ELSEVIER

International Journal for Parasitology 31 (2001) 815–821

The Australian Society for Parasitology Inc.
INTERNATIONAL Journal for PARASITOLOGY
www.parasitology-online.com

Research note

Differentiation of two locally sympatric *Protopolystoma* (Monogenea: Polystomatidae) species by temperature-dependent larval development and survival

J.A. Jackson[a], R.C. Tinsley[a,*], L.H. Du Preez[b]

[a]*School of Biological Sciences, University of Bristol, Bristol BS8 1UG, UK*
[b]*School of Environmental Science and Development, Potchefstroom University, Private Bag X6001, Potchefstroom 2520, South Africa*

Received 2 January 2001; received in revised form 27 February 2001; accepted 27 February 2001

Abstract

The developmental response of egg stages to different environmental temperature regimes was studied in *Protopolystoma xenopodis* and *Protopolystoma orientalis* (Monogenea: Polystomatidae) isolates from southern Africa. Eggs failed to develop at 10°C, whilst at 15°C only *P. xenopodis* completed larval development, hatching 49–88 days post-collection. Respective hatching windows were 26–34 (*P. xenopodis*) and 37–49 (*P. orientalis*) days at 20°C, and 18–26 and 27–37 days at 25°C. Continuous maintenance at 30°C was lethal for eggs of both species. There were no consistent interspecific differences in the response of egg stages to low and high temperature shocks during early embryonic development. © 2001 Australian Society for Parasitology Inc. Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Protopolystoma*; Monogenea; Larval development; Hatching; Temperature; *Xenopus*

The polystomatid monogeneans *Protopolystoma xenopodis* and *Protopolystoma orientalis* are specific to pipid anurans, respectively *Xenopus laevis* and *Xenopus muelleri* (eastern form) (see Tinsley and Jackson, 1998), which occur naturally in sub-Saharan Africa. Parasite transmission is effected by swimming oncomiracidia that hatch from freely deposited eggs (Tinsley and Owen, 1975; Tinsley and Jackson, 1998). The two hosts have largely allopatric geographical distributions, although peripheral overlaps have been documented in a number of areas (Kobel et al., 1996; Tinsley et al., 1996). Whilst the eastern form of *X. muelleri* occurs in lowland and coastal zones of east and southern Africa (Tanzania south to eastern South Africa), *X. laevis* is found at variable altitudes through a very wide range that extends to the Cape in the south, Nigeria in the north-west, and Sudan and Kenya in the north-east (Kobel et al., 1996; Tinsley et al., 1996). *Xenopus muelleri* is always distributed in biotypes characterised by relatively elevated environmental temperatures, whilst the range of *X. laevis* includes much cooler climatic zones (Poynton, 1964; Tinsley et al., 1996). In many areas where *X. laevis* and *X. muelleri* co-occur (e.g. eastern South Africa) populations of the former species are centred at higher elevations (Passmore and Carruthers, 1995). However, this segregation does not result from a simple altitudinal or thermal constraint on *X. laevis* which, in some areas, can occupy lowlands with temperature regimes as hot as those in typical *X. muelleri* habitats (Tinsley et al., 1996). The relationships of the two species in sympatric zones have not been studied in detail, but published field observations suggest there is a high degree of separation at the scale of individual habitats (e.g. Poynton and Broadley, 1985; Lambiris, 1988), with both species rarely sharing the same water bodies. However, in at least some areas of south-eastern Africa, local sympatry and hybridisation may occur (Fischer et al., 2000). The reproductive biology and embryonic development of *P. xenopodis* were described by Tinsley and Owen (1975), but data are lacking for *P. orientalis*. Given the variation of thermal environment occurring across the distributions of *X. laevis* and *X. muelleri*, populations of their respective parasite species could, perhaps, have been selected to perform optimally at distinct environmental temperatures and tolerate different temperature ranges. Apart from adaptation to prevailing thermal conditions, it is also possible that embryonic developmental and larval hatching characteristics may, for many other reasons, have diverged between species or between populations of the same species. The present study aimed to assess intra- and inter-

* Corresponding author. Tel.: +44-117-928-8660; fax: +44-117-925-7374.

E-mail address: r.c.tinsley@bristol.ac.uk (R.C. Tinsley).

