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Hatchability and Survival of *Lamproglena clariae* Fryer, 1956 Exposed to Increasing Concentrations of Aqueous Aluminium

Marilie Pretorius and Annemariè Avenant-Oldewage *

Department Zoology, University of Johannesburg, Auckland Park, P.O. Box 524, Johannesburg 2006, South Africa
* Correspondence: aoldewage@uj.ac.za

Abstract: The effect of increased aluminium concentration on the hatchability and survival of *Lamproglena clariae* is unknown. During October 2019 and October 2020, infected *C. gariepinus* were collected from the Vaal River and transferred to a controlled aquarium room. Parasite infection was established on acclimated *C. gariepinus*. Adult female parasites (F2 and F3 generation) on live fish were inspected for egg strings. Viable egg strings were removed, leaving females attached to produce more eggs. Bioassays were performed in varying concentrations of Al (control, 5 µg/L, 30 µg/L, 60 µg/L, and 120 µg/L). Egg development was monitored. In situ physical and chemical water quality parameters were measured, and water samples were collected every 24 h for metal analysis using inductively coupled mass spectrometry. The experiment terminated when all juveniles perished. Five percent of exposed eggs did not hatch in the control solution, compared to 26% in 120 µg/L Al. Hatchability and survival of *L. clariae* were negatively affected by increased Al concentrations. By removing this ectoparasite from living fish, the need to euthanise the host organism is eliminated, emphasising the usefulness of *L. clariae* as a bioindicator for metal pollution.

Keywords: ectoparasite; aluminium; toxicity; survival; hatching; bioindicator; metal pollution

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1. Introduction

Aluminium is one of the most abundant elements in nature, yet its biological function in ecosystems is still poorly known [1]. Awareness of its toxicity in aquatic and terrestrial environments has prompted various studies [2–4]. Aluminium is present in aquatic environments in relatively low concentrations due to the natural weathering of rocks; however, anthropogenic influences tend to increase the mobility of Al [1]. Aluminium is insoluble at a pH range of 6 to 8; however, it becomes soluble in more acidic or alkaline environments. Aluminium in soil can become soluble when in contact with acidic water and can cause the leaching of Al into streams, dams, or rivers [5]. In aquatic environments, this element's speciation changes are complex, making it challenging to define survival criteria for fish species in acidic environments [6]. Aqueous Al consists of various Al hydroxy species, including Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^+$, $Al(OH)_3^o$ $Al(OH)_4^-$ forming inorganic complexes with fluoride (F^-), and sulphates (SO_4^{2-}). Moreover, aluminium can bind with organic materials such as humic acids ($C_{187}H_{186}O_{89}N_9S$) and fulvic acids ($C_{14}H_{12}O_8$) associated with plant matter in the soil [1,7].

Evidence of Al bioaccumulation has been found in macrophytes that absorb most of their mineral requirements directly from the water through submerged roots in acidic environments [1]. Algae, lichen, and fungi in aquatic environments contribute to the solubility of Al by releasing citrates, oxalates, acetates, and salicylates [1]. Previous studies indicate that dissolved Al can be toxic to the diatoms *Ceratoneis closterium*, *Minutocellus polymorphus*, and *Phaeodactylum tricornutum* [8]. Similarly, the adverse effects of Al in acidic water on vertebrates such as fish populations are well known [6,9–11]. Exposing juvenile Atlantic Salmon (*Salmo salar*) to Al and zinc mixtures in a low pH environment (pH between 4.5 and 5.2), Roy and Campbell, (1995) [12] indicated a significant shortening of survival

time when zinc was present, indicating that the presence of other metals in a freshwater environment can contribute to the toxicity of Al in acidic conditions. Moreover, Adams et al. (2018) [13] concluded that both dissolved and precipitated Al on the gills of *S. salar* were toxic to the fish.

Compared with vertebrates, fewer studies have investigated Al uptake, accumulation, and toxicity in invertebrates. It has been suggested that invertebrates are generally less sensitive to Al than fish [1,10,14]. It was previously reported that aluminium does not biomagnify in aquatic invertebrates such as Cladocera, Decapoda, Mollusca, and Insecta [1,10,12,15]. High Al concentrations can lead to malfunctioning of ion regulation in both vertebrate and invertebrate organisms; for example, Havens (1993) [16] reported that survival of *Skistodiaptomus oregonensis* (Copepoda) decreased when they were exposed to 200 µg/L Al at a pH of 6. This was due to Al reacting antagonistically toward osmoregulatory effects of H^+ and the resulting loss of Na. According to Havas (1986) and Gensemer and Playle (1999) [1,17], their staining of chloride cells in crustaceans *Branchinecta paludosa* and *Daphnia magna* with hematoxylin showed that Al accumulated on the anal papillae, where ion-regulation occurs. In other aquatic invertebrates such as dragonfly nymphs (*Somatochlora cingulata*), oxygen uptake and ammonia excretion decreased at high Al concentrations (10.30 mg/L^{-1}) and low pH (4.2 and 3.6) [18]. Moreover, it was determined that exposure to high concentrations of aqueous Al in neutral pH harms the immunocompetence in the crayfish *Pacifasticus leniusculus* [18].

