



Biom mineralisation and metal sequestration in a crustacean ectoparasite infecting the gills of a freshwater fish

Lutfiyya Latief¹ · Beric M. Gilbert¹ · Annemarië Avenant-Oldewage¹

Received: 3 February 2023 / Revised: 23 March 2023 / Accepted: 14 April 2023 / Published online: 11 May 2023
© The Author(s) 2023

Abstract

It has been suggested that parasites are effective bioindicators as they are sensitive to environmental changes and, in some cases, accumulate trace elements in higher concentrations than their hosts. Accumulated elements sequester in different organs. In monogenean and crustacean ectoparasites, sclerotised structures and egg yolk appear to be the preferred site for element sequestration. In this study, the sequestration of trace elements; Mg, Al, Ca, Fe, Cu, and Zn in *Lamproglena clariae* was studied from two rivers. Adult *L. clariae* were collected from the gills of *Clarias gariepinus* from Lake Heritage in the Crocodile River and in the Vaal River below the Vaal Dam, South Africa. Collected parasites were flash frozen in liquid nitrogen and sectioned with a cryomicrotome. Sections were treated with Phen-Green to observe fluorescent signals. Trace elements in the parasite were analysed using a scanning electron microscope with an energy-dispersive spectroscope (SEM–EDS). Results showed more intense fluorescence signals in the exoskeleton compared to tissues, and in the egg yolk. Analysis by SEM–EDS confirmed the presence of elements in the parasite from both sites. Levels of Al were higher in *L. clariae* from the Vaal River than those from Lake Heritage, and Fe was higher in *L. clariae* from Lake Heritage. Element distribution patterns in the parasite matched those in the water from the sites. Unlike other crustaceans, regulation of metals in adult females of *L. clariae* does not occur through moulting, but high levels occurred in the yolk.

Keywords Acid mine drainage · Bioindicator · *Clarias gariepinus* · *Lamproglena clariae* · Life below water

Introduction

Aquatic organisms are exposed to a mixture of trace elements and chemicals which are of natural and anthropogenic origin. Some elements may become toxic when accumulated above the threshold (Hassaan et al. 2016). As a counter mechanism, organisms regulate levels by binding (or sequestering) elements to proteins in organs or inert tissues, such as bone in vertebrates or the exoskeleton of invertebrates (Mertz 1981; Seymore et al. 1995; Degger et al. 2009; Haug et al. 2011; Martiniaková et al. 2012; Callender 2013). Metal sequestration studies have shown that sequestration patterns are variable between species of organisms (Weeks et al. 1992) and between metal species (Kataria et al. 2022).

One of the principal elements found in the hard structures of organisms is calcium. Specifically, in invertebrates the exoskeleton is variably mineralised with calcium carbonate (Bentov et al. 2016). Some metals are able to substitute calcium in the exoskeleton, and in this way, the structure is a main sequestration site for metals (Mergelsberg et al. 2019).

In aquatic and terrestrial invertebrates, sclerotised structures of the exoskeleton such as ovipositors (Quicke et al. 1998), mandibles (Schofield et al. 2002), and tarsal claws (Schofield et al. 2003) sequester higher levels of trace elements including metals. In crustaceans, a significant fraction of the element burden is present in the exoskeleton; distributed between the exoskeleton and the inner matrix (Zanders and Rojas 1996; Munger and Hare 1997). Therefore, trace elements in the exoskeleton are lost during moulting, and this has been suggested as an important regulatory pathway in these organisms (Weeks et al. 1992; Keteles and Fleeger 2001; Bergey and Weis 2007).

Studies on metal accumulation in parasites have shown that some parasites accumulate metals in higher concentrations than in their hosts' tissues, and because of this, they are

Communicated by B. Pelster.

✉ Annemarië Avenant-Oldewage
aoldewage@uj.ac.za

¹ Department of Zoology, University of Johannesburg, P. O. Box 524, Auckland Park 2006, South Africa

considered to be effective bioindicators (Sures 2004; Retief et al. 2006; Nachev et al. 2013; Sures et al. 2017; Pretorius and Avenant-Oldewage 2021). In some instances, parasites are used as effect indicators and others as accumulation indicators. Exposure to poor water quality may result in a reduction in parasite infection (Avenant-Oldewage 1994). Most studies involving accumulation indicators have focused on endoparasites, particularly acanthocephalans and cestodes. Sures et al. (1994) found that the acanthocephalan, *Pomphorhynchus laevis* infecting chub (*Leuciscus cephalus*), accumulated Pb in higher concentrations compared to the intestinal, muscle and liver tissues of the host. Similarly, *Acanthocephalus anguillae* accumulated Ag, Cd, Cu, Mn, and Pb in higher concentrations than their perch host (*Perca fluviatilis*) (Filipović et al. 2014). Retief et al. (2006) and Retief et al. (2009) in *Schyzocotyle acheilognathi* and Malek et al. (2007) in *Acanthobothrium* sp. and *Paraorigmatobothrium* sp. showed that concentrations of Cd and Pb were also higher in cestodes than in their hosts. Studies comparing trace-metal accumulation in ectoparasites are comparatively fewer. In two studies in two different monogenean species, Fe and Zn were accumulated in higher concentrations than in hosts' muscle tissue (Gilbert et al. 2022; Nachev et al. 2022).