0020-7519/01/$20.00 © 2001 Australian Society for Parasitology Inc. Published by Elsevier Science Ltd. All rights reserved.
PII: S0020-7519(01)00185-0

specific variability in the egg development and hatching of *P. xenopodis* and *P. orientalis* under different thermal regimes. Significant interspecific variation in thermal tolerance and/or the timing of embryogenesis and larval emergence might provide a tool by which parasite eggs from in vivo studies on hybrid hosts could be distinguished. Data on temperature-dependence in egg stages would also identify possible seasonal constraints on the transmission of *Protopolystoma* species in nature.

Protopolystoma spp. eggs were collected from pooled groups of their respective host species (maintained at 23°C in plastic aquaria) by passing the water in which these had been maintained through a 55 μm nylon filter (Nitex). The parental parasites were laboratory-maintained isolates (isolated breeding groups) derived from field-collected groups of *Xenopus laevis laevis*, *Xenopus laevis petersii* and *X. muelleri* taken at different localities in southern Africa (see Table 1).

Eggs for use in the experiments were transferred to petri dishes (50 mm, Sterilin) containing 10 ml of dechlorinated tap water. Egg batches were incubated at constant temperatures in cooled incubators (Gallenkamp) (10, 15, 20, and 30°C) or a controlled environment room (25°C) with a 12:12 L/D photoperiod. Water temperature was maintained to within ±0.5°C at 20–30°C and ±1.0°C at 10–15°C. In order to distribute possible positional effects evenly within incubators or controlled environment rooms, dishes from each individual assay or experimental temperature treatment were placed in marked positions in stacked arrays of 4 × 6 dishes (25 × 35 cm). Dish positions were randomised daily using random numbers generated by Microsoft Excel 97 or a Sharp EL-531GH scientific calculator. The viability of egg batches was assessed as the proportion developing fully-formed larvae (% embryonation), irrespective of whether these hatched.

Five incubation temperatures were selected, 10, 15, 20, 25, and 30°C, which might represent much of the combined temperature ranges experienced by the host species (see Tinsley et al., 1996). At each temperature, a different incubation facility was used to maintain egg batches (replicates) from isolates Px1, Px2, Px3, and Po2 (see Table 1; three replicates, of approximately equal sample size, per isolate). Material of Po1, Px4, and Px5 was also included at 20°C. Eggs for each set of replicates were collected over the same 24 h period. After 10 days at 30°C, and 16 days in other conditions, each batch was removed daily, in random order, from its controlled temperature facility and examined under a stereo microscope (briefly exposing the dish to ambient air temperature). Hatched eggs were recorded, removed with a Pasteur pipette, and water was added to compensate for evaporation. After hatching had finished, or all eggs were ascertained to be dead, unhatched capsules were squashed in a small drop of water under a coverslip and examined with a high power microscope to determine the presence of fully-formed larval hooklets (evidence of embryonation without hatching). Most *P. xenopodis* and *P. orientalis* larvae will emerge spontaneously in total darkness (unpublished data), but hatching may also be precipitated by mechanical stimuli, increased light levels or increasing environmental temperature (Tinsley and Owen, 1975). It is assumed that randomisation procedures (see above) were sufficient to distribute variations in such stimuli evenly between replicates at each temperature. However, it is also possible that the effects of variability in non-thermal environmental stimuli between maintenance facilities may have been confounded with temperature-dependent effects. To test this possibility a comparison of median hatch times in three isolates (Px1, Px3, and Po2) was carried out in the maintenance facilities used for 15 and 25°C in the experiments (respectively an incubator and controlled environment room). Incubation was at 25°C, with three replicates/isolate per facility (18–268 eggs/replicate). There was no

Table 1
Details of *P. xenopodis* and *P. orientalis* isolates used in the experiments

Isolate	Species	Origin	Elevation (m)	Distribution (host)[a]	Date of isolation	No. of infected hosts[b]
Px1	*P. xenopodis*	Cape, RSA[c]	< 100	Allopatric (*X. l. laevis*)	Oct. 1997	13
Px2	*P. xenopodis*	Cape, RSA[c]	< 100	Allopatric (*X. l. laevis*)	Feb. 2000	3
Px3	*P. xenopodis*	Piggs Peak area, Swaziland; 26° 03′ 29″S, 31° 26′ 30″E	490	Sympatric (*X. l. laevis*)	Dec. 1999	5
Px4[d]	*P. xenopodis*	Nkambeni area, Swaziland; 26° 03′ 05″S, 31° 39′ 35″E	360	Sympatric (*X. l. laevis*)	Dec. 1999	1
Px5	*P. xenopodis*	Okavango delta, Botswana; 18° 58′ 25″S, 22° 34′ 26″E	980	Sympatric (*X. l. petersii*)	Dec. 1999	2
Po1	*P. orientalis*	Malelane area, RSA; 25° 30′ 44″S, 31° 26′ 27″E	430	Sympatric (*X. muelleri*)	Dec. 1999	2
Po2[d]	*P. orientalis*	Nkambeni area, Swaziland; 26° 03′ 05″S, 31° 39′ 35″E	360	Sympatric (*X. muelleri*)	Dec. 1999	21

