Polystoma australis (Monogenea): Development and reproduction in neotenic parasites

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Development and reproduction of neotenic *Polystoma australis* were investigated in natural anuran *Kassina* and *Semnodactylus* hosts in South Africa. Newly established parasites attached mainly inside the left branchial chamber. They then migrated so that parasites older than eight days occurred almost exclusively in the right branchial chamber. Mortality was high and continuous. In young worms age could be readily determined from haptoral structure. At 23°C the developing reproductive system was discernible at seven days. Egg production started at 16 days and could be maintained at constant rates of around 10–20 eggs/parasite/day for up to 40 days. No crowding effect influencing parasite development or reproduction could be established. These developmental standards could be used to measure the success of parasites subjected to experimental conditions.

Ontwikkeling en reproduksie van neoteniese *Polystoma australis* is ondersoek in natuurlike *Kassina*- en *Semnodactylus*-gashere in Suid-Afrika. Pas gevestigde parasiete het hoofsaaklik in die linker kieukamer vasgeheg. Daarna het migrasie plaasgevind sodat parasiete ouer as agt dae byna uitsluitlik in die regter kieukamer voorgekom het. Mortaliteit was hoog en deurlopend. In jong wurms kon ouderdom geredelik bepaal word op grond van veranderinge in opisthaptorstruktuur. By 23°C was die voortplantingstelsel reeds onderskeibaar na sewe dae. Eierproduksie het op 16 dae reeds begin en kon volgehou word teen 'n konstante tempo van nagenoeg 10–20 eiers/parasiet/dag vir tot so lank as 40 dae. Daar was geen aanduiding van 'n opeenhopingseffek wat parasietontwikkeling of -reproduksie beïnvloed het nie. Die vasgestelde maatstawe van ontwikkeling kan gebruik word om die sukses te meet van parasiete wat onderwerp is aan eksperimentele toestande.

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The African anuran Polystomatidae has received attention from several researchers, and the mature stages of many central African species have been described (Maeder, Euzet & Combes 1970; Maeder 1973; Murith 1981a). Immature stages have received less attention and although valuable information has been published on the role played by the various gill and bladder stages (Maeder 1973; Murith 1981b; Murith 1982), the present knowledge is hardly comprehensive (*cf.* Tinsley 1983).

In the present study the development, maturation and reproduction of neotenic *Polystoma australis* Kok & Van Wyk, 1986, occurring in tadpoles of South African *Kassina senegalensis* (Dumeril & Bibron, 1841) and *Semnodactylus wealii* (Boulenger, 1882), are described. The objectives were to define normal development, location preferences, mortality rates and reproductive potential. Data on the performance of *P. australis* in its natural tadpole hosts is necessary to establish a normal reference against which parasite success under experimental conditions can be measured.

Materials and Methods

Uninfected *K. senegalensis* tadpoles were either caught around Bloemfontein, where polystome infections do not occur (Kok & Van Wyk 1986), or raised from eggs laid by frogs, during the mating season (October to February).

Naturally infected tadpoles of *K. senegalensis* and *S. wealii* were collected during January and February in a wetland area on the farms Excelsior 62 and Pleasant View 343 in the Ladybrand district of the Orange Free State (29°14'S/27°28'E). Neotenic parasites harboured by these tadpoles served as a source of freshly laid *P. australis* eggs. Tadpoles were kept and eggs harvested as described by Kok & du Preez (1987).

For routine experimental infections, single or grouped tadpoles to be infected were transferred to 10 cm^3 water/tadpole and exposed to from four to eight newly hatched oncomiracidia/tadpole. Tadpoles were transferred to larger containers after 24 h and were kept at 23°C (± 1,5°C).

In the case of experimental infections aimed at the study of morphological development, the hosts used were 40-day-old laboratory-raised *K. senegalensis* tadpoles. From the second day onwards, following experimental infection, tadpoles were necropsied at two-day intervals. The number, location and level of development of all parasites were determined separately for each age group. The oldest parasites of this particular series were 19-day-old, egg-producing neotenics. Data on older neotenics were obtained from parasites recovered from experimentally infected *K. senegalensis* tadpoles not forming part of the above series, as well as from naturally infected *K. senegalensis* and *S. wealii*, tadpoles. All of these older parasites were at least 25 days old and some were more than 60 days old.

Before necropsy, tadpoles were anaesthetized with MS 222 (Sandoz). Live parasites were either fixed and stained in Borax Carmine before permanent mounting or were mounted directly in lactophenol.

