Drought-associated absence of alien invasive anchorworm, *Lernaea cyprinacea* (Copepoda: Lernaeidae), is related to changes in fish health

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A B S T R A C T

Recently, Mozambique tilapia (*Oreochromis mossambicus* Peters, 1852) were listed on the IUCN Red List as near-threatened as their populations are at risk due to hybridization. Another factor that potentially contributes to their population decline is that they are regularly infected by the invasive parasitic copepod anchorworm, *Lernaea cyprinacea* Linnaeus, 1758. Considering anchorworm-infected Mozambique tilapia are common, understanding their condition with respect to infection is difficult as uninfected fish from the same localities have been unavailable for comparison. A severe drought in southern Africa has created hypersaline environments in the Phongolo River floodplain of north-eastern South Africa, such that freshwater parasites cannot survive and uninfected fish are now found. To determine how infection influences host health, infected and uninfected Mozambique tilapia were collected before and during drought conditions, from Nyamiti pan of the Phongolo River floodplain. Anchorworm-infected fish prevalence was recorded, and anchorworms were collected from hosts and identified to the species level using molecular data of the 18S rRNA gene. For each fish, intensity of anchorworm infection, total length, and weights of the gutted body, liver, spleen, and gonads were recorded. Gutted condition factor, hepato-, spleeno-, and gonado-somatic index values per fish, and prevalence of infection per collection were determined. A rapid health assessment was also conducted to determine a health score for each fish. Molecular analyses confirmed the anchorworm studied was *L. cyprinacea*. Prior to and during drought, prevalence of infection was 100%, and 0%, respectively. Before drought, fish had significantly reduced hepato-, spleeno-, and gonado-somatic index values, and higher health assessment scores, yet significantly higher gutted condition. Anchorworm intensity was indirectly correlated with fish liver and gonad condition. This study demonstrates that host condition and health varies greatly with respect to drought and infection, and provides the necessary data for follow-up studies in post-drought conditions.

1. Introduction

The copepod, *Lernaea cyprinacea* Linnaeus, 1758, commonly known as the anchorworm, is considered one of the most invasive ectoparasites of freshwater fish. *L. cyprinacea* is a host generalist (Shariff et al., 1986), and thrives in nearly all lentic freshwater ecosystems (Demaree, 1967; Bulow et al., 1979; Medeiros and Maltchik, 1999). Infection by this parasite has been associated with reduced host weight, growth, and fecundity (Kabata, 1982,

1985; Khan et al., 2003). Considering that the distribution of anchorworm-infected fish is cosmopolitan (e.g. Avenant-Oldewage, 2012; Acosta et al., 2013; Sharifian, 2015), and the effects of infection on hosts can be severe (Woo and Shariff, 1990), *L. cyprinacea* infection may negatively influence host dynamics at the individual, population, and community level (Poulin, 1999; Mouritsen and Poulin, 2002; Hudson et al., 2006).

The Mozambique tilapia (*Oreochromis mossambicus* Peters, 1852) is a freshwater omnivorous cichlid that can tolerate and acclimate to brackish habitats and extreme temperatures (Trewevas, 1983; Stickney, 1986; Uchida et al., 2000). Despite their ability to adapt to a variety of environmental conditions, they are listed as near threatened on the IUCN Red List (Cambray and

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Swartz, 2007). The contributing factor for assigning this species as near threatened is that it frequently hybridises with alien tilapia species such as *Oreochromis niloticus* Linnaeus, 1758 (D'Amato et al., 2007; Zengeya et al., 2013). Fishing pressure by anglers and subsistence fishers (e.g., Van der Waal, 2000) may also threaten this species. Mozambique tilapia serve as an important protein supplement to small scale farmers in rural South Africa and especially in the Lower Phongolo region of northeastern KwaZulu-Natal province, where these wild-caught fish are consumed on average twice a week (Van den Berg, 2013; Coetzee et al., 2015). From comparing historical and recent studies examining South African fish abundance and diversity, Mozambique tilapia catches have declined from approximately 90% to 65% over the last 2 decades (Merron et al., 1993; de Swardt, 2015). Given the ecological and socioeconomic importance of Mozambique tilapia, a better understanding of their health and condition is warranted to aid in their management and to inform future environmental assessments (Retief et al., 2016).

There is a paucity of available data on wild-caught Mozambique tilapia, *L. cyprinacea*, and their interactions within the hosts' native range in South Africa. Information on this host-parasite interaction, and in particular how infection influences host condition is limited because *L. cyprinacea* prevalence is commonly reported as 100% and intensity of infection is consistently high throughout the year (Dalu et al., 2012). Thus, collecting uninfected fish to serve as a standard for host condition and health has been a logistical challenge. Another challenge to studying this host-parasite interaction is that *L. cyprinacea* infecting Mozambique tilapia have only been identified using morphological techniques (Demaree, 1967; Robinson and Avenant-Oldewage, 1996). Confirmation of the anchorworm species using molecular tools could provide an alternative and quantitative means of parasite identification, which would help insure the correct species of anchorworm is under study. This is especially important since there are at least 16 native *Lernaea* species reported from Africa (Oldewage and Avenant-Oldewage, 1993), and it is critical to know the exact species being examined. Considering the lack of available data, it is not surprising that anchorworm infection has not been evaluated as a potential factor contributing to the conservation status of Mozambique tilapia.

Since 2014 (to present, 2017), South Africa has been under drought conditions (Humphries and Baldwin, 2003; El Chami and El Moujabber, 2016). One effect of drought is that freshwater and brackish environments become hypersaline due to reduced water flow and rain input (Bond et al., 2008). Currently, floodplain pans of the Phongolo River in the northeastern KwaZulu-Natal province of South Africa have low to no flow, and are at record minimal depths and hypersaline (Dube et al., 2017). From previous hydrological studies of this floodplain, the total inundated area immediately following a flood is approximately 10,265 ha, and within 6 weeks, only 25% of the inundation remains and reaches a series of discrete lakes and channels (Merron et al., 1993). A historical two-year drought (1982–1884) left only 5% of the floodplain inundated (White et al., 1984), and this disturbance greatly influenced the overall fish diversity and abundance within this ecosystem (Merron et al., 1993). The effect of this drought on the spatial and temporal patterns of parasite diversity and abundance were not reported (Merron et al., 1993). The lack of these data demonstrates that the importance of parasites in ecosystem dynamics was not well-understood during the time at which the Merron et al. (1993) study was conducted. The significance of parasites in ecosystem dynamics has only become recognized and increasingly studied within the last two decades (Marcogliese, 2003; Lafferty et al., 2008; Dunne et al., 2013; Britton, 2013).

A recent comprehensive study on the ecology and biodiversity of the lower Phongolo River and associated floodplains reported that the number of observations of anchorworm-infected fish in the floodplain pans of the Phongolo River has recently declined (Smit et al., 2016), and this is likely associated with the indirect effects of drought. *Lernaea cyprinacea* intensity has been positively correlated with dissolved oxygen and pH (Dalu et al., 2012), and the reduced prevalence of anchorworm-infected fish is likely related to hypersaline water. Hypersalinity can subject freshwater parasites to osmotic shock, which they cannot tolerate, and this is a common treatment method to remove them from fish hosts (Stoskopf, 1993; Noga, 1996).

