (Martin & Randal, 1987). Even though the SE plays an important

environmental role, it is under considerable pressure from increasing development (Baird et al., 1988; Wallace et al., 1984). The area surrounding the SE is highly urbanised with municipal areas such as Uitenhage, Despatch, KwaNobuhle, Ibhayi and the northern part of Port Elizabeth (Binning & Baird, 2001; Odume, 2014). The SE's water quality is influenced by agricultural run-off of pesticides and fertilizers, wastewater treatment plants, tanneries, a carbon-black factory, automobile and auto parts factories, and other anthropogenic activities (Eggleton & Thomas, 2004; Lord & Thompson, 1988; Odume, 2014; Suiçmez et al., 2006). These sources all have the potential to release metallic elements into the environment via improperly treated wastewater, industrial processes, and run-off from waste and polluted soils. Binning & Baird (2001) concluded that the highest concentration of metallic elements entering the SE was from run-off, industries, and informal settlements along the banks of the river. Analyses of sediment, crustaceans, and fish from the SE indicated that cobalt (Co), chromium (Cr), Hg, strontium (Sr), uranium (U), vanadium (V) and zinc (Zn) in the SE might pose risk as the concentrations approached or exceeded norms of safety for human intake (see Nel et al., 2015). These elements are also listed in the substance priority list (ATSDR, 2017).

This study focuses on the Kelp Gull *Larus dominicanus* (Laridae) found throughout many parts of the southern hemisphere (Crawford et al., 1982; Crawford et al., 1997; Sepulveda & Gonzalez-Acuna, 2014; Whittington et al., 2009; Whittington et al., 2016). They occur along the coast of South Africa where they are common – their conservation status is Least Concern (IUCN, 2016). They capture live prey but are also scavengers, feeding on food scraps such as fish offal and discards from waste dumps (Nel et al., 2015; Sepulveda & Gonzalez-Acuna, 2014), making them susceptible to uptake of metals via their food (Ayaş, 2007; Mansouri et al., 2012b) that can be monitored using their eggs.

The aim of this study was to determine the metallic element concentrations in Kelp Gull eggs, comparing the concentrations with published levels of concern, and to assess whether the shells can be used as a proxy for pollutants contained in the egg contents, thereby negating the need to collect potentially viable eggs by sampling shells of hatched eggs only. As far as we are aware, this would be the first study of metallic elements in egg contents and eggshells of gulls from anywhere in the southern hemisphere.

2. Materials and methods

2.1. Ethical approval

The necessary permits needed for the collection of freshly laid eggs were obtained from the Department of Economic Development, Environmental Affairs and Tourism, Eastern

Cape (Permit No. CRO 124/12CR). Bird egg collection was approved by the ethics committee of the North-West University (NWU) (NWU-00055-07-S3).

2.2. Sampling

Kelp Gulls breed in colonies on sand islands in the SE (Fig. 1). Kelp Gulls lay one to four eggs, usually two to three (Hockey et al., 2005). Sixteen eggs were collected from eight nests from a large breeding colony on a sand island in the mouth of the SE (Fig. 1) during the Austral spring of 2012. If more than two eggs were available per nest, the “oldest” eggs were chosen due to size as the smallest egg is expected to be laid last (Quinn, 2010). The eggs were covered with pre-cleaned aluminium foil, placed on ice, and frozen on the same day at -20°C until analysis (Nel et al., 2015).

Fig. 1. Location of the Swartkops Estuary on the coast of South Africa (left), and a representation of residential and industrial factors that can influence the Swartkops Estuary, right.

2.3. Sample treatment and analysis

In the laboratory, the eggs were carefully rinsed with deionised water to remove any adhered materials from the shell. The eggs were divided into egg contents and eggshells. The mass of each sub-sample was determined before ultrasonic homogenising. Between every sample, the probe of the homogeniser was thoroughly washed with warm water and soap, then, rinsed in sequence with double-distilled water, 96% ethanol, acetone, and hexane. The samples were stored in separate pre-cleaned high-density polyethylene (HDPE) 50 mL centrifuge tubes, freeze-dried, and powdered for further analysis. Eggshells were washed to separate the membrane from the eggshell (since it can have an effect on

the eggshell thickness measurements and metallic element concentration). The eggshells were left for three months to air dry and then measured with a digital calliper (Kroeplin B2R20S) accurate to 0.01 mm.

