Polystoma umthakathi (Monogenea): establishment, mortality and reproduction of neotenic parasites in experimentally infected *Natalobatrachus bonebergi* tadpoles

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The development of *Polystoma umthakathi* oncomiracidia were studied in experimentally infected *Natalobatrachus bonebergi* tadpoles to establish a normal reference of parasite performance in the natural host. The anteroventral body wall is transparent in these tadpoles and it was possible to follow the destiny of each parasite throughout its life span of neotenic development. Success of establishment was 27,9% and mean parasite intensity 2,1 at 48 h. After parasite larvae initially attached to the gills on the left side, they gradually migrated to the right side and mature parasites only occurred inside the right branchial chamber. Parasite mortality was high, prevalence had declined to 72,4% after 12 days and 48,3% after 20 days while mean parasite intensity had declined to 1,8 and 1,6 during the same period. Egg production was around 13 to 15 eggs/parasite/day for parasites in burdens of one and two but significantly lower (5,24) in the case of three-parasite burdens. Tadpoles harbouring two or more parasites became anaemic but recovered when most or all parasites were lost.

Die ontwikkeling van *Polystoma umthakathi* onkomirasidia is bestudeer in eksperimenteel geïnfekteerde *Natalobatrachus bonebergi* larwes ten einde 'n normale verwysingsraamwerk op te stel van parasietprestasie in die natuurlike gasheer. Die anteroventrale liggaamswand is deursigtig by hierdie larwes, en dit was moontlik om die bestemming van elke parasiet te volg gedurende sy hele leeftyd van neoteniese ontwikkeling. Die sukses van vestiging was 27,9% en die gemiddelde besmettingsintensiteit 2,1 op 48 h. Nadat parasietlarwes aanvanklik aan die linkerkieue vasgeheg het, het hulle geleidelik na die regterkant gemigreer en geslagsryp parasiete is uitsluitlik in die regterkieukamers aangetref. Parasietmortaliteit was hoog, gasheerbesmetting het afgeneem tot 72,4% na 12 dae en 48,3% na 20 dae terwyl die gemiddelde besmettingsintensiteit gedurende dieselfde periode afgeneem het tot 1,8 en 1,6. Eierproduksie was nagenoeg 13 tot 15 eiers/parasiet/dag vir parasiete in ladings van een en twee maar beduidend laer (5,24) wanneer drie parasiete saam voorgekom het. Paddalarwes wat twee of meer parasiete gehuisves het, het anemies geword maar het herstel wanneer hulle die meeste of al die parasiete verloor het.

The greatest contribution to the knowledge of the polystomes of anurans in Africa has been taxonomic while their ecology has also received some attention (Maeder, Euzet & Combes 1970; Maeder 1973; Tinsley 1974; Murith 1981a; Murith 1981b; Murith 1982). Studies on the development of different stages within the life cycle have received less attention and hardly anything is known of interspecific differences in this regard. Kok & du Preez (1989) described the development, maturation and reproduction of neotenic *Polystoma australis* in an attempt to establish a normal reference against which parasite success under experimental conditions can be measured. A comparable study was undertaken on *P. umthakathi* Kok & Seaman, 1987), in its natural host *Natalobatrachus bonebergi*.

The transparency of the body wall ventral to the gills in *N. bonebergi* tadpoles made it possible to follow the destiny of each parasite from establishment up to its loss from the branchial chambers. The present study provides information on the establishment of oncomiracidia during experimental infection, the subsequent migration of parasites from left to right branchial chambers, survival during development and the egg production of mature neotenic parasites at different parasite burdens.

Materials and Methods

Parasite-free *Natalobatrachus bonebergi* tadpoles were hatched in the laboratory from egg masses collected in the Vernon Crookes Nature Reserve (30°16'S / 30°37'E). Egg masses are laid on vegetation overhanging streams in the indigenous coastal forests of southern Natal and eastern Cape (*cf.* Kok & Seaman 1988). Tadpoles were kept in aerated, chlorine-free, tap water at 23°C (± 1°C), and were fed on pulverized alfalfa and goldfish flakes.

N. bonebergi tadpoles infected with *P. umthakathi* were collected from streams in the eastern part of the nature reserve. They were kept and fed as described above.

P. umthakathi eggs, laid by neotenic parasites, were harvested from water in which naturally or experimentally infected *N. bonebergi* tadpoles were kept. Eggs were harvested by sieving the water through 112 µm plankton netting and were kept in chlorine-free tap water at 23°C (±1°C) in small petri dishes. Oncomiracidia hatched after 10 to 11 days.

For experimental infection, tadpoles between five and twenty days old (less than 20 mm total length) were exposed individually or in pairs, to five or six newly hatched oncomiracidia / tadpole in 20 ml of water. Tadpoles were transferred to larger containers after 24 h.

