Parasite adaptation to extreme conditions in a desert environment

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SUMMARY

Deserts represent universally recognized extreme environments for animal life. This paper documents the highly specialized adaptations of *Pseudodiplorchis americanus*, a monogenean parasite of the desert toad, *Scaphiopus couchii*. Building on a long-term record of parasite population ecology (continuing since the early 1980s), field studies focus on the effects of severe drought in the Sonoran Desert, Arizona, in the mid 1990s. This provides a test of the ability of the host-parasite system to tolerate exceptional perturbation. The analysis provides new insight into parasite infection dynamics in a natural wildlife system through integration of host and parasite population age structure. The environmental check interrupted host recruitment in 1993–95 and parasite recruitment in 1995–97. This produced an imprint in age structure and infection levels recognizable over several years: parasite recruitment failure reduced transmission 2–3 years later. The host (maximum life span 17 years) tolerated the disruption but the impact was more serious for the parasite (life span 3 years) leading to extinction of some previously stable populations. Despite this demonstration of a rare event exacerbating external environmental constraints, experimental studies suggest that the internal (host) environment normally creates the most severe conditions affecting *P. americanus*. Only about 3 % of parasites survive from invasion until first reproduction. Post-invasion factors including host immunity, characteristic of most parasite life cycles, constitute a greater constraint upon survival than external conditions, even in a desert environment.

Key words: Age analysis, population ecology, Monogenea, Polystomatidae, *Pseudodiplorchis*, life cycle adaptation.

INTRODUCTION

Deserts provide some of the harshest environmental conditions on Earth. Life in deserts requires adaptations to extremes of water deficit (a combination of low precipitation and high evaporation) and temperature. For most desert organisms there is also a requirement to withstand prolonged periods of starvation for which adaptations frequently involve dormancy. In most of the world's hot deserts, there are periodic weather patterns that, often very briefly, provide favourable conditions for life. As is well-documented, the passage of rainstorms across deserts may be followed by a sudden flourishing of organisms whose key attribute is the ability to wait, typically in a dormant state, until these conditions arise. Therefore, desert organisms show a suite of adaptations alongside specializations to tolerate or avoid drought, temperature fluctuations and periodic starvation. Characteristically, these include a rapid response to unpredictable opportunities and a lifestyle geared to rapid growth, reproduction and accumulation of reserves to enable survival through the next period of hostile conditions. By any ecological and physiological criteria, deserts represent extreme environments and animals that survive such conditions would be expected to have major specializations.

The extreme environment associated with hot deserts precludes a wide spectrum of animals that are unable to cope with the constraints. Amongst vertebrates, the class Amphibia should automatically be excluded because of limitations for terrestrial life including a highly permeable skin, inability to concentrate excretory products, and the requirement for breeding and early development to occur in water. Amongst parasites, the platyhelminth class Monogenea should equally be a 'non-starter'. These are typically ectoparasites of fishes, they possess a ciliated, swimming infective stage and lack a resting, resistant stage and tolerance of desiccation. Nevertheless, this study is based on a host–parasite association involving representatives of these two groups: their occurrence in deserts is a result of quite exceptional characteristics. The host is an anuran superbly adapted to the arid conditions in the southwestern deserts of North America, the spade-foot toad, *Scaphiopus couchii*. The parasite is *Pseudodiplorchis americanus*, a polystomatid monogenean. This system provides a special challenge to understanding the evolution of adaptations to extreme constraints: many of the components of the parasite's life cycle strategy have no known precedent within the platyhelminths and represent unique solutions to specific problems.

This paper has two aims. First, it presents a review of studies that document the specializations of *P. ·americanus* to the extreme conditions experienced during the life cycle. A previous account (Tinsley, 1995) has focused on the host–parasite interactions in this system concerned with pathology

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and regulation of disease. The first part of this paper represents a complementary overview of life cycle adaptations. The second aim is to explore the effects of extreme environmental perturbation on population biology, specifically the occurrence of severe drought in the Sonoran Desert study sites in 1993–1995. These conditions, associated with the lowest rainfall in a 34 year weather record for the area, provide a test of the ability of the host and parasite to tolerate environmental effects even more severe than those normally encountered. It would be predicted that there should have been major negative effects on host and parasite recruitment and, further, that extreme limitation of the host feeding season could have perturbed the interaction with its pathogenic, blood-feeding parasite, potentially contributing to more severe disease. This study aims to provide new insight into host-parasite ecology by applying techniques of age determination to both host and parasite in order to reconstruct the dynamic changes affecting the respective populations.

THE LIFE CYCLE ADAPTATIONS OF PSEUDODIPLORCHIS AMERICANUS

The Polystomatidae comprises an atypical family of monogeneans that has radiated within a diverse assemblage of tetrapod vertebrates, including lungfish, anuran and urodele amphibians, chelonians and one mammal (the hippopotamus) (Tinsley, 1990a). In the best known example, Polystoma integerrimum, there is an exact synchrony of host and parasite life cycles. The adult parasites, in the urinary bladder, produce eggs for about one week each year during the spawning of the European frog, Rana temporaria, and the target of invasion, when the eggs hatch some weeks later, is the tadpole. The same principle of reproductive synchrony applies to Pseudodiplorchis americanus. However, the timing is much more precise. The spadefoot toad hibernates below ground for at least 10 months each year; it has a brief activity season during the period of summer rainfall when it feeds intensively to accumulate energy reserves to survive the next long hibernation. Transmission involving the aquatic infective stage, the oncomiracidium, is focused on the period – normally a single night – when the toads enter temporary ponds to breed. The target of invasion is the adult host. Pseudodiplorchis americanus has a long uterus in which fully-developed oncomiracidia accumulate during host hibernation; these are discharged into water, hatch instantly, and invade via the host's nostrils. Juveniles migrate during the first week post-infection to the lungs where they develop for two weeks and then return to the oral cavity before migrating along the length of the alimentary tract to the urinary bladder. The worms reach sexual maturity about 1 month post-migration and then

begin to accumulate offspring in preparation for the next opportunity for transmission during the following summer (Fig. 1).

The general pattern of this life cycle, particularly the synchrony of host and parasite oviposition, resembles that of P. integerrimum but the details differ. Indeed, there is a closer correspondence with other polystomatids that show adaptations for transmission in 'dynamic' environments including the fast-moving water of mountain torrents or the floodwaters of tropical forest. In both of these situations, polystomatids are specialized for 'instantaneous' host-to-host transmission (Tinsley, 1983, 1990a): this may be inferred from the anatomy of their reproductive systems but none of these life cycles has been investigated experimentally. Until the start of the present investigations, P. americanus was known only from a single brief taxonomic description (Rodgers & Kuntz, 1940). A series of studies has now provided comprehensive information on the biology of this parasite. These form the basis of the following review which examines critically the constraints of the desert environment and the corresponding parasite adaptations.

