

Research Article

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# Seasonal occurrence and microhabitat specificity of *Paradiplozoon ichthyoxanthon* Avenant-Oldewage in Avenant-Oldewage et al., 2014 (Monogenea: Diplozoidae) infecting *Labeobarbus aeneus* (Burchell) (Teleostei: Cyprinidae) from the Vaal Dam, South Africa: water quality and host size as determining factors?

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**Abstract:** This is the first study detailing the microhabitat specificity of the monogenean parasite, *Paradiplozoon ichthyoxanthon* Avenant-Oldewage in Avenant-Oldewage et al., 2014. Samples of the monogenean were collected from the gills of the smallmouth yellowfish *Labeobarbus aeneus* (Burchell) from the Vaal Dam, South Africa, over four seasons with the use of gill nets. Host condition factor fluctuated between the different seasons, with the highest values recorded in summer. Adults and diporpas of *P. ichthyoxanthon* were found in all four seasons. Adult parasites predominantly infect the first gill arch, whereas diporpas were found on the second and fourth arches. *In utero* eggs were also found throughout the sampling seasons and their number negatively correlated with the condition factor of the host. The occurrence of adults and diporpas of *P. ichthyoxanthon* correlated with the size of the host but not the condition factor. Water quality of the study site also had little correlational relationship with the occurrence of the parasites and fluctuations were related to season. The main factor that may drive the microhabitat specificity of *P. ichthyoxanthon* is the flow of water over the surfaces of the gills and chances of the parasites becoming dislodged. Size of the attachment organs has also been shown to play a role in this specificity, because smaller attachment structures favour stronger holdfast to areas where flow might be stronger compared to larger attachment structures.

**Keywords:** ectoparasite, ecology, seasonality, smallmouth yellowfish, gill arch, epidemiology

Monogenea display a noticeably high degree of host specificity, predominantly infecting the skin and the gills of fishes (Euzet and Combes 1998) and in so doing partitioning the infection sites into smaller microhabitats (Buchmann and Lindenstrøm 2002). Site selectivity of monogeneans varies with some species attaching to the distal margins of gill filaments, whereas others attach to the proximal ends. The compact nature of the branchial chamber further increases the chances of locating a potential mate (Kearn 1994).

Monogenea show preference for certain gill arches, particularly the first and second gill arches which are more parasitised than the third and fourth arches, and has been linked to water flow over the gill surface, differences in surface area of the gill arches (Buchmann 1989), increased chances of reproducing and avoidance of interspecific competition (Šimková et al. 2000). The habitat and behaviour of the host (Le Brun et al. 1990) as well as physico-chem-

ical conditions of the environment (Anderson 1974, Raymond et al. 2006) further contribute to the migration of monogeneans across the branchial chamber.

Diplozoid species demonstrate particular preference for attachment to the medial third of the first and second gill arches. Both adult and fused diporpas are nestled between hemibranchs and attach proximally to the interbranchial septum with their anterior ends extending downstream of the direction of water flow (Owen 1963, Kagel and Taraschewski 1993). Attachment of diporpas is more random, initially on the outer surface and mostly on the distal portion of the primary lamellae (Owen 1963, Kagel and Taraschewski 1993).

Most literature pertaining to attachment of diplozoids to the gills of cyprinid fishes deals with European species (e.g. Anderson 1974, Bagge and Valtonen 1999). In Africa, biology (Mashego 2000, Avenant-Oldewage et al. 2014) and ecology (Chapman et al. 2000, Raymond et al. 2006) of diplozoid parasites have been studied. In South

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Africa, two diplozoid species, namely *Paradiplozoon ichthyoxanthon* Avenant-Oldewage in Avenant-Oldewage et al., 2014 and *P. vaalense* Dos Santos, Jansen van Vuuren et Avenant-Oldewage, 2015 have recently been described from *Labeobarbus aeneus* (Burchell) and *Labeo umbratus* (Smith), respectively. Little is known about the microhabitat specificity of these parasites.

We hypothesise that the distribution of *P. ichthyoxanthon* on the gills of *L. aeneus* will be similar to that described for other diplozoid species and that the infection site will not change with the quality of the environment. This study focuses on *P. ichthyoxanthon* and represents the first report on the spatial distribution of the parasite on gills of the smallmouth yellowfish in relation to host size and sex, and physico-chemical conditions of the macroenvironment.