Regarding the regulation and detoxification of trace elements in parasites, compartmentalisation and sequestration occurred in hardened, sclerotised structures and eggshells in some taxa (Riggs et al. 1987; Sures et al. 1994; Shinn et al. 1995; Degger et al. 2009; Khalil et al. 2009; Gilbert and Avenant-Oldewage 2017, 2018). In endoparasites, metals became sequestered mostly on the eggshells. For instance, Riggs et al. (1987) in *S. acheilognathi* and Sures et al. (1997) in *Bothriocephalus scorpii* showed higher levels of Se, Cd, and Pb, respectively, in the gravid proglottids. Degger et al. (2009) and Khalil et al. (2009) confirmed that the metals bind on the eggshells of *S. acheilognathi* using fluorescence microscopy and X-ray microanalysis, respectively. As for the crustaceans, *Argulus japonicus* and *Argulus foliaceus*, metals are not sequestered on the eggshells, but instead in the sclerotised structures of the carapace (Haug et al. 2011; Gilbert and Avenant-Oldewage 2018). Furthermore, for another crustacean *Lamproglana clariae*, it was observed that exposure to metals resulted in a change to their metalloprotein expression (Ndaba et al. 2022) and a reduction in infection intensity in natural environments (Tsoetsi et al. 2004; Crafford and Avenant-Oldewage 2009; Pretorius and Avenant-Oldewage 2021). All previous studies have focused on attached adult female *L. clariae* (Ndaba et al. 2022; Pretorius and Avenant-Oldewage 2022). This can be attributed to the fact that only gravid female *L. clariae* parasitise the host fish, while all other life stages and adult males are free-living (Madanire-Moyo and Avenant-Oldewage 2013).

The current study reports on the biomineralisation and sequestration of elements in female *L. clariae* including the

eggs and egg membrane. Specimens were collected from two different freshwater habitats in South Africa, which are variably impacted by pollution. The aims of the study were therefore to determine if metals accumulated by *L. clariae* become sequestered. Furthermore, if metals are sequestered, are they distributed variably through the body, and reflect levels in the environment.

Materials and methods

Samples of adult female *L. clariae* were collected from the gills of *Clarias gariepinus* from Lake Heritage, along the Crocodile River (S25°57'16.923" E 27°51'47.486") and in the Vaal River (S26°52'12.53" E 28°7'14.09") below the Vaal Dam (Fig. 1). The Vaal River is known to be polluted (Crafford et al. 2011; Pretorius and Avenant-Oldewage 2021; Ndaba et al. 2022) and Lake Heritage is affected by acid mine drainage (Windisch et al. 2022). Gill nets were used to collect 10 *C. gariepinus* (stretched mesh size: 60–170 mm) per sampling site and transported to the field laboratory where they were weighed, measured, and euthanised by severing the spinal cord. The gills were removed and placed into a Petri dish with water from the site. The gills were inspected for *L. clariae* using a Zeiss Stemi 305 dissection microscope (Jena, Germany). Handling of the fish was done in accordance with guidelines by the South African National Animal Ethics Council and approved by the University of Johannesburg Ethics committee (Reference Number: 2021-04-01/Latief_Oldewage). All collections of the fish were done in accordance with the conditions of the permit issued by the Gauteng Department of Agriculture and Rural Development (permit number: CPE2-000140). Five *Lamproglana clariae* were collected, placed into 2 mL microcentrifuge tubes, flash frozen in liquid nitrogen, and later transferred for storage in a – 80 °C freezer (Evosafe-series VF720-86, Snijders Labs, The Netherlands) until sectioning.

Cryosectioning

Specimens ($n = 5$ per site) were embedded in optimal cutting temperature compound (Scigen Scientific, California, USA) and sectioned at 6 μm using a Reichert–Jung CryoCut E cryomicrotome operated at – 20 °C. Sections were mounted on cleaned microscope slides. Slides were cleaned with acid alcohol to remove trace elements. Sections on slides were air dried at room temperature for 30 min and stored at – 80 °C.

Fluorescence microscopy

Sections were photobleached with a UV bulb (Philips TUV 30W, Holland), mounted in Phen-Green™ FL