We hypothesize that the effects of the current drought may have induced a natural parasite removal treatment in the Nyamiti floodplain pan of the Phongolo River, thereby providing an opportunity to sample anchorworm-infected and uninfected Mozambique tilapia. Thus the objectives of this study were three-fold: (1) to identify the *Lernaea* spp. infecting Mozambique tilapia using molecular analyses and quantify their prevalence before and during drought; (2) to compare the health and condition of infected and uninfected Mozambique tilapia before and during drought, and (3) to determine if there were any significant correlations between the abundance of alien invasive anchorworm, *L. cyprinacea*, infecting hosts and host health/condition.

2. Materials and methods

2.1. Description of study area and sampling methodology

Nyamiti pan forms part of the extensive floodplain of the lower Phongolo River and is situated in the Ndumo Game Reserve, close to the South Africa-Mozambique border, and 65 km downstream of the Pongolapoort dam (S26°53″29.7′, E32°17″30.9′) (Fig. 1). Nyamiti pan is the second largest pan within the borders of the reserve, and is a perennial pan with a sandy substrate and mud bottom. This pan is 1 m−1.5 m deep during base flow periods, and it sits on a marine cretaceous bed, such that the groundwater is typically saline. During low water flow, hypersalinity occurs as salts will leach from the ground more easily than during high water flow (South African Wetlands Conservation Programme, 1996).

Oreochromis mossambicus were collected before the drought in September 2013, and during the drought period in August 2016. In 2013, fish were caught using a 35 m seine net, and in 2016, fish were caught using a cast net. To determine the salinity of the Nyamiti pan at the time of collection, the conductivity of a depth-integrated water sample was measured using an AZ water quality meter (Model 8602 handheld IP67) with a combination pH, conductivity and dissolved oxygen meter. Following collection, all fish were immediately transported in an aerated 80 L thermally-insulated plastic container filled with water from the pan to a mobile field station inside Ndumo Game Reserve. In preparation for the host condition and health assessment index analyses (see below), the fish were humanely euthanized by severing the spinal cord (McHugh et al., 2011; Gerber et al., 2017). Anchorworms were removed with forceps from host fish and preserved in 80% ethanol for molecular analyses. This study received the relevant ethical approval (North-West University ethics approval no: NWU-00440-16-S5).

2.2. Molecular identification of Lernaea cyprinecea

Ethanol-preserved samples (n = 2) were used for molecular work. Genomic DNA was extracted from the samples using a rapid DNA extraction method as detailed in the KAPA Express Extract Kit (Kapa Biosystems, Cape Town, South Africa). Polymerase chain reactions (PCR) were used to amplify a 900 nt fragment of the 18S rRNA gene using the primer sets 1F and 5R (see Giribet et al., 1996).
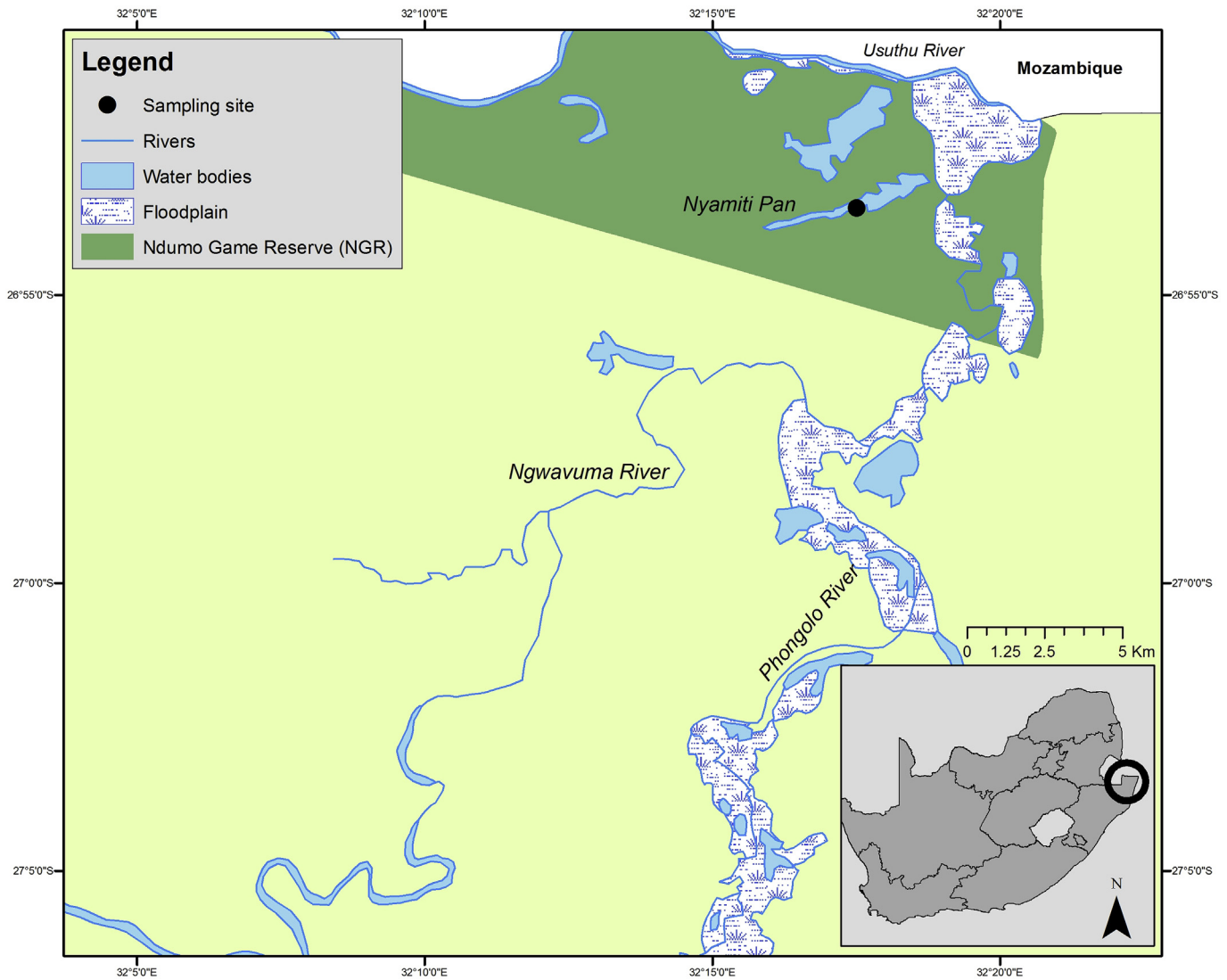
Fig. 1. Map of Nyamiti pan situated on the Phongolo River floodplain.

PCR was performed with volumes of 25 μl, using 12.5 μl Thermo Scientific DreamTaq PCR master mix (2×) (2×DreamTaq buffer, 0.4 mM of each dNTP, and 4 mM MgCl2), 1.25 μl of each primer, and 1 μl DNA. The final reaction volume was made up with PCR-grade nuclease free water (Thermo Scientific, Vilnius, Lithuania). The PCR reactions were carried out using a ProFlex™ PCR thermal cycler (applied biosystems by life technologies), following the PCR conditions as detailed in Boyer et al. (2007). PCR products were sent to a commercial sequencing company (Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa) for purification and sequencing in both directions. Resultant sequences were assembled, and chromatogram-based contigs were generated and trimmed using Geneious Ver. 9.1.