## MATERIALS AND METHODS

Four samples of smallmouth yellowfish were taken from the Vaal Dam (26°52'34"S; 28°10'26"E) during four seasons, namely June 2013 (winter), February 2014 (summer), August 2014 (winter/spring) and February 2015 (summer). *Labeobarbus aeneus* were collected with the use of gill nets (mesh size 45–170 mm) with the aim to collect fish of variable size. Nets were checked every three hours and live fish were removed from the nets and placed into wells, then weighed and measured. They were euthanised by severing the spinal cord according to accepted protocols set out by the South African National Animal Ethics Guidelines following ethical approval by the University of Johannesburg Ethical committee and dissected to determine their sex. Condition factor (CF) of the host organisms was calculated according to Carlander (1969) for each of the sampling excursions according to the equation  $CF = (W \times 10^5)/L^3$  where W is the weight of the fish in grams and L is the standard length in millimetres. Physico-chemical water quality variables (i.e. pH, temperature, conductivity, dissolved oxygen and oxygen saturation) were recorded with a YSI 556 Multi-Probe meter (YSI Inc., Yellow Springs, USA) for the sampling surveys at three intervals per day and mean values for each sample calculated.

Gills were then removed and assessed for *Paradiplozoon ichthyoxanthon* using a Zeiss DV4 Stereo microscope. The left and right pairs were separated and each gill arch examined. The infection site of individual adult *P. ichthyoxanthon*, diporpas and the number of *in utero* eggs at each site were recorded. The number of eggs *in utero* recorded during the surveys were easily determined as a result of the brown colouration of the egg shell, making them identifiable. The branchial chamber microhabitat was divided into left and right sides (Fig. 1), and gill arches were numbered 1–4 (anterior to posterior) and subdivided into dorsal, medial and ventral areas following Gelnar (1987).

Based on data obtained for host morphometrics, fish were divided into three groups based on their standard lengths, namely small (< 20 cm), medium (21–29 cm) and large (30–40 cm). The mean intensity of parasite infections was then calculated for each group (Bush et al. 1997). The mean intensity was similarly calculated for host sexes and the left and right sides of the branchial basket, gill arches and infected regions. This was calculated as the total number of parasites per subdivision analysed divided by the total number of infected compartments in the sample.



**Fig. 1.** Gills of *Labeobarbus aeneus* (Burchell) infected with *Paradiplozoon ichthyoxanthon* Avenant-Oldewage et al., 2014. **A** – division of the branchial chamber into left and right sides, number of gills; **B** – division of gill arches into dorsal, medial and ventral regions.

Statistical analyses of the data were performed with SPSS Version 22 (Statistical Package for the Social Sciences, SPSS, Inc., USA) and Microsoft Excel (2013). Seasonal fluctuations in physico-chemical water quality variables, host morphometrics and condition factor were evaluated using one-way ANOVA with a Tukey post hoc test and Kruskal-Wallis test, respectively. Variance in the mean intensity of infection with *P. ichthyoxanthon* between seasons, host sizes and sex, and microhabitat were determined using one-way ANOVA and Tukey post hoc tests, as well as Mann-Whitney U and Kruskal-Wallis tests. Pearson's chi-square test was used to assess the trends in microhabitat specificity between left and right sides of the branchial chamber, preference for attachment to specific gill arches as well as the dorsal, medial and ventral regions of the gill arches. Kendall's tau was calculated to determine if there were any correlations between the number of parasites infecting each microhabitat compartment and fluctuations in physico-chemical water quality variables. All significance values were set at 0.05 level for non-parametric tests and 0.01 level for two tailed comparisons of Kendall's tau correlation coefficient.

## RESULTS

Values of the physico-chemical water quality variables (Table 1) were higher during summer months compared to winter ( $p < 0.05$ ). Host data (Table 2) indicated that female fish were heavier, larger and had a greater condition factor compared to males ( $p < 0.05$ ).

**Table 1.** Physico-chemical variables of water quality measured for each of the sampling surveys for winter 2013 – summer 2015 in the Vaal Dam, South Africa.

Variable	Winter 2013	Summer 2014	Winter 2014	Summer 2015
pH	7.9 ± 0.3	9.9 ± 0.1	8.1 ± 0.9	8.6 ± 0.2
T (°C)	16.1 ± 2.4	22.9 ± 0.5	12.8 ± 1.7	23.5 ± 1.8
C (µS)	164.9 ± 2.7	191.4 ± 1.9	169.6 ± 0.9	176.3 ± 0.5
DO (mg/l)	8.1 ± 0.6	9.0 ± 0.5	6.6 ± 1.8	5.0 ± 0.5
OS (%)	83.9 ± 6.4	105.1 ± 5.9	61.7 ± 16.2	57.7 ± 5.1

T – temperature; C – conductivity; DO – dissolved oxygen; OS – oxygen saturation.