Table 1 Range of increase and variation in some measurements of *Polystoma australis* parasites, developing towards neotenic maturity in experimentally infected *Kassina senegalensis* tadpoles

	$\bar{x}$ dimensions at			
	2 days	19 days	$\bar{x}$ C.V.%[a](*S.E.*)	Best fit model
Total length mm	0,33	2,52	9,61 (2,57)	Exponential (r^2 = 0,985)
Haptor length mm	0,09	0,47	13,57 (2,36)	Exponential (r^2 = 0,985)
Greatest width mm	0,18	0,94	14,69 (2,39)	–
Haptor width mm	0,18	1,19	10,35 (2,28)	–
Diameter S1[b] µm	–	186	6,38 (1,79)	Linear (r^2 = 0,968)
Diameter S2 µm	–	185	9,03 (1,52)	Linear (r^2 = 0,974)
Diameter S3 µm	–	180	8,40 (4,21)	Linear (r^2 = 0,916)
Pharynx length µm	43	182	9,81 (1,99)	Linear (r^2 = 0,979)
Pharynx width µm	47	203	7,69 (0,99)	Linear (r^2 = 0,981)

[a] C.V.% = Coefficient of variation expressed as a percentage.

[b] S1 to S3 = Haptoral sucker pairs, numbered from posterior to anterior.

Results

Morphological development

Most of the measurements taken in an attempt to quantify gradual morphological changes, had limited value because of large variation. Total length, haptoral sucker diameter and pharynx dimensions were the most useful measurements, and seldom showed an average coefficient of variation (C.V.) of more than 10% (*cf.* Table 1) for all age groups of parasites (*n* varying from 2–7 within age groups).

Length measurements

Increase in total and opisthaptoral length was exponential during early development up to 20 days (Figure 1). The more rapid increase in total length resulted in a gradual decrease of the haptoral length/total length ratio from 0,27 at two days to 0,19 at maturity (*cf.* Figure 1). A group of fully mature, egg-producing neotenic parasites (n = 11), varying in age from 29 days to 66 days, was recovered from experimentally infected *K. senegalensis* tadpoles. The relevant measurements for the group are given in Table 2. Evidently a marked size increase, associated with an increase in variation, occurred after 20 days. There was no apparent correlation between age and size of this group. The mean body dimensions of another group of egg-producing neotenics (n = 20), recovered from naturally infected *S. wealii,* tadpoles, were even larger and showed much more variation. The mean total length of the group was 5,08 mm and the C.V. 30,3%.

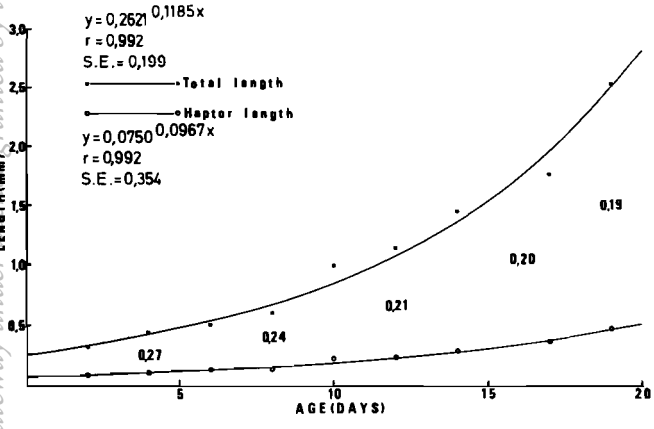

Figure 1 *Polystoma australis* development. Graph showing increase in total and opisthaptoral length, from establishment in the host gills up to neotenic maturity. The values given indicate the decrease, with increasing age, of the haptoral length/total length ratio. Each point represents the mean for the age group, n varied from 2 to 6.

Table 2 Measurements of fully mature, neotenic specimens (n = 11) of *Polystoma australis,* recovered from the gills of experimentally infected *Kassina senegalensis* tadpoles

	Mean	*S.D.*	C.V.%
Total length (T.L.) mm	3,66	0,67	18,3
Haptor length (H.L.) mm	0,70	0,13	18,6
Ratio HL/TL	0,19	–	–
Greatest width mm	1,49	0,35	23,5
Haptor width mm	1,74	0,30	17,0
Diameter S1 µm	285	23,5	9,1
Diameter S2 µm	284	28,5	10,0
Diameter S3 µm	278	27,5	9,9
Pharynx length µm	281	27,4	9,8
Pharynx width µm	311	40,8	13,1
Ovary length µm	734	156,7	21,4
Ovary width µm	206	50,7	24,6

Abbreviations as for Table 1.