Adaptations to the constraints operating during host invasion: reproductive preparation

The environmental constraints that restrict the opportunity for transmission of P. americanus are rigidly defined. Larval invasion must occur in water and the appropriate circumstances are provided by the spawning of S. couchii in pools created by torrential summer rainfall. However, this allows a maximum timeframe of only 7 h since each episode is limited by the host's nocturnal habits. Instant host-to-host transfer is achieved by the production of larvae that complete development to the infective stage within the parent. Typically, the platyhelminth egg capsule is a relatively rigid structure designed to protect the embryo in the environments encountered after deposition. At the point of formation, the entire nutrient provision for larval development is packaged within the shell in the form of vitelline cells. With this reproductive pattern, output is constrained by parasite body size (of both the parent and the offspring) because of the requirement for storage space, and by the nutrient requirements of the developing larvae. Some platyhelminths adapted for ovoviviparity have modifications of this basic organisation (reviewed by Tinsley, 1983, 1990a), but the adaptations exhibited by P. americanus are without precedent amongst platyhelminths. Recently-formed embryos in the proximal uterus are surrounded by a thin capsule, about 100 μm in diameter, apparently with little potential for expansion. However, during passage along the uterus there is a progressive increase in egg capsule and embryo size. The fully-formed oncomiracidium is
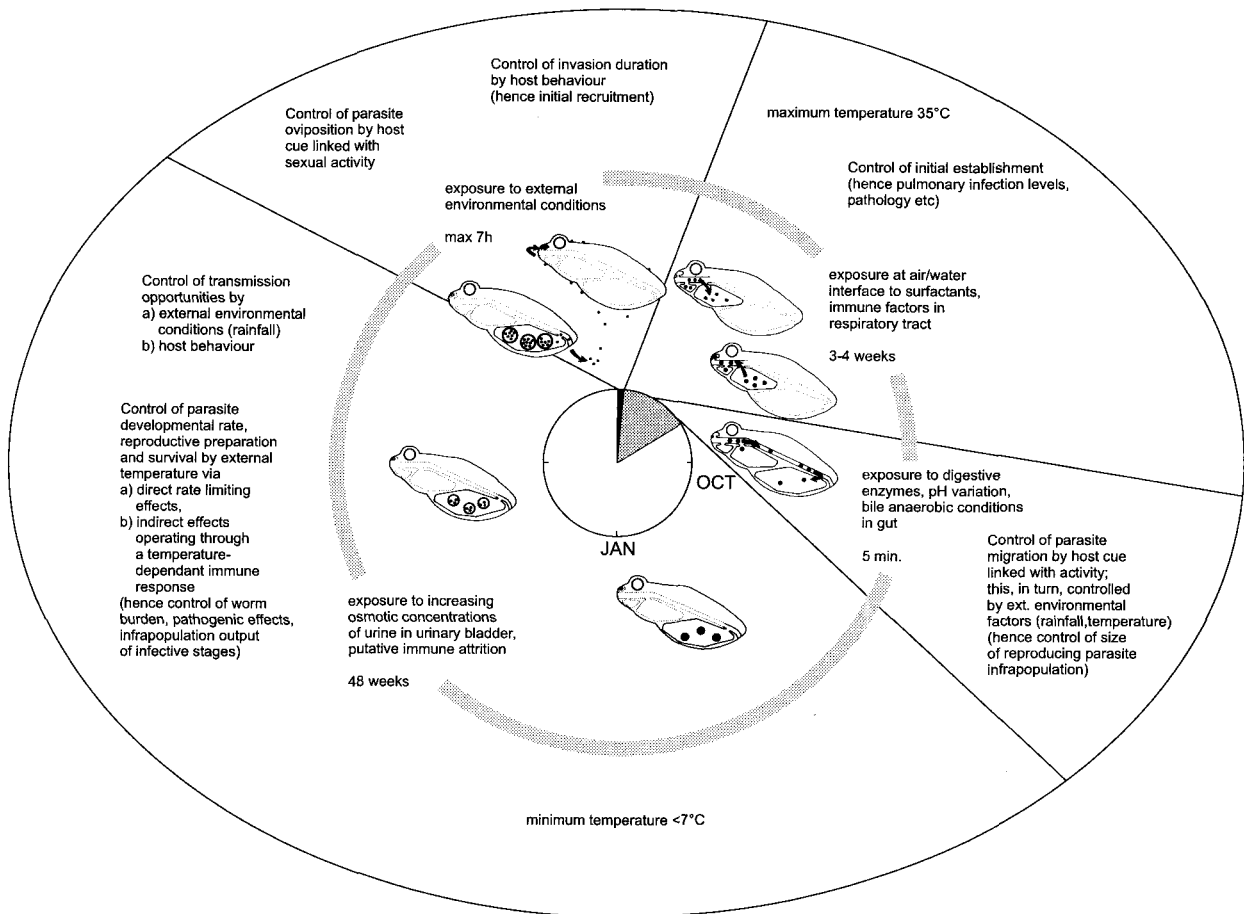
Control of invasion duration by host behaviour (hence initial recruitment)

Control of parasite oviposition by host cue linked with sexual activity

maximum temperature 35°C

exposure to external environmental conditions

max 7h

Control of initial establishment (hence pulmonary infection levels, pathology etc)

Control of transmission opportunities by
a) external environmental conditions (rainfall)
b) host behaviour

exposure at air/water interface to surfactants, immune factors in respiratory tract

3-4 weeks

Control of parasite developmental rate, reproductive preparation and survival by external temperature via
a) direct rate limiting effects,
b) indirect effects operating through a temperature-dependant immune response
(hence control of worm burden, pathogenic effects, infrapopulation output of infective stages)

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exposure to digestive enzymes, pH variation, bile anaerobic conditions in gut

5 min.

Control of parasite migration by host cue linked with activity; this, in turn, controlled by ext. environmental factors (rainfall,temperature) (hence control of size of reproducing parasite infrapopulation)

exposure to increasing osmotic concentrations of urine in urinary bladder, putative immune attrition

48 weeks

minimum temperature <7°C

Fig. 1. Summary of the constraints operating during successive phases of the life cycle of *Pseudodiplorchis americanus*. Inner ring of annotations lists the variations in environmental conditions experienced by the parasite; outermost annotations identify the accompanying environmental controls on parasite biology. Central diagram correlates parasite life cycle events with the annual activity cycle of the host, *Scaphiopus couchii* (based on Tinsley & Jackson, 1986): transmission occurs during host spawning (▼); juvenile development and internal migration during host feeding (▽); maturation and accumulation of embryos *in utero* during host hibernation (▽). The cycle follows one cohort of parasites, but adult worms (●) producing infective stages (·····) in one season's transmission may also survive to reproduce in the next year(s). Additionally, juveniles failing to migrate may remain in the respiratory tract throughout hibernation and then migrate to the bladder when the host again becomes active.

about 600 μm long and 250 μm wide. At this stage, the egg capsule forms a thin, loosely-fitting, transparent shroud wrapped around the oncomiracidium and, when distended, is over 800 μm in diameter. The highly flexible nature of this capsule, which is composed of elastin (Cable, Tocque & Tinsley, 1997), maximizes the numbers of oncomiracidia that can be stored *in utero*. The 'egg shell' is constructed with concentric layers of membranes. These are derived from the uterus wall and appear to become plastered onto the capsule surface. The resulting stacks of membranes can slide over one another, providing a mechanism by which the capsule can expand to accommodate the growth of the enclosed larva (Cable & Tinsley, 1991 a).