**Table 2.** Summary of *Labeobarbus aeneus* (Burchell) sampled from the Vaal Dam, South Africa for winter 2013 – summer 2015 surveys.

Variable	Number collected	Mean weight (g) ± SD	Mean standard length (cm) ± SD	Mean condition factor ± SD
Winter 2013				
Male	42	312.4 ± 178.8	24.7 ± 4.5	1.92 ± 0.26
Female	41	338.9 ± 184.5	25.8 ± 4.6	1.84 ± 0.48
Total	83	325.2 ± 179.9	25.2 ± 4.5	1.88 ± 0.38
Summer 2014				
Male	30	296.7 ± 123.1	24.4 ± 2.7	1.99 ± 0.34
Female	11	338.2 ± 200.5	25.5 ± 3.9	1.92 ± 0.33
Total	41	307.8 ± 146.3	24.7 ± 3.1	1.97 ± 0.33
Winter 2014				
Male	24	226.3 ± 115.5	23.8 ± 4.1	1.53 ± 0.25
Female	11	252.7 ± 85.5	24.8 ± 2.8	1.58 ± 0.16
Total	35	234.6 ± 106.4	24.1 ± 3.8	1.55 ± 0.22
Summer 2015				
Male	10	306.0 ± 84.2	26.0 ± 2.0	1.70 ± 0.13
Female	20	461.0 ± 227.9	29.2 ± 4.3	1.72 ± 0.16
Total	30	409.3 ± 204.3	28.2 ± 4.0	1.71 ± 0.15

Adult parasites of *Paradiplozoon ichthyoxanthon* were found attached between the hemibranchs of gill arches with haptors positioned proximally to the interbranchial septum. The anterior ends of the parasites were directed distally from the branchial septum. Attachment of diporparas to gill arches was variable, with some specimens attached to the outer faces of hemibranchs and others between hemibranchs.

Pooled data for winter and summer comparisons between the number of adults and diporparas of *P. ichthyoxanthon* and number of *in utero* eggs indicated significantly higher numbers of adult parasites ( $p < 0.05$ ) during summer ( $n = 300$ ) compared to winter ( $n = 165$ ). The number of diporparas and *in utero* eggs were not significantly ( $p > 0.05$ ) higher during winter (diporpara:  $n = 54$ ; eggs:  $n = 42$ ) compared to summer (diporpara:  $n = 29$ ; eggs:  $n = 21$ ). This trend was consistent when comparing the number of parasites between the different surveys. However, if fecundity is expressed as the average number of eggs per worm in summer 21/300 (0.07) and in winter 42/165 (0.25), it becomes clear that the few adults that are present in winter have a higher fecundity.

Differences in the number of adult parasites and diporparas collected from left and right sides of the branchial chamber were not significant ( $p > 0.05$ ) even though more para-

sites were present on the left side ( $n_{adults} = 242$ ;  $n_{diporpara} = 42$ ) compared to the right ( $n_{adults} = 223$ ;  $n_{diporpara} = 41$ ). Seasonal differences in the total number of adults, diporparas and *in utero* eggs for the separate surveys are indicated in Table 3.

More diporparas were found on the right side of the chamber in summer 2014, but no difference in the number of diporparas between left and right sides of the branchial chamber was detected in summer 2015. Following distribution of adults, the number of *in utero* eggs was also highest on the left side of the branchial chamber during winter 2014 and summer 2015 survey, but the opposite occurred for winter 2013 and summer 2014 surveys (Table 3) where higher egg numbers were recorded for the right side of the branchial chamber.

Preference for specific gill arches was found for both adult and diporpara stages. The number of adults were significantly higher ( $p < 0.05$ ) on the first arch ( $n_{adults} = 181$ ), followed by the fourth arch ( $n_{adults} = 117$ ) and lowest for the middle arches ( $n_{adults}$ : second arch = 98; third arch = 69). Distribution of diporparas across the gill arches indicated significantly more diporparas on the second gill arch ( $n_{diporpara} = 34$ ) and first arch ( $n_{diporpara} = 20$ ) compared to the third and fourth arches ( $n_{diporpara}$ : third arch = 16; fourth arch = 13) and more *in utero* eggs were present in adult *P. ichthyoxanthon* on the first arch ( $n_{eggs} = 25$ ) compared to the others ( $n_{eggs}$ : second arch = 13; third arch = 15; fourth arch = 10); this distribution was significantly ( $p < 0.05$ ) different between gill arches (Table 3).