Opisthaptoral morphology

Of all features, the early developmental changes in opisthaptoral morphology were the most useful in parasite age determinations. The distinct haptor of the oncomiracidium (Figure 2A) is movable and capable of attachment even to a glass surface. Its 16 larval hooklets can be protruded (Figure 2B) and are used in early

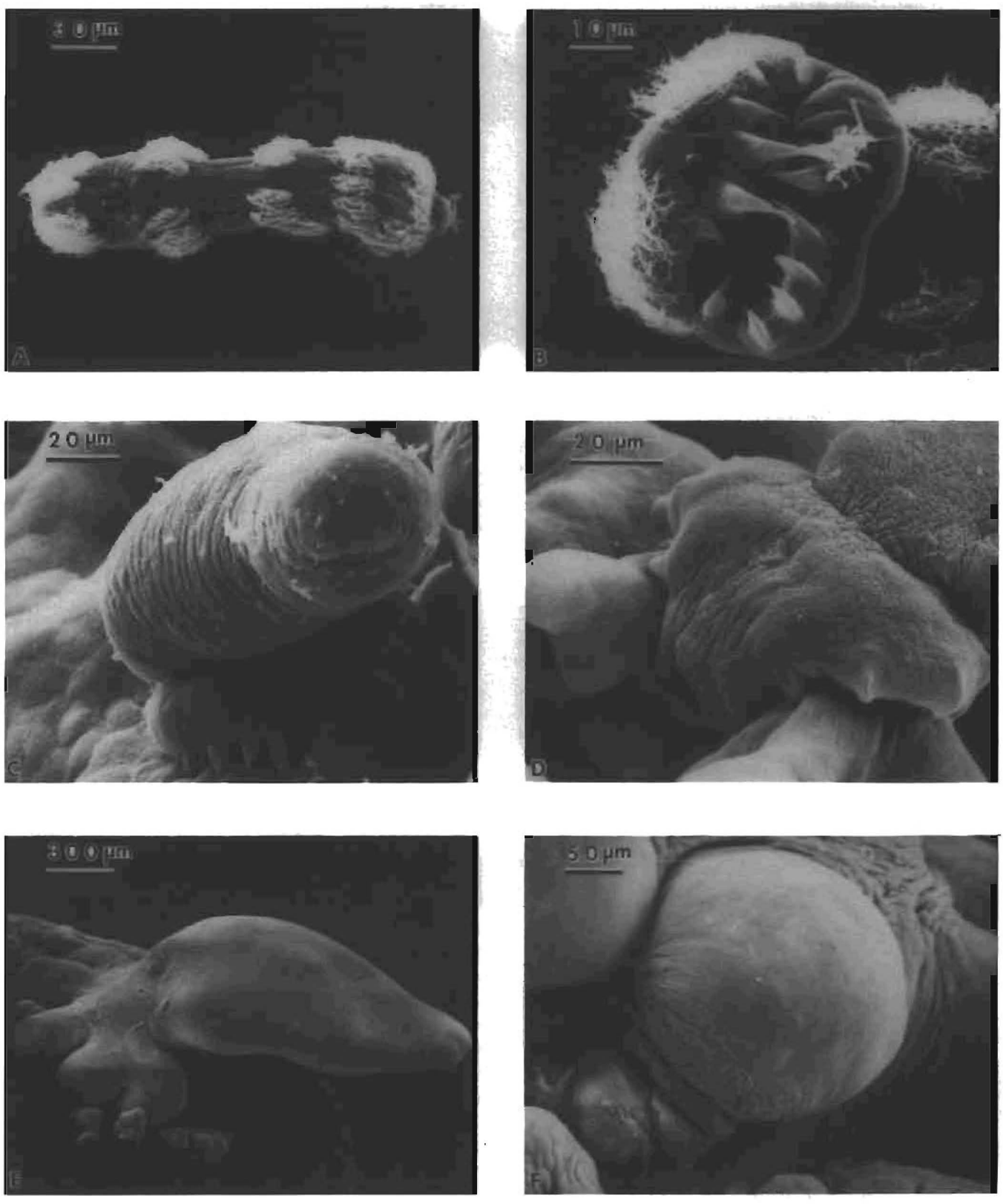

Figure 2 Scanning electron micrographs of *Polystoma australis* oncomiracidium and branchial stages developing towards neotenic maturity. A: Ventral view of a newly hatched oncomiracidium, with head towards the right. B: Oncomiracidium opisthaptor with protruding larval hooklets. C: Two days after establishment on tadpole gill. D: Haptor of a two-day-old parasite, attached to the tadpole gill. E: Mature neotenic parasite attached to tadpole gill. F: Haptoral sucker of a mature neotenic.