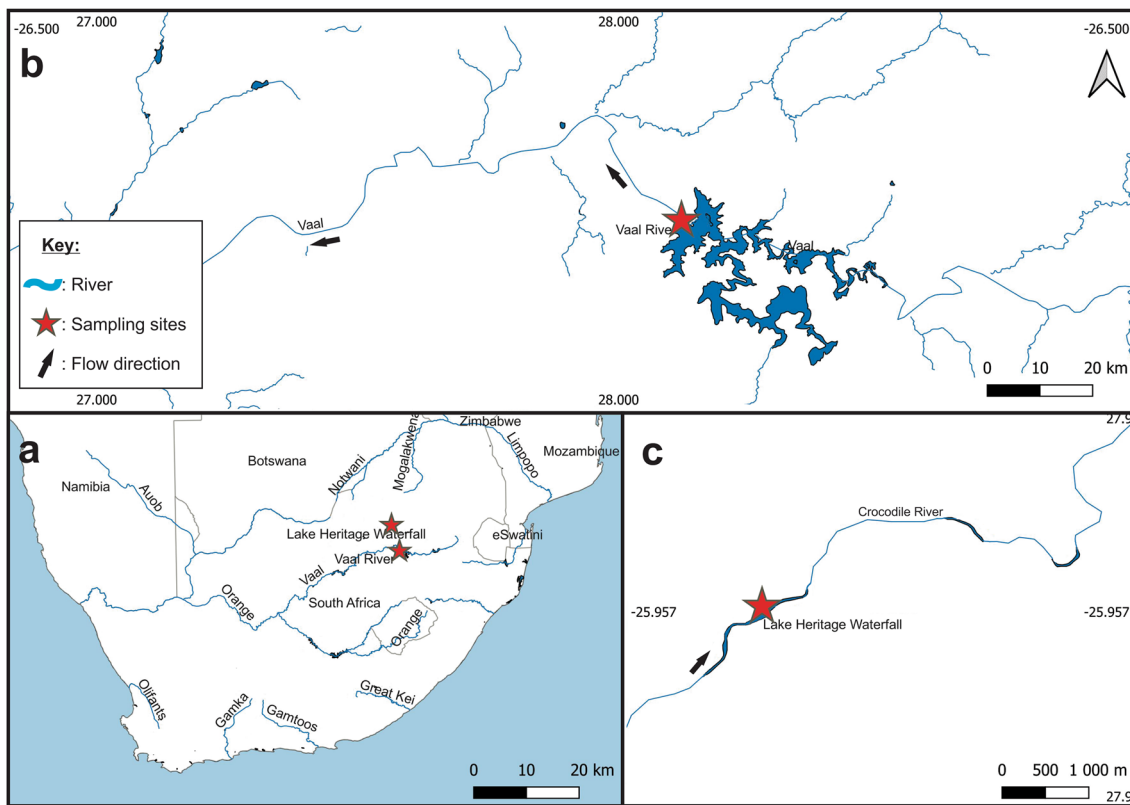


Fig.1 Sampling site locations in South Africa (**a**) for the Vaal River (**b**) and Crocodile River (**c**). Red stars (★) indicate sampling sites and black arrows (→) showing the direction of water flow in the rivers

cell-permeant diacetate (Molecular Probes, Eugene, Oregon, United States of America) and sealed with clear nail varnish (Revlon) in the dark to prevent photodegradation. Sections were studied using a Zeiss Axioplan 2 epifluorescence microscope operated with Axiovision 4.3 software with rhodamine and DAPI band-pass filters (BP 365/12; excitation 490 nm; emission 520 nm).

SEM–EDS analysis

Additional sections used for analysis of elements and to correlate with fluorescence results were dried in a desiccator cabinet (Kita-Ku, Osaka, Japan) and thereafter coated with carbon. The sections were analysed by point analysis at 20 keV using a Tescan Vega 3 scanning electron microscope (SEM) (Brno, Czech Republic) equipped with a X-Max50 energy-dispersive X-ray spectrometer (EDS) (Oxford Instruments, Halifax, England), operated by Aztec 2.1 software (Oxford Instruments, Halifax England) for Windows. Body tagmata and eggs were scanned for all elements. Only Mg, Al, Ca, Fe, Cu, and Zn were

detected and levels were expressed in weight percentage (wt %).

Statistical analysis

For the statistical analysis, IBM SPSS Version 28 Statistical package for Windows was used. To assess normality of the data for elements in wt %, the Levene's test and histograms were used. As the data were not normally distributed, comparisons of element levels among tagmata were done using the Kruskal–Wallis test. In cases where there was a significant difference between tagmata a Mann–Whitney *U* test was used for pairwise comparisons. To test for differences in element levels between the exoskeleton and tissue in adults, and between egg contents and egg membrane, a Mann–Whitney *U* test was used. Differences in element levels in parasites from each site were tested using a Mann–Whitney *U* test. Bar charts were constructed for SEM–EDS data by log transforming the data ($\ln[\text{concentration} + 1]$), with the error bars displaying 95% confidence intervals. All statistical tests were assessed at a confidence level of 95%.

Results

Fluorescence microscopy

Sections of adult female *L. clariae* collected from Lake Heritage and the Vaal River showed brighter fluorescence in the exoskeleton compared to the tissue of all tagmata (Fig. 2b–g). Sections through the egg sacs indicated brighter fluorescence of the egg yolk than the egg membrane (Fig. 2h–i), within eggs brighter signals were observed for yolk droplets (Fig. 2j). Furthermore, eggs within the thorax showed a weaker fluorescence signal in the membrane and yolk than those in the egg sacs (Fig. 2d, e, h and i). The egg sacs from Lake Heritage showed intense yellow fluorescence, whereas eggs from the Vaal River showed an intense green signal (Fig. 2h and i). Although specimens were collected from different sites, no differences were observed when comparing fluorescence signals of body sections.

EDS analysis

The presence of Mg, Al, Ca, Fe, Cu, and Zn was confirmed in all sections of tagmata and eggs (Fig. 3). The distribution of elements among the different tagmata, and the exoskeleton and tissue of the head, thorax, and abdomen varied. It also varied between the sites. Element concentrations in samples from Lake Heritage differed significantly in tissue between tagmata (Kruskal–Wallis for all elements; $p < 0.05$). While, in the exoskeleton, only levels of Fe, Cu, and Zn were significantly different among tagmata (Kruskal–Wallis Fe, Cu and Zn; $p < 0.05$). Comparing the exoskeleton to the tissue of all tagmata Al (Mann–Whitney U test: $Z = -2.12$, $p = 0.034$), Mg (Mann–Whitney U test: $Z = -4.29$, $p < 0.001$) and Ca (Mann–Whitney U test: $Z = -4.48$, $p < 0.001$) were higher. Whereas Cu (Mann–Whitney U test: $Z = -0.11$, $p = 0.916$) and Zn (Mann–Whitney U test: $Z = -1.78$, $p = 0.075$) were higher in the tissue than the exoskeleton for all tagmata, and Fe (Mann–Whitney U test: $Z = -1.94$, $p = 0.052$) was only higher in the exoskeleton of the thorax.