For microscopical examination tadpoles were narcotized with MS 222 Sandoz (2 mg / 10 ml water). The period of narcotization seldom exceeded 5 min. In *N. bonebergi* tadpoles the body wall ventral to the branchial chambers remains almost completely free of pigment up to metamorphosis. The gills can therefore be examined microscopically throughout the life of the tadpole and

the complete history of each parasite present can be followed. For each tadpole the numbers and locations of established parasites were determined on every second day following experimental infection. From the sixteenth day onward daily numbers of eggs produced were also recorded.

Results

Establishment and migration

Success of establishment, determined by counting numbers of parasites present 48 h after exposure to oncomiracidia, was 27,9% (n = 215). Parasites were established in 29 of the 38 tadpoles exposed (76,3%), although in some subgroups all tadpoles became infected. The mean parasite intensity (number / infected host) at 48 h was 2,1 (SD = 1,4; n = 29) and the highest intensity, observed in two tadpoles, was 6.

Oncomiracidia entered through the left-sided spiracle. Parasites initially attached to the left gill filaments but soon started migrating towards the right (closed) branchial chamber. At two days only 35% of the parasites were found still attached to the left gill (Figure 1). This migration was largely completed after 10 to 12 days and older parasites were found almost exclusively within the right branchial chamber (Figure 1). Out of 25 egg producing neotenic parasites (18 days and older) recorded, none was located in the left branchial chamber. Young parasites were attached to the gill filaments but older parasites mostly attached to the more rigid anterior limb buds developing within the branchial chambers.

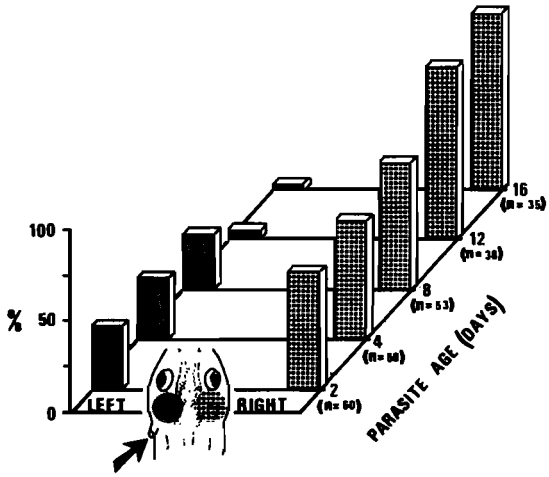

Figure 1 Percentage of *Polystoma umthakathi* in each branchial chamber of *Natalobatrachus bonebergi* tadpoles at various periods following the entrance of oncomiracidia through the left-sided spiracle (arrow).

Mortality

Figure 2 indicates the percentage survival of branchial parasites from the second day following establishment until all parasites were lost. None of these losses was due to tadpole metamorphosis. It is evident that there was a rapid and continuous decline in parasite numbers and none survived for longer than 46 days. Prevalence (% of host population infected), taken as 100% at 48 h, declined to 72,4% at 12 days and 48,3% at 20 days. During the same period the initial mean intensity of 2,1