attachment to the host. In a newly established parasite the haptor had no suckers, but was distinctly outlined and capable of strong attachment to the gills (Figures 2C & D). The subsequent unequal development of the three sucker pairs (Figure 3), provided a set of parameters which could, owing to limited variation (*cf.* Table 1), be used for fairly accurate age determination. The size difference between sucker pairs disappeared at about 20 days (Figure 2E). Individual suckers were highly movable and of characteristic shape (Figure 2F).

Alimentary canal development

The intestine developed from a simple sac-like structure with two posteriorly joined crura, through a gradual increase in diverticula to a widespread structure filling most of the body (Figure 4A). In live parasites up to 16 days old, the eight to ten medial diverticula stretched to beyond the midline but did not anastomose. At later stages the vitelline glands obscured the intestinal outlines and it became increasingly difficult to distinguish between medially overlapping diverticula and possible anastomoses. In one mature, live parasite, anastomoses were distinctly absent (Figure 4B). Anastomoses probably never occur in neotenic *P. australis*.

Sexual maturation

At six to eight days after establishment, a compact, darkly staining area developed in midbody behind the pharynx. At 10 days oocytes were distinctly visible in this region, now vaguely outlined as ovary and about 100 µm long. Copulatory spines, still very thin and only about 12 µm long, were already present at 10 days. At 14 days all parts of the genital system were clearly outlined, and in many of the parasites egg production had started as early as 16 days after establishment. Ovary length and width were variable (Table 2) but very large numbers of oocytes were always present.

Migration and mortality during development

During establishment, oncomiracidia entered through the left-sided spiracle and attached mostly inside the left

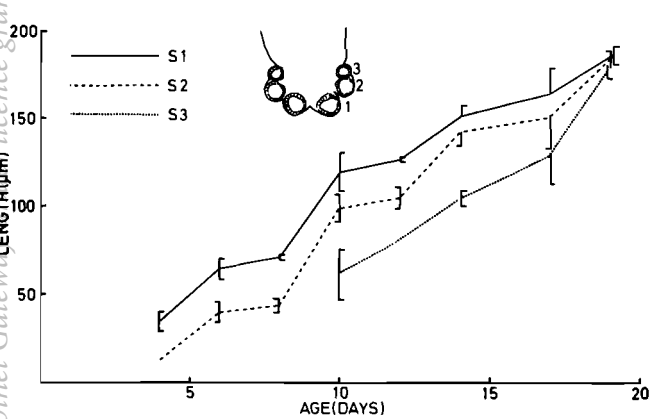

Figure 3 Graph showing the increase in mean diameter of the three haptoral sucker pairs of *Polystoma australis* developing towards neotenic maturity. *S.D.* indicated.

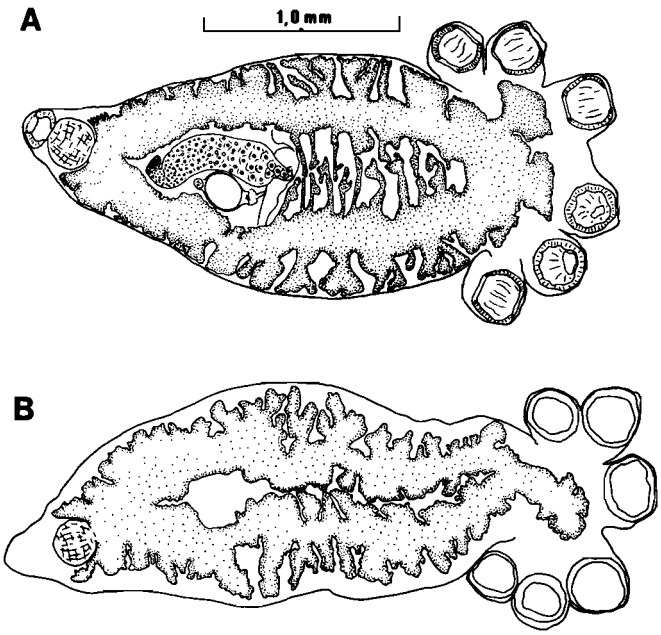

Figure 4 *Polystoma australis*, sexually mature, neotenic parasites. A: Camera lucida drawing of a stained and permanently mounted specimen. B: Drawn from a micrograph of a live specimen with intestinum clearly outlined.