[a] *Xenopus muelleri* and *X. laevis* allopatric or regionally sympatric in area of isolate origin.
[b] Number of infected hosts during period of egg collection.
[c] Parasites isolated from *X. l. laevis* in commercial shipments collected from Cape flats area, South Africa; exact locality unknown.
[d] Derived from individuals occurring in same pond.

significant effect ($P > 0.05$) of constant temperature facility on median hatch time (ANOVA). Accordingly, the effects of different incubators or rooms are assumed to be negligible in the following analysis. In low temperature groups, where embryonic development was arrested but egg contents had not visibly deteriorated by 120 days, dishes were finally raised to 25°C to confirm embryo death.

To study the sensitivity of early stage embryos to high temperature shocks, parasite eggs from 72 h collections (four isolates: Px1, Px2, Px3, and Po2) were transferred to glass test tubes containing dechlorinated tap water at 25°C (final volume 3.5 ml), and then placed in a water bath at a set temperature for 1 h. After cooling, eggs were transferred to fresh dechlorinated water and incubated at 25°C in replicates of approximately 50 to assess their viability. Three separate assays were performed to identify the upper lethal temperature limit for 1 h exposure and compare the relative responses of different isolates as this limit was approached. The first assay covered exposures at 25–50°C (5°C intervals: three replicates/isolate per temperature), the second assay exposures at 35–40°C (1°C intervals: one replicate/isolate per temperature), and the third assay exposure at 36°C (five replicates/isolate).

To assess the effect of a cold shock on embryonic development, parasite eggs from a 72 h collection (four isolates: Px1, Px2, Px3, and Po2) were placed in a refrigerator at 5°C for 18 h, and then incubated alongside untreated controls at 25°C. There were five replicates/isolate per group, each of approximately 50 eggs.

Proportion data (for viability or hatching failure) were analysed by generalised linear models (GLM) specifying binomial errors (GLIM 4.0). The significance of terms (on the basis of χ^2 values) was assessed by successive deletion. In cases of overdispersion, an adjusted scale parameter was used (with significance assessed by *F*-tests) (Crawley, 1993). Because hatching time increased and became more variable in colder conditions (producing heteroscedastic data), analysis of larval emergence timings over different temperatures was by a GLM using gamma errors (GLIM 4.0). All significant differences reported were at $P < 0.01$, unless otherwise indicated.

Eggs of *P. xenopodis* (Px1, Cape; Px3, Swaziland) and *P. orientalis* (Po2, Swaziland) ($n = 150$ eggs per isolate) died without evidence of embryonation when maintained at 10°C. At 15°C all *P. orientalis* eggs (Po2, $n = 151$) died without hatching, although some showed initial signs of development. This was limited to a reduction in the volume occupied by vitelline cells and the formation of an embryonic mass, changes that are characteristic of early postzygotic development at higher temperatures (Tinsley and Owen, 1975). Embryos did not survive to the stage of larval hooklet formation. *Protopolystoma xenopodis* eggs were capable of full development at 15°C: the overall hatching window was 49–79 days post-collection ($n = 150$) for Px1 and 56–88 days ($n = 91$) for Px3 (see Fig. 1), and per batch median hatching (MH) times were 61–64 and 62–65 days, respectively. The frequency distribution of larval emergence time in these samples was skewed to the right, with a small number of larvae emerging after 70 days post-collection (Fig. 1). Hatching oncomiracidia observed later than 70 days were slow-swimming, morphologically abnormal and probably of compromised infectivity. Eggs of *P. orientalis* isolate Po2 developed at 20°C and hatched 37–49 days post-collection ($n = 67$) (MH 40–44 days). This hatching window did not overlap with that of Px1 (26–33 days, $n = 141$; MH 28–30 days) nor Px3 (29–34 days, $n = 45$; MH 29–31 days) at the same temperature (Fig. 2). There was also a non-overlapping hatching window at 25°C for Po2 (27–37 days, $n = 45$; MH 31–33 days) compared with Px1 (18–26 days, $n = 169$; MH 20–21 days) and Px3 (19–25 days, $n = 61$; MH 21–22 days) (Fig. 2). Variations in MH times for Po2, Px1, and Px3 at 20–25°C were analysed by a GLM (gamma errors) with temperature and isolate as factors. The effects of temperature ($F_{1,14} = 349.9$) and isolate ($F_{2,14} = 235.7$) were highly significant, but there was no significant interaction ($F_{2,12} = 1.99$). Factor levels for the conspecific isolates could be combined without a significant reduction in deviance from the model ($F_{1,14} = 1.99$). A second analysis excluding Po2, and including data for Px1 and Px3 at 15°C, also found a highly significant effect of temperature ($F_{2,14} = 2176.3$) but no significant effect of isolate ($F_{1,14} = 3.86$) or interaction ($F_{2,12} = 0.41$). This suggests that there is only limited variation in the temperature-dependent embryonic developmental schedule of the two allopatric *P. xenopodis* isolates. To further assess geographical variability in time to hatching, samples of eggs from other isolates were also assayed at 20°C. *Protopolystoma xenopodis* isolates Px4 (Swaziland; $n = 23$) and Px5 (Okavango, Botswana; $n = 37$) and *P. orientalis* isolate Po1 (South Africa; $n = 20$) (see Table 1) showed hatching windows falling within the overall ranges observed above: 26–34 days for *P. xenopodis* and 37–49 days for *P. orientalis* (Fig. 2).