There is no provision of stored nutrients within the egg capsule; instead, the cytoplasmic lining of the capsule is modified to create a totally novel mechanism for the supply of nutrients. Cytoplasmic connections form placenta-like processes which

'plumb in' to the tegument of the developing larva. Transmission electron microscopy (TEM) shows that these are packed with glycogen rosettes and provide a route for the continuous transfer of nutrients from parent worm to offspring. The intestine of the adult parasite is highly branched, with diverticula passing between the folds of the uterus, ensuring close proximity between the sites of digestive uptake and the sites of nutrient delivery to the developing larvae (Tinsley, 1990 a; Cable & Tinsley, 1991 a).

Thus, the long uterus of *P. americanus*, which may accommodate up to 300 larvae, represents a remarkable adaptation (Fig. 2). In contrast to its presumed role in most platyhelminths as an inert storage chamber and passageway to the exterior, the uterus of *P. americanus* is a dynamic structure, responsible for the continuous production of new capsule membranes and for the transfer of nutrients to the accumulated embryos.
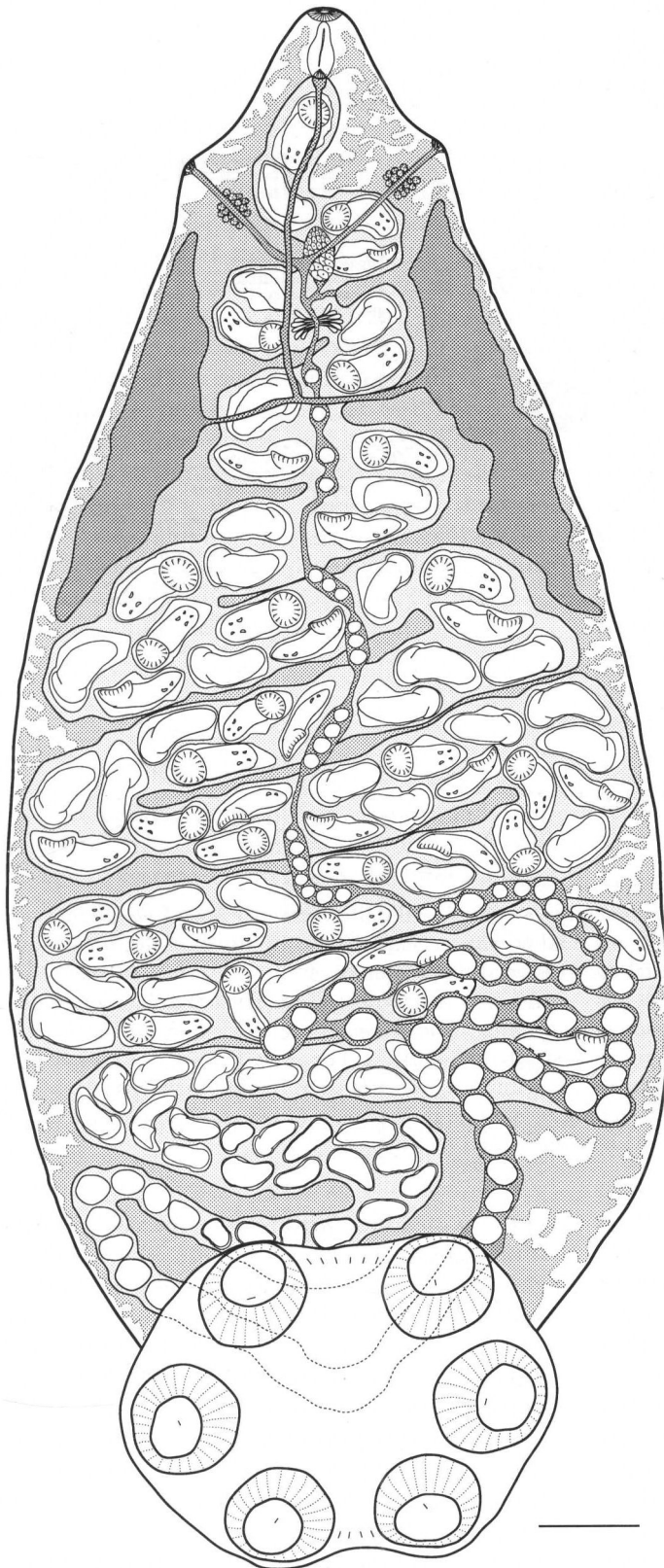
Fig. 2. *Pseudodiplorchis americanus*: diagrammatic representation of gravid adult showing extensive uterus with developing embryos in the descending limb and fully-developed encapsulated oncomiracidia in the ascending coils. Scale bar 0·5 mm.

Every aspect of this suite of adaptations complements the exacting demands of the life cycle. Parasites reach sexual maturity quickly, soon after migration to the urinary bladder, and begin to produce embryos while they are still relatively small (only 30 % of the size of a one year old adult). Thus, accumulation of offspring proceeds in tandem with adult body growth. Larvae are produced continu-

ously and numbers increase with time prior to transmission. The membranous egg capsule enables the close packing of maximum numbers of oncomiracidia within the uterus. Direct parent-to-offspring transfer of nutrients means that resources can be produced and supplied gradually over an extended period of time, instead of having to be stockpiled in the adult reproductive system before egg assembly can begin.

Probably the most important advantage of these reproductive adaptations relates to the unpredictable timing of transmission. Tocque & Tinsley (1991 *a*) recorded data showing that, in successive years, the interval between transmission opportunities – and hence the period for production and storage of infective stages – varied from 11 to 13 months. The oncomiracidia of *P. americanus* must therefore be prepared for the earliest opportunity for transmission yet still remain in constant readiness if this is delayed. Continuous channelling of nutrients from the parent guarantees an adequate provision up to the moment of discharge and hatching. The process is efficient for the parent since transuterine nutrition requires investment of resources only for the precise period of need, and it also ensures that, when oncomiracidia are eventually released, they have maximum energy reserves for host invasion (Tinsley, 1990 *a*; Cable & Tinsley, 1991 *a*).