branchial chamber. Migration towards the right branchial chamber then took place over the first few days following establishment. In a group of experimentally infected tadpoles (n = 17), necropsied at different times following exposure to oncomiracidia, only 32,5% of the parasites recovered (n = 43) during the first six days were attached inside the right branchial chamber. During the next 13 days 86,4% of the parasites recovered (n = 22) were located inside the right branchial chamber. Field studies confirmed these observations in that 97,4% of egg-producing neotenics recovered (n = 78) from naturally infected *K. senegalensis* and *S. wealii* tadpoles, were attached inside the right branchial chamber. Early developmental stages usually attached to the gills (Figures 2C & D), but mature neotenic parasites almost invariably attached to the more rigid anterior limb bud protruding into the right branchial chamber.

In the experimentally infected tadpoles mentioned above, a mean parasite intensity (= parasites/infected host) of 5,4 for the first six days decreased to 2,4 for the following 13 days. Parasite mortality was continuous and even mature neotenics, possessing a particularly well developed attachment system (Figure 2E), were often found in the water during egg harvesting. A group of ± 70 naturally infected *S. wealii* tadpoles, used as a source of parasite eggs, initially yielded 2000–3000 eggs/24 h but within 3 weeks the harvest had decreased to less than 200 eggs/24 h. Most of the parasites lost during this time were not found and were presumably eaten by the tadpoles.

Egg production

In experimental infections egg production usually started at a parasite age of 16 to 17 days and then gradually

increased to reach its full potential in parasites 20 to 25 days old. The maximum period over which eggs were harvested from a single infected tadpole before it reached metamorphosis was 39 days, day one being 25 days after experimental infection.

Eggs are laid by neotenic parasites at the same rate at which they are produced since there is no uterus in which eggs can be accumulated. A major problem during the study on egg-production rates, was to determine the numbers of egg-producing neotenics with reasonable accuracy. Detached parasites were seldom found, with the result that times of detachment were often unknown and estimates of total parasite numbers were then unreliable.

Figures 5A & B represent the daily cumulative totals of eggs harvested from two experimentally infected tadpoles. One of these yielded relatively low numbers of eggs (mean = 34,7/day; *S.D.* = 16,2) over an extended period (Figure 5A) and the other very high numbers of eggs (mean = 357,3/day; *S.D.* = 127,1) over a short period (Figure 5B). Three parasites were eventually recovered from the first tadpole and this gave a rate of production of 11,6 eggs/parasite/day. Only five parasites were recovered from the other tadpole during metamorphosis — two from the surrounding water, two disintegrating in the gills and one in the host's stomach. Other parasites must have been lost without trace, since a rate of production of 70 eggs/parasite/day was considered highly unlikely. The largest number of egg-producing neotenics recovered from one tadpole host, necropsied before the onset of metamorphosis, was nine. They had produced 21,93 eggs/parasite/day (*S.D.* = 6,4) for three days.