Egg hatching time in *P. xenopodis* showed a strong non-

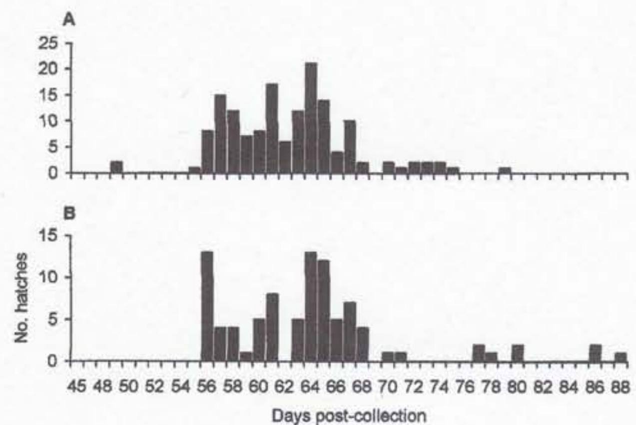

Fig. 1. Frequency distribution of hatching time in *P. xenopodis* isolates at 15°C. (A) Px1 (Cape, South Africa; $n = 150$). (B) Px3 (Piggs Peak, Swaziland; $n = 91$). Pooled data for three replicates per isolate.

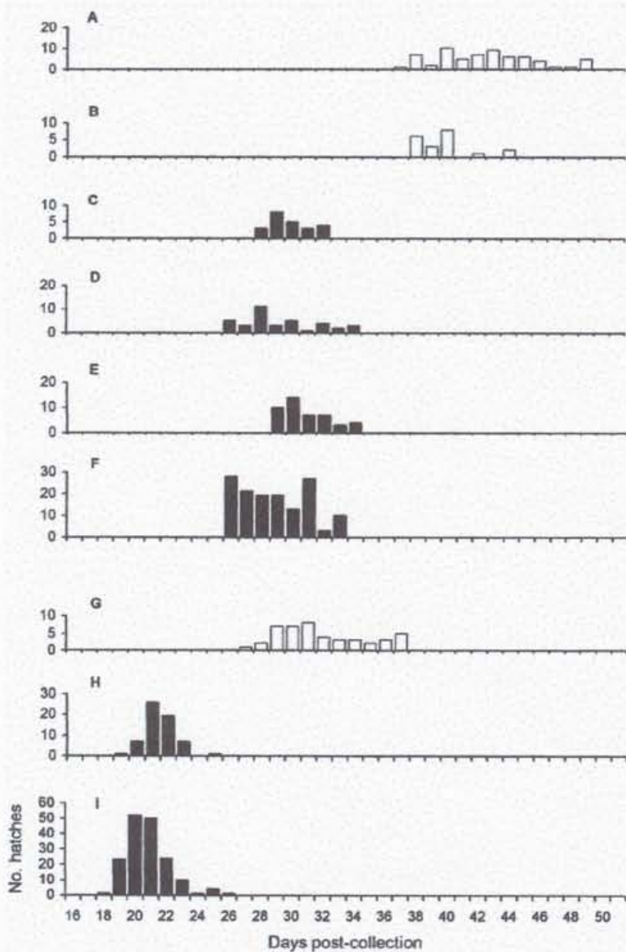

Fig. 2. Frequency distribution of hatching time in *P. orientalis* (light bars) and *P. xenopodis* (dark bars) isolates at 20 (A–F) and 25°C (G–I). (A) Po2 (Nkambeni, Swaziland; $n = 67$). (B) Po1 (Malelane, South Africa; $n = 20$). (C) Px4 (Nkambeni, Swaziland; $n = 23$). (D) Px5 (Okavango delta, Botswana; $n = 37$). (E) Px3 (Piggs Peak, Swaziland; $n = 45$). (F) Px1 (Cape, South Africa; $n = 141$). (G) Po2 ($n = 45$). (H) Px3 ($n = 61$). (I) Px1 ($n = 169$). Pooled data for three replicates per isolate.

linear relationship with environmental temperature: development being disproportionately retarded below 20°C (Fig. 3A). This effect was not observed in *P. orientalis*, but might occur at unstudied temperatures between 20 and 15°C (see Fig. 3A).

Over the ranges 15–25°C for *P. xenopodis*, and 20–25°C for *P. orientalis*, egg viability (percentage embryonation) was relatively high (>80–90%) (see Fig. 3B). However, maintenance at 30°C was lethal for the eggs of *P. xenopodis* isolates Px1 ($n = 196$) and Px3 ($n = 103$), and *P. orientalis* isolate Po2 ($n = 150$). At all temperatures at