Although these adaptations for maintaining a prolonged state of readiness are remarkable, the stored infective stages of *P. americanus* do have a finite life-span. The data of Tocque & Tinsley (1991 *b*), based on laboratory experimental infections, suggest that uterine larvae die about 2 months after they complete development to the infective stage. These disintegrate within the uterus and their nutrients may be recycled across the uterine wall or via the genito-intestinal canal to the gut (Cable & Tinsley, 1991 *a*). In experimental infections (at 25 °C), Tocque & Tinsley (1991 *b*) found that, after 6 months post-migration to the bladder, the complement of infective larvae achieved a steady state – about 100 oncomiracidia/parasite – with the fraction reaching the end of their storage life balanced by the numbers completing development to the infective stage. This corresponds with the output of 2 or 3 year old worms in the field, but output is also significantly affected by worm age and intraspecific competition (see below).

Adaptations to the constraints operating during host invasion : oviposition

The environmental events preceding transmission typically involve a long period of drought and steadily increasing temperatures during early summer. The 'monsoon' rains often arrive suddenly and spadefoot toads are aroused from hibernation below ground where they have remained inactive since the previous September. The stimulus for emergence is provided by the low frequency vibrations of rainfall drumming on the surface (Dimmitt & Ruibal, 1980) and the toads migrate after darkness to rapidly-forming pools. Assemblies of several hundred individuals gather in pools a few metres in diameter; the chorus of male mating calls, which may be heard by the human ear over distances of 1–2 km, attracts animals from a considerable area and spawning is rapid. At dawn, all the toads leave the water and bury themselves in shallow burrows in the desert soil to escape the daytime heat.

Given the preparation of immediately-infective larvae within the uterus of gravid parasites, the key requirement for exploiting this brief opportunity for transmission is a mechanism for synchronising discharge of oncomiracidia. Clearly this stimulus for parasite oviposition must be very precise and absolutely reliable: if gravid parasites discharge their larvae at any time outside the host's immersion in water the oncomiracidia will be lost. Environmental events accompanying entry of the toads into water could provide distinct signals for recognition by the parasites and Tinsley (1990 *a*) discussed how various host physiological changes could represent the trigger, including the osmotic and chemical changes accompanying rehydration. However, the actual cue is associated with host sexual activity – a guarantee of the presence both of water, required for transmission by the ciliated infective stages, and of other potential hosts. Tinsley (1990 *a*) summarized data recording the exact synchrony of larval discharge with amplexus by males and oviposition by females. Nevertheless, discharge is not linked with host gamete release. Indeed, it is important that the cue is disengaged from spawning *per se* because, with a male:female ratio of between 2:1 and 10:1 (Tinsley, 1990 *b*), a majority of males in a given assembly may not actually mate. Instead, field and laboratory experiments demonstrate that the parasites (including those in chorusing unmated males) release their accumulated larvae in response to intense sexual excitement.

This general response can be confirmed by hormone treatment and behavioural manipulations (Tinsley 1990 *a*, *b*), but the best evidence for the proximal mechanisms regulating oviposition in polystomatid monogeneans has been provided by experimental studies on the related *Polystoma nearcticum*, a urinary bladder parasite of the north American gray treefrog, *Hyla versicolor*. This system has the same requirement for synchrony of host and parasite oviposition, and the same exact link between host sexual excitement and the parasite's response. However, in this case, the stimulated parasite rapidly manufactures eggs from accumulated components (ova, sperm, vitelline cells and associated secretions) rather than the simple discharge of already-prepared larvae. Immunocytochemical studies by Armstrong

et al. (1997) have monitored immunoreactivity of a FMRFamide-related peptide (FaRP), GYIRF-amide, in the neurons associated with the ootype musculature (the chamber in which egg assembly occurs). Intense immunostaining in this nerve plexus occurred during periods of rapid parasite egg laying (coinciding with host sexual activity), whereas in hosts examined post-spawning, when parasite oviposition had ceased, there was little or no GYIRF-amide immunoreactivity. These findings suggest that regulatory peptide expression at the egg assembly site is switched on and off to coincide with the short periods of *P. nearcticum* egg production. The FaRP may therefore serve as the trigger that coordinates the muscle contractions required for egg manufacture, and this control meets the ecological requirements for a very precise oviposition response by the parasite.

Survival in the external environment: characteristics of transmission

The problem of host-to-host transfer in a desert environment by a parasite with an aquatic infective stage appears to represent the most challenging demand for adaptation to extreme environmental constraints. Paradoxically, field evidence indicates that this segment of the *P. americanus* life cycle is accomplished easily because of two key factors. First, the characteristics of host behaviour provide a very specific opportunity and, second, the remarkable adaptations of the parasite's reproductive strategy achieve a finely-tuned response. The mating of anuran amphibians generates a mass assembly of animals which are otherwise solitary. For a short time each year, normally dispersed anuran populations become highly aggregated. The explosive breeding of *Scaphiopus*, triggered by intense rainfall and restricted to a single night after each storm, serves to produce maximum densities of potential hosts congregated in water which is essential for monogenean transmission (Tinsley, 1989, 1990*b*). Newly-formed temporary pools become foci for intense infection. However, whilst these characteristics serve to maximize transmission success, departure of the toads at dawn abruptly terminates invasion. Thus, each episode of infection is limited by the numbers of parasite larvae that can invade before this deadline. Since larvae are discharged at intervals throughout the night, especially because hosts must migrate varying distances to the newly-formed pools and may arrive relatively late within the total time-window, the exposure duration for many infective stages – and many potential hosts – is far less than the maximum 7 h. Males tend to remain in water from the variable time of their arrival until the assembly disperses, whereas each female remains only for the period of her own spawning, limiting involvement to around 4 h.

With these constraints restricting each transmission episode, larval characteristics promoting rapid host invasion should have selective advantage. The observations of Tinsley & Earle (1983) show that *P. americanus* has 'super-larvae', the largest recorded in the Monogenea, with a body length of 600 μm and a swimming life more than twice that typical of other monogeneans: oncomiracidia swim continuously for over 4 h at 25–27 °C. Uniquely, the ciliated stages are also resistant to desiccation. They survive drying on the skin of the host for up to 1 h (at 32 °C, r.h. 45 %), and the unimpaired swimming ability of these larvae, if returned to water, demonstrates that the cilia are not affected. The tegument is tough and, whereas most monogenean oncomiracidia disintegrate rapidly after death, dead *P. americanus* larvae retain their integrity in water for several days (Tinsley & Earle, 1983; Cable & Tinsley, 1992*a*). The oncomiracidia possess four photoreceptors that are adapted for very low light intensity. Their fine structure, employing a quarter wavelength reflector, is found in some other polystomatids but in no other platyhelminths (Cable & Tinsley, 1991*b*). Tocque (1990) measured the energy reserves which contribute to the remarkable longevity and showed that glycogen content at hatching averages 0·216 μg/larva and declines exponentially during swimming life. At 22 °C, close to night-time water temperatures in the desert, 50 % of the oncomiracidial population survives 29 h, with maximum longevity 96 h (Tinsley, unpublished). The survivorship curve is characterized by negligible mortality for the first 15 h of life, a period much greater than the entire transmission episode. Therefore, successful invasion is unlikely to be limited by larval survivorship. Rather, the exceptional attributes of the larva may reflect the energetic and other physiological demands of initial establishment in the host.

