Distribution of *Contracaecum* (Nematoda: Anisakidae) larvae in freshwater fish from the northern regions of South Africa

Sareh Tavakol*, Willem J Smit, Joseph R Sara, Ali Halajian and Wilmien J Luus-Powell

Department of Biodiversity, University of Limpopo, Sovenga, South Africa

* Corresponding author, e-mail: sareh_tav58@yahoo.com

A total of 1 847 fishes (16 species) from 14 reservoirs in northern and north-eastern regions of South Africa were collected and examined for larval *Contracaecum* spp. between 2005 and 2013. This study, the first to examine several potential second intermediate hosts, found *Clarias gariepinus*, *Coptodon rendalli*, *Cyprinus carpio*, *Hydrocynus vittatus*, *Laboobarbus marequensis*, *Marcusenius macrolepidotus*, *Micropterus salmoides*, *Oreochromis mossambicus* and *Schilbe intermedius* infected with the third-stage larvae. *Coptodon rendalli*, *Marcusenius macrolepidotus* and *Micropterus salmoides* are new host records for South Africa. A generalised linear model identified locality as the main factor affecting parasite burden.

Keywords: fish parasite, mean abundance, mean intensity, nematode, prevalence

Online supplementary information: Supporting information for this paper is available as online supplementary material at http://dx.doi.org/10.1080/15627020.2015.1052302

Introduction

Nematodes of the family Anisakidae Skrjabin & Karokhin, 1945 naturally parasitise fish, cephalopods, marine mammals and piscivorous birds with humans becoming accidental hosts when ingesting raw fish infected with the third-stage larvae (L3). Larval infection by species of the anisakid genus *Contracaecum* RAILLET & Henry, 1912 commonly occurs in the body cavity of several fish species that serve as a second intermediate/paratenic host, while the adult stage of the parasite occurs in the gut of the definitive piscivorous host (Mashego and Saayman 1981; Boomker 1982; Moravec 1998; Barson and Marshall 2004; Nadler et al. 2005; Barson and Avenant-Oldewage 2006; Moravec 2009; Villegas and González-Solís 2009). Although species of *Contracaecum* are among the most prevalent fish nematodes in Africa (Khalil and Polling 1997), with several studies having investigated infection levels in fish within southern Africa, most studies have primarily focused on a single fish species within a specific locality. For example, in South Africa the infection of *Contracaecum* larvae was investigated in *Schilbe intermedius* Ruppell, 1832 from the Nwanedi–Luphephe dams (Smit and Luus-Powell 2012), in *Oreochromis mossambicus* (Peters, 1852) from the Olifants River (Madanière-Moyo et al. 2012), in *Clarias gariepinus* (Burchell, 1822) from the Elands and Olifants rivers (Prudhoe and Hussey 1977), Rietvlei Dam (Barson and Avenant-Olde-dewage 2006) and in Zimbabwe from Lake Chivero (Barson 2004).

According to Skelton (2001), 16 families of freshwater fish from nine orders occur in the northern region of South Africa where several large reservoirs are located in the Olifants and Limpopo River systems, the tributaries of which are inhabited by different endemic and alien species important for both recreational and subsistence fishing. To date no investigations have been done on the prevalence of *Contracaecum* larvae in multiple fish species from various localities. For this reason, data derived from a number of short-term surveys conducted between 2005 and 2013 were compiled to establish the infection status of *Contracaecum* spp. in several freshwater fish species collected from different reservoirs situated in the northern regions of South Africa. The effects of season, gender, locality and body length on parasite burden in infected fish are also reported.

Materials and methods

Selection of sampling localities

Samples were collected from 14 reservoirs, including three small tailing dams, situated in the Limpopo and Mpumalanga provinces, South Africa (Figure 1) from January 2005 to April 2013.

Fish collection

Fish were collected in waters <2 m deep by electrofishing, angling and seine nets. Gill nets (2 m wide and 25 m long, subdivided into 5 m sections having stretched mesh sizes of 30, 50, 70, 90 and 110 mm) were used to sample deeper waters (≥2 m). Live specimens were transported to a field laboratory in containers with well-aerated water and housed until they could be examined.