Content and shell samples, as well as a standard reference material (SRM 1944) were analysed for 30 metallic elements, using EPA 3050B for inductively coupled plasma mass spectrometry (ICP-MS) analyses, by Eco-Analytica at the North-West University. Each sample was acid- and heat-digested using 65% nitric acid, 50% hydrogen peroxide, and 33% hydrochloric acid (all reagent grade; Associated Chemical Enterprises). An Agilent 7500 CE ICP-MS fitted with a Micromist-type nebulizer and a standard quartz chamber was used, optimised with a solution containing lithium (Li), yttrium (Y), cerium (Ce), and thallium (Tl) (1 µg/L) to reduce interference ($\leq 1.5\%$).

External calibrations were with ULTRASPkEC-certified multi-element standard solutions with elements at different, certified, concentrations (De Bruyn Spectroscopic Solutions). Calibration series standards were run separately for content and shell samples. Standard reference material concentrations were within 25% of certified values. Concentrations are expressed as mg/kg dry mass (dm).

2.4. Statistical analysis

Summary statistics and comparisons were done using GraphPad Prism 7.04 (www.graphpad.com). We used linear regression of elemental concentrations between shells (log-transformed) and contents (normally distributed data) to investigate whether elemental concentrations in the contents can be predicted from the shell. We used each egg as an individual sample for statistical analysis as each embryo is exposed to the metals inside each egg as 16 individual exposures. Multivariate comparisons of the profiles ('fingerprints') of egg contents and eggshells were done using PC-Ord version 6.20 (MjM Software Design, www.pcord.com). The concentrations were relativised per element to compensate for the large concentration differences. Non-metric multidimensional scaling (NMS) was used, with Sørensen as the distance measure. A maximum of four axes were allowed with 200 maximum iterations, random starting conditions, and 50 runs with real data. Fifty runs with randomised data were done to stress test the final ordination.

3. Results

3.1 Elemental concentrations and associated hazards

All elements had quantifiable concentrations in all samples (Table 1 for a summary of comparative data, and Table S1 in supplementary materials for summary statistics arranged by increasing %CV values for egg contents). Although 30 elements were analysed, we concentrate on Cr, Zn, Co, V, Sr, U, and Hg in the SE, due to concentrations posing potential risks found in fish and sediment (based on the findings of Nel et al., 2015).

Table 1: Comparison of concentrations (mg/kg dm) found in literature and from our own data for 18 elements in gull and tern egg contents and eggshells, as well as concentrations in sediment, mud prawn and fish from Swartkops Estuary.