In Norway, controversy followed the use of rotenone, a non-species-specific parasiticide, to eradicate *Gyrodactylus salaris* on Atlantic salmon [19] as it also killed infected fish, other fish species, and gill-breathing invertebrates [19]. Therefore, there is a search for species-specific parasiticides that can effectively treat parasite infections in economically valuable fish species [19,20]. In the pursuit of finding a less controversial method, aluminium has been identified as a possible alternative [19]. Aqueous Al effectively eliminates *G. salaris* in four days (202 µg/L) on Atlantic salmon (*S. salar*) under laboratory conditions [21].

In the Vaal River, South Africa, parasite infection is negatively impacted by increased metal concentrations in the water [22–24]. In a previous study, the crustacean *L. clariae* was identified as a potential bioindicator [24] of the impact of metal, particularly Al pollution, on biodiversity at six sites along the Vaal River (Supplemental Figure S1).

Adult *L. clariae* females attach to the gill filaments of *C. gariepinus.* Gravid specimens bear two egg strings (mean of 52 eggs) and are directly exposed to the environment [25]. Eggs hatch randomly from the egg strings [25]. The first naupliar stage (NI) emerges from the eggs, moults into nauplius II (NII) after two days, and then moults into nauplius III (NIII) after five days [25]. After extreme morphological transformation [25], copepodid I (CI) emerges eight to 15 days after hatching followed by copepodid II and III (CII and CIII), which live for two days after moulting. Thereafter, male and female cyclopoid stages follow, and, after copulation, the females attach to a host and feed on its blood [25,26].

This study records the effect of increasing aluminium concentrations on *L. clariae* hatching and survival in a controlled environment.

2. Materials and Methods

Aluminium was chosen as the exposure element through a discriminant analysis in SPSS to determine the probability of group membership, firstly between sites based on metals (Al, V, Cr, Cu, Mn, Fe, Zn, As, Cd, Pb) as predictor variables and, secondly, group membership between metal concentrations based on metal concentrations recorded in water (Supplemental Figure S1).

Infected fish and additional egg strings, collected from the Vaal River, 1.6 km below the Vaal Dam wall (−26.87060, 28.12506), were introduced to the research aquarium at the University of Johannesburg. A colony was established, and exposures were performed with second and third generation *Lamproglena* eggs previously exposed to borehole water.

2.1. Collection and Hatching of Lamproglena clariae Eggs

During field collection, all egg strings removed from *L. clariae* gravid females were placed into an acid-washed 200 mL PYREX® glass beaker filled with water from the collection site. The water was continuously aerated in the field with a small electric-powered single-way aquarium air pump (K.W., DOPHIN AP1301 manufactured by McMerwe, Bloemfontein, South Africa). To prevent turbulence affecting the eggs, a slow-flowing stream of tiny air bubbles was created by attaching a glass pipette tip to the outflow point.

The eggs were transported to the parasitology laboratory at the University of Johannesburg and kept at room temperature (±22 °C) while hatching. In the laboratory, daily water changes were conducted with borehole water. Development to the first copepodid stage occurred in the glass beaker, confirming the identification of the various naupliar stages according to Madanire-Moyo and Avenant Oldewage (2013) [25]. The copepodid larvae were transferred into 200-litre fish tanks to attach to the gills of *C. gariepinus*, and the environmental room was set to a summer day/night cycle (14 h light and 10 h dark) and 25 °C ambient temperature, resulting in 20–22 °C water temperature.

2.2. Collection and Maintenance of Clarias gariepinus

Infected *C. gariepinus* (n = 13) collected with gill nets during fieldwork in the Vaal River (October 2019, October 2020) were placed into holding tanks at the University of Johannesburg's aquarium and fed 1% of their body weight daily in the form of pellets. They were acclimated prior to infection with additional *L. clariae*. Permits to catch, transport, and use the fish for experimental procedures were obtained from the Department of Nature Conservation, Gauteng Province (CPE2 No.000127 and No.0112, CPE3 No.000150 and No.000362, CPE4 No.000003, CPF6 No.000193).