*African Zoology* is co-published by NISC (Pty) Ltd and Taylor & Francis
**Fish examination**

The standard length (SL; mm), total length (TL; mm), mass (g), sex and locality of each specimen were recorded. Fishes were sacrificed by severing their spinal cords, dissected, and their body cavities and gastrointestinal tracts examined. Nematodes were removed and placed into petri dishes containing a physiological saline solution for cleaning and their numbers recorded. The majority of parasites were fixed by placement in 70% ethanol heated to 70 °C and thereafter, when uncoiled, preserved in 70% ethanol. For morphological and identification purposes some specimens were temporarily mounted on glass slides in lactophenol. All specimens were identified to genus level based on morphological characters using keys by Bykhovskaya-Pavlovskaya (1964) and Moravec (1998). Identification was possible based on the presence of three anterior lips and the structure of the anterior alimentary tract comprising the visible ventriculus, ventricular appendix and an intestinal caecum. Infection parameters, i.e. prevalence (P), mean intensity (MI) and mean abundance (MA), were calculated in accordance with Bush et al. (1997).

**Statistical analysis**

Data used for this study were a composite of several unrelated surveys done from 2005 to 2013. Data pertaining to the five most infected fish, i.e. *C. gariepinus*, *Coptodon rendalli* (Boulenger, 1896), *Micropterus salmoides* (Lacepede, 1802), *O. mossambicus* and *S. intermedius* (see Supplementary Table S1), were subjected to analysis. Analysis of variables common to all sampling episodes such as season, fish gender, sampling locality and fish length were used to determine which variables affected parasite burden. Rows missing any data for variables tested were omitted from the analysis process. To evaluate which variable had an effect on parasite burden a generalised linear model was performed. All analyses were conducted in R 3.1.1 (R Development Core Team 2014).

*Figure 1:* Names and location of various reservoirs in Limpopo and Mpumalanga provinces sampled to establish the prevalence and distribution of *Contracaecum* spp. from 2005 to 2013. The insert illustrates the position of the Phalaborwa Barrage and the three tailing dams (P1, P2 and P3) in relation to the town of Phalaborwa
Interpretation of \( p \)-values was in accordance with Wild and Seber (2000).

**Results**

A total of 1847 specimens from 16 fish species, nine families and five orders were examined. Of these, 531 (28.74%) harbour L3 *Contracaecum* larvae. Amongst the fish examined, *Barbus trimaculatus* Peters, 1852, *Glossogobius giuris* (Hamilton-Buchanan, 1822), *Hypophthalmichthys molitrix* (Valenciennes, 1844), *Labeo roae* Steinacher, 1894, *Mugil cephalus* (Peters, 1852), *Synodontis zambezensis* Peter, 1852 and *Tilapia sparrmanii* Smith, 1840 were not infected (Supplementary Table S2). Of the fish species that were infected, *C. gariepinus*, *C. rendalli*, *M. salmoides*, *O. mossambicus* and *S. intermedius* had a higher recorded parasite prevalence and MA than *Cyprinus carpio* Linnaeus, 1758, *Hydrocynus vittatus* Castelnau, 1861, *Labeobarbus marequensis* (Smith, 1841) and *Marcusenius macrocephalotus* (Peters, 1852). Individual surveys represented independent studies; sampling routines were therefore not standardised, resulting in inconsistent recording of water parameters. Consequently, water variables that may have driven seasonal differences in parasite burden could not be established, thus data pertaining to each species and locality surveyed were pooled (Table 1).

The mean TL of female *C. gariepinus*, *M. salmoides* and *S. intermedius* (60.28 ± 19.33 cm, 27.63 ± 8.93 cm and 29.22 ± 5.47 cm, respectively) were larger than males (53.01 ± 19.38 cm, 26.14 ± 10.64 cm and 22.89 ± 4.45 cm, respectively). Of the variables tested, locality had a significant effect \( (p < 0.001) \) on parasite burden for the five species most infected (Table 2). In addition, parasite burden in *C. gariepinus*, *M. salmoides* and *S. intermedius* was also affected at some level by all the other variables tested, while body length \( (p < 0.001) \) was the only other variable to affect parasite burden in *C. rendalli*.