For specimens from the Vaal River, distribution of metals among the tagmata varied; for the tissue Al, Mg, Ca, and Zn (Kruskal–Wallis; $p < 0.05$), while for the exoskeleton, Al, Fe, Mg, and Ca (Kruskal–Wallis; $p < 0.05$) were significantly different. Higher levels of Al (Mann–Whitney U test: $Z = -0.31$, $p = 0.755$) and Fe (Mann–Whitney U test: $Z = -1.13$, $p = 0.258$) were detected in the exoskeleton of the head and thorax, but in the abdomen levels for both elements were higher in the tissue. The only element which showed similar accumulation patterns was

Ca (Mann–Whitney U test: $Z = -1.10$, $p = 0.272$) which was consistently higher in the exoskeleton. Levels of Mg (Mann–Whitney U test: $Z = -1.78$, $p = 0.075$) showed opposite trends to Al and Fe, in that higher levels were detected in the tissue of the head and thorax, and in the abdomen levels were also higher in the exoskeleton than the tissue. For Zn (Mann–Whitney U test: $Z = -0.79$, $p = 0.431$), higher levels were detected in the exoskeleton of the head than in the tissue, but in the thorax and abdomen levels were higher in the tissue than the exoskeleton. Levels of Cu (Mann–Whitney U test: $Z = -0.62$, $p = 0.537$) showed no differences between exoskeleton and tissue in all body tagmata.

Element distribution within the eggs from both sampling sites showed no difference in levels of Cu (Mann–Whitney U test: $Z = -0.04$, $p = 0.970$) between the egg membrane and the egg contents. However, Al (Mann–Whitney U test: $Z = -0.09$, $p = 0.929$), Fe (Mann–Whitney U test: $Z = -0.12$, $p = 0.902$), and Zn (Mann–Whitney U test: $Z = -1.28$, $p = 0.199$) were higher in the yolk than the egg membrane for *L. clariae* from Lake Heritage, while at the Vaal River, these metals were higher in the egg membrane than the yolk. Mg (Mann–Whitney U test: $Z = -0.01$, $p = 0.990$) and Ca (Mann–Whitney U test: $Z = -0.90$, $p = 0.366$) were higher in the yolk in parasites collected at the Vaal River, whereas at Lake Heritage, these metals were higher in the egg membrane.

Discussion

In *L. clariae* treatment with Phen-Green, resulted in more intense fluorescence of the exoskeleton compared to internal tissues. The intensity of the fluorescent signal of Phen-Green is related to the concentration and type of ion present (Johnson and Michelle 2010), and therefore, areas of the body which are more mineralised will show brighter fluorescence. Similarly, in *A. japonicus*, Gilbert and Avenant-Oldewage (2018) reported a more intense fluorescence signal in the sclerotised parts of the exoskeleton. Higher metal levels in the exoskeleton of *L. clariae* were confirmed using SEM–EDS and Mg, Al, Ca, and Fe were higher in the exoskeleton compared to the internal tissues. It is well known that the sclerotised parts of the exoskeleton in invertebrates incorporates elements (Quicke et al. 2004) and offers protection to the internal structures of crustaceans (Olesen 2013). Metals, such as Zn, occur in the cutting edges of the mandibles and maxillae of *Atta sexdens* (leaf-cutting ants) and the jaws of *Nereis limbata* (marine polychaete) (Schofield et al. 2002; Lichtenegger et al. 2003).

In addition to the protective and functional aspects associated with biomineralisation of the exoskeleton, it functions in reducing trace element and metal burdens (Keteles and