For the regions of the gills the number of all parasite stages studied was significantly ( $p < 0.05$ ) higher on the medial area ( $n_{adults} = 233$ ;  $n_{diporpara} = 36$ ;  $n_{eggs} = 35$ ) of the gill arch compared to the dorsal ( $n_{adults} = 152$ ;  $n_{diporpara} = 18$ ;  $n_{eggs} = 16$ ) and ventral areas ( $n_{adults} = 77$ ;  $n_{diporpara} = 24$ ;  $n_{eggs} = 12$ ). The distribution of adult worms was uniform between gill regions, with a notable preference for the medial region of the gills; diporparas were more randomly distributed between medial and ventral areas. To test the predilection of *P. ichthyoxanthon* for specific sides of the branchial chamber, gill arches and gill region probabilities of finding the parasites on each aspect of the branchial apparatus were assessed (Table 3).

For the preference of either the left or right side of the gill chamber, the null hypothesis was that there is a 50% chance of locating the parasite on either the left or right side of the branchial chamber. Pearson's chi-square indicated that there was no preference for either the left or right side of the branchial chamber for either adult or diporparas and the null hypothesis that there is a predilection for a specific side of the chamber was therefore rejected.

For the distribution of the parasite on the gill arches, it was hypothesised that there is a 25% chance of locating either stage on the first through to the fourth gill arch. Pearson's chi-square indicated that the number of adults is significantly greater on the first gill arch, whereas for diporparas there is a significant preference for the second gill arch. Similarly, for the probability of locating the parasite on either the dorsal, medial or ventral region of the gill, the null hypothesis that there is no preference for one specific region was rejected for all the life stages studied. Pearson's

**Table 3.** Summary of the total number of *Paradiplozoon ichthyoxanthon* Avenant-Oldewage in Avenant-Oldewage et al., 2014 adults, diporpas and in utero eggs collected from the Vaal Dam, South Africa for winter 2013 – summer 2015 period and preference for specific regions within the branchial chamber.

Variable	Stage	Winter 2013	Summer 2014	Winter 2014	Summer 2015	Overall preference (Chi-square)	<i>p</i> – value
		Preferred	Preferred	Preferred	Preferred		
Side of chamber (left/right)	Adults	Left (64)	Right (100)	Left (27)	Left (57)	Left (0.78)	0.38
	Diporpas	Left (12)	Right (7)	Left (16)	-	Left (0.05)	0.83
	Eggs	Right (18)	Right (12)	Left (4)	Left (2)	Right (0.14)	0.71
Gill arch (first, second, third, fourth)	Adults	First (49)	First (70)	First (19)	First (43)	First (60.05)	<b>0.000</b>
	Diporpas	First (10)	Second & Fourth (4)	Second (18)	Second (7)	Second (13.12)	<b>0.004</b>
	Eggs	First (12)	First (8)	First (4)	First & Fourth (1)	First (8.05)	<b>0.045</b>
Position on gill (dorsal, medial, ventral)	Adults	Medial (55)	Medial (104)	Medial (31)	Dorsal (47)	Medial (79.05)	<b>0.000</b>
	Diporpas	Medial (12)	Medial (5)	Medial (13)	Ventral (7)	Medial (6.46)	<b>0.038</b>
	Eggs	Medial (19)	Medial (12)	Dorsal & Medial (3)	Dorsal & Medial (1)	Medial (13.72)	<b>0.001</b>

Bold values indicate significant difference ( $p < 0.05$ ); dash (-) indicates no difference between chamber sides, gill arches and regions.

**Table 4.** Summary of *Paradiplozoon ichthyoxanthon* Avenant-Oldewage in Avenant-Oldewage et al., 2014 adult and diporpas mean intensity, and number of *in utero* eggs in relation to *Labeobarbus aeneus* (Burchell) size and sex for surveys conducted in the Vaal Dam, South Africa.