Species	Year	Place	V	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Sr	Mo	Ag	Cd	Sb	Hg	Tl	Pb	U	Reference
Arctic tern	2008	Canada															2.11				Akearok et al., 2010
Arctic tern	2004-2005	Canada															1.15				Peck et al., 2016
Bridled Tern	2002	Hong Kong	0.096	1.949	2.636	0.026		3.92	47.62	1.384	4.386	1.966		0.021	0.002	0.006	0.562	0.001	0.01		Lam et al., 2005
Bridled Tern	2011	Iran	0.096			0.009	0.055			0.076	1.082				0.004				0.286		Khademi et al., 2015
Bridled Tern	2012	Persian Gulf	0.129																		Avazpour et al., 2014
Common tern	2000	USA		0.045	2.29					0.195	2.046				0.004		1.241		0.164		Burger, 2002
Common tern	2010-2012	Poland															1.2394				Grajewska et al., 2015
Common tern	2000-2002	USA		0.1077	2.499					0.26133	2.5167				0.006		1.0463		0.0953		Burger & Gochfeld, 2003
Forster's tern	2000	USA		0.028	1.702					0.19	1.688				0.002		1.939		0.056		Burger, 2002
Lesser Crested tern	2011	Iran		0.088		0.009	0.038			0.051	1.133				0.006				0.181		Khademi et al., 2015
Lesser Crested tern	2012	Persian Gulf	0.03																		Avazpour et al., 2014
Sandwich tern	2010-2012	Poland															1.1542				Grajewska et al., 2015
Whiskered Tern	2008	Iran															0.433				Aliakbari et al., 2010
White-cheeked Tern	2012	Persian Gulf	0.01																		Avazpour et al., 2014
Black-tailed Gull	2008	South Korea			1.99			2.67	73.4						0.31				0.92		Kim & Oh 2014
Black-tailed Gull	1999-2001	Japan,	0.041	0.78	1.82	0.043		4.14	65.3		3.3	21.3	0.237	0.008	0.021	0.03	0.38	0.012	0.023		Agusa et al., 2005
Glaucous Gull	2004-2005	Canada															1.63				Peck et al., 2016
Glaucous-winged Gull	2005	USA		0.248	1.08					0.126	1.57				0.0841		0.703		0.107		Burger et al., 2009
Great Black-backed Gull	2000	USA		0.021	1.651					0.1	1.543				0.005		0.43		0.227		Burger, 2002
Great Black backed Gull	2012	USA		0.0553						0.159	2.164				0.0045		1.049		0.0627		Burger & Elbin, 2015a
Great Black backed Gull	2013	USA		0.0963						0.0982	1.864				0.0032		0.504		0.26		Burger & Elbin, 2015a
Great Black-backed Gull	2012	USA		0.0055						0.159	2.164				0.0045		1.049		0.0627		Burger & Elbin, 2015a
Herring Gull	2012	USA		0.0852						0.0574	1.822				0.0053		0.708		0.138		Burger & Elbin, 2015a
Herring Gull	2000	USA		0.11	1.622					0.126	1.836				0.005		0.419		0.273		Burger, 2002
Herring Gull	2013	USA		0.0164						0.0497	1.323				0.0005		0.229		0.244		Burger & Elbin, 2014
Herring Gull	2010-2012	Poland															0.56				Grajewska et al., 2015
Herring Gull	2012	USA		0.0852						0.00574	1.822				0.0053		0.706		0.138		Burger & Elbin, 2015a
Ivory Gull	2004-2005	Canada															6.4				Peck et al., 2016
Ross's Gull	2004-2005	Canada															0.96				Peck et al., 2016
Sabine's Gull	2004-2005	Canada															0.53				Peck et al., 2016
Thayer's Gull	2004-2005	Canada															1.02				Peck et al., 2016
Kelp Gull	2012	South Africa	1.3	18	1.4	0.02	0.12	4.8	62	0.26	2	12	0.25	0.28	0.0015	0.05	0.38	0.00022	0.014	0.000084	This study
Bridled Tern	2002	Hong Kong		0.241	1.112	0.398		1.235	2.351	0.397	15.58	289.6			0.002		0.004	0.002	0.06		Lam et al., 2005
Bridled Tern	2012	Persian Gulf	0.45																		Avazpour et al., 2014
Common tern	2010-2012	Poland															0.0536				Grajewska et al., 2015
Lesser Crested tern	2012	Persian Gulf	0.12																		Avazpour et al., 2014
Sandwich tern	2010-2012	Poland															0.0501				Grajewska et al., 2015
Whiskered Tern	2008	Iran															0.0026				Aliakbari et al., 2010
White-cheeked Tern	2012	Persian Gulf	0.05																		Avazpour et al., 2014
Black-tailed Gull	2008	South Korea			2.76			2.8	53.6						0.45				3.1		Kim & Oh 2014
Black-tailed Gull	1999-2001	Japan	0.011	0.4	1.13	0.74		0.535	0.778		0.42	1650	0.025	0.005	0.013	0.03		0.023	0.061		Agusa et al., 2005
Kelp Gull	2012	South Africa	170	3.8	0.68	1.7	1.4	13	2.1	0.26	0.3	880	0.1	0.5	0.0004	0.032	0.02	0.00017	0.022	0.000057	This study
Sediment*	2012	South Africa	10	22	84	3	7	7	28	5	1	300	1	0.003	0.1	0.2	0.01	0.1	11	1	Nel et al., 2015
Knysna sand goby*	2012	South Africa		10	10	0.2	0.5	2.8	110	2.4	1.7	52			0.07		0.02	0.02	0.4		Nel et al., 2015
Estuarine round herring*	2012	South Africa		5.2	14	0.5	0.7	4.5	67	6.6	1.7	240			0.05		0.02	0.03	0.5		Nel et al., 2015
Mud prawn*	2012	South Africa	2	8	100	1	2	150	77	7	4	700	1	2	0.2	0.2	0.2	0.04	2	0.1	Nel et al., 2015
Garrick liver*	2012	South Africa		26	5	2	2	2000	200	10	100	3			1		1	0.1	1		Nel et al., 2015

*Dry mass and means from original (Nel et al., 2015) data.