Based on molecular evidence, 18S rDNA sequences were identified as *Lernaea cyprinacea* using the Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/blast). Uncorrected pair-wise distances (p-distance) and base pair differences were determined with the MEGA7 bioinformatics software program (http://www.megasoftware.net). The sequences obtained in the current study were aligned and compared to all available 18S rDNA sequences of *L. cyprinacea* [Genbank: DQ107554 - DQ107557; KM281816; KP235363; KX258625] (Table 1). The analysis involved

eight nucleotide sequences with an alignment length of 618 nt. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. A single sequence was deposited in the NCBI GenBank database under the accession number: KY435939.

2.3. Condition scoring and health assessment indices

Standard morphometric data were collected to assess various condition factors. For each fish, total length (mm) and weight (g) of the gutted body were obtained using a standard ruler and digital calibrated lip-grip scale (Berkley, United States of America). Weights of the liver, spleen, and gonads were determined with the aid of a R200D Sartorius research scale (Zeiss, West Germany). Using these data, gutted condition factor (GCF), and the hepatosomatic (HSI), spleenosomatic (SSI), and gonadosomatic (GSI) indices were calculated following McHugh et al. (2011). The total length of fish collected in 2013 and 2016 were significantly different (df = 1, chi-sq = 6.929, p = 0.009). Accordingly, for statistical analyses, HSI, SSI, and GSI were standardized for the total length of the fish, such that it was [(organ weight (g)/gutted body weight (g))

Table 1
List of partial 18S rDNA sequences from *Lernaea cyprinacea* used for molecular analysis, with accession numbers, host species, country, locality and references.

Accession number	Species	Host	Country	Locality	Reference
KY435939	*L. cyprinacea*	*Oreochromis mossambicus*	South Africa	Nyamithi Pan	Current study
DQ107554	*L. cyprinacea*	*Hemiculter leucisculus*	China	Dangjiangkou	Song et al., 2008
DQ107555	*L. cyprinacea*	*Cultrichthys erythropterus*	China	Dongxi Lake	Song et al., 2008
DQ107556	*L. cyprinacea*	*Cyprinus carpio*	China	Dongxi Lake	Song et al., 2008
DQ107557	*L. cyprinacea*	*Opsariichthys bidens*	China	Dangjiangkou	Song et al., 2008
KM281816	*L. cyprinacea*	Cyprinid fish	Iran	n/a	Unpublished
KP235363	*L. cyprinacea*	*Plecoglossus altivelis*	Japan	Shonai River	Yoshimine et al., 2015
KX258625	*L. cyprinacea*	*Carassius auratus*	Egypt	n/a	Unpublished

*100]/total length (mm).

Concurrent with the condition factor data collection, a rapid health assessment, designed to measure fish health and condition in the field, was conducted following Heath et al. (2004). Using this protocol, the health assessment index (HAI) scores of each fish were determined. This assessment included observations and rank scores of the condition of the eyes, skin, fins, opercules, gills, liver, spleen, hindgut, and kidney. Some modifications to this assessment were made. Only blood-related variables, heamatocrit and plasma protein, were included. Pseduobranch condition was not applicable and excluded. Parasite condition was scored following the rank system from Adams et al. (1993) such that ranks combined counts of ecto- and endo-parasites. Following Heath et al. (2004), parasite intensity was scored as none (0 parasites), few (1–10 parasites), moderate (11–20 parasites), and numerous ($\geq$21 parasites), and assigned 0, 10, 20, 30 values, respectively. The HAI score of each fish was calculated from the sum of the rank scores from each HAI factor. Increasing HAI scores are associated with declining health. The prevalence of anchorworm-infected fish per year, and the number of anchorworm infections per fish, hereafter referred to as anchorworm intensity, were also recorded for later analyses.

2.4. Statistical analyses

To describe the health of fish, the average $\pm$ 1. s.d. for GCF, HSI, SSI, GSI, and HAI values were calculated once for data collected prior to drought, and once for data collected during drought, as prevalence of infected fish was 100% before drought, and 0% during drought. To better describe differences in fish health before and during drought, the mode and range of rank scores for each variable are also reported.

To determine if there was a relationship between fish condition and health, and the drought period, individual generalized models were calculated. The dependent variables were the condition factor, organosomatic index (GCF, HSI, SSI, GSI) and HAI values. The independent variable was drought period, and categorized into two levels, before and during drought. All dependent variables met the assumptions of a normal distribution by infection status except GCF and GSI. The GCF and GSI were log transformed to meet the assumptions of a normal distribution.

To examine if there were associations between anchorworm intensity and GCF, HSI, SSI, GSI, and HAI, individual generalized linear models were calculated using the before drought data, where the dependent variable was the standardized condition factor, organosomatic index (GCF, HSI, SSI, GSI) or HAI value, and the independent variable was anchorworm intensity. For the dependent variables that were significant predictors of anchorworm intensity, Pearson correlation coefficients were determined.

3. Results

3.1. Molecular identification of Lernea cyprinecea

The BLAST results from the 18S rDNA sequence fragment (932 nt) obtained in the current study revealed a 99–100% identity to all the available 18S rDNA sequences of *L. cyprinacea* [Genbank: DQ107554 - DQ107557; KM281816; KP235363; KX258625] from the GenBank database. Additionally, the genetic comparisons (uncorrected p-distance and base pair differences) based on a 618 nt alignment length confirmed that the *Lernaea* species from the current study was *L. cyprinacea* [GenBank:] (Table 2).

3.2. Fish condition, organosomatic indices and health assessment index scores

In September 2013, 13 *O. mossambicus* (178.095 mm $\pm$ 40.626 s.d.; 103.327 g $\pm$ 85.404 s.d.) were collected from water with a conductivity of 2.15 mS/cm. In August 2016, 16 *O. mossambicus* (148.563 mm $\pm$ 19.054 s.d.; 41.490 g $\pm$ 18.480 s.d.) were collected from water with a conductivity of >13 mS/cm. Before drought, all fish were infected with anchorworms, and the average anchorworm intensity was 22 anchorworms $\pm$ 10 s.d. (Fig. 2). During drought, none of the fish collected were infected (Fig. 2). With the exception of GCF, uninfected fish collected during drought were in better health than infected fish collected before drought. On average, compared to fish collected during drought, fish collected before drought had a 0.033 greater GCF value and an HSI value that was approximately 50% less. The SSI values of all fish were similar. The average GSI value for fish collected prior to drought was 6% that of fish collected during drought (Fig. 3). The average HAI scores for fish collected before and during drought were 110.476 $\pm$ 10.235 s.d. and 34.375 $\pm$ 5.123 s.d., respectively. The mode score for fish collected pre-drought was 3.67 times greater than that of fish collected during drought (Table 3), indicating that fish collected pre-drought were considered in poorer health relative to those collected during drought.