**Discussion**

*Contracaecum* spp. are widely distributed globally with their larvae recorded in various fish species from different continents (Aloo 1999; Wharton et al. 1999; Farahnak et al. 2002; Lymbery et al. 2002; Martins et al. 2005; Barson et al. 2008; Al-Zubaidy 2009; see Supplementary Table S3 for South African records). In this study *Contracaecum* spp. were recorded in nine of the 16 fish species and seven of nine families examined. In the life cycle of *Contracaecum* spp., the first intermediate host is a copepod and the second a fish. After an infected copepod is ingested by a fish, the nematode migrates along the alimentary canal and makes its way into the host’s body cavity by burrowing through the intestinal wall where it attains a specific length while encapsulated within a transparent sheath. Further development occurs only if the fish host is consumed by a final host and maturation and reproduction occurs in the host’s gut (Moravec 1998). Hence the probability of a fish being infected with *Contracaecum* spp. will largely depend on the feeding behaviour and presence of all hosts necessary to complete the life cycle of this parasite (Boomker 1994a, 1994b; Bergmann and Motta 2004).

In this study a high prevalence of *Contracaecum* larvae in *C. gariepinus* and *S. intermedius* was inferred to be due to both species being opportunistic and omnivorous feeders (Skelton 2001). Conversely, infection levels in *C. carpio*, *M. salmoides*, *O. mossambicus* and *C. rendalli* were far lower. For example, a single parasite was found in the 28 *C. carpio* examined. Findings in this study for *C. carpio* and *O. mossambicus* are in agreement with those reported by Boomker (1994b) and Barson et al. (2008), and the likelihood of these and *M. salmoides*, and *C. rendalli* ingesting the first intermediate host may be coincidental. For example, *O. mossambicus* may ingest infected copepods incidentally as they both feed on phytoplankton, whereas *C. rendalli* may consume copepods that shelter among their macrophyte food. Of interest is that none of the 116 *S. zambezensis* specimens examined were infected, even though this species shares a similar feeding preference to those of *C. carpio*, *C. gariepinus* and *S. intermedius* (Skelton 2001).

Although female *C. gariepinus* were larger than males, significantly higher levels of infection occurred in males. Differences can be attributed to males having a MA of 71 as opposed to 37 for females, despite both genders sharing a parasite prevalence of 78%. Although female specimens were larger, growth in male *C. gariepinus* exceeds that of female fish after three years (Skelton 2001). Higher growth rates in fish equate to a higher food demand and hence higher ingestion rates, which in turn can increase the likelihood of ingesting the first intermediate host.

By contrast, a prevalence of 38% and a MA of 2.5 were reported for female *M. salmoides* as opposed to 19% and 0.7, respectively, for males. Here the higher food requirement of females increases the prospect of ingesting infected hosts. In addition, males are restricted to the proximity of breeding nests in order to safe-guard eggs and newly hatched larvae during periods when copepod numbers are expected to swell, thereby greatly reducing the likelihood of consuming infected copepods. Similarly, *S. intermedius* females had a higher prevalence of 80% and a MA of 39 compared to 60% and 20, respectively, for males. Given that *S. intermedius* are opportunistic predators that feed from the mid-depth and surface waters (Skelton 2001), a higher food demand by females will increase the possibility of ingesting infected free-swimming copepods.

Despite Mashego and Saayman (1981) and Barson (2004) not finding an effect of host gender on burden of *Contracaecum* spp., studies on *M. salmoides* by Aloo (1999) and on *Liza abu* (Heckel, 1843) by Al-Zubaidy (2009) indicated a higher prevalence in females. Findings in this study with regard to *M. salmoides* concur with those of Aloo (1999). However, higher infection levels in females is thought by Thomas (2002) to be attributed to testosterone immunosuppression with a fluctuation of hormone levels occurring during the breeding season (Desjardins et al. 2005). Hence variations of parasite burden in male and female fish during pre- and post-spawning should be investigated.
| Species                      | AP   | FBD  | HR   | KPT  | LD   | MO   | ND   | NL   | PB   | P1   | P2   | P3   | TS   | TZ   |
|------------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Clarias gariepinus           | 16   | 17   | 14   | 9    | –    | –    | 18   | –    | 56   | 18   | 21   | 18   | –    | –    |
| P (%)                        | 81.25| 64.71| 50.00| 33.33| –    | –    | 100  | 100  | 100  | 100  | 100  | 100  | –    | –    |
| Lower CL                     | 54.35| 38.33| 23.04| 7.49 | –    | –    | 58.58| –    | 91.19| 81.47| 83.89| 81.47| –    | –    |
| Upper CL                     | 95.95| 85.79| 76.96| 70.07| –    | –    | 96.42| –    | 100  | 100  | 100  | 100  | –    | –    |
| MI                           | 43.84| 38.54| 22.14| 10.33| –    | –    | 42.86| –    | 23.90| 95.75| 81.47| 81.47| –    | –    |
| (12.10) (92.31) (3.17)       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| IR                           | 2–150| 50–950| 2–76 | 1–20 | –    | –    | 1–120| –    | 3–176| 20–702| 50–604| 50–1009| –    | –    |
| MA                           | 35.62| 249.41| 11.07| 3.44 | –    | –    | 35.72| –    | 23.90| 81.47| 314.38| 314.38| –    | –    |
| (12.97) (74.61) (4.05)       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |

| Coptodon rendalli            | 26   | 14   | 5    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | 1    |
| P (%)                        | 25.00| 14.28| 25.00| –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | 0.00 |
| Lower CL                     | 10.69| 1.78 | 0.63 | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | 0.00 |
| Upper CL                     | 44.87| 80.59| 12.00| –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | 97.50|
| MI                           | 2.00 | 1.50 | 12.00| –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | 0.00 |
| (0.22) (0.19)                |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| IR                           | 1–4  | 1–2  | 12   | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | 0    |
| MA                           | 0.50 | 0.21 | 3.00 | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | 0.00 |
| (0.20) (0.15)                |      |      |      |      |      |      |      |      |      |      |      |      |      |      |

| Cyprinus carpio              | 11   | 28   | 2    | 6    | –    | –    | –    | –    | –    | –    | –    | –    | –    | 1    |
| P (%)                        | 0.00 | 3.57 | 0.00 | 0.00 | –    | –    | –    | –    | –    | –    | –    | –    | –    | 0.00 |
| Lower CL                     | 0.00 | 0.09 | 0.00 | 0.00 | –    | –    | –    | –    | –    | –    | –    | –    | –    | 0.00 |
| Upper CL                     | 28.49| 84.19| 45.93| –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | 97.50|
| MI                           | 0.00 | 1.00 | 0.00 | 0.00 | –    | –    | –    | –    | –    | –    | –    | –    | –    | 0.00 |
| (0.00)                       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| IR                           | 0    | 0    | 0    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | 0    |
| MA                           | 0.00 | 0.04 | 0.00 | 0.00 | –    | –    | –    | –    | –    | –    | –    | –    | –    | 0.00 |
| (0.04)                       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |

| Hydrocynus vittatus          | 20   | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    |
| P (%)                        | –    | –    | –    | –    | –    | 30.00| –    | –    | –    | –    | –    | –    | –    | –    |
| Lower CL                     | –    | –    | –    | –    | –    | 11.89| –    | –    | –    | –    | –    | –    | –    | –    |
| Upper CL                     | –    | –    | –    | –    | –    | 54.28| –    | –    | –    | –    | –    | –    | –    | –    |
| MI                           | –    | –    | –    | –    | –    | 3.33 | –    | –    | –    | –    | –    | –    | –    | –    |
| (0.46)                       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| IR                           | –    | –    | –    | –    | –    | 2–37 | –    | –    | –    | –    | –    | –    | –    | –    |
| MA                           | –    | –    | 1.00 | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    |

| Marcusenius macrolepidotus   | 10   | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | 1    |
| P (%)                        | 10.00| –    | –    | –    | –    | 0.00 | –    | –    | 7.14 | –    | –    | –    | –    | 0.00 |
| Lower CL                     | 0.25 | –    | –    | –    | –    | 0.18 | –    | –    | 0.00 | –    | –    | –    | –    | 0.00 |
| Upper CL                     | 44.50| –    | –    | –    | –    | 97.50| –    | –    | 33.87| –    | –    | –    | –    | 0.00 |
| MI                           | 1.00 | –    | –    | –    | –    | 6.00 | –    | –    | 40.96| –    | –    | –    | –    | 0.00 |
| (0.00)                       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| IR                           | 1    | –    | –    | –    | –    | 0    | –    | –    | 0    | –    | –    | –    | –    | 0    |
| MA                           | 0.10 | –    | –    | –    | –    | 0.00 | –    | –    | 0    | –    | –    | –    | –    | 0.00 |
| (0.10)                       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |

Table 1: Prevalence (P), mean intensity (MI), intensity range (IR) and mean abundance (MA) of Contracaecum larvae in fish species sampled from different impoundments in north and north-eastern regions of South Africa between 2005 and 2013. The upper and lower confidence levels (CL) for prevalence and the standard error about the mean, in parentheses, for MI and MA are given. \( n \) = the total number of fish collected per locality.
Table 1 (cont.)