Variable	Stage	Winter 2013	Summer 2014	Winter 2014	Summer 2015	Chi-square	<i>p</i> - value
		Preferred	Preferred	Preferred	Preferred		
Host size (small, medium, large)	Adults	Medium (3.57 ± 0.2)	Large (7.00 ± 0.4)	Small (3.5 ± 0.3)	Medium (6.23 ± 0.4)	7.064	<b>0.029</b>
	Diporpas	Large (1.50 ± 0.1)	Medium (1.00 ± 0.1)	Medium (2.21 ± 0.2)	Large (1.40 ± 0.2)	0.878	0.645
	Eggs	Medium (25)	Medium (19)	Medium (7)	Medium & Large (1)	1.537	0.464
Host sex (male, female)	Adult	-	Male (6.46 ± 0.4)	Female (4.75 ± 0.3)	Male (5.40 ± 0.3)	2.344	0.126
	Diporpas	Male (1.54 ± 0.1)	-	Female (2.60 ± 0.2)	Female (1.44 ± 0.2)	1.026	0.311
	Eggs	Male (21)	Male (15)	Female (5)	Female (2)	0.582	0.561

Bold values indicate significant difference ( $p < 0.05$ ); dash (-) indicates no difference between host size and sex

chi-square indicated a statistically significant preference for the medial region of the gills for adult parasites and ventral region for diporpas ( $p < 0.05$ ).

The number of *in utero* eggs corresponded to the distribution of adult parasites across the gills, when numbers of adults were higher for a particular gill arch or region, the numbers of eggs correspondingly were also high for the same region. This pattern was, however, lacking for the number of adults on the left or right sides of the branchial chamber with more eggs present on the right side ( $n = 33$ ) versus the left ( $n = 30$ ). This difference was insignificant ( $p > 0.05$ ).

Seasonal differences were also recorded and indicated that smaller fish were more frequently infected compared to larger ones during winter months, compared to summer, which indicated the opposite (Table 4). These trends were statistically significant and overall preference of adult *P. ichthyoxanthon* was found for medium-sized fish. Numbers of diporpa were not significantly different between the host size groups, even though larger fish were more heavily infected (higher values of mean intensity) compared to smaller ones. The number of parasites, eggs and mean intensity of infections did not significantly differ ( $p > 0.05$ ) between male and female hosts. The number of *in utero* eggs was highest for medium-sized male hosts in winter 2013. No significant differences were recorded for *in utero* eggs between the host size groups or sexes.

Significant positive correlations were present between the number of adult *P. ichthyoxanthon* and the physico-chemical variables studied, with the strongest correlation to the conductivity of the water (Table 5). Correlations for diporpas and *in utero* eggs were weak and insignificant in all comparisons. The number of diporpas was negatively correlated with pH, conductivity and oxygen level of the water. Number of *in utero* eggs was positively correlated with all water quality variables except for pH, which was negatively correlated. Standard length, weight and condition factor weakly correlated with the numbers of adult parasites, diporpas and *in utero* eggs. All variables were positively correlated with adult *P. ichthyoxanthon* numbers, whereas, the number of *in utero* eggs was negatively correlated with the standard length and weight of the hosts. The number of diporpas was negatively correlated with host condition factor but positively correlated with standard length and weight, but in both these cases the relationship was not significant ( $p > 0.001$ ).

## DISCUSSION

Aggregation of monogeneans in particular microhabitats increases the chance of locating mates and is especially required in diplozoids which only attain sexual maturity once two diporpas fuse. Significantly higher numbers of adult *Paradiplozoon ichthyoxanthon* were recorded on the first gill arch, whereas diporpas were significantly more

**Table 5.** Correlations for differences in the number of *Paradiplozoon ichthyoxanthon* Avenant-Oldewage in Avenant-Oldewage et al., 2014 adults, diporpas and *in utero* eggs compared to physico-chemical water quality and host morphometrics for winter 2013 – summer 2015 surveys from Vaal Dam, South Africa.