2.3. Egg Collection from Infected Aquarium Fish

The infection intensity on each infected aquarium fish (n = 5) was checked at three-week intervals, allowing time for free-living stages to attach to the gill filaments and reproduce. After an initial parasite life cycle was established, eggs from the adult parasites (F2 and F3 generation) were harvested from the gill filaments.

Each fish was removed from its tank individually and inspected by two people. One person held the fish by the lower jaw, immobilising the fish while exposing the ventral part of the fish. The second person opened the right and left operculum while inspecting the gill arches individually with the help of a watchmaker's headband with magnifying glasses and an adjustable LED light (1 × 5 times magnification). Egg strings were carefully removed from the female parasite with tweezers, and the parasite was left attached to the gill filament of the fish to produce more eggs.

Removed eggs were counted, each egg string halved, and ±10 eggs were placed into individual PYREX® glass beakers. Each Al exposure concentration was prepared in triplicate, resulting in a total of ±30 eggs exposed per concentration. The levels of exposure were chosen to represent environmentally relevant concentrations in the Vaal River, as reported in a previous article related to the current study [24].

2.4. Serial Dilutions of Aluminium Exposures

A stock solution of 10 ppm Al was prepared by dissolving 1 mg aluminium nitrate nonhydrate 99.997% (Sigma-Aldrich, St. Louis, MO, USA) in 100 mL reverse osmosis (RO) water. The stock solution was aerated 24 h before the serial dilutions were prepared for the exposures: 120, 60, 30, 5 µg/L. The control solution contained RO water with no added aluminium. All exposures were prepared in triplicate in acid-washed 200 mL PYREX® glass beakers. Mean in situ water quality parameters (pH, DO, temperature, conductivity, TDS) measured at the beginning of each 24 h exposure interval, and again after, indicated that laboratory conditions remained constant throughout the experiment, with low standard deviations recorded within each parameter.

Eggs in exposure beakers were monitored at 24 h intervals by inspecting them with a dissection microscope (Carl Zeiss Stemi 305, Carl-Zeiss-Promenade 10, 07745 Jena, Germany). Hatching, juvenile life stages, swimming pattern, mortalities, and abnormalities were noted during each inspection. Hatching in any concentration (control, 5, 30, 60, 120 µg/L) was recorded as day 1. Micrographs of initial hatching and larval stages were taken with a dissection microscope (Carl Zeiss Stemi 305, Carl-Zeiss-Promenade 10, 07745 Jena, Germany) during exposures and additional micrographs to study temporarily mounted specimens were taken with a stereomicroscope (Carl Zeiss, Carl-Zeiss-Promenade 10, 07745 Jena, Germany).

In situ physiochemical water quality parameters were measured every 24 h using a YSI 556 Multi-Probe meter (YSI Incorporated, Yellow Springs, OH, USA) and a 10 mL water sample from each beaker taken for metal analysis using ICP-MS (NexION® 300 Series Inductively Coupled Plasma-Mass Spectrometer, Perkin Elmer, Boston, MA, USA).

Exposure concentrations were prepared in aerated RO water and replaced every 24 h. This process was repeated until all juvenile stages perished.

Water samples were pipetted from each concentration (10 mL) from each beaker, daily. The samples were acidified to 1% with Suprapur® Nitric acid (HNO_3) in preparation for ICP-MS analysis for Al. Water samples were stored at room temperature and analysed twice per sample, and Al recovery rates of 80% to 133% were recorded (level of detection for Al (LOD) = 0.24 µg/L). The instrument's calibration was verified using quality proven Standard Reference Material 1643e, obtained from the National Institute of Standards and Technology in Gaithersburg, Maryland, United States.

2.5. Statistical Analyses

2.5.1. Aluminium Exposure Concentrations

Daily Al values were used to calculate an average for each of the exposure concentrations (control, 5 µg/L, 30 µg/L, 60 µg/L, 120 µg/L) with their respective standard deviations over the entire exposure period of 24 days.

2.5.2. Survival of *Lamproglena clariae*

Outliers were determined with box plot analysis followed by histograms and QQ plots to check the data for normality. Levene's test was used to determine the homogeneity of variance. If $p < 0.05$, the Welch robust test of equality of means (reporting F-statistic and p-value) was used as an alternative to one-way ANOVA to determine a significant difference for percentage mortalities during each life stage between exposure concentrations.