Chromium: Mean Cr concentrations of 4 and 18 mg/kg dm were found in eggshells and egg contents of the Kelp Gull (Table 1). The highest concentrations recorded in eggshell and content were 6.4 and 20 mg/kg dm, respectively (Table S1). This Cr concentration was the highest found in literature for any larid egg or shell (Table 1). The %CV of Cr in egg contents was low at 8%, compared with other elements (Table S1).

Zinc: Mean Zn concentration of 2.1 and 62 mg/kg dm was found for eggshells and egg content of the Kelp Gull, respectively (Table 1). A relatively low %CV of 12% was found for egg content (Table S1).

Cobalt: Mean Co concentrations of 1.7 and 0.002 mg/kg dm were quantified in eggshells and egg contents of the Kelp Gull, respectively (Table 1). A relatively low %CV of 8.2% were found for eggshells concentrations, in comparison to other elements (Table S1).

Vanadium: Mean concentrations of V at 170 and 1.3 mg/kg dm were found in eggshell and egg content of the Kelp Gull, respectively (Table 1), with a relatively low %CV of 19% for eggshells (Table S1). Higher concentrations of V were associated with lower concentrations of Cr in eggshell ($p = 0.0232$), respectively (Table S1). Higher concentrations of Cr (18 mg/kg dm) occurred in egg contents, coinciding with higher concentrations of V (170 mg/kg dm) in eggshells, suggesting antagonism (Avazpour et al., 2014).

Strontium: Mean Sr concentrations of 880 and 12 mg/kg dm were determined for eggshells and egg contents, respectively (Table 1). Eggshell concentrations from this study ranged between 360-1299 mg/kg dm with a relatively low %CV of 33% (compared with the other elements) (Table S1). There was a statistically significant positive linear regression between Ca and Sr within eggshells (Fig. 2h; $p = 0.0006$), but not in contents.

Uranium: Mean concentrations of 0.00006 and 0.00008 mg/kg dm were found in eggshells and egg contents of the Kelp Gull, respectively (Table 1). A relatively low %CV of 31% and 9% were found for eggshells and egg content, respectively, when compared with the other elements (Table S1).

Mercury: Mean Hg concentrations of 0.015 and 0.375 mg/kg dm were found in eggshells and egg contents, respectively (Table 1). The highest Hg concentration we determined within egg content was 2.1 mg/kg dm, with a relatively high %CV of 130% (Table S1).

3.3 Associations between elemental concentrations in egg contents and eggshells

Figure 2: Linear regressions representing log-transformed shell concentration versus content concentration (mg/kg dm) for chromium (a), zinc (b), cobalt (c), vanadium (d), strontium (e), uranium (f), and mercury (g). The linear regression for Sr shell concentration versus Ca shell concentration (mg/kg dm) is presented in (h).

The content concentrations were normally distributed while the shell concentrations had to be log-transformed. A positive but not significant linear association between concentrations in egg contents and eggshells was found for Cr (Fig. 2a; $p = 0.822$) and Sr (Fig. 2c; $p = 0.0141$). Vanadium (Fig. 2d; $p = 0.0003$) and Co (Fig. 2e; $p = 0.0151$) had significant negative linear regressions. Although the regression for Zn was not statistically significant (Fig. 2b; $p = 0.2584$), a positive association between egg contents and eggshells was apparent. Uranium and Hg also had negative linear associations (Fig. 2f and 2g) which were not statistically significant. Care should be taken when interpreting 30 regressions, as some might be spurious, at $p < 0.05$.