Variable	Adult		Diporpas		Eggs	
	R <sup>2</sup> value	<i>p</i> - value	R <sup>2</sup> value	<i>p</i> - value	R <sup>2</sup> value	<i>p</i> - value
pH	<b>0.172</b>	<b>0.000</b>	-0.009	0.840	-0.010	0.808
Temperature (°C)	<b>0.259</b>	<b>0.000</b>	-0.046	0.284	0.023	0.596
Conductivity (µS/cm <sup>3</sup> )	<b>0.294</b>	<b>0.000</b>	0.046	0.288	0.063	0.144
Oxygen saturation (%)	<b>0.146</b>	<b>0.000</b>	-0.056	0.192	0.067	0.116
Dissolved oxygen (mg/L)	0.071	0.072	-0.053	0.209	0.035	0.415
Standard length (cm)	0.022	0.573	0.024	0.566	-0.051	0.229
Weight (g)	0.045	0.252	0.021	0.615	-0.025	0.550
Condition factor	0.071	0.069	-0.021	0.612	0.090	0.032

Bold values indicate significant differences at  $p < 0.001$  level (2 – tailed).

numerous on the second and fourth gill arches. The number and mean intensity of adults gradually decreased from the first to the fourth gill arch. Similarly, Zargar et al. (2012) found that *Diplozoon kashmirensis* Kaw, 1950 favoured attachment to the first gill arch and numbers gradually decreased toward the fourth arch. Chapman et al. (2000) and Soylu (2007) indicated preference for the second gill arch in *Afrodiplozoon polycotyleus* (Paperna, 1973) and *P. homoion* (Bychowsky et Nagibina, 1959). Selection of specific microhabitat compartments could be further influenced by variability in water flow across the gills of the fish, allowing adults to attach to sites where flow would otherwise be strong and prevent infection.

Crafford et al. (2014) indicated that site preference was linked to season, with significant differences in site preference of monogeneans from *Labeo umbratus* during summer but not winter. Le Roux et al. (2011) indicated site preference in *Cichlidogyrus philander* Douëllou, 1993 for the third and second gill arches, concluding that due to a lack of correlation between gill filament length and number of primary and secondary lamellae with parasite prevalence, site selection is governed by water flow over the gills. The distribution of diporpas of *P. ichthyoxanthon* indicates greater numbers attached to the middle arches and can be linked to large volumes of water washing over the middle gill arches facilitating attachment (Wootten 1974, Dzika 1999). Thereafter, diporpas migrate to the first and fourth arches and develop into adults. Suydam (1971) found *Diclidophora maccallumi* (Price, 1943) more frequently on arches 1 through 3 and also related this to water flow. Both adult and diporpas of *P. ichthyoxanthon* favoured attachment to the medial regions of the gills. For adults this was followed by the dorsal aspect while diporpas were found second to the medial area on the ventral section of the gills. This has similarly been observed by Raymond et al. (2006) and Zargar et al. (2012) for two diplozoids from Uganda and India, respectively.

Owen (1963) and Kagel and Taraschewski (1993) found diporpas of *Diplozoon paradoxum* (Nordmann, 1832) attached mostly to the distal extremities and outer faces of hemibranchs, whereas adult worms were found proximally near or at the level of the interbranchial septum. The attachment by adults and diporpas is most likely

a result of the free swimming oncomiracidium. Buchmann (1989) indicated that differences in the attachment site of *Pseudodactylogyrus bini* (Kikuchi, 1929) and *P. anguillae* (Yin et Sproston, 1948), was related to size of the hamuli. Parasites with smaller hamuli attached distally along the primary lamellae, whereas parasites with larger hamuli attached proximally toward the base. Furthermore, adult parasites attach and nestle between hemibranchs, further reducing the chances of being dislodged (Owen 1963). This was also observed in this study. The larger body size of the adult would experience greater drag from water flow than smaller life stages and is a response to water flow across the gills and a means of reducing the chance of being washed off.

The number of parasites fluctuates with host size (Patterson and Ruckstuhl 2013) and has largely been related to differences in age related immune response of fish and also increased surface area (Buchmann and Lindenstrøm 2002). This may be valid also for *P. ichthyoxanthon* infecting *Labeobarbus aeneus*. However, the heaviest infections with *P. ichthyoxanthon* was found in medium-sized fish and the lowest in small fish, which could be related to the available surface area (greater in larger fish). However, this trend is probably driven by the synergistic effects of surface area and host size/age. Furthermore, this can be enhanced by the schooling behaviour of the hosts and feeding preferences (de Villiers and Ellender 2007). According to O'Brian et al. (2011), juvenile smallmouth yellowfish tend to school more than larger fish and remain near the substrate where they feed and find refuge from predators.