3. Results

3.1. Physiochemical Water Quality

Mean total dissolved solids (ppm) measured in all exposure concentrations reduced by almost half after 24 h of exposure (Figure 1). Mean total dissolved solids (from this point forward, mean reported as mean, ±SD) ranged between 30.9 (30 µg/L, ±1.2) and 28.5 ppm (5 µg/L, ±5.4) when the exposure period started, and 18.2 (5 µg/L, ±3.2) and 16.4 ppm (30 µg/L, ±0.9) after 24 h. Mean conductivity ranged between 62.4 (5 µg/L, ±3.2) and 59.7 µS/cm^3 (60 µg/L, ±2.2, and 120 µg/L, ±3.8) freshly prepared. Similarly, mean conductivity also decreased by half after 24 h, with the highest mean concentration measured to be 120 µg/L (34.9 µS/cm^3, ±3.1) and the lowest to be 60 µg/L (32.8 µS/cm^3, ±1.2). In contrast, mean pH, mean water temperature (°C), and mean DO (%) calculated from measurements taken at the beginning and end of each 24 h interval remained relatively stable in exposure vessels. Mean pH ranged between 7.19 (5 µg/L, ±0.1) and 7.24 (60 µg/L, ±0.2) freshly prepared and 7.20 (120 µg/L, ±0.3) and 7.25 (5 µg/L, ±0.1) after 24 h. Mean water temperature (°C) remained constant in the prepared concentrations and throughout the experimental period, ranging between 23.9 and 24.3 °C, ±0.1. Mean DO (%) was sufficient to sustain the juvenile *L. clariae* ranging from 76.8 to 80% throughout

the experiment, decreasing slightly (±1.1) after 24 h of exposure in all concentrations (5 µg/L = 0.4%, 30 µg/L = 2.6%, 60 µg/L = 1.1%, 120 µg/L = 2.0%).

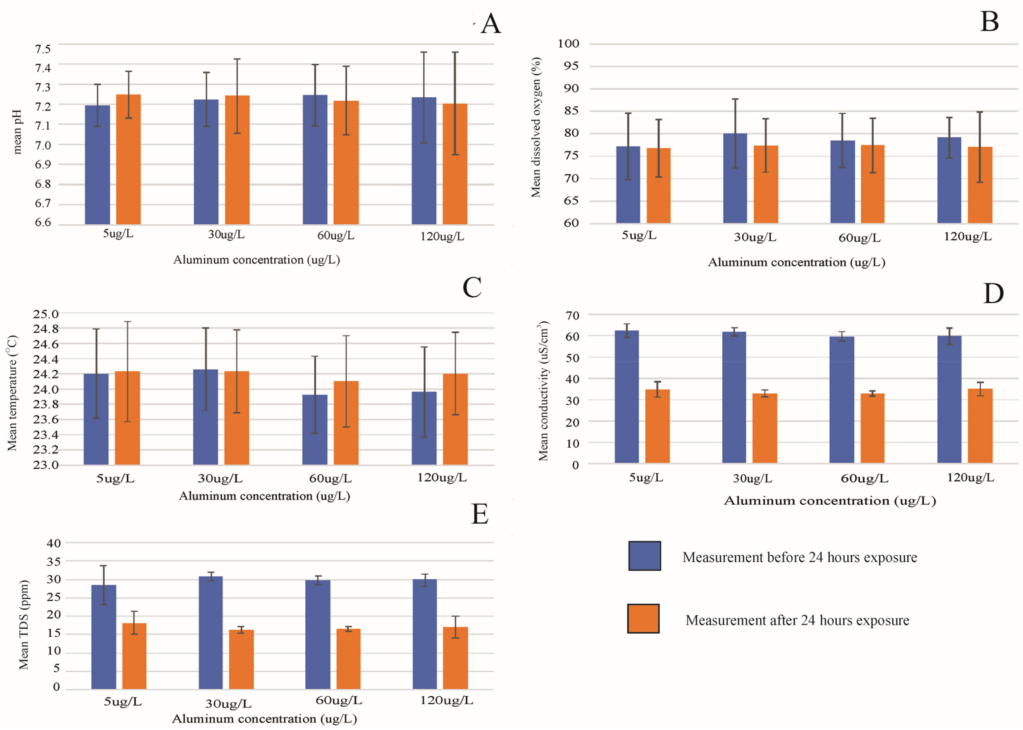

Figure 1. Mean ± SD water quality measurements for pH (**A**), dissolved oxygen (%) (**B**), temperature (°C) (**C**), conductivity (µS/cm^3) (**D**), and total dissolved solids (ppm) (**E**), in different aluminium concentrations: 5, 30, 60, and 120 µg/L. SD: standard deviation; TDS: total dissolved solids.