3.2 Eggshell thinning

The thinnest eggshell measured was 0.25 mm; the thickest was 0.31 mm. This is a 19% difference between the thickest and thinnest shells. Linear regressions showed no significant ($p < 0.05$) associations between elements in both egg contents and eggshells, with eggshell thickness for any of the 30 elements analysed. However, since 60 regressions were run, one out of twenty regressions (5%) may be spurious and care should be taken when interpreting the results.

3.3 Relative compositional profiles of elements in eggshells and egg contents

The NMS ordination (Fig. 3), showing the relative compositions ('fingerprints') between eggshell and egg contents needed only two of the four allowed dimensions after 49 iterations to achieve a final stress of 6.15, with a final instability of 0.0000. The final instability means that after 49 iterations, no further improvement will be achieved with more iterations. The stress value shows the measure of alteration needed to project the ordinations within only two dimensions. A final stress value between five and ten provides an excellent representation of the relationships between samples (McCune & Grace 2002). Individual samples that are more closely grouped together are more similar and elements grouped further from each other are less similar, based on their relative proportional composition.

The two convex hulls indicate the separation of metallic elements in association with eggshells and egg content ('fingerprints' are distinct). Egg contents had relatively higher relative compositions of HG, Rb, Zn, and B, while the contents had relatively higher compositions of Co, Pd, Ni, Cu, and Ba. Lead and Ag ordinated perpendicular to axis 1, indicating no association with either matrix. Lead and Ag also ordinated opposite each other, indicating an increase in relative composition of one is concomitant with a decrease of the other. These two elements, and Cd to a lesser extent, seem to be contributing the most

towards axis 2 (Fig. 3), irrespective of their relative concentrations in eggshells or egg contents.

Figure 3: Nonmetric Multidimensional Scaling illustrating relative compositional profiles of metallic elements in Kelp Gull eggshells and egg contents with Sørensen as distance measure. Only two dimensions were needed, with a final stress of 6.15 reached after 49 iterations to achieve a final instability of 0.0000. Axis 1 explained 81.9% of the variation, and axis 2 explained 12.4%.

4. Discussion

4.1 Elemental concentrations, comparisons with literature, and possible effects

Chromium: Chromium is present naturally in the earth's crust and water, but elevated concentrations can be traced to anthropogenic activities (Mansouri et al., 2012b; Movalli, 2000). Concentrations of Cr can vary between 1 to 10 µg/L in river waters; in soil the concentrations can vary from 1 to 3000 mg/kg (Aslam & Yousafzai; 2017). Chromium is

associated with a wide variety of activities such as stainless steel welding, tanning, dyes and pigments, preservation of wood, sewage treatment, mining, and use in agriculture (Aslam & Yousafzai, 2017; Egwumah et al., 2017; Mansouri et al., 2012b), all of which are present in the SE catchment.

Trivalent and hexavalent chromium are the two most abundant forms of Cr in the environment (Aslam & Yousafzai, 2017). Trivalent chromium seems essential to at least some organisms for, *inter alia*, insulin regulation of carbohydrates, activation of specific enzymes, and the stabilisation of proteins (Pechova & Pavlata, 2007; Simonetti et al., 2015; Živkov Baloš et al., 2016). However, hexavalent chromium can have neurological, respiratory, hepatic, and behavioural effects (Burger & Elbin, 2015b; Wang et al., 2017) due to its ability to easily cross through biological membranes, impacting cell metabolism (Alijagic et al., 2018; Aslam & Yousafzai, 2017). Furthermore, Wang et al. (2017) found that hexavalent chromium inhibits growth and reduce body mass of birds.

This study reports the highest Cr concentration found in literature for any larid egg or shell (Table 1). The high concentration coupled with the low %CV would suggest physiological regulation on the one hand, but the high concentrations compared with the other larids (Table 1) suggests a possible environmental overload. Without knowledge of the valence of Cr in the environment and food of the Kelp Gull, no assessment of its toxicity can be made, but further investigation seems warranted based on the high concentrations found.

Zinc: Although an essential element, it can be toxic to birds when ingested in excess. As an essential element, Zn helps with metabolic processes and gene expression. Furthermore, Zn also plays a role in eggshell formation (Mansouri et al., 2012^a; Simonetti et al., 2015). Zinc is mainly released into the environment through mining and industrial effluent, but Zn can also be found in food packaging and transported via air. Zn is known to leach into water systems through leaking galvanized pipes from households (Klaassen & Watkins, 2015; Nordberg et al., 2014). Furthermore, approximately half of the zinc that is transported by rivers is deposited in estuaries (Nordberg et al., 2014).