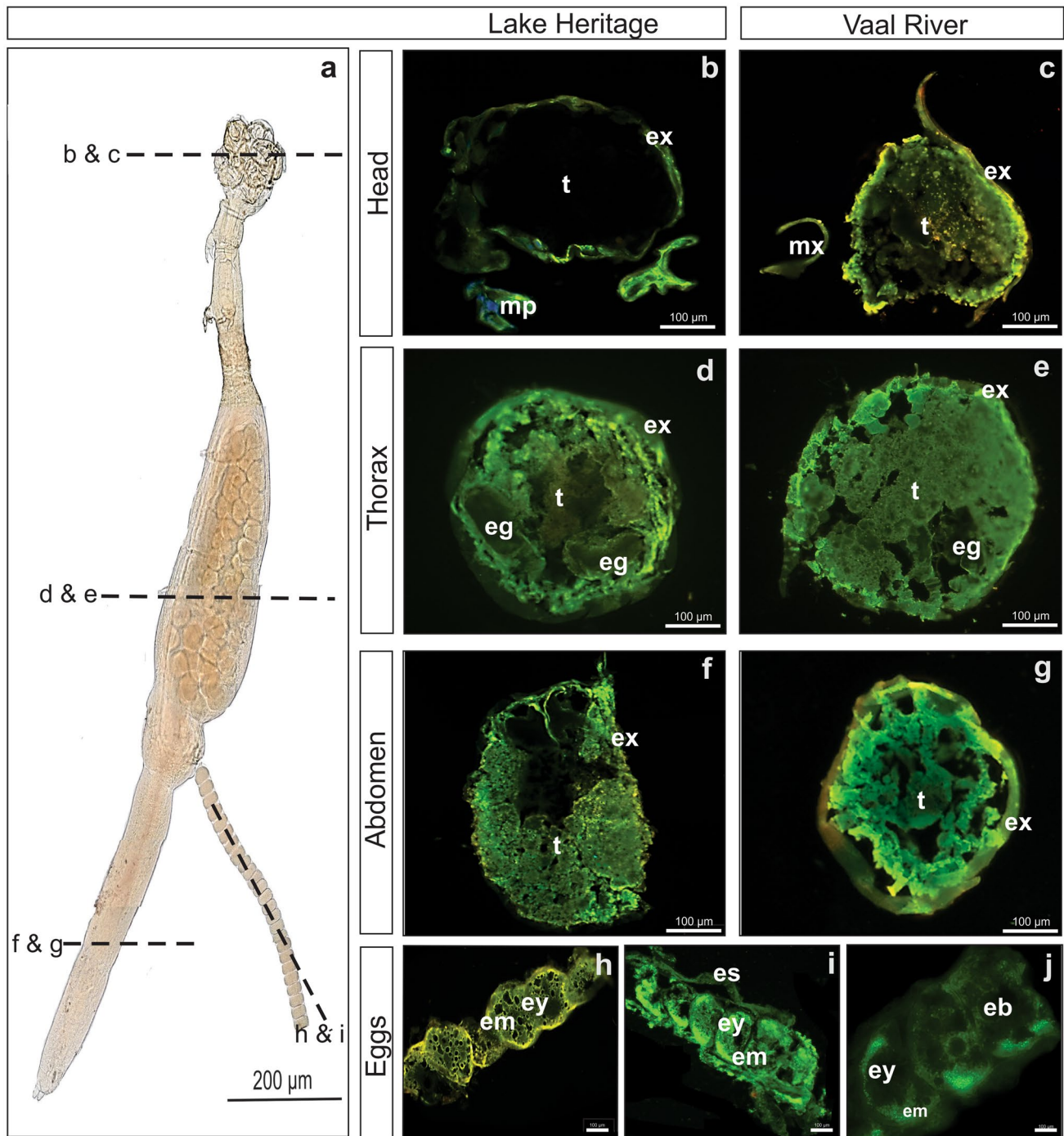


Fig.2 Light (a) and fluorescence (b–i) micrographs of *Lamproglena clariae* showing a whole mount (a) and sections through adults (b–g) and eggs (h–i). Dotted lines in a indicate section planes for micrographs b–i. Transverse sections through the head (b and c), thorax (d and e), and abdomen (f and g) show exoskeleton (ex), soft tissue (t), maxilliped (mp), maxilla (mx), and developing eggs (eg). Longi-

tudinal sections through the eggs (h–j) show the egg yolk (ey), egg membrane (em), egg sac (sc), and embryo (eb). Fluorescence of the Phen-green indicate a positive reaction for divalent cations and trace elements. Micrographs were taken utilizing the Zeiss band-pass filter set 01 (BP 365/12) at 490–520 nm

Fleeger 2001; Bergey and Weis 2007). Keteles and Fleeger (2001) reported that Cd concentrations were reduced in the grass shrimp, *Palaemonetes pugio*, following ecdysis.

Similarly, Bergey and Weis (2007) showed that crab, *Uca pugnax* from contaminated sites had higher Pb concentrations in the exoskeleton compared to the internal tissues and

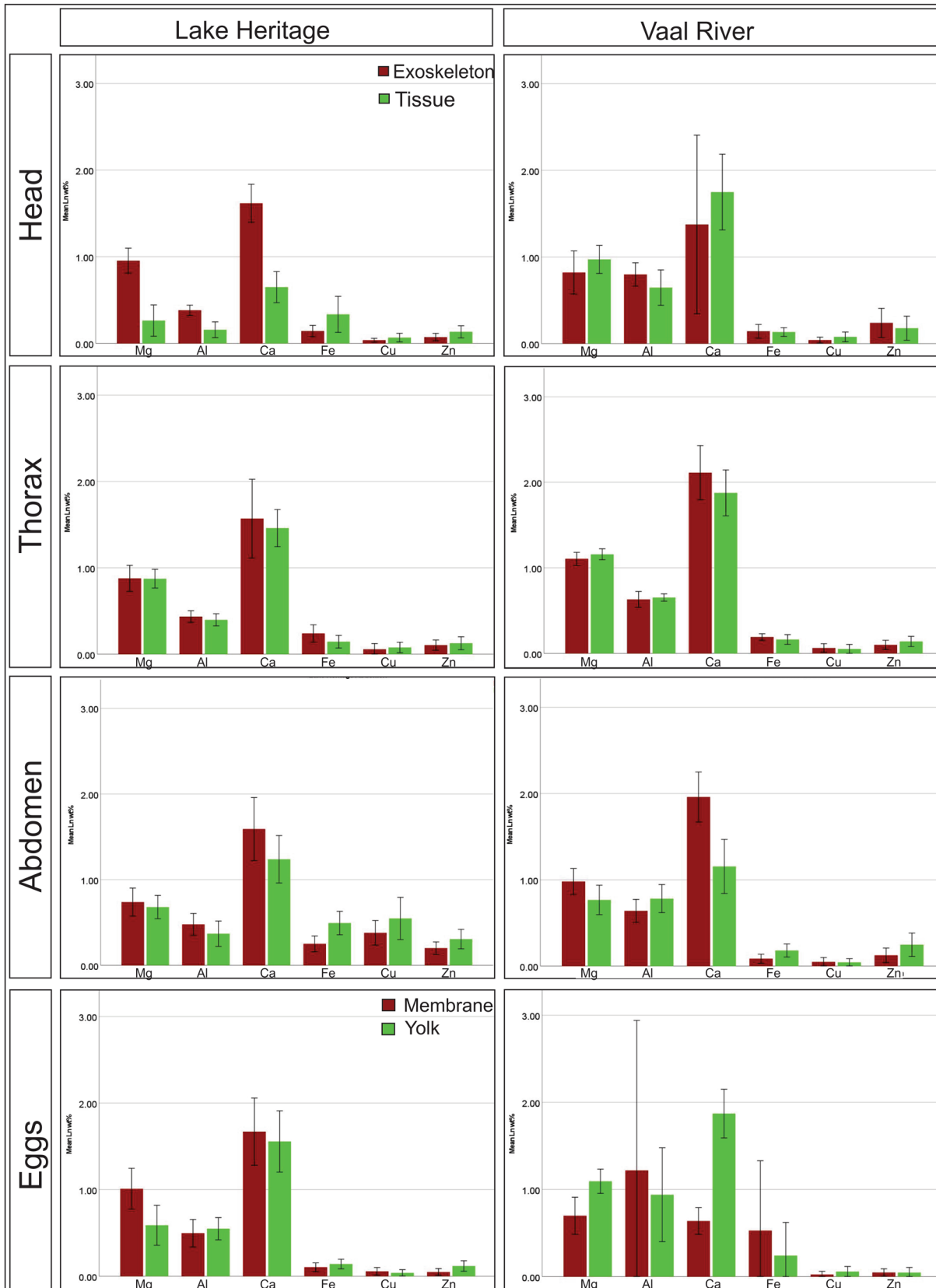


Fig. 3 Bar graphs showing the mean for the log transformed weight percentages (wt%) for elements detected in sections of the head, thorax, abdomen, and eggs of *Lamproglena clariae* by SEM/EDS analysis for samples collected at Lake Heritage and the Vaal River. Red bars indicate elements found in the exoskeleton, while green bars indicate elements found in the tissue. The error bars indicate 95% confidence intervals