Ultimately, a parasite transmission stratagem inextricably linked with host reproduction is guaranteed to be successful – as long as the hosts do reproduce. The consequences for the parasite of host reproductive failure are considered below, under the heading host-parasite population ecology.

Survival in the external environment: transmission efficiency

Although the exposure is very brief, events occur so rapidly that dynamic changes can be recorded over a time scale of a few hours. Samples of mating populations of *S. couchii* taken at intervals during one night show a more or less exponential increase in infection levels during the 7 h window. Ten per cent of the night's total invasions occur in the 3 h until midnight, but more than 30 % occur in the final hour before dawn (03·00–04·00 h) (Tinsley, 1989). Field studies have demonstrated the consistency of events. Infection levels of newly-invaded larvae are zero at
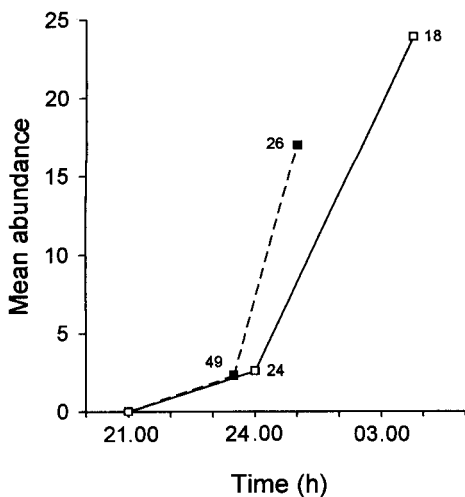
Fig. 3. *Pseudodiplorchis americanus*: short-term dynamics of invasion of *Scaphiopus couchii* illustrated by two different transmission episodes; (☐—☐) data from Tinsley & Jackson (1988), (■---■) data from Tinsley (1989, Fig. 2). Time (Standard Mountain Time, Arizona) represents the duration of the exposure from dusk until dawn. Sample sizes shown adjacent to respective points.

about 21·00 h on the first night of breeding (with animals having just emerged from hibernation). Data recorded by Tinsley (1989) show that mean abundance reached 2·3 worms/toad at 23·30 h, increasing to 17·0 worms/toad only 90 minutes later at 01·00 h. Comparable data were recorded by Tinsley & Jackson (1988) showing a mean abundance of 2·6 worms/toad at midnight, increasing to 23·9 worms/toad at 03·45 h (Fig. 3). Parasite population terms used in this account follow Bush *et al.* (1997).

In both these studies, a second torrential storm led to a repeat aggregation on the following night, producing a second input of invading larvae. In Tinsley & Jackson's (1988) study, the cumulative burdens acquired after the two exposures produced a mean abundance of 44 worms/toad. Exceptionally, in this season, there was a third storm and another transmission episode 11 days later. A record of cumulative recruitment for the population recorded 100% prevalence and mean abundance 81 worms/toad, but this was taken at 03·00 h and would have underestimated final levels. Based on the relationship of recruitment with time in other studies, the last hour before dawn would be expected to have increased the final total to over 100 larvae/toad (Tinsley & Jackson, 1988). In the separate study of Tinsley (1989), a small sample taken at dawn showed that an equivalent final total was achieved by the end of the second night's exposure: a prevalence of 100% and mean abundance 107 worms/toad.

These and other unpublished studies confirm a consistent, virtual saturation of the host population each season by invading *P. americanus* larvae. Prevalence in males is normally 100% and around half of the individuals in populations acquire burdens exceeding 100 worms, up to a maximum of 400/host (Tinsley, 1989; Tinsley & Jackson, 1988). These field studies provide a detailed view of factors influencing individual invasion levels, including the effects of behavioural differences between males and females, and between mated and unmated males (see Tinsley, 1989, 1990*a*, *b*). Other studies have included a more precise analysis of the output of infective stages from the reproducing parasite population. Tocque & Tinsley (1991*b*) showed that the contribution of oncomiracidia by adult worms is age-dependent: 2nd year worms produce nearly 10 times the oncomiracidial output achieved by 1st year's; 3rd year's produce over twice the *per capita* output of 2nd year's; and, in this study, a rare 4 year old worm produced almost the same larval output as the combined total from the cohort of 73 1st year worms. Reproductive investment is also significantly affected by temperature and the duration of the host hibernation period (Tocque & Tinsley, 1991*a*), and by competitive interactions that reduce larval production at high worm burdens (Tocque & Tinsley, 1991*b*). Thus, larval output in transmission assemblies is influenced by the proportion of each age class present in the parasite suprapopulation, by environmental conditions preceding transmission, and by the distribution of infection levels within the host population and its effects on density-dependent parasite interactions.

A detailed analysis of *P. americanus* population dynamics is therefore considerably more complex than envisaged in the initial correlation of adult parasite population size with recruitment success (Tinsley & Jackson, 1988). Nevertheless, these field studies provide a useful estimate of invasion efficiency. Assuming a mean abundance of gravid parasites of 3·0 worms/toad, a mean output of 100 oncomiracidia/gravid worm, and a mean input of invading worms at the end of the season of around 100 larvae/toad, the probability of individual invasion success is about 0·3 (Tinsley & Jackson, 1988). This calculation may underestimate actual invasion probability. Comprehensive studies suggest that a mean of 100 larvae/gravid worm is achieved under only the most favourable conditions. In most seasons, output is considerably less. Tocque & Tinsley (1991*b*) recorded an overall mean of 39 larvae/adult measured 11 months p.i., indicating a mean of about 60 at the time of transmission. On the other hand, previously-published records of the mean input of invasions/host also reflect best circumstances, based on years where there was repeated transmission. In many years the final burdens are lower. Tocque & Tinsley (1994*a*) recorded invasion data for one population sample of mean abundance 59 worms/toad. Actually, therefore, these alternative (more conservative) data – mean output and input both around 60, with

an adult suprapopulation of 3 worms/toad – produce exactly the same probability of invasion by individual larvae of around 0·3.