It is known that birds in general have some tolerance towards high concentrations of Zn, but they may still be affected at elevated exposures (Mansouri et al., 2012^a; Movalli, 2000; Yohannes et al., 2017). Although a high concentration of Zn was found within this study, it was not the highest concentrations yet reported for larid eggs (Table 1). Zinc may therefore be physiologically regulated as well as pose an ecotoxicological issue for larids in particular, and for aquatic animals in general, in the SE.

Strontium: Strontium is known to occur naturally within the environment as an inorganic element. The main input of Sr into the environment occurs from natural weathering of rocks and through atmospheric deposition (Kitowski et al., 2014). Even though chemically similar to Ca, an increase in Sr can result in increased embryotoxicity affecting the transport

of Ca and vitamin D resulting in lower hatching success, increased eggshell thinning, and shell breakage as Sr can cause physical changes of eggshells (Kitowski et al., 2014; Lam et al., 2005; Mora., 2003; Mora et al., 2007). An increase in Ca concentration will result in a decrease of Sr toxicity (Lam et al., 2005; McPherson et al., 2014). With little comparable information available from elsewhere, this is the second highest eggshell and egg content concentrations for gulls, with the highest concentration in Black-tailed Gull *Larus crassirostris* eggs from Japan (Table 1).

Despite the relatively low %CV, the high concentrations we found suggests that Sr is occurring at elevated levels within the SE, but the specific source(s) are unknown. The Sr concentration within the eggshells was higher than the toxic reference value (TRV) determined by Meyer et al. (2015) for bird eggshells (between 360-1299 mg/kg dm). The egg content TRV (176-507 mg/kg dm) was not exceeded (Meyer et al., 2015).

Figure 2(h) illustrates a direct positive linear regression between Ca and Sr eggshell concentrations, suggesting interaction between these two elements. Strontium and Ca are divalent alkaline earth metals and mimic each other. Because of its atomic similarity to Ca, Sr accumulates in milk and bone (Nordberg et al., 2015), as well as eggshells (du Preez et al., 2015). Although no clear evidence of eggshell thinning was seen (based on regressions; Section 3.2), there is still warrant for concern about Sr.

Vanadium: Vanadium occurs naturally in the environment, but is also anthropogenically added to the environment through fossil fuel burning (Nordberg et al., 2014). This element is physiologically essential, playing a role in bone formation, kidney development, and reproduction (Henry & Miles, 2001; Metcheva et al., 2011). In low concentrations, V does not display any toxic effects, but an increase can lead to inhibition of enzymes, decreased hatching success of birds, and lysis of cells (Henry & Miles, 2001). Henry & Miles (2001) found that when laying hens were fed with a concentration of 10 mg/kg V in their food, kidney and bone development was impaired. They also determined a toxic threshold for V between 8-50 mg/kg dm for egg contents. Živkov Baloš et al. (2016) reported that albumin quality and growth developments started to occur in poultry fed with concentrations of 6 mg/kg dm feed and hatching success started to decrease from 40 mg/kg dm within feed. Although the concentrations of V in gull egg contents (1 mg/kg dm) did not exceed those found by Henry & Miles (2001) and Živkov Baloš et al. (2016), within egg content V concentrations warrants concern as they were higher than reported by others in literature for larid eggs (Table 1). This suggests elevated environmental levels, possibly from pollution.

Cobalt: Occurring naturally and in its oxidised form, Co forms complex associations with other metallic elements such as arsenic (As), Ni, copper (Cu), and iron (Fe) (DWAf, 1996; Gál et al., 2007; Gault et al., 2009). Cobalt is considered an essential element in

animal metabolism, associated with the vitamin B₁₂ complex (Gál et al., 2007; Gault et al., 2009; Norouzi et al., 2012). Cobalt can enter the environment through natural processes but also through anthropogenic releases such as fossil fuel combustion, production of cobalt alloy, mining, burning of fossil fuels, and from refuse, sewage effluent, and agricultural run-off (Gál et al., 2007; Gault et al., 2009). All these sources are present at the SE.