3.3. Relationships among drought period and condition, organosomatic indices, and health assessment index scores

Drought (which was tied to the presence/absence of anchorworms) was a significant predictor of GCF, HSI, SSI, GSI, and HAI (Table 4). Prior to drought when anchorworm-infected fish were collected, anchorworm intensity was only a significant predictor for HSI and GSI, and trended towards significant for the HAI (Table 4). HSI and GSI values decreased with increasing anchorworm intensity, but the strength of these relationships were weak (HSI, $r^2 = 0.294$; GSI, $r^2 = 0.397$).

Table 2
Matrix showing ranges for uncorrected p-distances per site between sequences (below diagonal) and ranges for nucleotide differences per site between sequences (above diagonal). Alignment length 618 nt. The bolded species indicates the sequence is from the current study.

| Species	1.	2.	3.	4.	5.	6.	7.	8.
1. KY435939 *Lernaea cyprinacea*		1	1	1	0	0	0	0
2. DQ107554 *Lernaea cyprinacea*	0.00		0	0	1	1	1	1
3. DQ107555 *Lernaea cyprinacea*	0.00	0.00		0	1	1	1	1
4. DQ107556 *Lernaea cyprinacea*	0.00	0.00	0.00		1	1	1	1
5. DQ107557 *Lernaea cyprinacea*	0.00	0.00	0.00	0.00		0	0	0
6. KM281816 *Lernaea cyprinacea*	0.00	0.00	0.00	0.00	0.00		0	0
7. KP235363 *Lernaea cyprinacea*	0.00	0.00	0.00	0.00	0.00	0.00		0
8. KX258625 *Lernaea cyprinacea*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
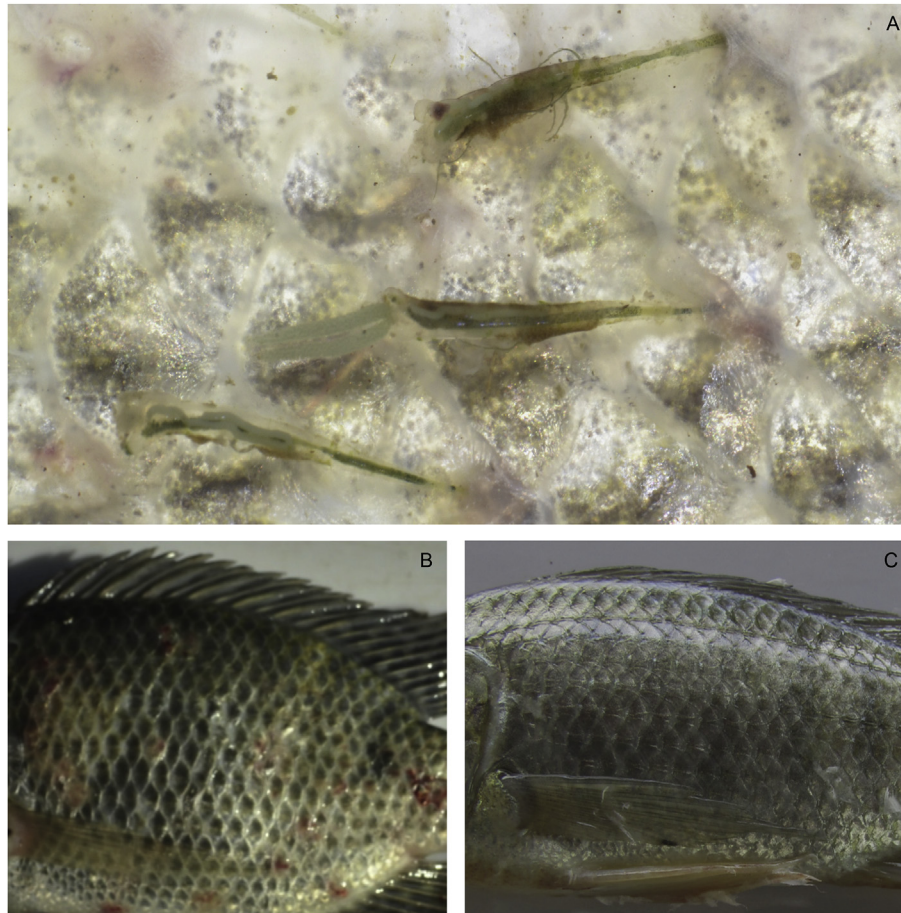
Fig. 2. A) *Lernaea cyprinacea* infecting *Oreochromis mossambicus*. B) *O. mossambicus* skin damage from *L. cyprinacea* infection, before drought. C) Uninfected *O. mossambicus*, during drought.

4. Discussion

4.1. Molecular confirmation of Lernaea cyprinacea

This study provides the first molecular data of the invasive copepod *L. cyprinacea* from South Africa and confirms the species identity as *L. cyprinacea*. Genetic comparisons of 18S rDNA sequences from the current study were compared to all the available 18S rDNA sequences from the GenBank database (see Table 1), including a sequence of *L. cyprinacea* [GenBank: DQ107556] from the type host, *Cyprinus carpio* from China (see Song et al., 2008; McAllister et al., 2015). Although L. *cyprinacea* has a cosmopolitan distribution, very limited molecular data from individuals in the native (including the type locality, Europe) and invasive ranges are available. Thus, the current study provides molecular data for future studies examining the confirmation, identification, and distribution of this parasite species across its type locality, native and invasive ranges.

4.2. Lernaea cyprinacea infection and Mozambique tilapia health and condition

This study is the first to evaluate the associations between drought condition, anchorworm infection, and the health of wild Mozambique tilapia, and the first to do so by examining the changes in health of a fish population that was subjected to a natural experimental parasite removal treatment associated with the current drought conditions. Drought can subject fish to a
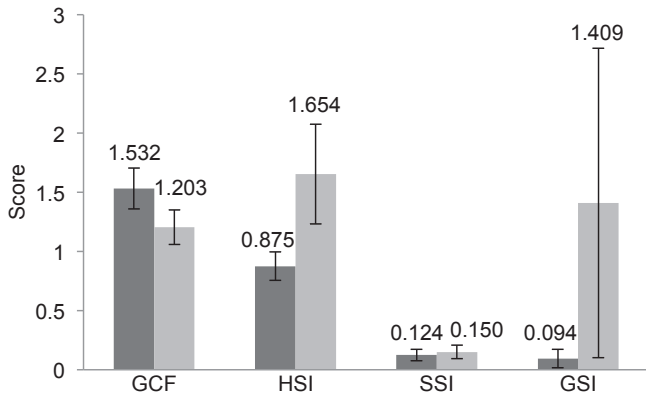
Fig. 3. Mean ± 1 s.d. condition factor, somatic indices, and health assessment index values from raw data for *Lernaea cyprinacea* infected and uninfected *Oreochromis mossambicus*. All infected fish were collected prior to drought in 2013 and indicated in dark gray. All uninfected fish were collected during drought in 2016 and indicated in light gray. GCF = gutted condition factor, HSI = hepatosomatic index, SSI = spleenosomatic index, and GSI = gonadosomatic index. The mean value of each variable is reported above the corresponding bar.

Table 3
The mode score and range for each HAI value and the total index value by infection and drought period. HAI values increase with decreasing health.