which hatching occurred, a small proportion of fully formed *P. xenopodis* larvae (Fig. 3C) failed to emerge (<3% overall). In these cases oncomiracidia often showed morphological signs of compromised viability (shrinkage or malformation) before dying. There was sometimes evidence of intermittent motility or an unsuccessful attempt to hatch before death. Larval non-emergence may be related to faults in the egg hatching mechanism, or to the depletion of oncomiracidial energy resources near the end of the normal hatching window. It is also possible that some eggs remain unhatched due to the strength or timing of environmental stimuli received. All unhatched *P. xenopodis* eggs at 20 and 25°C had died or were clearly incapable of emergence by the start of the *P. orientalis* hatching window. Hatching failure was very significantly more common in *P. orientalis* than *P. xenopodis* ($\chi^2_2 = 160.70$) (Fig. 3C), sometimes exceeding 50% frequency in egg batches of the former species. It was

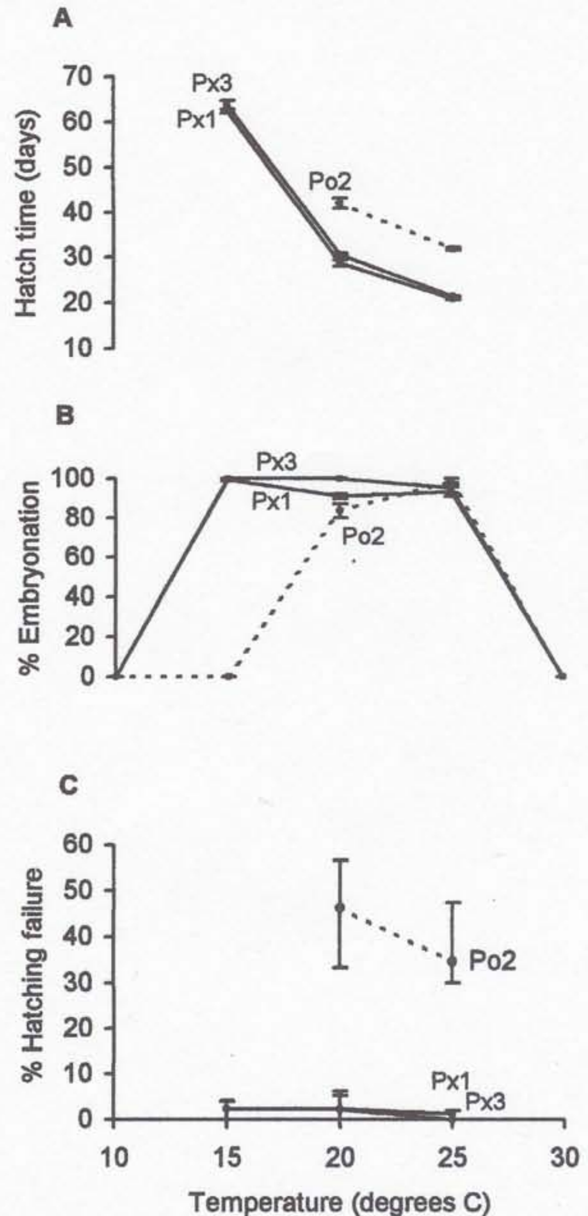

Fig. 3. Relationships of egg hatching time, viability and hatching failure with environmental temperature in isolates of *P. orientalis* (Po2) and *P. xenopodis* (Px1, Px3). (A) Average MH time. (B) Mean viability (percentage embryonation). (C) Mean hatching failure (as a percentage of total embryonated). Bars indicate one standard error above and below the mean (A,B) or range around the mean (C) for three replicates.