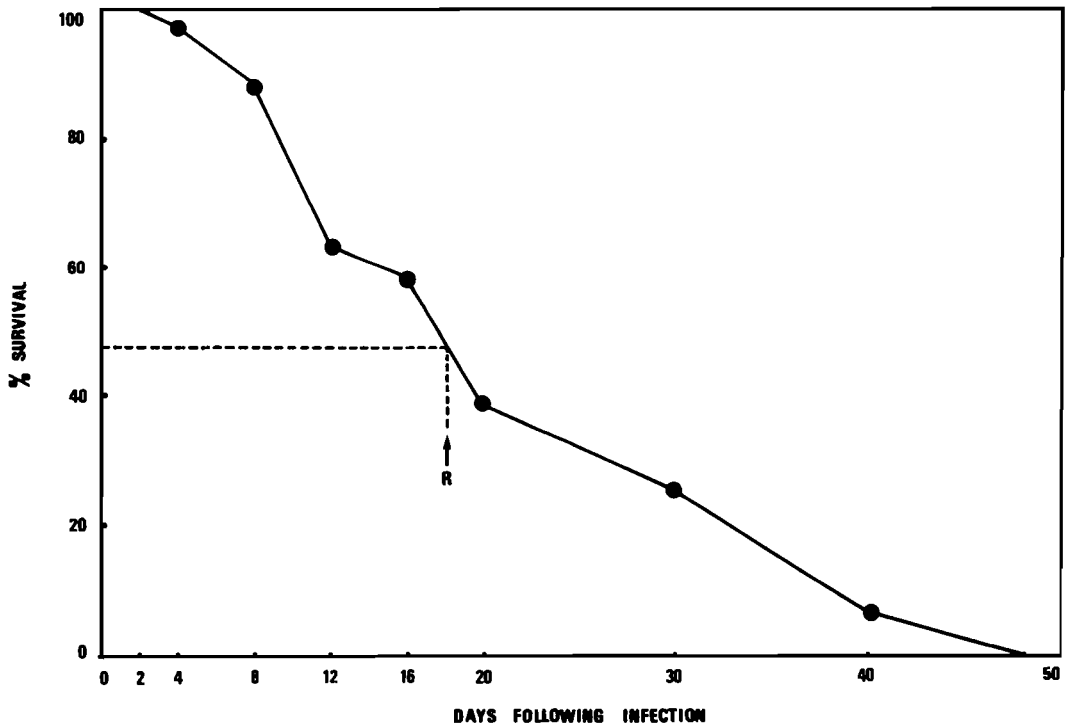

Figure 2 Survival in percentage of branchial *Polystoma umthakathi* parasites in *Natalobatrachus bonebergi* tadpoles at various times following the establishment of oncomiracidia. Neotenic maturity and the onset of reproduction (R) are reached at an approximate age of 18 days (arrow) by which time at least 50% of parasites have been lost.

(vide supra) declined to 1,8 (SD = 1,2) and 1,6 (SD = 0,9). About 50% of the parasites were already lost at the time when neotenic reproduction began (*cf.* Figure 2).

Parasite losses from left and right branchial chambers were similar and there was no evidence of a greater loss of those parasites actively migrating from the left to the right branchial chamber.

Although differences in activity, size and fecundity of parasites were observed, parasite losses could in no way be associated with such differences.

Tadpoles harbouring three or more egg-producing parasites became anaemic to such an extent that the normally pink to red gills lost all trace of colour. Gill colour returned to normal only when one or no parasites remained.

Reproduction

Onset of egg production

Eggs were often seen in the ootype of 16-day old parasites but were seldom found in the water before 17 to 18 days after infection. First eggs laid were normal in appearance and developed normally. Egg production was slow during the first five or six days after which it became more stable.

Rate of egg production

Daily rates of egg production were easily determined since numbers of egg-producing parasites were precisely known throughout the study. However, difficulties were encountered during the interpretation of the data, owing to the variation in egg production between individual parasites and the continual changes in numbers of egg-producing parasites / host (= parasite intensity) because of parasite mortality *(vide supra)*.

In view of these difficulties, cases of one-, two- and three-parasite burdens were selected for comparison only if the number of parasites remained unchanged before and during the period of egg production taken into account. The period of egg production was selected for each case to fall within the peak 10-day period of production and periods shorter than seven consecutive days were not taken into account. The data were tabulated (Table 1) and where applicable daily averages were used to calculate the mean egg production / parasite /day for the 10-day period.

It is evident from Table 1 that egg production was very similar for parasites in single and double infections. In 3-parasite burdens significantly less eggs / parasite / day were produced. In one case, not forming part of the present series of experimental infections and therefore not shown in Table 1, a single neotenic parasite produced a mean of 25,9 eggs / day at a fairly constant rate (SD = 4,0 = 15,7%) over a period of 15 days.

Development of eggs laid by single parasites, i.e. where self-fertilization occurred, was similar to the development of eggs laid by parasites in worm burdens of 2 and 3. In different batches of eggs the success of development varied from 70% to 98% and was mostly above 80%. Fully developed eggs hatched at 10 to 11 days but hatching success was variable. Unhatched, 40-day old eggs were broken by light pressure and the swimming movements of the escaped oncomiracidia were apparently normal.

Table 1 Rates of egg production of individual neotenic parasites occurring as one-, two- or three-parasite burdens in *Natalobatrachus bonebergi* tadpoles. Means are based on daily production of eggs during seven- to ten-day periods of continuous production

Parasites/ host	Day d	d+1	d+2	d+3	d+4	d+5	d+6	d+7	d+8	d+9
1	14	12	9	12	11	11	17	27	26	–
1	7	9	12	9	8	6	5	7	10	14
1	20	23	26	20	15	15	25	28	26	10
1	16	7	15	7	10	10	19	–	–	–
1	25	30	20	15	31	14	11	13	–	–
1	11	10	8	9	15	4	6	–	–	–
$\bar{x}$e/par/d	15,5	15,2	15,0	12,0	15,0	10,0	13,8	18,8	20,6	12,0
$\bar{x}$ = 14,97 eggs/parasite/day (SD = 3,16 = 21,4%)										
2	11	25	25	29	26	24	12	35	37	50
e/par/d	5,5	12,5	12,5	14,5	13	12	6	17,5	18,5	25
$\bar{x}$ = 13,7 eggs/parasite/day (SD = 5,8 = 42%)										
3	13	12	21	19	15	17	20	23	23	17
3	14	15	11	14	14	17	12	10	18	16
$\bar{x}$e/par/d	4,5	4,5	5,3	5,5	4,8	5,7	5,3	5,5	6,8	5,5
$\bar{x}$ = 5,24 eggs/parasite/day* (SD = 0,67 = 12,8%)										

*Significantly different ($p < 0,05$) from means for single and double infections (Tukey's test, $Q_{0,05}$ = 4,34).