HAI Variable	Before drought (Anchorworm present)		During drought (Anchorworm absent)	
	Mode	Range	Mode	Range
Eyes	0	0	0	0
Skin	30	0	10	0
Fins	20	0	0	0
Opercules	0	0	0	0
Gills	10	10	0	0
Liver	10	0	0	0
Spleen	10	0	0	0
Hindgut	0	0	0	0
Kindney	0	0	0	0
Haematocrit	0	10	10	0
Blood plasma	0	10	10	0
Parasites	30	0	10	10
Total Index Value	110	40	30	10

whereas those collected during drought were in better health despite living in a drought-effected system, where water quality and access to resources are greatly altered (Lake, 2003; Balcombe et al., 2005). The most conspicuous difference between fish collected before and during drought, was that all fish collected before drought were infected with anchorworms, whereas all fish collected during drought were not infected with anchorworms. Thus it is likely that anchorworm infection contributed to the reduced health and condition of Mozambique tilapia.

Relative to uninfected fish, infected fish had reduced hepatosomatic condition yet higher gutted condition, and inhabited a more resource rich environment (Carrasco et al., 2012; Dyer et al., 2013; Dube et al., 2017). Both hepatosomatic and gutted condition factors of fish are indicators of the quality and availability of their prey resources, as these organs both store energy. The rate at which energy is metabolized varies with respect to the organ and a hosts' infection status. Fish hosts more readily use energy from the liver than muscle (de la Higuera et al., 1999; de la Higuera, 2001), and parasites may process host nutrients from different host tissues at different rates (Demopoulos and Sikkel, 2015; Welicky et al., 2017, in press). Thus, the hepatosomatic and gutted condition results of this study are neither surprising nor contradictory. The reduced liver condition of infected fish likely reflects the short-term effects of infection, where hosts are metabolizing lipid and carbohydrate more readily than protein (de la Higuera et al., 1999; de la Higuera, 2001). Declines in glycogen levels have been correlated with reduced liver size in previous studies (Maes et al., 2005; Gerber et al., 2017), and Barber (2005) determined that copepod parasite infection reduced fish liver size. The higher gutted condition of infected fish likely reflects that changes in gutted condition with respect to parasite infection occur over a longer duration of time as compared to changes in hepatosomatic condition. Moreover, higher gutted condition of infected fish may reflect that prey resources were different, more readily available for hosts to intake, and/or easier to locate, if overall water quality and turbidity were better before drought as compared to during drought (Semyalo et al., 2011; Dyer et al., 2013). Further analyses that account for the duration of anchorworm infection and regularly monitor changes in water quality would aid in a more complete understanding of temporal changes in host condition that are associated with drought and infection.

Since infected fish had reduced short-term energetic reserves, it is likely that they had less energy to allocate towards reproduction (Hall et al., 2007; Heins et al., 2014). This may explain the reduced gonad condition of anchorworm-infected Mozambique tilapia that we observed. Reduced fecundity of fish in association with parasitism is well-documented (e.g. Lafferty and Kuris, 2009; Fogelman et al., 2009), and the fish gonad condition and associated correlation analyses conducted in this study support these previous findings. Nevertheless, Barson et al. (2008) did not find a correlation between *L. cyprinacea* intensity and Mozambique tilapia gonad

variety of abiotic and biotic stressors (Magoulick and Kobza, 2003; Matthews and Marsh-Matthews, 2003; Crook et al., 2010), thereby negatively and indirectly influencing their health and condition. Parasitism can also negatively influence the health and condition of fish (e.g. Berry Jr. et al., 1991; Paperna, 1996; Barber et al., 2000). Generally, fish collected before drought were in reduced health despite inhabiting a more typical floodplain pan ecosystem,

Table 4
Results of the generalized linear models (GLM) conducted for the response variables, GCF = gutted condition factor, HSI = hepatosomatic index, SSI = spleenosomatic index, GSI = gonadosomatic index, and HAI = Health Assessment index. Separate GLMs were conducted for each independent variable. Bold values indicate statistical significance at the p = 0.05 level.

Independent Variable		Response Variable				
		GCF	HSI	SSI	GSI	HAI
Drought (Before/During Drought)	Df	1	1	1	1	1
	X^2 value	27.250	30.454	5.823	31.491	56.032
	P value	**0.000**	**0.000**	**0.016**	**0.000**	**0.000**
Anchorworm Intensity (Before Drought Data Only)	Df	1	1	1	1	1
	X^2 value	0.064	4.179	0.021	3.965	3.126
	P value	0.800	**0.041**	0.885	**0.018**	0.077

condition in Zimbabwean river impoundments, despite having a similar sample size. These contrasting results may highlight the natural variability that occurs as a result of site and individual fish differences (e.g. Barber et al., 2000; Blanchet et al., 2010). In this study, the reduced gonad condition but higher gutted condition of infected fish compared to uninfected fish may demonstrate an energetic trade-off between growth and maintenance, and reproductive effort (Tytler and Calow, 1985; Wootton, 1985; Mangel and Stamps, 2001).

Although our statistical analyses suggest uninfected fish had reduced spleen condition compared to infected fish, the recorded observations on spleen condition suggest this is not the case. All uninfected fish had spleens that were normal in size and not swollen, and some of the infected fish had spleens that were enlarged and swollen. Both smaller and larger spleens may be indicative of infection (Adams et al., 1993; Kortet et al., 2003), and large spleens may be positively associated with a fish's immune response to infection, and overall reduced condition (Kortet et al., 2003; Ottová et al., 2005; Lamková et al., 2007). Our confounding spleen condition statistical analyses may suggest uninfected fish are also responding to infection, but this infection is likely not parasite related. The severity of this unknown infection may be less than that of anchorworm infection given that low haematocrit values are associated with disease (Adams et al., 1993), and infected fish had lower haematocrit values than uninfected fish.

The overall HAI values indicated that infected fish were in significantly poorer health than uninfected conspecifics. There was also a positive correlation between HAI value and anchorworm intensity, but this result was not statistically significant. This may be an artefact of small sample size, as the majority of our observations provide support for this relationship. For example, compared to uninfected fish, the frequency and intensity of skin lesions and fin fraying was greater for infected fish. Moreover, the gills, liver, and spleen were discoloured in infected fish. Such aberrations are typical of anchorworm-infected fish (Berry Jr. et al., 1991; Avenant-Oldewage, 2012; Hangan et al., 2013). Interestingly, the haematocrit and blood plasma health assessment scores of uninfected fish were consistently greater or equal to that of infected fish. High haematocrit values and low plasma protein concentrations in fish are associated with dehydration and impaired water balance (Wedemeyer and Yasutake, 1977; Novotny and Beeman, 1990). Although laboratory studies have demonstrated that short-term (hours to 1 month) exposure to hypersalinity does not influence the blood parameters of aquaculture reared tilapia (Sardella et al., 2004; Kammerer et al., 2010), studies examining the chronic (months to years) and natural exposure of wild Mozambique tilapia to hypersalinity for a period of months to years have not been conducted. It is likely that the higher scores of uninfected fish are in part a physiological response to living in a hypersaline environment for an extended period of time, but long-term studies are necessary to validate this postulate.