therefore concluded that moulting can significantly reduce body burdens. Despite the current results showing that the exoskeleton of *L. clariae* is more enriched with some elements than the internal tissues, it is unlikely that the exoskeleton in adult females plays a significant role in metal regulation. This is because it is unlikely that moulting occurs in permanently attached adult females of *L. clariae*. Therefore, elements are likely deposited as a means of providing support to the exoskeleton, but this may be a limited means of regulating metals.

Metals are deposited on the eggs as a means of reducing body burdens in some parasites (Sures et al. 1997; Degger et al. 2009; Khalil et al. 2009; Gilbert and Avenant-Oldewage 2017, 2018). However, in adult female *L. clariae* fluorescence and SEM–EDS results confirmed that elements were deposited inside the egg, specifically in the yolk. Sequestration in the eggs is supported by the fact that elements, such as Al, Mg, Ca, Fe, and Zn, were higher in the eggs compared to sections of adult parasites from both sites. In cestodes, Sures et al. (1997) showed that gravid proglottids had higher Pb levels, and this was later confirmed to be bound onto the eggshells (Degger et al. 2009; Khalil et al. 2009).

However, in ectoparasites, Gilbert & Avenant-Oldewage (2017, 2018) showed that the eggshells in *P. ichthyoxanthon* and *A. japonicus* lack elements, rather metals sequestered to the vitellaria in *P. ichthyoxanthon* (Gilbert and Avenant-Oldewage 2017) and gelatinous layer around the eggshell in *A. japonicus* (Gilbert and Avenant-Oldewage 2018). In the present study, a positive signal for yolk droplets within the eggs of *L. clariae* suggest that similar to *P. ichthyoxanthon*, elements become associated with yolk.

Comparisons between parasites from Lake Heritage and the Vaal River showed variable element levels in sections. Differences corroborated published results on dissolved element levels from each site for some elements. For instance, Zn and Fe levels in the eggs of *L. clariae* from Lake Heritage were higher than in specimens from the Vaal River, which matched the differences in levels of these elements in the water and was conducted parallel to this study (Ndaba et al. 2022; Windisch et al. 2022). Such similarities can be related to the fact that *L. clariae* is an ectoparasite and therefore is exposed to and accumulates elements present in the water and/or host blood.

Therefore, in conclusion, results from the present study demonstrated that *L. clariae* accumulates elements present in the macroenvironment, and reflect levels dissolved in the

water. Like other crustaceans, *L. clariae* deposits metals in the exoskeleton where they assist in providing support to the exoskeleton. In terms of regulating metals, it is unlikely that elements are released during moulting of the exoskeleton in adult female *L. clariae*. Rather, the current results suggest that elements become sequestered to yolk. Higher levels of elements in the yolk may become transferred to and accumulated by the nauplii. These elements could then bind to the exoskeleton of the nauplii and be released when they moult. Therefore, one way that adult *L. clariae* females could regulate element body burdens is through sequestration into the egg.

Acknowledgements We would like to thank and acknowledge the University of Johannesburg (URC) for the Special URC Scholarship for MSc degree funding to L.L. The National Research Foundation (NRF), and University of Johannesburg (FRC and URC) for project funding to AAO; The NRF for providing postdoctoral funding to BMG (grant 120683). Furthermore, we acknowledge the University of Johannesburg FRC SPECTRUM analytical facility for equipment and facilities.

Author contributions Conceptualisation: AA-O and BMG. Validation: LL, AA-O, and BMG. Sample preparation and analysis: LL. Investigation: LL, AA-O, BMG. Data curation: LL. Writing—original draft: LL. Writing—review and editing: LL, AA-O, and BMG. Supervision: AA-O and co-supervision BMG. All authors have read and agreed to the submitted version of the manuscript.

Funding Open access funding provided by University of Johannesburg. This research was funded by the University Research Committee, University of Johannesburg, the Faculty Research Committee and the National Research Foundation to AA-O Grant number [UGN 116067]. BMG received PDRF from SPECTRUM and the National Research Foundation Bursary number [120683] and LL a bursary from the University Research Committee, University of Johannesburg.

Data availability All data generated or analyzed during this study are included in the published article. Raw data can be requested from the first author on reasonable request.