| Species                                      | Locality* |
|----------------------------------------------|-----------|
|                                              | AP  | FBD | HR | KPT | LD | MO | ND | NL | PB | P1 | P2 | P3 | TS | TZ |
| Micropterus salmoides*                       | n   |     |    |     |    |    |    |    |    |    |    |    |    |    |
| P (%)                                        | 100.0 | 50.0 | 33.3 | 14.28 | - | - | 1 | 21 | - | - | - | - | - | - |
| Lower CL                                     | 2.50 | 1.26 | 0.84 | 0.36 | - | - | 0.00 | 0.45 | - | - | - | - | - | - |
| Upper CL                                     | 100.0 | 98.74 | 90.57 | 57.87 | - | - | 97.50 | 41.91 | - | - | - | - | - | - |
| MI                                           | 7.00 | 3.00 | 1.15 | 1.00 | - | - | 0.00 | 2.50 | - | - | - | - | - | - |
|                                              | (0.00) | (0.00) | (9.39) | (0.00) | - | - | (0.00) | (0.28) | - | - | - | - | - | - |
| IR                                           | 7 | 3–7 | 23 | 1 | - | - | 0 | 1–4 | - | - | - | - | - | - |
| MA                                           | 7.00 | 1.50 | 7.67 | 0.14 | - | - | 0.00 | 0.48 | - | - | - | - | - | - |
|                                              | (0.00) | (1.50) | (2.03) | (0.14) | - | - | (0.00) | (0.25) | - | - | - | - | - | - |
| Oreochromis mossambicus*                     | n   |     |    |     |    |    |    |    |    |    |    |    |    |    |
| P (%)                                        | 0.00 | 13.64 | 68.75 | 10.20 | 0.00 | 0.00 | 14.29 | 5.00 | 12.50 | 5.00 | 19.56 | 50.00 | 8.69 | - |
| Lower CL                                     | 0.00 | 6.43 | 41.34 | 0.00 | 3.40 | 0.00 | 6.75 | 0.13 | 3.51 | 0.61 | 9.36 | 35.23 | 2.42 | - |
| Upper CL                                     | 8.22 | 24.31 | 88.98 | 15.44 | 22.23 | 13.72 | 25.39 | 24.87 | 28.99 | 16.92 | 33.91 | 64.77 | 20.79 | - |
| MI                                           | 0.00 | 1.78 | 1.09 | 0.00 | 2.60 | 0.00 | 1.78 | 1.00 | 1.25 | 2.00 | 9.22 | 7.00 | 1.00 | - |
|                                              | (0.10) | (0.08) | (0.30) | - | (0.08) | (0.09) | (0.09) | (0.22) | (3.37) | (3.90) | (0.00) | - | - | - |
| IR                                           | 0.00 | 0.24 | 0.75 | 0.00 | 0.27 | 0.00 | 0.25 | 0.05 | 0.16 | 0.10 | 1.80 | 3.50 | 0.09 | - |
| MA                                           | 0.00 | 0.24 | 0.75 | 0.00 | 0.27 | 0.00 | 0.25 | 0.05 | 0.16 | 0.10 | 1.80 | 3.50 | 0.09 | - |
|                                              | (0.08) | (1.48) | (1.41) | - | (0.08) | (0.05) | (0.08) | (0.08) | (1.52) | (2.42) | (0.04) | - | - | - |
| Schilbe intermedius                          | n   |     |    |     |    |    |    |    |    |    |    |    |    |    |
| P (%)                                        | 0.00 | 98.57 | - | - | - | - | - | - | - | - | - | - | - | - |
| Lower CL                                     | 0.00 | 92.30 | - | - | - | - | - | - | - | - | - | - | - | - |
| Upper CL                                     | 0.00 | 99.96 | - | - | - | - | - | - | - | - | - | - | - | - |
| MI                                           | 0.00 | 68.47 | - | - | - | - | - | - | - | - | - | - | - | - |
|                                              | (7.78) | (5.50) | (5.50) | - | (5.50) | (5.50) | (5.50) | (0.02) | (0.73) | - | - | - | - | - |
| IR                                           | 0.00 | 4–465 | - | - | - | - | - | - | - | - | - | - | - | - |
| MA                                           | 0.00 | 67.50 | - | - | - | - | - | - | - | - | - | - | - | - |
|                                              | (7.79) | (6.56) | (6.56) | (6.56) | - | - | (1.58) | (6.66) | (6.66) | (6.66) | (6.66) | (6.66) | (6.66) | (6.66) | - |