5. Conclusions

Studies associated with drought are often constrained with regards to sample size and data collection. Whereas before drought data are often overlooked as their relative importance in studies related to drought are unforeseen, the collection of data after drought can be difficult to plan for and obtain (e.g. Crook et al., 2010; Lake, 2011). In this study, we examined before drought and during drought data. Post drought data are not yet possible to collect. Although this study is unique in the fact that it has both before and during drought data, the dataset is confounded because of drought. The hypersaline conditions associated with drought, resulted in the collection of only infected fish before drought, and only uninfected fish after drought. Thus, this study had a natural sampling bias and limited our ability to examine the effects of infection on host condition without the confounding variable of drought. Nevertheless, anchorworm infection in southern Africa is pervasive and observing uninfected fish under more typical hydrological regimes is challenging (Dalu et al., 2012). Accordingly, while this study has sampling bias, the effects of drought provided an opportunity to locate uninfected fish. If anchorworms return post drought, then complementary laboratory experiments would be worthwhile to determine to what extent changes in salinity, anchorworm intensity, and resource availability directly influence Mozambique tilapia condition.

Thus, this is the first study to determine the associations between wild native Mozambique tilapia health and condition, drought, and invasive *L. cyprinacea* infection, and to confirm using molecular data that the parasite examined is *L. cyprinacea*. Notably, this is also the first report on the localized 'disappearance' of an alien fish parasite in association with drought condition. The baseline data presented herein can be used in future studies examining the potential re-establishment of *L. cyprinacea* and its effects on host health and condition.

Conflict of interest

The authors declare there is no conflict of interest.

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References

Acosta, A.A., Carvalho, E.D., da Silva, R.J., 2013. First record of *Lernaea cyprinacea* (Copepoda) in a native fish species from a Brazilian river. Neotrop. Helminthol. 7, 7–12.

Adams, S.M., Brown, A.M., Goede, R.W., 1993. A quantitative health assessment index for rapid evaluation of fish condition in the field. Trans. Am. Fish. Soc. 122, 63–73.

Avenant-Oldewage, A., 2012. *Lernea cyprinacea* and related species. In: Woo, T.K., Buchmann, K. (Eds.), Fish Parasites: Pathobiology and Protection. CABI, Wallingford and Cambridge, pp. 337–349.

Balcombe, S.R., Bunn, S.E., McKenzie-Smith, F.J., Davies, P.M., 2005. Variability of fish diets between dry and flood periods in an arid zone floodplain river. J. Fish. Biol. 67, 1552–1567.

Barber, I., 2005. Parasites grow larger in faster growing fish hosts. Int. J. Parasitol. 35, 137–143.

Barber, I., Hoare, D., Krause, J., 2000. Effects of parasites on fish behaviour: a review and evolutionary perspective. Rev. Fish. Biol. Fish. 10, 131–165.

Barson, M., Mulonga, A., Nhiwatiwa, T., 2008. Investigation of a parasitic outbreak of *Lernaea cyprinacea* Linnaeus (Crustacea: Copepoda) in fish from Zimbabwe. Afr. Zool. 43, 175–183.

Berry Jr., C.R., Babey, G.J., Shrader, T., 1991. Effect of *Lernaea cyprinacea* (Crustacea: Copepoda) on stocked rainbow trout (*Oncorhynchus mykiss*). J. Wildl. Dis. 27, 206–213.

Blanchet, S., Rey, O., Loot, G., 2010. Evidence for host variation in parasite tolerance in a wild fish population. Evol. Ecol. 24, 1129–1139.

Bond, N.R., Lake, P.S., Arthington, A.H., 2008. The impacts of drought on freshwater ecosystems: an Australian perspective. Hydrobiologia 600, 3–16.

Boyer, S.L., Clouse, R.M., Benavides, L.R., Sharma, P., Schwendinger, P.J., Karunarathna, I., Giribet, G., 2007. Biogeography of the world: a case study from

cyphophthalmid Opiliones, a globally distributed group of arachnids. J. Biogeogr. 34, 2070–2085.

Britton, J.R., 2013. Introduced parasites in food webs: new species, shifting structures? Trends Ecol. Evol. 28, 93–99.

Bulow, F.J., Winningham, J.R., Hooper, R.C., 1979. Occurrence of the copepod parasite *Lernaea cyprinacea* in a stream fish population. Trans. Am. Fish. Soc. 108, 100–102.

Cambray, J., Swartz, E., 2007. Oreochromis mossambicus. The IUCN Red List of Threatened Species 2007: e.T63338A12659743. http://dx.doi.org/10.2305/IUCN. UK.2007.RLTS.T63338A12659743.en.

Carrasco, N.K., Perissinotto, R., Nel, H.A., 2012. Diet of selected fish species in the freshwater-deprived St Lucia Estuary, South Africa, assessed using stable isotope analysis. Mar. Biol. Res. 8, 701–714.

Coetzee, H.C., Nell, W., Van Eeden, E.S., De Crom, E.P., 2015. Artisanal fisheries in the Ndumo area of the lower Phongolo River floodplain, South Africa. Koedoe 57, 1–6.

Crook, D.A., Reich, P., Bond, N.R., McMaster, D., Koehn, J.D., Lake, P.S., 2010. Using biological information to support proactive strategies for managing freshwater fish during drought. Mar. Freshw. Res. 61, 379–387.

D'Amato, M.E., Esterhuyse, M.M., Van Der Waal, B.C., Brink, D., Volckaert, F.A., 2007. Hybridization and phylogeography of the Mozambique tilapia *Oreochromis mossambicus* in southern Africa evidenced by mitochondrial and microsatellite DNA genotyping. Conserv. Genet. 8, 475–488.

Dalu, T., Nhiwatiwa, T., Clegg, B., Barson, M., 2012. Impact of *Lernaea cyprinacea* Linnaeus 1758 (Crustacea: Copepoda) almost a decade after an initial parasitic outbreak in fish of Malilangwe Reservoir, Zimbabwe. Knowl. Manag. Aquat. Ecosyst. 406, 1–9.

de la Higuera, M., 2001. In: Houlihan, D., Boujard, T., Jobling, M. (Eds.), Effects of Nutritional Factors and Feed Characteristics on Feed Intake. Blackwell Science Ltd., Oxford, pp. 250–268.

de la Higuera, M., Akharbach, H., Hidalgo, M.C., Peragon, J., Lupianez, J.A., Garcıa-Gallego, M., 1999. Liver and white muscle protein turnover rates in the European eel (*Anguilla anguilla*): effects of dietary protein quality. Aquaculture 179, 203–216.

de Swardt, J.J.S., 2015. Ecosystem Health of the Phongolo Floodplain, South Africa, Based on Fish Diversity, Community Structure and Health of Selected Species. Masters Thesis. North-West University, Potchefstroom, 167pp.

Demaree, R.S., 1967. Ecology and external morphology of *Lernaea cyprinacea*. Am. Midl. Nat. 78, 416–427.

Demopoulos, A.W., Sikkel, P.C., 2015. Enhanced understanding of ectoparasite–host trophic linkages on coral reefs through stable isotope analysis. Int. J. Parasitol. Parasites Wild 4, 125–134.