also marginally temperature-related ($\chi^2_1 = 4.33$, $P \sim 0.05$), with more larvae of both species remaining unhatched at 20 than at 25°C (Fig. 3C).

In an initial assay to determine tolerance to a short exposure at elevated temperature, *P. orientalis* isolate Po2 and *P. xenopodis* isolates Px1–3 were exposed to 25, 30, 35, 40, 45, and 50°C for a period of 1 h and subsequently incubated at 25°C. Embryonation only occurred in the 25–35°C exposure groups. Over this temperature range, an analysis of viabilities by a GLM (binomial errors), with isolate and temperature as factors, showed that when non-significant terms for interaction ($F_{6,24} = 0.76$) and isolate ($F_{3,30} = 1.02$) were removed, temperature was only marginally significant ($F_{2,30} = 3.51$, $P \sim 0.05$). Further 1 h incubations of eggs at 1°C intervals between 35 and 40°C identified a marked decline in viability between 35 and 36°C, whilst temperatures of 37°C or above were lethal (Fig. 4). In a final assay of egg survival at 36°C, Po2 showed relatively low percentage embryonation (Fig. 4), but the effect of isolate was not statistically significant ($F_{3,16} = 2.57$). Overall, these results suggest that viability is not severely depressed by high temperature shocks unless these approach within 1°C of the upper lethal limit (36–37°C), which is the same for both species. At near-lethal temperatures (36°C) *P. xenopodis* eggs do not have a consistently different level of tolerance to those of *P. orientalis*.

Eggs of *P. orientalis* isolate Po1 and *P. xenopodis* isolates Px1–3 were exposed to temperatures of 5°C for 18 h and then incubated, alongside untreated controls, at 25°C. The effects of cold shock on viability were of limited magnitude. All treatment groups showed relatively high viability (above 75%) (see Table 2), and the maximum difference between cold shock and control group means for any isolate was 18.7%. Significant differences were found for Px1 and Px2, but not Px3 nor Po2 (table-wide significance level = 0.05; Rice, 1989). This suggests that *P. xenopodis* isolates were variable in their egg viability response to cold

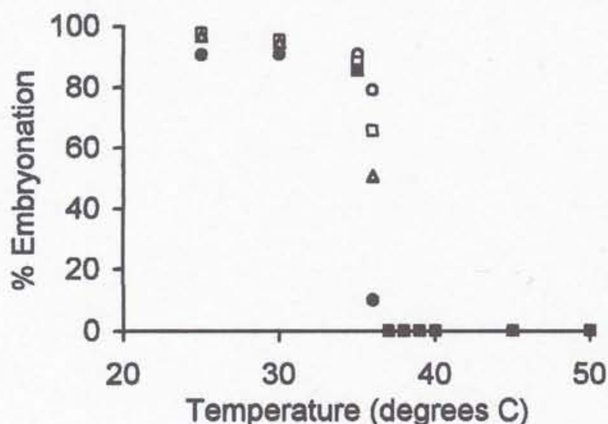

Fig. 4. Effect of high temperature shocks on percentage embryonation in the eggs of *P. xenopodis* (Px1, □; Px2, △; Px3, ○) and *P. orientalis* (Po2, ●) isolates. The exposure temperature was maintained for 1 h, with eggs subsequently incubated at 25°C.

Table 2
Response of egg viability to cold shock in *P. xenopodis* and *P. orientalis* isolates[a]

	Px1	Px2	Px3	Po2
Cold-shocked	79.8 (6.1)[b]	91.4 (3.0)[b]	99.4 (0.3)	80.8 (2.1)
Control	98.5 (0.7)	99.6 (0.4)	96.0 (2.2)	75.7 (3.4)

[a] Mean percentage embryonation (for five replicates of approximately 50 eggs), with standard error in parentheses. Cold-shocked groups were exposed to 5°C for 18 h, and then incubated at 25°C alongside untreated controls.