* As referred to in Figure 1; AP = Anglo Platinum, FBD = Flag Boshielo Dam, HR = Hout River Dam, KPT = Komatiport Reservoir, LK = Loskop Dam, MO = Molepo Dam, ND = Nandoni Dam, NL = Nwanedi–Luphephe Dams, P1–P3 = tailing dams, TS = Tompi Seleka, TZ = Tzaneen Dam
* Despite a recent study by Maake et al. (2014), Marcusenius macrolepidotus specimens collected in this study were reported to be of the same species
* Alien species

Table 2: Degrees of freedom (df), F-value and p-value for variables used to test for significant differences using a generalised linear model with regard to parasite burden in fish species most infected by Contracaecum spp.

| Variable                  | Fish species         | Claris gariepinus | Coptodon rendalli | Micropterus salmoides | Oreochromis mossambicus | Schilbe intermedius |
|---------------------------|----------------------|-------------------|-------------------|----------------------|------------------------|---------------------|
|                           | df | F | p  | df | F | p  | df | F | p  | df | F | p  | df | F | p  | df | F | p  |
| Gender                    | 1.00 | 6.12 | * | 1.00 | 3.21 | ns | 1.00 | 8.78 | *** | 1.00 | 0.001 | ns | 1.00 | 3.16 | * |
| Locality                  | 8.00 | 18.03 | *** | 4.00 | 45.70 | *** | 2.00 | 27.98 | *** | 12.00 | 10.01 | *** | 5.00 | 60.61 | *** |
| Season                    | 3.00 | 4.05 | * | 3.00 | 1.30 | ns | 3.00 | 63.67 | * | 3.00 | 1.81 | ns | 3.00 | 6.71 | *** |
| Total length              | 1.00 | 9.23 | ** | 1.00 | 177.05 | *** | 1.00 | 3.10 | * | 1.00 | 0.07 | ns | 1.00 | 30.86 | *** |

*p < 0.001; ** p < 0.01; * p < 0.05; • p < 0.1 (non-significant); ns, p > 0.05 (non-significant)

Data analysis revealed that differences in parasite infection levels (P, MI and MA) between localities were significant for the five most infected species. This is similar to previous studies (Supplementary Table S3) where infection levels for a given species varied between localities. This variation may be due to several factors such as the presence and density of the intermediate and definitive hosts (Hockey et al. 2005), water quality (Madanire-Moyo et al. 2012), climatic conditions, geological parameters, the implementation of various sampling procedures and the size or the impoundment investigated. From the different localities surveyed, C. rendalli, M. macrolepidotus and M. salmoides are new host records for Contracaecum larvae in South Africa.

Statistical analyses revealed seasonal variations of parasite burden to be significant in C. gariepinus, M. salmoides and S. intermedius, but not in the cichlids examined. Seasonal variation in parasite burden can be attributed to a number of factors such as fish activity, feeding behaviour and feeding rate, all of which depend on water temperature. As temperatures increase, so do the metabolic rates of fish and the demand for food which, in
turn, leads to an increase in food intake and the probability of ingesting infected sources (Rohlénova et al. 2011). For example, Barson (2004) suggested that low prevalence in C. gariepinus occurred in winter due to a reduction in feeding activity and because Contracaecum spp. larvae optimally hatch at temperatures ≥21°C. According to Mashego (1989) and Smit and Luus-Powell (2012), parasite infection by Contracaecum spp. in hosts occupying the northern region of South Africa show no seasonal trends as climatic fluctuation in the region are slight and because the definitive hosts, such as White-breasted cormorant Phalacrocorax lucidus (Lichtenstein, 1823) and African Darter Anhinga rufa (Daudin, 1802), are present all year round (Hockey et al. 2005). Conversely, Amin (1985) and Scholz (1986) reported seasonal variation in the infection of helminth species in fish inhabiting temperate waters. Findings in this study concur with those of Mashego (1989), Szalai and Dick (1990) and Smit and Luus-Powell (2012) with regard to the cichlids tested, but for C. rendalli, C. gariepinus and S. intermedius, results are in agreement with those of Amin (1985) and Scholz (1986).