Dube, T., de Necker, L., van Vuren, J.H., Wepener, V., Smit, N.J., Brendonck, L., 2017. Spatial and temporal variation of invertebrate community structure in flood-controlled tropical floodplain wetlands. J. Freshw. Ecol. 32, 1–15.

Dunne, J.A., Lafferty, K.D., Dobson, A.P., Hechinger, R.F., Kuris, A.M., Martinez, N.D., McLaughlin, J.P., Mouritsen, K.N., Poulin, R., Reise, K., Stouffer, D.B., 2013. Parasites affect food web structure primarily through increased diversity and complexity. PLoS Biol. 11, e1001579.

Dyer, D.C., Perissinotto, R., Carrasco, N.K., 2013. Post-flood dietary variation in the Mozambique tilapia *Oreochromis mossambicus* in the St Lucia Estuary, South Africa. Mar. Ecol. Prog. Ser. 476, 199–214.

El Chami, D., El Moujabber, M., 2016. Drought, climate change and sustainability of water in agriculture: a roadmap towards the NWRS2. S. Afr. J. Sci. 112, 1–4.

Fogelman, R.M., Kuris, A.M., Grutter, A.S., 2009. Parasitic castration of a vertebrate: effect of the cymothoid isopod, *Anilocra apogonae*, on the five-lined cardinalfish, *Cheilodipterus quinquelineatus*. Int. J. Parasitol. 39, 577–583.

Gerber, R., Wagenaar, G.M., Smith, W., Ikenaka, Y., Smit, N.J., 2017. Insights into the drivers of histopathological changes and potential as bio-indicator of riverine health of an aquatic apex predator from a premier conservation area: a multiple lines of evidence and multivariate statistics approach. Ecol. Indic. 72, 530–544.

Giribet, G., Carranza, S., Baguna, J., Riutort, M., Ribera, C., 1996. First molecular evidence for the existence of a Tardigrada + Arthropoda clade. Molec. Biol. Evol. 13, 76–84.

Hall, S.R., Becker, C., Cáceres, C.E., 2007. Parasitic castration: a perspective from a model of dynamic energy budgets. Integr. Comp. Biol. 47, 295–309.

Hangan, M., Urdes, L.D., Ianitchi, D., 2013. The prevalence and pathogenicity of the "anchor Worm"(*Lernea spp*, Phylum Arthropoda) within the finfish inhabiting the Danube Delta area. Sci. Pap. Anim. Sci. Biotechnol. 46, 258–262.

Heath, R., du Preez, H., Genthe, B., Avenant-Oldewage, A., 2004. Freshwater Fish and Human Health Reference Guide. WRC Report No. TT213/04. Water Research Commission, Pretoria.

Heins, D.C., Barry, K.A., Petrauskas, L.A., 2014. Consistency of host responses to parasitic infection in the three-spined stickleback fish infected by the diphyllobothriidean cestode *Schistocephalus solidus*. Biol. J. Linn. Soc. 113, 958–968.

Hudson, P.J., Dobson, A.P., Lafferty, K.D., 2006. Is a healthy ecosystem one that is rich in parasites? Trends Ecol. Evol. 21, 381–385.

Humphries, P., Baldwin, D.S., 2003. Drought and aquatic ecosystems: an introduction. Freshw. Biol. 48, 1141–1146.

Kabata, Z., 1982. Copepoda (Crustacea) parasitic on fishes: problems and perspectives. Adv. Parasitol. 19, 1–71.

Kabata, Z., 1985. Parasites and Diseases of Fish Cultured in the Tropics. Taylor & Francis Ltd, London and Philadelphia.

Kammerer, B.D., Cech, J.J., Kültz, D., 2010. Rapid changes in plasma cortisol, osmolality, and respiration in response to salinity stress in tilapia (*Oreochromis mossambicus*). Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 157, 260–265.

Khan, M.N., Aziz, F., Afzal, M., Rab, A., Sahar, L., Ali, R., Naqvi, S.M., 2003. Parasitic infestation in different fresh water fishes of mini dams of Potohar region. Pak. Pak. J. Biol. Sci. 6, 1092–1095.

Kortet, R., Taskinen, J., Sinisalo, T., Jokinen, I., 2003. Breeding related seasonal changes in immunocompetence, health state and condition of the cyprinid fish, *Rutilus rutilus*, L. Biol. J. Linn. Soc. 78, 117–127.

Lafferty, K.D., Allesina, S., Arim, M., Briggs, C.J., De Leo, G., Dobson, A.P., Dunne, J.A., Johnson, P.T., Kuris, A.M., Marcogliese, D.J., Martinez, N.D., 2008. Parasites in food webs: the ultimate missing links. Ecol. Lett. 11, 533–546.

Lafferty, K.D., Kuris, A.M., 2009. Parasitic castration: the evolution and ecology of body snatchers. Trends Parasitol. 25, 564–572.

Lake, P.S., 2003. Ecological effects of perturbation by drought in flowing waters. Freshw. Biol. 48, 1161–1172.

Lake, P.S., 2011. Drought and Aquatic Ecosystems: Effects and Responses. John Wiley & Sons, Oxford, West Sussex and Hoboken.

Lamková, K., Šimková, A., Palíková, M., Jurajda, P., Lojek, A., 2007. Seasonal changes of immunocompetence and parasitism in chub (*Leuciscus cephalus*), a freshwater cyprinid. Parasitol. Res. 101, 775–789.

Maes, G.E., Raeymaekers, J.A.M., Pampoulie, C., Seynaeve, A., Goemans, G., Belpaire, C., Volckaert, F.A.M., 2005. The catadromous European eel *Anguilla anguilla* (L.) as a model for freshwater evolutionary ecotoxicology: relationship between heavy metal bioaccumulation, condition and genetic variability. Aquat. Toxicol. 73, 99–114.

Magoulick, D.D., Kobza, R.M., 2003. The role of refugia for fishes during drought: a review and synthesis. Freshw. Biol. 48, 1186–1198.

Mangel, M., Stamps, J., 2001. Trade-offs between growth and mortality and the maintenance of individual variation in growth. Evol. Ecol. Res. 3, 611–632.

Marcogliese, D.J., 2003. Food webs and biodiversity: are parasites the missing link. J. Parasitol. 89, 106–113.

Matthews, W.J., Marsh-Matthews, E., 2003. Effects of drought on fish across axes of space, time and ecological complexity. Freshw. Biol. 48, 1232–1253.

McAllister, C.T., Bursey, C.R., Martin, S.D., 2015. *Lernaea cyprinacea* (Crustacea: Copepoda: Lernaeidae) anchorworms from two larval aquatic insects (Ephemeroptera: Baetidae: Trichoptera: Hydropsychidae) in northeastern Oklahoma. Proc. Okla. Acad. Sci. 91, 37–40.

McHugh, K.J., Smit, N.J., van Vuren, J.H.J., van Dyk, J.C., Bervoets, L., Covaci, A., Wepener, V., 2011. A histology-based fish health assessment of the tigerfish, *Hydrocynus vittatus* from a DDT-affected area. Phys. Chem. Earth 36, 895–904.

Medeiros, E.S., Maltchik, L., 1999. The effects of hydrological disturbance on the intensity of infestation of *Lernaea cyprinacea* in an intermittent stream fish community. J. Arid. Environ. 43, 351–356.