The transfer from external to internal environments: invasion

Uniquely, amongst monogenean life cycles so far described, the target for host invasion by the swimming oncomiracidium – the nostrils – is above the water surface. To reach this point of entry, larvae must break through the surface film of the water and migrate over exposed skin. Under normal conditions in mating aggregations, the skin is kept wet by movements of the toads in water, but larvae are nonetheless able to migrate over damp skin where only their ventral surface is in contact with moisture (Tinsley & Earle, 1983). Remarkably, as mentioned above, oncomiracidia can tolerate short-term drying on the skin. From the time of entry into the nostril chamber and sinuses, larvae live at an air/water interface. The ciliated cells are shed 1–2 h post-invasion (Cable & Tinsley, 1992*a*); all locomotion subsequent to initial host contact is by vigorous muscular crawling.

Internal environmental conditions: survival in the respiratory tract

As with other aspects of the biology of *P. americanus*, the environmental conditions experienced during initial establishment within the host have few precedents elsewhere in the Monogenea. Worms remain in the host nostrils and sinuses for 24 h after invasion, and during the ensuing days they migrate into the mouth and associated cavities (sub-lingual space, eustachian tubes, glottis and the vocal sac of males). They begin to feed on blood 24–48 h p.i. (Tinsley & Earle, 1983), but mucus and epithelial cells may be ingested by recently-invaded larvae. Progressively they migrate through the glottis and into the lungs and all occur in this site after 1 week p.i., remaining for 7–14 days. At approximately 3 weeks p.i., juvenile worms return to the buccal cavity where they accumulate prior to migration to the urinary bladder (Tinsley & Jackson, 1986).

The natural temperature regime experienced during this phase of parasite development is determined by host behaviour: this buffers the extreme characteristic of the desert habitats where soil surface temperatures can exceed 70 °C (Tocque & Tinsley, 1991*a*). During the day, the toads bury themselves about 5 cm below the surface (Ruibal, Tevis & Roig, 1969). Field records at this depth in July and August indicate that parasites experience a cycle with a mean daytime temperature of 34 °C for 7 h, falling at night to a mean of 22 °C for 5 h (Tocque & Tinsley, 1991*a*). On damp nights, when the toads emerge to forage, soil surface temperature varies between 18 and 24 °C.

Few monogeneans inhabit air-filled passageways where, even if the parasite's ventral surface is in contact with moist epithelia, the dorsal surface is at the air/water interface. Species of *Polystomoides* and *Neopolystoma* occur in the oral cavity and pharynx of chelonians (Tinsley & Earle, 1983), and *Gyrdicotylus gallieni* inhabits the oral membranes of *Xenopus laevis*. In this latter case, worms invade via the nostrils (as in *P. americanus*) but this portal is underwater at the time of entry (Harris & Tinsley, 1987; Jackson & Tinsley, 1994; Tinsley, 1996). *Pseudodiplorchis americanus* and the closely related *Neodiplorchis scaphiopodis* are unique amongst monogeneans in infecting vertebrate lungs (Tinsley & Earle, 1983). Here, the acid mucosubstance lining and phospholipid surfactant, together with the pulmonary immune defences, create a highly hostile environment for the juvenile worms. The environmental conditions parallel those experienced by pentastomid parasites in reptilian lungs. Riley & Henderson (see this volume) have described a remarkable defence mechanism based on massive membrane production and the secretion of lipids that apparently mimic host surfactant. Nothing is known of the responses of *P. americanus* in equivalent circumstances. Cable & Tinsley (1992*a*, *b*) found no obvious ultrastructural correlates of the hostile conditions in the respiratory tract: the tegument of juveniles is similar to that of oncomiracidia and there are no structural differences between the dorsal and ventral body surfaces. Membrane-bound tegumental vesicles probably contribute to the glycocalyx which, together with glandular secretions, may provide protection. However, in contrast to this lack of obvious adaptations to life in the respiratory tract, there are unique specializations that enable survival in the digestive tract.

Internal environmental conditions: migration through the alimentary tract

During the period when *P. americanus* occurs in the mouth, ingestion of prey by the host includes a majority of hard-bodied, abrasive desert invertebrates (beetles, ants, crickets) together with a remarkable range of noxious animals such as scorpions, solpugids, centipedes and pogonomyrmid ants (Tocque, Tinsley & Lamb, 1995). Exposure of parasites to a variety of toxic chemicals, especially acids, seems inevitable.

Migration through the stomach and intestine to the cloaca and urinary bladder is first possible at 4 weeks p.i. (at 25 °C), normally at the peak of the host's activity season when the gut is packed with digesting contents (see Tocque *et al.* 1995). Experimental trials indicate that movement is rapid, taking as little as 5 min for worms to traverse the 80–100 mm length of the alimentary tract. Tinsley & Jackson (1986) demonstrated that migration and

survival in the potentially lethal conditions in the gut is dependent on a trigger factor: this both controls the moment of migration and activates the mechanisms providing protection. The cue is associated with host activity. In toads that enter dormancy before the time of migration, the parasites remain in the respiratory tract and undergo no more growth and development than that achieved prior to normal migration at 4 weeks p.i. Indeed, if hosts remain undisturbed during hibernation in the wild, or during laboratory maintenance simulating natural conditions, worms fail to migrate and remain in an arrested state for more than 1 year. These nevertheless retain the ability to migrate: when the host is aroused from dormancy, the delayed migrants are stimulated to undertake the rapid journey and then begin normal reproductive development leading to maturation around 1 month later.

Whilst the cue triggering migration has not been identified, it has emerged that anaesthetization of the host with tricaine methane sulphonate (MS222) provides a highly effective artificial stimulus facilitating experimental studies (Tinsley & Jackson, 1986). Worms transferred directly from the host respiratory tract to stomach and intestinal contents at varying stages of digestive activity all died within 2 min, and TEM showed that the tegument was stripped away. In starved toads, including those just emerged from hibernation, the mid-intestine contains a section of accumulated bile fluid and this is also lethal to transferred worms within 2 min. In complete contrast, worms that have begun migration, either naturally or in response to host anaesthetization with MS222, survive for 4–8 h in conditions that are lethal to non-migrants. Ultrastructural studies show that survival is conferred by the mass discharge of tegumental vesicles (Cable & Tinsley, 1992b). During development in the respiratory tract, the juvenile worms accumulate two types of electron-dense, membrane-bound vesicles, one PAS-positive and the other PAS-negative. These increase in density in the distal cytoplasm up to 28 d p.i. but then remain unaltered in numbers and appearance until migration, even if this is delayed for over 1 year. During transfer through the gut, there is a progressive discharge of vesicles from the surface layers and an initial compensatory transfer of vesicles from the perikarya into the distal cytoplasm. After depletion of the store of perikarya vesicles, the density in the surface layer also reduces. This dynamic flux suggests that mass release of vesicles contributes to rapid turnover of an outer coat that provides protection against digestion. If migrants are left for prolonged periods in gut contents (normally over 8 h), the store of tegumental vesicles is eventually exhausted and, at this point, the tegument begins to disintegrate and the worms die (Cable & Tinsley, 1992b; Cable, Harris & Tinsley, 1998).