Declarations

Conflict of interest The authors have no conflicts of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

Avenant-Oldewage A (1994) Parasites as indicator organisms for heavy metal pollution. *J S Afr Vet* 65:154

- Bentov S, Afalo ED, Tynyakov J, Glazer L, Sagi A (2016) Calcium phosphate mineralization is widely applied in Crustacean mandibles. *Sci Rep* 6:22118. <https://doi.org/10.1038/srep22118>
- Bergey L, Weis J (2007) Molting as a mechanism of depuration of metals in the fiddler crab, *Uca pugnax*. *Mar Environ Res* 64:556–562. <https://doi.org/10.1016/j.marenvres.2007.04.009>
- Callender E (2013) Heavy metals in the environment - historical trends. *Treatise Geochem* 9:67–105
- Crafford D, Avenant-Oldewage A (2009) Application of a fish health assessment index and associated parasite index to *Clarias gariepinus* (Teleostei: Clariidae) in the Vaal River system, South Africa. *Afr J Aquat Sci* 34(3):261–272. <https://doi.org/10.2989/AJAS.2009.34.3.8.984>
- Crafford D, Avenant-Oldewage A (2011) Uptake of selected metals in tissues and organs of *Clarias gariepinus* (sharp-tooth catfish) from the Vaal River System - Chromium, copper, Iron, manganese and Zinc. *Water SA* 37(2):181–200. <https://doi.org/10.4314/wsa.v37i2.65864>
- Degger N, Avenant-Oldewage A, Greenfield R (2009) Innovative fluorescence detection technique for metals in cestode egg-shells. *Afr Zool* 44(2):204–207
- Filipović MV, Vardić SI, Raspor B (2014) Does fish reproduction and metabolic activity influence metal levels in fish intestinal parasites, acanthocephalans, during fish spawning and post-spawning period? *Chemosphere* 112:449–455
- Gilbert BM, Avenant-Oldewage A (2017) Trace element and metal sequestration in vitellaria and sclerites, and reactive oxygen intermediates in a freshwater monogenean *Paradiplozoon ichthyoxanthon*. *PLoS ONE* 12:e0177558. <https://doi.org/10.1371/journal.pone.0177558>
- Gilbert BM, Avenant-Oldewage A (2018) Trace element biomineralisation in the carapace in male and female *Argulus japonicus*. *PLoS ONE* 13:e0197804. <https://doi.org/10.1371/journal.pone.0197804>
- Gilbert BM, Jirsa F, Avenant-Oldewage A (2022) First record of trace element accumulation in a freshwater ectoparasite, *Paradiplozoon ichthyoxanthon* (Monogenea: Diplozoidae), infecting the gills of two yellowfish species, *Labeobarbus aeneus* and *Labeobarbus kimberleyensis*. *J Trace Elem Med Biol* 74:1–7. <https://doi.org/10.1016/j.jtemb.2022.127053>
- Hassaan MA, El Nemr A, Madkour FF (2016) Environmental assessment of heavy metal pollution and human health risk. *Am J Water Sci Eng* 2(3):14–19. <https://doi.org/10.11648/j.ajwse.20160203.11>
- Haug JT, Haug C, Kutschera V, Mayer G, Maas A, Liebau S, Castellani C, Wolfram U, Clarkson ENK, Waloszek D (2011) Autofluorescence imaging, an excellent tool for comparative. *J Microsc* 244(3):259–272. <https://doi.org/10.1111/j.1365-2818.2011.03534.x>
- Johnson ID, Michelle T (2010) *The molecular probes handbook: a guide to fluorescent probes and labeling technologies*. Eugene, Oregon
- Katarina N, Chauhan AK, Garg VK, Kumar P (2022) Sequestration of heavy metals from contaminated water using magnetic carbon nanocomposites. *J Hazard Mater* 6:100066
- Keteles KA, Fleeger JW (2001) The contribution of ecdysis to the fate of copper, zinc and cadmium in grass shrimp *Palaemonetes pugio* Holthuis. *Mar Pollut Bull* 42(12):1397–1402
- Khalil M, Furness D, Polwart A, Hoole D (2009) X-ray microanalysis (EDXMA) of cadmium-exposed eggs of *Bothriocephalus acheilognathi* (Cestoda: Bothriocephalidae) and the influence of this heavy metal on coracidial hatching and activity. *Int J Parasitol* 39:1093–1098. <https://doi.org/10.1016/j.ijpara.2009.02.023>
- Lichtenegger HC, Schöberl T, Ruokolainen JT, Cross JO, Heald SM, Birkedal H, Waite JH, Stucky GD (2003) Zinc and mechanical prowess in the jaws of *Nereis*, a marine worm. *PNAS* 100(16):9144–9149
- Madanire-Moyo GN, Avenant-Oldewage A (2013) On the development of a parasitic copepod, *Lamproglana clariae* Fryer, 1956 (Copepoda, Lernaeidae) infecting the sharp-tooth catfish, *Clarias gariepinus*. *Crustaceana* 86:416–436. <https://doi.org/10.1163/15685403-00003165>
- Malek M, Haseli M, Mobedi I, Ganjali MR, MacKenzie K (2007) Parasites as heavy metal bioindicators in the shark *Carcharhinus dussumieri* from the Persian Gulf. *Parasitology* 134:1–4. <https://doi.org/10.1017/S0031182007002508>
- Martiniaková M, Omelka R, Stawarz R, Formicki G (2012) Accumulation of lead, cadmium, nickel, iron, copper, and zinc in bones of small mammals from polluted areas in Slovakia. *Pol J Environ Stud* 21(1):153–158
- Mergelsberg ST, Ulrich RN, Xiao S, Dove PM (2019) Composition systematics in the exoskeleton of the American Lobster, *Homarus americanus* and implications for Malacostraca. *Front Earth Sci* 7:69. <https://doi.org/10.3389/feart.2019.00069>
- Mertz W (1981) The essential trace elements. *Science* 213:1332–1338. <https://doi.org/10.1126/science.7022654>
- Munger C, Hare L (1997) Relative importance of water and food as cadmium sources to an aquatic insect (*Chaoborus punctipennis*): implications for predicting Cd bioaccumulation in nature. *Environ Sci Technol* 31(3):891–895
- Nachev M, Schertzing G, Sures B (2013) Comparison of the metal accumulation capacity between the acanthocephalan *Pomphorhynchus laevis* and larval nematodes of the genus *Eustrongylides* sp. Infecting Barbel (*Barbus barbus*). *Parasit Vectors* 6(21):1–8. <https://doi.org/10.1186/1756-3305-6-21>
- Nachev M, Rozdina D, Michler-Kozma D, Raikova G, Sures B (2022) Metal accumulation in ecto- and endoparasites from the anadromous fish, the Pontic shad (*Alosa immaculata*). *Parasitology*. <https://doi.org/10.1017/S0031182021002080>
- Ndaba J, Gilbert BM, Avenant-Oldewage A (2022) Metallothionein expression in a parasitic crustacean, *Lamproglana clariae* (Crustacea: copepoda), on *Clarias gariepinus* (Teleostei: Clariidae) corresponds to water quality. *J Parasitol* 108(1):10–21. <https://doi.org/10.1645/21-62>
- Olesen J (2013) *The crustacean carapace: morphology, function, development and phylogenetic history*. Functional morphology and diversity. Oxford University Press, pp 103–139
- Pretorius M, Avenant-Oldewage A (2021) Parasites as biological indicators: the impact of environmental quality on the infections of *Lamproglana clariae* (Crustacea) on *Clarias gariepinus* along the Vaal River, South Africa. *Biol Trace Elem Res* 200:2937–3947. <https://doi.org/10.1007/s12011-021-02899-5>
- Pretorius M, Avenant-Oldewage A (2022) Hatchability and survival of *Lamproglana clariae* Fryer, 1956 exposed to increasing concentrations of aqueous aluminium. *Appl Sci* 13(4):2145. <https://doi.org/10.3390/app13042145>
- Quicke DLJ, Wyeth P, Fawke JD, Basibuyuk HH, Vincent JFV (1998) Manganese and zinc in the ovipositors and mandibles of hymenopterous insects. *Zool J Linn Soc* 124:387–396
- Quicke DLJ, Palmer-Wilson J, Burrough A, Broad GR (2004) Discovery of calcium enrichment in cutting teeth of parasitic wasp ovipositors (Hymenoptera: Ichneumonidae). *Afr Entomol* 12(2):259–264
- Retief NR, Avenant-Oldewage A, du Preez H (2006) The use of cestode parasites from the largemouth yellowfish, *Labeobarbus kimberleyensis* (Gilchrist and Thompson, 1913) in the Vaal Dam, South Africa as indicators of heavy metal bioaccumulation. *Phys Chem Earth* 31:840–847. <https://doi.org/10.1016/j.pce.2006.08.004>
- Retief NR, Avenant-Oldewage A, du Preez HH (2009) Seasonal study on *Bothriocephalus* as an indicator of metal pollution in yellowfish, South Africa. *Water SA* 35(3):315–322. <https://doi.org/10.4314/wsa.v35i3.76769>