Merron, G., Bruton, M., Lalouviere, D., La Hausse, P., 1993. Changes in fish communities of the Phongolo floodplain, Zululand (South Africa) before, during and after a severe drought. Regul. Rivers Res. Manag. 8, 335–344.

Mouritsen, K.N., Poulin, R., 2002. Parasitism, community structure and biodiversity in intertidal ecosystems. Parasitology 124, 101–117.

Noga, E.J., 1996. Fish Disease: Diagnosis and Treatment. Mosby, St. Louis.

Novotny, J.F., Beeman, J.W., 1990. Use of a fish health condition profile in assessing the health and condition of juvenile chinook salmon. Prog. Fish. Cultur. 52, 162–170.

Oldewage, W.H., Avenant-Oldewage, A., 1993. Checklist of the parasitic Copepoda (Crustacea) of African fishes. Zool. Doc./Koninklijk Mus. Midden-Afrika 23, 1–28.

Ottová, E., Šimková, A., Jurajda, P., Dávidová, M., Ondračková, M., Pečínková, M., Gelnar, M., 2005. Sexual ornamentation and parasite infection in males of common bream (*Abramis brama*): a reflection of immunocompetence status or simple cost of reproduction? Evol. Ecol. Res. 7, 581–593.

Paperna, I., 1996. Parasites, Infections and Diseases of Fishes in Africa: an Update. FAO/CIFA Technical Paper No. 31. FAO Publications, Rome.

Poulin, R., 1999. The functional importance of parasites in animal communities: many roles at many levels? Int. J. Parasitol. 29, 903–914.

Retief, F., Bond, A., Pope, J., Morrison-Saunders, A., King, N., 2016. Global mega-trends and their implications for environmental assessment practice. Environ. Assess. Rev. 61, 52–60.

Robinson, J., Avenant-Oldewage, A., 1996. Aspects of the morphology of the parasitic copepod *Lernaea cyprinacea* Linnaeus, 1758 and notes on its distribution in Africa. Crustaceana 69, 610–626.

Sardella, B.A., Matey, V., Cooper, J., Gonzalez, R.J., Brauner, C.J., 2004. Physiological, biochemical and morphological indicators of osmoregulatory stress in California Mozambique tilapia (*Oreochromis mossambicus × O. urolepis hornorum*) exposed to hypersaline water. J. Exp. Biol. 207, 1399–1413.

Semyalo, R., Rohrlack, T., Kayiira, D., Kizito, Y.S., Byarujali, S., Nyakairu, G., Larsson, P., 2011. On the diet of Nile tilapia in two eutrophic tropical lakes containing toxin producing cyanobacteria. Limnol. Ecol. Manag. Inland Waters 41, 30–36.

Shariff, M., Kabata, Z., Sommerville, C., 1986. Host susceptibility to *Lernaea cyprinacea* L. and its treatment in a large aquarium system. J. Fish. Dis. 9, 393–401.

Sharifian, I., 2015. On the occurrence of parasite, *Lernaea cyprinacea* from common carp, *Cyprinus carpio* farmed in ponds and rice fields. Comp. Clin. Pathol. 24, 1435–1438.

Smit, N.J., Vlok, W., van Vuren, J.H.J., Du Preez, L.H., Van Eeden, E., O'Brien, G.C., Wepener, V., 2016. Socio-ecological System Management of the Lower Phongolo River and Floodplain Using Relative Risk Methodology. Water Research Commission (WRC) Project K5-2185, Pretoria, 456pp.

South African Wetlands Conservation Programme, 1996. Ndumo Game Reserve South Africa. South African Wetlands Conservation Programme Document No 24/21/3/3/3/15, pp. 1–34.

Song, Y., Wang, G.T., Yao, W.J., Gao, Q., Nie, P., 2008. Phylogeny of freshwater parasitic copepods in the Ergasilidae (Copepoda: Poecilostomatoida) based on 18S and 28S rDNA sequences. Parasitol. Res. 102, 299–306.

Stickney, R.R., 1986. Tilapia tolerance of saline waters: a review. Prog. Fish-Cult. 48, 161–167.

Stoskopf, M.K., 1993. Fish Medicine. W.B. Saunders, Philadelphia.

Trewevas, E., 1983. Tilapiine Fishes of the Genera *Sarotherodon, Oreochromis*, and *Danakilia*. Comstock Publishing Associates, Ithaca.

Tytler, P., Calow, P., 1985. Fish Energetics: New Perspectives. Springer, Netherlands.

Uchida, K., Kaneko, T., Miyazaki, H., Hasegawa, S., Hirano, T., 2000. Excellent salinity tolerance of Mozambique tilapia (*Oreochromis mossambicus*): elevated chloride cell activity in the branchial and opercular epithelia of the fish adapted to concentrated seawater. Zool. Sci. 17, 149–160.

Van den Berg, J., 2013. Socio-economic factors affecting adoption of improved agricultural practices by small scale farmers in South Africa. Afr. J. Agric. Res. 8, 4490–4500.

Van der Waal, B.C.W., 2000. Fish as a resource in a rural river catchment in the Northern Province, South Africa. South. Afr. J. Aquat. Sci. 25, 56–70.

Wedemeyer, G.A., Yasutake, W.T., 1977. Clinical Methods for the Assessment of the Effects of Environmental Stress on Fish Health. US Fish Wildl. Serv. 89.

Welicky, R.L., Demopoulos, A.W.J., Sikkel, P.C., 2017. Host-dependent differences in resource use associated with *Anilocra* spp. parasitism in two coral reef fishes, as revealed by stable carbon and nitrogen isotope analyses. Mar. Ecol. http://dx.doi.org/10.1111/maec.12413 (in press).

White, P.N., Merron, G.S., Quick, A.J.R., la Hausse de lalouviere, P., 1984. The impact of sustained drought conditions on the fishes of the Phongolo floodplain based on a survey in September 1983. J. L. B. Smith Inst. Ichthyol. Invest. Rep. 7, 39pp.

Woo, P.T., Shariff, M., 1990. *Lernea cyprinacea* L. (Copepoda: Caligidea) in *Helostoma temmincki* Cuvier & Valenciennes: the dynamics of resistance in recovered and naive fish. J. Fish. Dis. 13, 485–493.

Wootton, R.J., 1985. Energetics of reproduction. In: Tytler, P., Calow, P. (Eds.), Fish Energetics: New Perspectives. Springer, Netherlands, pp. 31–254.

Yoshimine, Y., Isshiki, T., Aino, S., Tun, K.L., Yoshinaga, T., 2015. Occurrence of *Lernaea cyprinacea* (Copepoda) in wild Ayu *Plecoglossus altivelis* and several other fishes in the Shonai River, Japan. Fish Pathol. 50, 81–84.

Zengeya, T.A., Robertson, M.P., Booth, A.J., Chimimba, C.T., 2013. A qualitative ecological risk assessment of the invasive Nile tilapia, *Oreochromis niloticus* in a sub-tropical African river system (Limpopo River, South Africa). Aquat. Conserv. Mar. Freshw. Ecosyst. 23, 51–64.