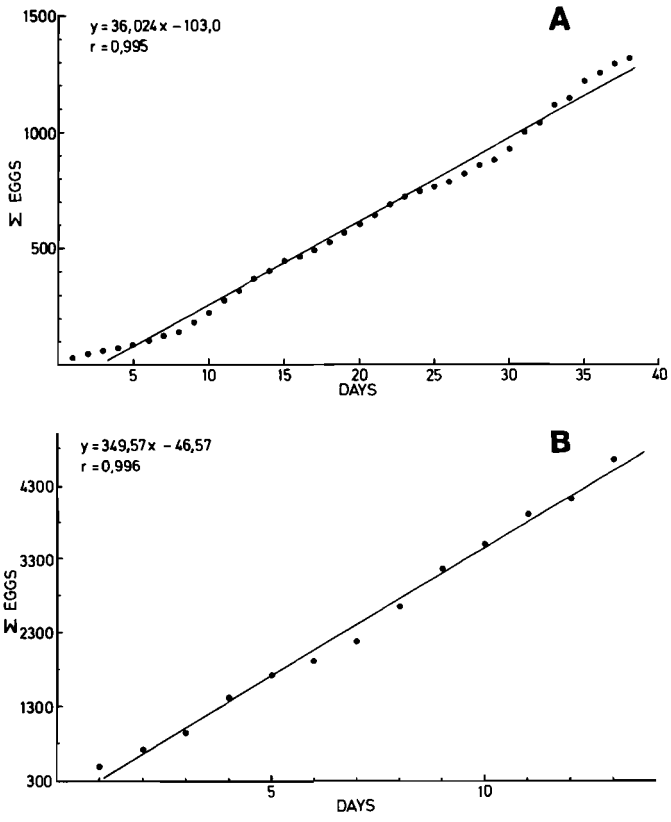

Figure 5 Cumulative daily egg production of neotenic *Polystoma australis*. A: Tadpole host harboured three parasites, releasing eggs over an extended period. B: Tadpole host harboured an unknown number of parasites, releasing large numbers of eggs over a shorter period.

Eggs produced by neotenic parasites were more pyriform in shape than those of the adult parasites (*cf.* Kok & Van Wyk 1986), and were consequently shorter (215,4 μm; *S.D.* = 12,4; *n* = 50) but somewhat wider (187,4 μm; *S.D.* = 13,97; *n* = 50) than those of the adults. Freshly laid eggs were golden brown and were ready to hatch within 10 to 11 days at 23°C (± 1,5°C). Darker coloured eggs, which did not develop, were often found. Egg darkening resulted from the passage of eggs through the host's intestine after ingestion. When tadpoles were prevented from feeding off the bottom of the container in which they were kept, darkened eggs practically disappeared from daily egg harvests, indicating only occasional swallowing of eggs directly from the branchial chambers. Oncomiracidia developed normally in a high percentage of the golden brown eggs. Out of a total of 2085 eggs harvested over an extended period, oncomiracidia developed in 1719 (83,5%) of the eggs. The eggs were laid in 24 batches of different sizes and the mean success of development per batch was 78,6% (*S.D.* = 17,4).

Discussion

Several authors have contributed to our knowledge of polystome development and the relationship between mode of transmission and host ecology (*cf.* Combes, Bourgat & Salami-Cadoux 1976; Murith 1981b, c), but there is still a lack of information on the details of embryonic and larval development of most species of African polystomes.

Regarding mortality of neotenic parasites, it is necessary to distinguish between a loss of parasites owing to host mortality as opposed to parasite losses from the branchial chambers of live tadpoles. Tinsley (1983) referred to heavy parasite mortality owing to enormous mortality of tadpoles, but did not indicate whether or not the parasites contributed in any way to host mortality. Savage (1950) indicated that *P. integerrimum* did not cause any damage to tadpole hosts. Heavily infected *K. senegalensis* tadpoles suffered no apparent ill effects under laboratory conditions.

Major losses of parasites from the branchial chambers of live tadpoles have been reported by several researchers (Savage 1950; Tinsley 1983) but a host immune response to the presence of polystomes has not been demonstrated (see Tinsley 1983). The loss of neotenic polystomes owing to an immune response is unlikely in that such a response probably would prevent the establishment of infective stages destined to become bladder parasites. Murith (1981b) mentioned the possibility of antigenic differences between neotenic and bladder-destined branchial parasites. Should such differences exist, then an immunological response to neotenic parasites would have little or no effect on bladder-

destined parasites. However, the mechanism underlying the loss of branchial parasites seems to be a mechanical one — the parasites become detached despite a well developed attachment system and are then washed out of the branchial chamber or swallowed by the tadpole.

The fecundity of neotenic *P. australis* is relevant because these parasites are responsible for the introduction of the bulk of the recruitable material into the host population. The reproductive life span of the neotenic parasite begins at an age of 16 to 20 days and ends with its death at tadpole metamorphosis. Under natural conditions, where metamorphosis can be expected to take place at a tadpole age of 60–70 days (Balinsky 1969 and personal observations), parasite reproductive life-span can hardly exceed 40 to 50 days. Rates of egg production during this period can vary around 10 to 20 eggs/parasite/24 h according to published estimates (Maeder 1973; Tinsley 1983; Tinsley & Owen 1975). Similar production rates were observed during the present study but in one case, where the actual number of parasites present was not known, a high estimate of 10 parasites still meant an above average output of 35 eggs/parasite/24 h. Combes (1972) reported an inverse relationship between parasite population density and egg production for adult *P. integerrimum*. Savage (1950), on the other hand concluded that *P. integerrimum* neotenics '...do not seem to have any injurious effect upon each other when several occur in the same gill chamber'. Our results would also indicate towards the absence of such a 'crowding effect', but more comprehensive studies are necessary in this regard.

Acknowledgements

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