At low concentrations, Co does not display any toxicity, but uptake of elevated concentrations can lead to DNA breakage, cardiomyopathy genotoxicity, reproductive defects, and mutagenicity (Gál et al., 2007; Gault et al., 2009). Although the egg content concentration was not the highest found in literature, the eggshell concentration was the highest concentration based on literature (Table 1) for terns and gulls.

Uranium: Uranium is found within rocks and soil in its oxidised form. With a concentration of 0.0003% and 0.0004% in the earth's crust, relatively low concentrations occur naturally in fresh and marine water (Bleise et al., 2003; Boryło et al., 2010). Uranium has severe toxic effects for humans and animals, including developmental defects, nephrotoxicity, and genotoxicity (Brugge et al., 2011). The relatively low concentrations of U combined with a relatively low %CV in Kelp Gull eggs suggests that physiological regulation is taking place, but no concern about effects is not warranted.

Mercury: Mercury is one of the best-documented toxic elements in literature (Bond & Lavers, 2011). It occurs naturally in the environment, but concentrations are increasing due to anthropogenic activities (Arcos et al., 2002). The transformation from inorganic to organic Hg is one of the major problems associated with Hg because methyl-mercury bioaccumulates in animals (Arcos et al., 2002). Mercury concentrations above 3 mg/kg dm in animals can influence growth, embryonic development, hatching success, neurological, behaviour, and cause developmental defects (Burger & Gochfeld, 2000, 2007; Mansouri, 2015^b; Yohannes et al., 2017). Seabirds seem to be more tolerant to higher Hg concentrations (Arcos et al., 2002; Burger & Gochfeld, 2007). Meyer et al (2015) calculated the TRV for Hg at 2 mg/kg dm in egg contents. Although the mean concentration of Hg did not exceed the TRV in eggs, one of the eggs had a concentration of 2.1 mg/g dm, therefore Hg-associated toxicological effects might be expected in the SE.

4.2 Trophic level increases

Table 1 includes previous data from Nel et al. (2015) for sediment, mudprawn *Upogebia Africana* (Malacostraca: Upogebiidae), and three fish species; estuarine round herring *Gilchristella aestuaria* (Pisces: Clupeidae), Knysna sand goby *Psammogobius knysnaensis* (Pisces: Gobiidae), and the garrick *Lichia amia* (Pisces: Carangidae).

The mud prawn is an important prey species for the Garrick, but the garrick is an unlikely prey species for gulls as it is quite large (up to 40 cm in length as used by Nel et al., 2015). However, when looking at the sediment – prawn – garrick trophic series (Table 1), Cr, Co, Cu, Zn, As, Se, and Hg increases in concentrations on a dry-mass basis. For the gull, the smaller estuarine round herring and Knysna sand goby (both about 7 cm in length) are important prey (Baird et al., 1988; Whitfield, 1998). When looking at the sediment – small fish – gull trophic series (Table 1), Zn, Se, and Hg, increases on a dry-mass basis. Because Zn and Hg increase in concentrations in the two SE food webs, they should be considered as elements of concern.

4.3 Can the relative compositional profiles be used to compare eggshell and egg content?

The NMS (Fig. 3) clearly shows differences in relative compositional profiles between egg contents and eggshells, as there was no overlap between their respective convex hulls. Relative higher proportions of rubidium (Rb), boron (B), Zn and Hg categorised egg contents, and higher relative proportions of Co, palladium (Pd), and nickel (Ni) categorised eggshells (Fig. 3). Based on these results in combination with results presented in Table S1 and linear regressions (Fig. 2), Kelp Gull eggshells are not a viable proxy for egg content. Kelp Gull egg contents (and therefore the collection of whole eggs) will be needed to monitor embryonic exposures to metallic elements.

4.4 Synthesis and conclusions

Although the eggs were collected in 2012, we do not know of any major changes in the release of metallic elements since then that would substantially have affected current concentrations. We showed that Hg, Cr, V, Co, and Zn approached and exceeded concentrations from elsewhere and certain levels of concern where available. We therefore suggest further research into other higher trophic animals such as herons, egrets, cormorants, and otters, within the SE system, as well as identifying possible sources and mitigation measures to reduce pollution.