Eggs of *P. ichthyoxanthon* lack filaments and are covered by a sticky substance. The highest numbers of *in utero* eggs were likely deposited in summer, in areas where hosts occur. When hosts frequent these areas a school of *L. aeneus* can swim through a swarm of oncomiracidia and become infected (Wiles 1970). Le Brun et al. (1990, 1992) suggested that the variable occurrence of *Diplozoon gracile* (Reichenbach-Klinke, 1961) was related to the behaviour and habitat of the host. Oncomiracidia of the parasite were more likely to be located near the bottom of a water body and fish found in this area were more likely to become infected (Le Brun et al. 1990, 1992).

The lack of correlation between the size of the host and adult *P. ichthyoxanthon* can possibly be related to the fact that once diporpas fuse, movement by the pair across the gill is restricted and therefore they remain in the same area. The number and mean intensity of adults and diporpas of *P. ichthyoxanthon* were significantly different between gill arches and sections of the gills. Comparisons between the right and left sides of the branchial basket were insignificant and correspond to other studies on diplozoids (Chapman et al. 2000, Raymond et al. 2006, Soylu 2007). The only exception to this was reported by Zargar et al. (2012) who found significantly higher numbers of *D. kashmirensis* in the left branchial chamber.

Mean intensity of *P. ichthyoxanthon* adults and diporpas between the different standard lengths of *L. aeneus* sampled was significantly higher for medium-sized hosts. Höglund and Thulin (1989) and Soylu (2007) indicated that mean intensity and prevalence of *P. homoion* increased with size of the hosts, with higher numbers recorded in large hosts. Kagel and Taraschewski (1993) observed an opposing trend for diporpas and pre-adults of *D. paradoxum* with the highest prevalence values on smaller fish, whereas adult worm prevalence increased with host size. Chapman et al. (2000) indicated that *A. polycotyleus* occurred more frequently on medium-sized hosts and Raymond et al. (2006) indicated weak and insignificant occurrence of the same species with regard to host size.

Host condition factor did not significantly correlate with the number or mean intensity of *P. ichthyoxanthon* but rather fluctuated seasonally, indicating that the parasite has a negligible relationship with the overall health of the host. The parasites would have to either reduce or increase the weight of the host and given the small size of the microhabitat and localisation in feeding, this is unlikely to occur even in high infections. Furthermore, given the weak correlation between *P. ichthyoxanthon* numbers and mean intensity with host weight, this further supports the fact that the parasites have little to no effect on the overall health of *L. aeneus*. Reduced condition during winter months may, however, increase susceptibility of the host to larval parasites (Crafford et al. 2014) and result in higher mean intensities. The negative correlation between *in utero* eggs and host condition factor could point to release of parasite eggs during host spawning, signalled by decreased condition factor, corroborating the finding of Šimková et al. (2005) that a parasite's reproductive cycle could be linked to that of the hosts.

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Infection biology of parasites change in relation to season and water quality. In the present study, higher mean intensity and number of adults were found during summer surveys, whereas mean intensity and number of diporpas were found to be highest during winter. Egg numbers were highest during summer months and such fluctuations are related to temperature (Anderson 1974, Šimková et al. 2000). Chapman et al. (2000) indicated that numbers of *A. polycotyleus* decreased during rainy seasons and were related to decreased host concentration. Negative correlation between temperature and number of diporpas of *P. ichthyoxanthon* support this assumption, similarly as those of Dzika (1987) who observed two peaks in the occurrence of *D. paradoxum* during one year.

Höglund and Thulin (1989) indicated that prevalence and density of *P. homoion* did not differ seasonally for a site with thermal pollution and higher temperatures resulted in earlier production and release of eggs compared to reference sites. These authors also assumed that higher temperatures increased the production of eggs and thus the number of adults of *P. homoion*. Even though the temperatures at the thermally altered site were higher compared to the reference, seasonal changes remained noticeable suggesting that temperature was not the only determining factor in their study site. MacDonald and Jones (1978) found that egg production by *D. paradoxum* under controlled conditions increased with temperature, but egg laying was independent of temperature. They found that the deposition of eggs was linked to daylight, with the highest number being deposited during dark periods.

Negative correlations between mean intensity of infection with *P. ichthyoxanthon* and water conductivity have previously been shown by Gilbert and Avenant-Oldewage (2016). Even though the water quality of the Vaal Dam did fluctuate during the present study, it is inferred that these small changes have little effect on microhabitat selection. Although the relationship between infection and water quality is correlational, the trends indicate that as the water quality fluctuates so do the numbers and mean intensity of parasite stages. In order to properly elucidate any causative relationships, laboratory study is required.

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