3.2. Aluminium Concentrations in Exposures

The lowest mean concentration of Al (0.3 µg/L ± 0.1) was recorded in the control, and the highest (95.5 µg/L ± 16.7) in the treatment with 120 µg/L. The average concentration for 5 µg/L was higher than expected, at 12.9 µg/L, ±2.3. For the 30 and 60 µg/L treatments, the actual average recorded concentrations were 30.1 µg/L, ±3.4 and 47.1 µg/L, ±4.7, respectively.

3.3. Influence of Al on Hatching and Survival

Eggs in the control hatched between days one and four, and eggs exposed to Al started hatching on day two, with hatching concluded after day 3. Increasingly lower percentages of *L. clariae* eggs hatched as Al concentration increased from 30 to 120 µg/L. Only 5% of the 38 eggs exposed to 5 µg/L Al did not hatch, followed by 7% of 43 eggs not hatching exposed to 30 µg/L, increasing to 10% that did not hatch in both the control (30 eggs) and 60 µg/L (42 eggs) concentrations. At the highest concentration of Al (120 µg/L), the percentage of unhatched eggs more than doubled, with a total of 26% of 38 eggs.

A rapid increase in mortalities was observed in NI between day five and day eight. The highest was observed in 120 µg/L (29%) and 5 µg/L (29%) and the lowest in the control (17%) and 60 µg/L (17%). Significant differences were noted between the control and 5 µg/L, and 30 and 120 µg/L, respectively (Welch F (4, df2 54.711) = 24.490, $p < 0.01$). Significant differences were also found between 5 and 60 µg/L ($p < 0.01$), 30 and 60 µg/L ($p < 0.01$), and 60 and 120 µg/L ($p < 0.01$).

Mortalities recorded for NII were 20% in the control, 15% in 60 µg/L, 11% in 5 µg/L, 8% in 120 µg/L, and 7% in 30 µg/L (Figure 2). Significant differences in NII mortalities were found between the various exposure concentrations (Welch F (4, df2 53.354) = 5.875, $p < 0.01$), specifically between the control concentration and 5, 30, and 120 µg/L ($p < 0.01$).