[b] Cold-shocked groups significantly different from control (table-wide significance level = 0.05).

shock, but did not differ consistently from the *P. orientalis* isolate studied.

There were clear differences in the embryonic developmental pattern of *P. orientalis* and *P. xenopodis* in relation to the environmental temperature regime. Timing of larval emergence was later in *P. orientalis*, with the two species showing non-overlapping hatching windows at 20 and 25°C. Embryonic growth was arrested by cold at higher constant maintenance temperatures in *P. orientalis* (15°C) than in *P. xenopodis* (10°C). Eggs of both species survived short exposures to very low temperatures (5°C), and subsequent incubation at 25°C, without a large effect on viability. It is therefore likely that stages whose development is inhibited by cold (at least for thermal conditions falling to 5°C) may, for some time, retain a capacity to embryonate normally if elevated to a higher temperature. However, arrested/retarded embryos (at 10°C for *P. xenopodis* and 10–15°C for *P. orientalis*) did not survive to 120 days post-collection, suggesting that this period of tolerance is less than 4 months. The greater capacity of *P. xenopodis* to develop at low temperatures may be related to the wide range of environmental conditions in which its host, *X. laevis*, can occur. This species has a greater altitudinal range than *X. muelleri*, the host of *P. orientalis*. In areas where their distributions converge, *laevis* populations are often displaced towards higher elevations (see Tinsley et al., 1996), as is the case in eastern South Africa and Swaziland (Passmore and Carruthers, 1995). Here, and within other parts of its normal range, *X. laevis* may experience colder climatic conditions than would ever be encountered by *X. muelleri*, whose lowland habitats are characterised by relatively elevated environmental temperature (Passmore and Carruthers, 1995; Tinsley et al., 1996). Despite its upland distribution in some areas, *X. laevis* is not limited to cooler climatic conditions across all of its range: in other regions (e.g. Namibia) this species survives lowland temperature regimes at least as hot as those in typical *X. muelleri* habitats (Tinsley et al., 1996). In contrast to the superior developmental capacity of *P. xenopodis* at lower temperatures, there was no evidence that *P. orientalis* eggs better tolerated elevated thermal conditions. Both species showed high viability at 25°C, but did not survive when

maintained at 30°C. It is possible that further studies of intermediate temperatures may have revealed differing performance. However, in the case of short exposures to extreme high temperature, survival of *P. xenopodis* was at least as great as for *P. orientalis*.

The developmental differences observed in this study support the biological distinctness of *P. xenopodis* and *P. orientalis*, which have previously been separated by adult morphological criteria (Tinsley and Jackson, 1998). *Protopolystoma xenopodis* isolates from widespread localities in southern Africa (five isolates from South Africa, Swaziland and Botswana) showed similar developmental schedules at 20°C, differing from *P. orientalis* isolates from one site in South Africa (Po1) and another in Swaziland (Po2) (Fig. 2). The *P. xenopodis* isolates included those derived from localities in the southerly distribution of this species where *X. muelleri* is absent (Cape, South Africa: Px1 and Px2), and others from where the distributions of *X. muelleri* and *X. laevis* are adjacent or sympatric (Botswana: Px5; Swaziland: Px3 and Px4) (see Table 1). Isolates Px4 and Po2 originated from hosts in the same pond (at Nkambeni, Swaziland) and showed characteristic interspecific differences in the timing of larval emergence (Fig. 2). There was also variation in the proportion of embryonated larvae which failed to hatch under experimental conditions. This was greater in *P. orientalis* than *P. xenopodis* and might reflect biological differences, either in hatching responses to environmental stimuli, or in the general physiology of egg stages.

It is possible that some of the characteristics documented here may vary in eggs produced by worms of different ages. However, routine observations made on hatching patterns during other studies in our laboratory suggest that this is unlikely to be a large effect. Eggs were periodically collected from the Px1, Px2, and Po2 infections shown in Table 1 over a 1 year period (for use in experimental cross-infections). Hatching windows at 25°C remained within the ranges observed above (samples of 5–200 eggs/isolate per 48 h collection) and hatching failure was consistently higher in *P. orientalis* than in *P. xenopodis* eggs. Most infections survived for less than 1 year. It is also possible that larval hatching and development may be affected by water chemistry. This might be significant given the very broad range of habitat conditions that *Xenopus* spp. are known to tolerate (Tinsley et al., 1996), although there is no reported difference in water quality preferences or requirements between *X. laevis* and *X. muelleri*.