Death of worms transferred to gut contents from the respiratory tract (without prior 'activation') occurs despite their store of tegumental vesicles, indicating that exposure to the hostile conditions in the gut does not trigger the protective secretions. Instead, vesicle discharge is stimulated in advance by a specific cue, presumably at the same point as activation of the migration response.

The arrested development of worms whose migration is delayed emphasizes the effect of environmental factors on this life cycle. Worms remaining in the respiratory tract are active and continue to feed (as indicated by the presence of haematin in the gut) but further growth and reproductive development is entirely inhibited. Worms from the same invading cohort that do migrate immediately begin rapid development. These developmental outcomes are unlikely to be determined by nutritional factors since parasites in the two sites have access to the same blood diet. The gut migration therefore has a central role in the life cycle, not only linking together the two phases of parasitization – of the respiratory and urinary tracts – but also initiating reproduction. Under experimental conditions at 25 °C, parasites reach sexual maturity one month post-migration and begin to accumulate embryos whilst their body growth continues. About 200 offspring are produced after 6 months, of which about half are fully-developed and infective (Tocque & Tinsley, 1991 a, b). This appears to represent a very efficient course of preparation for the next transmission episode. However, information on development at the temperatures actually experienced in the desert indicates that the potential for reproduction during the first year post-infection is almost completely eliminated by this natural constraint.

Constraints of external environmental conditions: direct and indirect effects on parasite development and reproduction

Parallel field and laboratory studies have demonstrated that temperature exerts a powerful regulatory influence over parasite growth, development and reproductive output: fastest rates occur around 25 °C but are reduced by half at 20 °C and virtually halted at 16 °C. Based on the annual temperature cycle in the field, Tocque & Tinsley (1991 a) showed that all parasite development is arrested from about October to April when temperatures at the soil depth occupied by hibernating toads are below 15 °C: this totally precludes growth and reproductive preparation for 6–7 months/year. Soil temperatures exceed 25 °C, providing optimum conditions for parasite development, for only about 3 months of the year. Temperatures exceed 20 °C for 4–5·5 months, i.e. during summer and early autumn following transmission in one year and late spring/early summer preceding transmission in the next year. For

each cohort of invading parasites, juvenile development in the lungs occupies the first month of these favourable temperatures, leaving only 3–4·5 months for maturation and embryo production. Tocque & Tinsley (1991*b*) showed that 4 months at 25 °C is necessary for the first embryos developing *in utero* to reach the infective stage, and adult worms accumulated only about 30 infective larvae after 5 months post-migration. It follows that, as a result of the combined constraints of temperature and time, 1st year worms may make only a very minor contribution to transmission. Body growth continues throughout life but 2nd year worms, having already achieved initial development to maturity, benefit from the full period above 20 °C and make a larger reproductive contribution. This trend is maintained by 3rd year and very rare 4th year worms. The field records of Tocque & Tinsley (1991*b*) (based on a suprapopulation sampled 11 months after the previous year's transmission) showed means for 1st, 2nd and 3rd year adults of 5, 43 and 96 infective larvae/worm respectively, and a single 4th year worm with 326 larvae.

Because of this strict constraint of temperature regime, the period of reproductive development is also regulated by the timing of the 'monsoon' rains, and hence transmission, in adjacent years. Short 'years' occur when a late monsoon in one year is followed by early rains in the next. The study of Tocque & Tinsley (1991*a*) included one such 11 month inter-transmission period (first rains on 27 July and 1 July in successive years): field temperature records showed that parasites experienced only 17 weeks above 20 °C, precluding any contribution to transmission by 1st year adults. This was followed by a 13 month 'year' (first rains on 1 July and 29 July in the adjacent years), including 23 weeks above 20 °C, and gave the potential to produce around 30 infective larvae/1st year worm. Late invasion, resulting from host breeding in early August (see, for instance, Tocque, 1993), allows a very reduced time for development at optimum temperatures. Worms migrating in early September may not reach maturity before temperatures decline below 20 °C; these resume growth in May but remain small, with little reproductive development, at the start of the summer transmission. If host activity is precluded (by dry conditions and declining temperatures) before parasite migration, then the pre-migrants remain undeveloped in the respiratory tract throughout hibernation and migrate only when the hosts become active and enter breeding assemblies in the following summer (Tinsley & Jackson, 1986, 1988). These arrested stages, now 1 year p.i., are only 4 weeks more advanced in development than the new season's invading larvae.

Tinsley & Jackson (1988) recorded one suprapopulation of *P. americanus* in which the normal synchrony of the life cycle was highly disrupted,

reflecting delayed migration and arrested development in the previous year. The host sample at the start of the transmission season (n = 92) contained a larger subset of parasites that had failed to complete reproductive preparation (mean abundance 3·37 worms/toad) than those prepared with infective stages *in utero* (mean abundance 2·95). About half of these 'failed' stages remained arrested in the respiratory tract, whilst the others that had migrated ranged from small immature worms to adults containing only developing embryos. Whilst these parasites had missed the once-per-year opportunity for transmission, it could be considered advantageous that they now had a full year to prepare an above average contribution for the next. However, given the normal 3 year life span, with an estimated 50 % mortality between years 2 and 3 (Tocque & Tinsley, 1991*b*), inability to contribute to one of the years would be expected to have significant consequences for overall transmission. The actual effects of such disrupted life history schedules are explored in a case study of parasite population ecology later in this paper.

It seems surprising that there should be such a narrow 'margin of safety' in this life cycle. Year-to-year variations in temperature and rainfall have effects of such magnitude that a major fraction of the surviving parasite population may fail to contribute to annual transmission. Part of this vulnerability is attributable to the necessity for migration through the extreme environment represented by the alimentary tract. There are two consequences. On the one hand, the unique survival mechanism requires a cue from a host factor which is, in turn, dependent on external environmental conditions (rainfall and temperature); this creates the risk of disruption by chance factors. On the other hand, reproductive development is inhibited until migration has occurred. This dual effect reinforces the key importance of gut migration in the biology *P. americanus* (see above). Moreover, the vulnerability of the life cycle to disruption by environmental conditions is a direct consequence of the novel characteristic of a respiratory phase.

Constraints of internal (host) environmental conditions: effects on parasite survival

Following the manifest efficiency of the transmission process, it has emerged that the reproductive potential, represented by the annual input of invading worms, fails to have a commensurate effect on future transmission because of the powerful constraint of the natural temperature cycle in the desert. This reflects a direct rate-limiting effect of the environment on reproductive output. However, there is a second, far more important, constraint that limits realisation of the potential of the life cycle: a remarkably high mortality rate within established

parasite infrapopulations. The major effects of this, too, are temperature dependent but, in this case, putatively operating through the host immune response.