Boomker (1994a) and Bergmann and Motta (2004) reported that nematode burden increases via ontogenic changes with infection being absent in juveniles but present in adults. Reasons for a greater presence of worms in larger individuals are attributed to a larger body cavity and/or the feeding preference of the second intermediate host (Alo 1999; Al-Zubaidy 2009). These assumptions are supported by previous studies done on Barbus spp. (Mashego 1989), M. salmoides (Szalai and Dick 1990) and S. intermedius (Smit and Luus-Powell 2012). Failure to obtain a wide size range of O. mossambicus to establish any ontogenic shift in parasite burden can be attributed to the sampling methods used. In conclusion, it is suggested that future surveys adopt a protocol whereby similar numbers of different species be sampled between localities within a reasonable period and that all potential variables be recorded in order to better interpret factors affecting parasite burden.

Acknowledgements — The authors thank Mr Hendrik Hattingh and Prof. Antoinette Jooste for logistical support and the University of Limpopo (Biodiversity Research Chair), National Research Foundation (NRF), Water Research Commission and Vlaamse Interuniversitaire Raad-Universitaire Ontwikkelingsaanwerking (VLIR-UOS) for funding various smaller projects from which the data set for this study was derived and compiled. Special thanks go to Dr Sean Marr and Dr Lourens Swanepoel for their assistance with analysing the data set. Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the NRF. Ethical clearance certificate T001-12 was used.

References

Aloo PA. 1999. Ecological studies of helminth parasites of the largemouth bass, Micropterus salmoides, from Lake Naivasha and the Oloiden Bay, Kenya. Onderstepoort Journal of Veterinary Research 66: 73–79.

Al-Zubaidy AB. 2009. Prevalence and densities of Contracaecum sp. larvae in Liza abu (Heckel, 1843) from different Iraqi water bodies. Marine Science 20: 3–17.

Amin O. 1985. The relationship between the size of some salmonid fishes and the intensity of their acanthocephalan infections. Canadian Journal of Zoology 63: 924–927.

Barson M. 2004. The occurrence of Contracaecum sp. larvae (Nematoda: Anisakidae) in the catfish Clarias gariepinus (Burchell) from Lake Chivero, Zimbabwe. Onderstepoort Journal of Veterinary Research 71: 35–39.

Barson M, Avenant-Oldewage A. 2006. Nematode parasites of Clarias gariepinus (Burchell, 1822) from the Rietvlei Dam, South Africa. Onderstepoort Journal of Veterinary Research 73: 87–94.

Barson M, Bray R, Ollevier F, Huyse T. 2008. Taxonomy and faunistics of the helminth parasites of Clarias gariepinus (Burchell, 1822) and Oreochromis mossambicus (Peters, 1852) from temporary pans and pools in the Save-Runde river floodplain, Zimbabwe. Comparative Parasitology 76: 228–240.

Barson M, Marshall, BE. 2004. First record of Contracaecum spp. (Nematoda: Anisakidae) in fish-eating birds from Zimbabwe. Journal of the South African Veterinary Association 75: 74–78.

Boomker GM, Motta PJ. 2004. Infection of anisakid nematodes Contracaecum spp. in the Mayan cichlid fish ‘Cichlasoma (Nandopsis) urophthalmus’ (Günther 1862). Journal of Parasitology 90: 405–407.

Boomker J. 1982. Parasites of South African freshwater fish. I. Some nematodes of the catfish [Clarias gariepinus (Burchell, 1982)] from the Hartbeespoort Dam. Onderstepoort Journal of Veterinary Research 49: 41–51.

Boomker J. 1994a. Parasites of South African freshwater fish. VI. Nematode parasites of some fish species in the Kruger National Park. Onderstepoort Journal of Veterinary Research 61: 35–43.

Boomker J. 1994b. Parasites of South African freshwater fish. VII. Nematodes of some scaled fishes from the Hartbeespoort Dam. Transvaal. Onderstepoort Journal of Veterinary Research 61: 197–199.

Bush AO, Lafferty KD, Lotz JM, Stockaw AK. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology 83: 575–583.

Bykhovskaya-Pavlovskaya IE. 1964. Key to parasites of freshwater fish of the U.S.S.R. Jerusalem: Israel Program for Scientific Translations.

Desjardins JK, Hazelden MR, van der Kraak GJ, Balshine S. 2005. Male and female cooperatively breeding fish provide support for the “Challenge Hypothesis”. Behavioral Ecology 17: 149–154.