Suspected hybrids between *X. muelleri* and *X. laevis* (see Poynton and Broadley, 1985; Fischer et al., 2000) are sometimes encountered in field collections from sympatric areas. Although these may be identified by a variety of biochemical means (Fischer et al., 2000), their susceptibility to parasites normally specific to either parental host might also provide information on possible hybrid constitution. In this context, the non-overlapping larval hatching windows of *P. xenopodis* and *P. orientalis* can be utilised as the basis of a bioassay to monitor infection identity in long-term laboratory studies on suspected *X. muelleri* × *X. laevis* hybrids (Tinsley et al., unpublished data).

Unlike the majority of anuran polystomatids, which have reproductive strategies targeted on the aquatic reproductive phases of terrestrial or semi-terrestrial hosts (Tinsley, 1995), *Protopolystoma* species infect a fully aquatic host and reproduce continuously (Tinsley and Owen, 1975). In such a life-history, the present data suggest that seasonal variation in the parasite transmission rate might be important where there is significant annual temperature variation. For example, aquatic habitats in the Cape area of South Africa may cool to below 10°C in winter (Tinsley and McCoid, 1996), conditions under which the embryonic development of *P. xenopodis* is arrested. Although *X. muelleri* occurs in relatively warmer biotypes, the eggs of *P. orientalis* show a comparatively increased sensitivity to cold (compromised development at 15°C) and might also develop more slowly at some times of the year. Combined with depressed egg production rates at lower temperatures (Jackson and Tinsley, 1998), thermal dependence in embryonic development has the potential to produce significant temperature-driven seasonal variability in the transmission rate of *Protopolystoma* species in southern Africa. These phenomena may also produce different transmission regimes in *Protopolystoma–Xenopus* populations occurring in distinct climatic zones.

Acknowledgements

This work was supported by research grants from BBSRC (7/S12169, to R.C.T.) and the South African National Research Foundation (to L.H.D.). Our field collections in Swaziland were greatly facilitated by the logistical support and local knowledge of Richard Boycott (Senior Warden, Malolotja Nature Reserve).

References

Crawley, M.J., 1993. GLIM for Ecologists, Blackwell Science, Oxford.

Fischer, W.J., Koch, W.A., Elepfandt, A., 2000. Sympatry and hybridization between the clawed frogs *Xenopus laevis laevis* and *Xenopus muelleri* (Pipidae). J. Zool. Lond. 252, 99–107.

Jackson, J.A., Tinsley, R.C., 1998. Effects of temperature on oviposition rate in *Protopolystoma xenopodis* (Monogenea: Polystomatidae). Int. J. Parasitol. 28, 309–15.

Kobel, H.R., Loumont, C., Tinsley, R.C., 1996. The extant species. In: Tinsley, R.C., Kobel, H.R. (Eds.), The Biology of *Xenopus*. Oxford University Press, Oxford, pp. 9–33.

Lambiris, A.J.L., 1988. A review of the amphibians of Natal. Lammergeyer 39, 1–191.

Passmore, N.I., Carruthers, V.C., 1995. South African Frogs, Witwatersrand University Press, Johannesburg.

Poynton, J.C., 1964. The Amphibia of southern Africa: a faunal study. Ann. Natal Mus. 17, 1–334.

Poynton, J.C., Broadley, D.G., 1985. Amphibia Zambesiaca 1. Scolecomorphidae, Pipidae, Microhylidae, Hemisidae, Arthroleptidae. Ann. Natal Mus. 26, 503–53.

Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223–5.

Tinsley, R.C., 1995. Parasitic disease in amphibians: control by the regulation of worm burdens. Parasitology 111, S153–78.

Tinsley, R.C., Jackson, J.A., 1998. Speciation of *Protopolystoma* Bychowsky, 1957 (Monogenea: Polystomatidae) in hosts of the genus *Xenopus* (Anura: Pipidae). Syst. Parasitol. 40, 93–141.

Tinsley, R.C., McCoid, M.J., 1996. Feral populations of *Xenopus* outside Africa. In: Tinsley, R.C., Kobel, H.R. (Eds.), The Biology of *Xenopus*, Oxford University Press. Oxford, pp. 81–94.

Tinsley, R.C., Owen, R.W., 1975. Studies on the biology of *Protopolystoma xenopodis* (Monogenoidea): the oncomiracidium and life-cycle. Parasitology 71, 445–63.

Tinsley, R.C., Loumont, C., Kobel, H.R., 1996. Geographical distribution and ecology. In: Tinsley, R.C., Kobel, H.R. (Eds.), The Biology of *Xenopus*. Oxford University Press, Oxford, pp. 35–59.