- Riggs M, Lemly D, Esch GW (1987) The growth, biomass, and fecundity of *Bothriocephalus acheilognathi* in a North Carolina cooling reservoir. *J Parasitol* 73(5):893–900
- Schofield RMS, Nesson MN, Richardson KA (2002) Tooth hardness increases with zinc-content in mandibles of young adult leaf-cutter ants. *Sci Nat* 89:579–583. <https://doi.org/10.1007/s00114-002-0381-4>
- Schofield R, Nesson M, Richardson K, Wyeth P (2003) Zinc is incorporated into cuticular “tools” after ecdysis: the time course of the zinc distribution in “tools” and whole bodies of an ant and a scorpion. *J Insect Physiol* 49:31–44
- Seymore T, du Preez H, van Vuren JH (1995) Manganese, lead and strontium bioaccumulation in the tissues of the yellowfish, *Barbus marequensis* from the lower Olifants River, Eastern Transvaal. *Water SA* 21(2):159–172
- Shinn AP, Gibson DI, Sommerville C (1995) A Study of the composition of the sclerites of *Gyrodactylus* Nordmann, 1832 (Monogenea) using X-ray elemental analysis. *Int J Parasitol* 25(7):797–805
- Sures B (2004) Environmental parasitology: relevancy of parasites in monitoring environmental pollution. *Trends Parasitol* 20(4):170–177. <https://doi.org/10.1016/j.pt.2004.01.014>
- Sures B, Taraschewski H, Jackwerth E (1994) Lead accumulation in *Pomphorhynchus laevis* and its host. *J Parasitol* 80(3):355–357
- Sures B, Taraschewski H, Rokicki J (1997) Lead and cadmium content of two cestodes, *Monobothrium wageneri* and *Bothriocephalus scorpii*, and their fish hosts. *Parasitol Res* 83:618–623
- Sures B, Nachev M, Selbach C, Marcogliese DJ (2017) Parasite responses to pollution: what we know and where we go in ‘Environmental Parasitology.’ *Parasit Vectors* 10(65):1–19. <https://doi.org/10.1186/s13071-017-2001-3>
- Tsotetsi AM, Avenant-Oldewage A, Mashego SN (2004) Aspects of the ecology of *Lamproglena clariae* (copepoda: Lernaeidae) from the Vaal River system, South Africa. *J Crustac Biol* 24(4):529–536
- Weeks JM, Rainbow PS, Moore PG (1992) The loss, uptake and tissue distribution of copper and zinc during the moult cycle in an ecological series of talitrid amphipods (Crustacea: Amphipoda). *Hydrobiologia* 245:15–25. <https://doi.org/10.1007/BF00008725>
- Windisch J, Gradwohl A, Gilbert BM, Dos Santos QM, Wallner G, Avenant-Oldewage A, Jirsa F (2022) Toxic elements in sediment and water of the crocodile river (West) system, South Africa following acid mine drainage. *Appl Sci* 12:10531. <https://doi.org/10.3390/app122010531>
- Zanders IP, Rojas WE (1996) Salinity effects on cadmium accumulation in various tissues of the tropical fiddler crab *Uca rapax*. *Environ Pollut* 94:293–299

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.