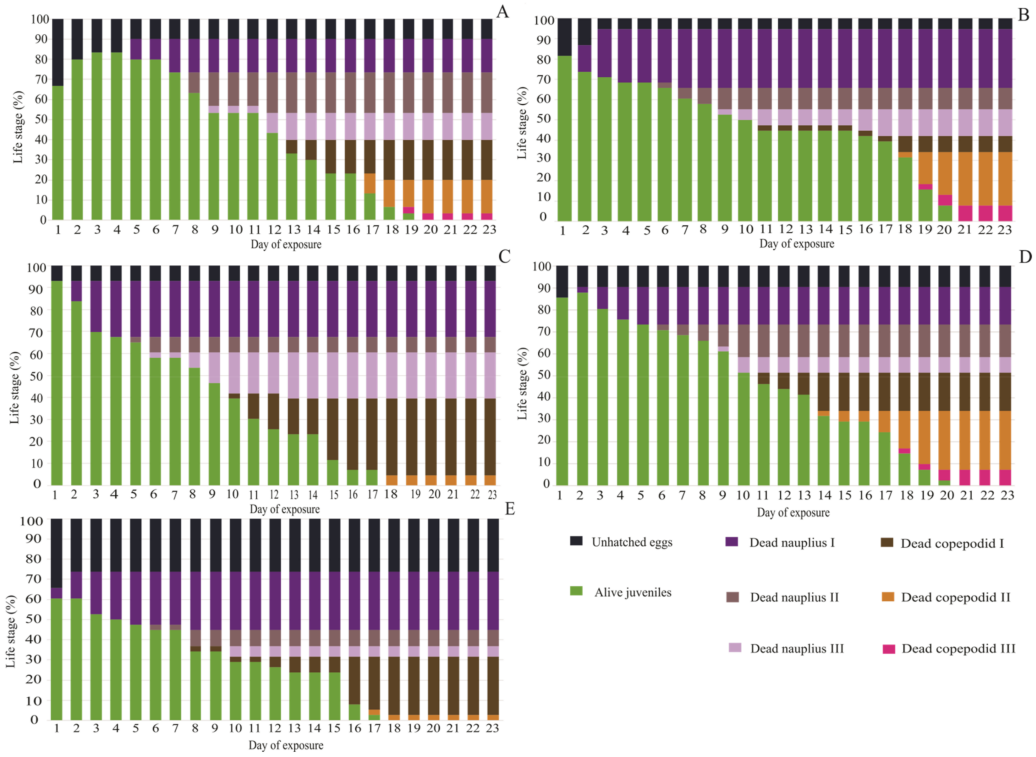

Figure 2. Percentage survival versus percentage dead juveniles in various stages of *Lamproglena clariae* development (black bar: unhatched eggs; green bar: alive juveniles; dark purple bar: dead nauplius I; medium purple bar: dead nauplius II; light purple bar: dead nauplius III; dark brown bar: dead copepodid I; orange bar: dead copepodid II; pink bar: dead copepodid III) during exposure in the control (**A**), 5 µg/L aluminium (**B**), 30 µg/L aluminium (**C**), 60 µg/L aluminium (**D**), 120 µg/L aluminium (**E**).

For NIII, 13% each died in the control and 5 µg/L exposures, 21% in 30 µg/L, 7% in 60 µg/L, and 5% in 120 µg/L (Figure 2). Specifically, significant differences (Welch F (4, df2 52.538) = 8.808, $p < 0.01$) for NIII mortalities were found between the 30 µg/L treatment and all other concentrations ($p < 0.01$).

A general increase in mortalities occurred in all the exposure concentrations when the transition was made from nauplius stages, leaving only a few individuals to develop into copepodid stages II and III. Twenty percent of juveniles perished as copepodid I in the control, 8% in 5 µg/L, 35% in 30 µg/L, 17% in 60 µg/L, and 29% in 120 µg/L (Figure 2). Significant differences for CI were found at a 0.5% level between the various exposure concentrations (Welch F (4, df2 49.831 = 7.766, $p < 0.01$). Significant differences for CI were found between 5 and 30 µg/L ($p < 0.01$).

Seventeen percent of juveniles died as copepodid II in the control, compared to 26% in 5 µg/L, 5% in 30 µg/L, 27% in 60 µg/L, and 3% in 120 µg/L (Figure 2). Significant differences for CII mortalities were found between the various exposure concentrations (Welch F (4, df2 49.786 = 4.083, $p < 0.01$), i.e., between 30 and 60 µg/L ($p < 0.01$), and 60 and 120 µg/L ($p < 0.01$).

A meagre percentage of individuals survived to CIII. Final mortalities were recorded at 3% in the control, 8% in 5 µg/L, 7% in 60 µg/L, and 0% in 30 and 120 µg/L (Figure 2). The percentage of juveniles that survived to CIII was low or 0% in some concentrations; therefore, the Welch robust test of equality of means could not be performed. Differences between the concentrations for CIII were not significant.

3.4. Survival of Juveniles

As expected, the percentage of surviving juveniles showed a negative relationship with exposure time and an increase in Al concentration.

It was anticipated that the copepodids would die due to starvation as they were deprived of sustenance. Additionally, the successful development (egg to nauplius I–III and copepodid I–III) was also distinctly influenced by increasing Al concentration. In the exposure concentrations below 60 µg/L, higher percentages of juveniles moulted to subsequent life stages until inevitable starvation caused mortalities between days 16 and 19. Fewer individuals survived to copepodid stage II in 120 µg/L (Figure 2). Juveniles exposed to 30 and 120 µg/L had an overall shorter life span (17 days) compared to 19 days in the control, and 20 days in 5 and 60 µg/L, respectively.

3.5. Hatching and Development of Juveniles during Exposure to Al

Individual eggs were white in colour (A) when exposed to varying levels of Al (Figure 3). Before hatching, the eggs became yellow, appearing denser and extending outwards; the nauplii moving inside the egg (B). Nauplius I wiggled out of their eggs (C), hatched at random, and swam away rapidly. In the highest concentration (120 µg/L), some of the nauplius I died immediately after hatching and started to decompose next to the egg string (D).