Farahnak A, Nobedi I, Tabibi R. 2002. Fish Anisakidae helminthes bodies. Veterinary Research 31: 129–132.

Hockey PAR, Dean WRJ, Ryan PG (eds). 2005. Larvae of Contracaecum sp. among inshore fish species of southwestern Australia. Diseases of Aquatic Organisms 79: 1–9.

Maake PA, Gon O, Swartz ER. 2014. Description of three new species of Marcusenius Gill, 1862 (Teleostei: Mormyridae) from South Africa and Mozambique. Zootaxa 3780: 455–480.

Madanire-Moyo GN, Luus-Powell WJ, Olivier PAS. 2012. Diversity of metazoan parasites of the Mozambique tilapia, Oreochromis mossambicus (Peters, 1825), as indicators of pollution in the Limpopo and Olifants River systems. Onderstepoort Journal of Veterinary Research 79: 1–9.

Martins ML, Onaka EM, Fenerick J. 2005. Larval Contracaecum sp. (Nematoda: Anisakidae) in Hoplias malabaricus and Hoploerythrus unitaeniatus (Osteichthyes: Erythrinidae) of economic importance in occidental marshlands of Maranhao, Brazil. Veterinary Parasitology 127: 51–59.

Mashego SN. 1989. Nematode parasites of Barbus species...
in Lebowa and Venda, South Africa. *South African Journal of Wildlife Research* 19: 35–37.

Mashego SN, Saayman JE. 1981. Observations on the prevalence of nematode parasites of the catfish, *Clarias gariepinus* (Burchell, 1822), in Lebowa, South Africa. *South African Journal of Wildlife Research* 11: 46–48.

Moravec F. 1998. *Nematodes of freshwater fishes of the neotropical region*. Praha: Academia.

Moravec F. 2009. Experimental studies on the development of *Contracaecum rudolphii* (Nematoda: Anisakidae) in copepod and fish paratenic hosts. *Folia Parasitologica* 56: 185–193.

Nadler SA, D’Amelio S, Dailey MD, Paggi L, Siu S, Sakanari JA. 2005. Molecular phylogenetics and diagnosis of *Anisakis, Pseudoterranova* and *Contracaecum* from northern pacific marine mammals. *Journal of Parasitology* 91: 1413–1429.

Prudhoe S, Hussey CG. 1977. Some parasitic worms in freshwater fishes and fish-predators from the Transvaal, South Africa. *Zoologica Africana* 12: 113–147.

R Development Core Team. 2014. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at http://www.r-project.org.

Rothlenová K, Morand S, Hyrst P, Tolarová S, Flajšhans M, Šimková A. 2011. Are fish immune systems really affected by parasites? An immunocological study of common carp (*Cyprinus carpio*). *Parasites and Vectors* 4: 120.

Scholz T. 1986. Observations on the ecology of five species of intestinal helminths in perch (*Perca fluviatilis*) from the Macha Lake fishpond system, Czechoslovakia. *Vestník Československé Společnosti Zoologické* 50: 300–320.

Skelton P. 2001. *A complete guide to the freshwater fishes of southern Africa*. Cape Town: Struik Publishers.

Smit WJ, Luus-Powell WJ. 2012. The occurrence of metazoan endoparasites of *Schilbe intermedius* Rüppell, 1832 from the Nwanedi-Luphephe Dams in the Limpopo River System, South Africa. *African Zoology* 47: 35–41.

Szalai AJ, Dick TA. 1990. *Proteocephalus ambloplitis* and *Contracaecum* sp. from largemouth bass (*Micropterus salmoides*) stocked into Boundary Reservoir, Saskatchewan. *Journal of Parasitology* 76: 598–601.

Thomas JD. 2002. The ecology of fish parasites with particular reference to helminth parasites and their salmonid fish hosts in Welsh rivers: a review of some of the central questions. *Advanced Parasitology* 52: 1–154.

Villegas A, González-Solís D. 2009. Gastrointestinal helminth parasites of the American crocodile (*Crocodylus acutus*) in southern Quintana Roo, Mexico. *Herpetological Conservation and Biology* 4: 346–351.

Wharton DA, Hassall ML, Aalders O. 1999. *Anisakis* (Nematoda) in some New Zealand inshore fish. *New Zealand Journal of Marine and Freshwater Research* 33: 643–648.

Wild C, Seber G. 2000. *Chance encounters: a first course in data analysis and inference*. New York: John Wiley and Sons.