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Comparing the metallic elemental compositions of Kelp Gull *Larus dominicanus* eggs and eggshells from the Swartkops Estuary, Port Elizabeth, South Africa

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Supplemental materials

Table S1: Concentrations in eggshells and egg content for 30 elements (mg/kg dm).

Element	Shells							Egg content						
	n	Mean	Median	Min	Max	SD	%CV	n	Mean	Median	Min	Max	SD	%CV
Be	16	0.00013	0.000009	0.0000007	0.0012	0.00034	260%	16	0.00032	0.000034	0.000002	0.0017	0.00048	150%
B	16	0.018	0.02	0.003	0.029	0.0083	46%	16	1	1	1	2	0.42	34%
Al	16	18	14	5	61	17	89%	16	3	3	2.5	6	1	26%
Ti	16	77	730	480	1100	200	27%	16	11	11	10	13	1	6%
V	16	170	170	110	246	32	19%	16	1	1	0.95	1.5	0.1455	11%
Cr	16	4	3.5	0.17	6	2	42%	16	18	19	15	20	1.5	8%
Mn	16	0.7	0.5	0.23	1.5	0.42	62%	16	2	1	1	2.5	0.44	31%
Fe	16	840	840	770	900	43	5%	16	101	105	63	140	21	21%
Co	16	2	2	1	2	0.14	8%	16	0.02	0.02	0.01	0.028	0.0043	23%
Ni	16	1.4	1.4	1.3	2	0.09	6%	16	0.12	0.11	0.08	0.27	0.06	47%
Cu	16	13	12	8	21	4	32%	16	5	3.5	3	24	5	110%
Zn	16	2.1	2	0.45	5	1	50%	16	62	63	47	75	7	12%
As	16	0.3	0.3	0.08	0.35	0.078	30%	16	0.26	0.25	0.1	0.45	0.1	40%
Se	16	0.3	0.3	0.09	0.58	0.13	43%	16	2	2	1.4	3	0.47	24%
Rb	16	0.1	0.07	0.05	0.17	0.037	44%	16	4	4	3	5	1	20%
Sr	16	880	960	360	1300	290	33%	16	12	11	8	17	3	24%
Mo	16	0.1	0.11	0.09	0.13	0.012	11%	16	0.25	0.25	0.19	0.3	0.03	13%
Pd	16	3	3	2	4	0.69	23%	16	0.22	0.2	0.17	0.35	0.05	24%
Ag	16	0.5	0.03	0.00028	7	2	350%	16	0.28	0.21	0.0017	1	0.3	98%
Cd	16	0.0004	0.000013	0.00000084	0.0018	0.00065	160%	16	0.0015	0.00044	0.0000022	0.015	0.004	250%
Sb	16	0.03	0.03	0.03	0.042	0.0049	15%	16	0.051	0.035	0.029	0.23	0.049	97%
Ba	16	5	4	2	10	2	47%	16	1	2	0.45	2	0.42	30%
Pt	16	0.01	0.003	0.00036	0.186	0.046	310%	16	0.041	0.028	0.0049	0.12	0.034	84%
Au	16	0.3	0.2	0.24	0.38	0.038	15%	16	0.32	0.26	0.24	0.73	0.12	40%
Hg	16	0.02	0.0001	0	0.13	0.039	270%	16	0.4	0.2	0.1	2	0.49	130%
Tl	16	0.0002	0.0002	0.00008	0.00022	0.000046	27%	16	0.00022	0.00022	0.00019	0.00023	0.000013	6%
Pb	16	0.02	0.0008	0.00009	0.17	0.045	210%	16	0.014	0.0008	0.0004	0.1	0.026	188%
Bi	16	0.09	0.08	0.08	0.085	0.00059	1%	16	0.089	0.085	0.084	0.13	0.011	13%
Th	16	0.2	0.16	0.15	0.16	0.0026	2%	16	0.2	0.2	0.16	0.35	0.046	25%
U	16	0.00006	0.00006	0.000024	0.00008	0.000018	31%	16	0.000084	0.000085	0.00007	0.000095	0.0000072	9%