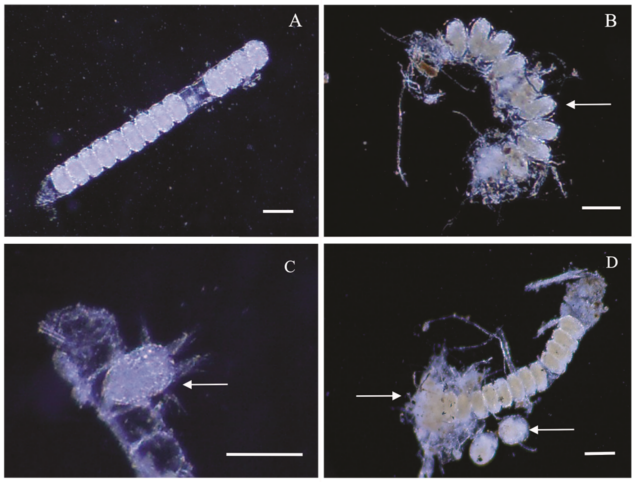

Figure 3. Egg string placed into exposure beaker (**A**), egg string with eggs becoming extended and nauplius moving (**B**), nauplius I hatching from egg (**C**) arrow points to hatching larva, and egg string with nauplia dying just after hatching (**D**) arrows point to dead larvae and decaying larvae respectively. Scale bars: (**A**) 225 µm; (**B**) 285.3 µm; (**C**) 490.8 µm; (**D**) 225 µm.

All three naupliar stages were observed in the various Al exposures. No obvious morphological deformities were observed at increased Al concentrations. However, critical stages for survival were identified: the nauplii appeared to be vulnerable while hatching and when moulting into the next life stage. Individuals that hatched successfully into NI (Figure 4) swam around rapidly with staccato movements, especially in the lower Al concentrations (control, 5 µg/L, and 30 µg/L). A large yolk (y) area was observed in their oval-shaped bodies (A). Movement of nauplia in 60 and 120 µg/L treatments was less coherent, and they briefly swam in circles before settling to the bottom of the beakers. Many nauplii died soon after hatching or moulting (B). They appeared to "rupture" anteriorly (r) with the yolk (y) distant from the body wall. If they survived to NIII (C), their bodies became more elongated, and the developing intestine was visible (i).

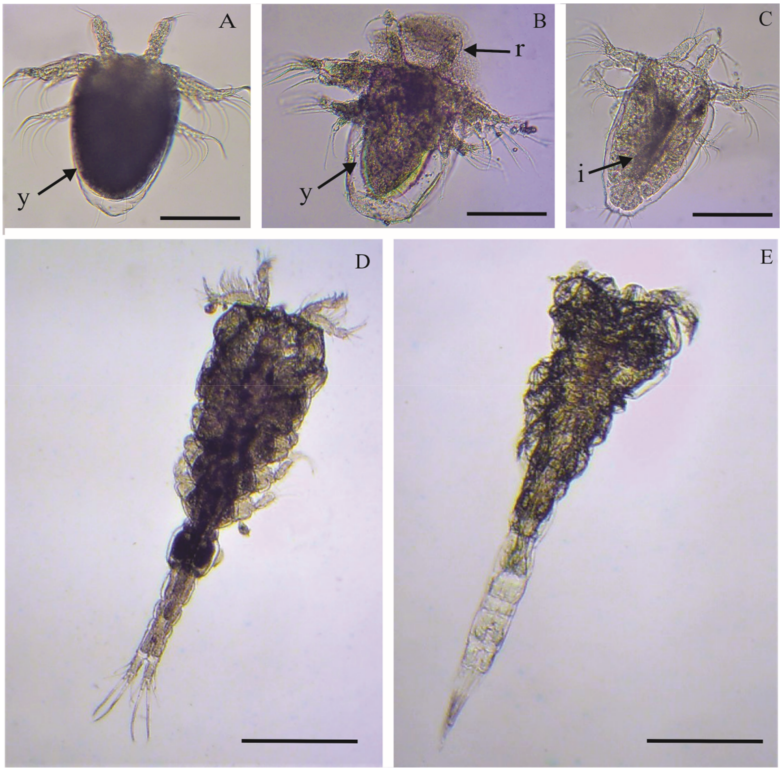

Figure 4. Oval-shaped nauplius I (**A**) with yolk (y) visible; NII carapace ruptured (r) and specimen dying (**B**) while moulting; and NIII (**C**) with developing intestine (i) becoming visible. Copepodid III (**D**) in the control; copepodid II died while moulting (**E**) in 120 µg/L. Scale bars (**A**–**C**) 250 µm, (**D**,**E**) 260 µm.

Copepodid stages have cyclopiform bodies, with elongated thoracic and abdominal segments, and more thoracopods, setae, and spines (Figure 4). In the control, the larvae reached the CIII stage (D), and four pairs of thoracopods were visible. In the 120 µg/L exposure, the copepodids did not survive past the CII stage (E), and only three pairs of thoracopods were visible. The specimen (E) also appears to be less dense when compared to the specimen from the control (D).