Discussion

The pattern of migration and mortality presently reported for neotenic *P. umthakathi* in *N. bonebergi* tadpoles, is closely similar to what has been reported by Kok & du Preez (1989) for neotenic *P. australis*. The loss of neotenic parasites from the branchial chambers of their tadpole hosts may be influenced by several factors:

- Factors resulting from the experimental conditions such as handling of tadpoles or the effect of MS 222.
- Host-determined factors which may include acquired immunity or host tissue responses such as inflammation or cell proliferation.
- Accidental detachment of parasites which are then washed out of the branchial chambers.

Tinsley & Jackson (1986) took care to prevent contact of monogenean larvae with 0,1% MS 222 because they considered it to be highly toxic to the monogeneans. Since *N. bonebergi* tadpoles had to be anaesthetized for microscopic examination of live parasites, MS 222 was extensively used during the present study. Although possible effects of MS 222 cannot be ruled out, it seems that the low concentration of 0,02% MS 222 used had no harmful effects. Many parasites remained active for long

periods and in those cases where it was possible to compare exposed to non-exposed infections no differences were evident.

In polystome parasites with a neotenic cycle in the tadpole host, the development of bladder-destined parasites, i.e. the completion of the life cycle, is dependent on the establishment of infective larvae in tadpoles which are close to metamorphosis. Should early losses of neotenic parasites be due to acquired immunity, such immunity would then prevent the completion of the life cycle. This would apply particularly under natural conditions. Tadpoles are then continuously exposed to infective oncomiracidia and possible immunity effects would thus be strengthened. Murith (1981b) suggested the possibility that bladder-destined parasites may be antigenically different from neotenic parasites. Only a thorough investigation would indicate whether an immune response against either the oncomiracidium, the parasite developing towards neotenic maturity or the bladder-destined parasite occurs at all.

The most likely explanation for parasite losses from the branchial chambers seems to be that of accidental detachment. In spite of its strong development the opisthaptor apparently does not always give the parasite the necessary power of attachment. Attachment problems would also explain the necessity for migration of branchial polystomes since parasites located in the closed right branchial chamber are less likely to be washed away after accidental detachment than those closer to the spiracle. Combes & Bremond (1988) reported virtually no loss of bladder-destined (i.e. 'slowly growing') *Polystoma integerrimum* during the period of attachment to the gills of *Rana temporaria* tadpoles. The opisthaptor of bladder-destined parasites is not better equipped for attachment than that of neotenic parasites. In fact, sucker development is slower in bladder-destined parasites than in neotenics and functional hamuli, which might assist in attachment, are only present during the later stages of development (unpublished observations, relevant to *P. umthakathi*). Neotenic forms are rare in *P. integerrimum* (Combes 1968; Combes & Bremond 1988) and further studies should be directed towards the population dynamics of bladder-destined larvae of other species such as *P. umthakathi*.

Experimentally infected *N. bonebergi* tadpoles during the present study never harboured more than three mature neotenic parasites. Among investigated naturally infected tadpoles (n = 26), 10 harboured four mature neotenies and one harboured five (Kok, unpublished). Even tadpoles harbouring only three mature neotenics became markedly anaemic and egg production was significantly lower than in single and double burdens. The problems of quantifying the relationship between rates of egg production and numbers of parasites / host were similar to those encountered by Jackson & Tinsley (1988). Amongst these are the small host samples available for larger parasite burdens. Nevertheless, a crowding effect, where there is an inverse relationship between parasite fecundity and number / host (Kennedy 1975), seems evident. Kok & du Preez (1989) reported the absence of a crowding effect for *P. australis*. Up to nine mature neotenic parasites / host were recorded with no apparent harmful effects to either host or parasites (Kok & du Preez 1989). The difference is perhaps mainly due to a marked difference in size of the tadpole hosts. In comparison with *N. bonebergi* (*cf.* Kok & Seaman 1988), *Kassina senegalensis*, and to a lesser extent also *Semnodactylus wealii*, have enormous tadpoles which can understandably support more parasites than the smaller *N. bonebergi*. On the other hand Jackson & Tinsley (1988) reported a crowding effect at the 'surprisingly low burdens' of two to four *Protopolystoma xenopodis*, bladder parasites which are of a size comparable with that of neotenic polystomes but occurring in a relatively gigantic host. A similar effect has been reported by Combes (1972) for mature *Polystoma integerrimum* in adult *Rana temporaria*.

Acknowledgements

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