4. Discussion

Increasing environmental pollution in the Vaal River impacts infections by *L. clariae* [22,24,27,28]. The toxic effect of Al on free-living aquatic organisms [5,6,12,29] and parasites [8,11,19] is well known.

The reduction in mean conductivity and mean TDS in exposure containers after the 24 h exposure period was due to the change in total Al concentration (combination of dissolved and precipitated Al) and behaviour [13]. Some of the available Al possibly settled to the bottom of the glass beakers or on the sides of the beakers, and was inaccessible for the *L. clariae* juveniles. Bonds between Al and organic matter form rapidly in water (humic and fulvic acids), reducing bioavailable Al [1]. Previous studies indicate that Al is insoluble at pH 6 to 8 but becomes more soluble in acidic or extreme alkaline water [1,5,6,13].

The egg strings harvested from infected aquarium fish were of the same maturity; eggs with a lighter coloration took longer to hatch than egg strings with a darker yellow appearance. Before hatching, the eggs became distended [25]. Eggs in this state hatch randomly into NI while the egg membrane breaks away from the cord membrane [25]. Many embryos died during hatching and remained attached to the egg membranes, even though they appear to be fully developed.

In the lowest concentrations (control and 5 µg/L), the N1 swam actively with rapid staccato movements. The percentage of nauplii that successfully hatched decreased as

the Al concentration increased (30, 60, and 120 µg/L). Nauplii I–III struggled to swim and displayed abnormal circling behaviour after they hatched or moulted in 120 µg/L. The dead nauplii appeared to have "ruptured" anteriorly, leaking internal contents. It is speculated that this was probably due to osmotic and ionic regulation being affected by high Al concentration, as Al is known to interfere with osmotic and ionic regulation [1,17].

The highest mortality of NI (29%) was observed in both 120 and 5 µg/L concentrations. In contrast, the lowest (17%) was observed in the control and 60 µg/L treatments. Soon after the nauplii died, they became necrotic, with debris sticking to their appendages, making them appear deformed. However, no structural abnormalities were observed when specimens mounted on slides were studied at 10 × 10 magnification.

Retarded development was also observed, which would result in a delay in host finding and would lead to starvation in nature. Furthermore, larvae were incapacitated and not able to swim to a potential host. Metals may possibly become integrated into the softened carapace of moulting larval stages of *L. clariae* g, similar to what was reported in adult *Argulus japonicus* [30].

It is therefore argued that the reduced population of *L. clariae* in the Vaal Barrage is the result of the continuous inflow of *L. clariae* larvae from the pristine sites upstream. It is, therefore, likely that at Yellowfish Paradise and other sites with poor water quality, high metal concentrations and specifically high Al concentrations, *L. clariae* will, in all probability, eventually become locally extinct. A previous study reported increased metallothionein (intercellular proteins that bind with metals) levels in *L. clariae* and *C. gariepinus* sampled from the more polluted site (YP), confirming that parasites are under stress in metal and organic polluted environments [23].

Lamproglena clariae can be used as biological indicators of effect and accumulation, and are sensitive to increased Al concentrations in water. The aluminium concentration range used for the exposures was determined by evaluating the mean minimum and mean maximum aluminium concentrations recorded in water samples collected from the six sampling sites during 2017 and 2018, when it ranged between 7.87 and 115.68 µg/L.

5. Conclusions

The current study adds to the continuous health evaluation of a critical river system in South Africa. Increasing concentrations of Al negatively affected the hatchability of *L. clariae* eggs and the functional morphology of larvae. Increased Al concentration reduced survival and delayed development, ultimately impacting host-finding time and ability. *L. clariae* can potentially become extinct in areas where Al pollution occurs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13042145/s1, Figure S1: Canonical discriminant functions between sites (GD, VD, YP, BD, VW, DW) (A) and between elements (Al, V, Cr, Cu, Mn, Fe, Zn, As, Cd, Pb) (B). GD: Grootdraai Dam; VD: Vaal Dam; YP: Yellowfish Paradise; BD: Bloemhof Dam; VW: Vaal-Harts Weir; DW: Douglas Weir.

Author Contributions: The first author (M.P.) collected specimens in 2018, conducted the laboratory exposures, prepared all specimens for analysis, conducted the statistical analysis, and compiled the first draft of the manuscript and took an active part in every other draft. The second author (A.A.-O.) conceptualized the research question, wrote the funding applications, and obtained funding for the project. She supervised the fieldwork and analysis and actively participated in writing of every draft of the manuscript. All authors have read and agreed to the published version of the manuscript.

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