Tinsley (1995) demonstrated that the virtual saturation of the mating host population by invading parasites is followed, each year, by a major pre-reproductive reduction in surviving worm numbers. The consistently high invasion levels, with prevalence 100 % and mean intensities of 60–100 worms/host, fall in the period preceding the next transmission opportunity to prevalence 50 %, mean intensity 5–6 worms/host. Maximum burdens following invasion, typically around 200 worms/host and sometimes 300–400, virtually never exceed 30 adults at the start of the next transmission season (Tinsley, 1995). The same effects can be demonstrated in laboratory experimental infections (Tinsley, 1989). Overall, 97 % of the larvae that successfully invade the host population die before they can contribute to transmission. The basis of this estimate derives from the converse of the simple calculation (above) that, given relatively stable year-to-year population levels, where the mean abundance of invaded parasites is around 100 worms/toad, the mean abundance of adults surviving to reproduce one year later is around 3·0 worms/toad (Tinsley, 1995). This may actually be a significant over-estimate: the adult reproducing populations are generally composed of 3 year classes of parasites, of which the 1st year adults – the survivors of the previous season's invasions – may comprise only 40 % of the suprapopulation (Tocque & Tinsley, 1991*b*). On the other hand, the data cited by Tinsley (1989, 1995) are based on gravid adult parasite suprapopulations (worms with infective oncomiracidia *in utero*) and in many years there may also be a significant number of developing (not yet gravid) worms, the product of the same invasion. With these qualifications, therefore, the overall probability of survival from invasion to first transmission is likely to be close to 0·03 (as recorded by Tinsley, 1995).

Tinsley (1993) identified a succession of factors involved in parasite mortality, including losses attributable to host mortality (influenced by life history characteristics including longevity, and by stochastic factors), parasite-induced host mortality, parasite intra-specific competition. Other hazards include, in experimental studies, around 10 % mortality during the course of gut migration (Cable & Tinsley, 1992*b*), and up to 10 % attributed to the pathogenic effects of a microsporidean hyperparasite (Cable & Tinsley, 1992*c*).

However, even in combination, these mortality factors cannot contribute to more than a part of the total, almost overwhelming, parasite losses. Tinsley (1995) has reviewed evidence that regulation of the *P. americanus* populations strongly resembles the operation of a host immune response. In summary, a number of regularly-observed features of the population biology of *P. americanus* support this hypothesis (although there is not yet any experimental immunological evidence). First, studies at the same field sites in successive years have recorded a striking constancy in the surviving adult suprapopulations of about 3 worms/toad. This occurs despite year-to-year variations in the environmental conditions that influence invasion success. Apparently, differences in annual recruitment are overridden by post-invasion factors which exert a dominant control over surviving parasite numbers. The more-or-less constant annual 50 % prevalence may represent the fraction of the host population that is susceptible to infection. Second, repeated annual infection by *P. americanus* should lead to progressively greater worm burdens during the host's life-time. However, infection levels rise to a plateau in toads aged about 6 years and then decline in the oldest age groups despite continued re-infection by parasites with a 3 year life span. This could provide evidence of acquired immunity. Third, experimental infection survived better (89 % prevalence) in field-caught hosts that had pre-existing adult infections compared with those without a pre-existing infection (31 % prevalence). This is suggestive of variation in susceptibility that predisposes to further infection (Tocque & Tinsley, 1994*a*). Fourth, there is comprehensive experimental evidence for an inverse relationship between parasite survival and temperature: this is consistent with the operation of an immune response that is temperature-dependent (as is characteristic of ectothermic vertebrates) (Tocque & Tinsley, 1994*a*). Finally, there is also indication that exceptionally high burdens of *P. americanus* in individual toads tend to co-occur with abnormally high burdens of other parasites (example cited in Tocque & Tinsley (1994*a*) and unpublished records). A behavioural influence is unlikely since the nematode, cestode and monogenean species involved have entirely different transmission routes. The findings are suggestive of pre-disposition to infection potentially reflecting impaired immune competence.

Evidence for the putative regulation of worm burdens is discussed in detail by Tocque & Tinsley (1994*a*) and Tinsley (1995). Based on experimental infections of *P. americanus* in wild-caught toads, Tocque & Tinsley (1994*a*) found that parasite survival was strongly temperature and time dependent. At 25 °C, there was no significant decline in the infections of 1st year worms for up to 5 months post-migration to the bladder (prevalence 94 %, mean intensity 13–18 worms/host, maximum 30 worms). Subsequently, prevalence and intensity fell rapidly to 50 %, maximum 19 worms after 6–8 months, and 25 %, maximum 9 worms after 10 months. Only 2·1 % of the initial experimental

invasion survived to 10 months post-migration. Survival of pre-existing, older age classes (acquired in the field before experimental infection) followed a similar pattern. At 25 °C, infection levels were virtually identical after 3–4 months to those recorded at the time of host capture: prevalence 52%, intensity 6·2 worms/host, maximum 32 worms. However, prevalence declined to 27% after 7 months and 11% after 8–10 months with a corresponding decrease in intensity, and no pre-existing adult worms survived after 10 months. In both 1st year and pre-existing adult worm infections, the decline in infection levels was accompanied by a loss of the heaviest burdens with a corresponding decline in the variance/mean ratio.

In marked contrast, at 15–20 °C there was no decline in prevalence or intensity of either 1st year or pre-existing adult parasites for the duration of the laboratory study (14 months), and there was no change in the variance/mean ratio confirming the persistence of higher worm burdens.

These data suggest that the losses of *P. americanus* occur only during periods when the environmental temperatures (at the soil depth occupied by buried *S. couchii*) exceed 20 °C, a period comprising 4–5·5 months of each year (Tocque & Tinsley, 1991*a*).

In these laboratory studies, experimentally-infected hosts did not experience temperature manipulations during the period when the parasites occur in the host lungs. Potentially, this is the most hostile site for immune attack and respiratory infection coincides with the period of highest annual temperatures. TEM studies on the pathology of this phase have revealed evidence of immune attack where macrophage-like cells are associated with tegumental damage (Cable *et al.* Unpublished). Major parasite attrition might be expected during lung infection, but available quantitative evidence relates to total parasite losses rather than those occurring in this specific segment of the cycle.

These overall data, from a series of field studies and laboratory experiments, point consistently to the conclusion that the internal environment of the host presents by far the most extreme conditions for parasite survival. Indeed, if it were not for the amelioration of these hostile conditions by the period of low temperatures each year, the parasite would not survive in the Sonoran Desert from one transmission season to the next.

HOST-PARASITE POPULATION ECOLOGY

The field and laboratory studies reviewed above provide quantitative assessment of the effects of environmental conditions on the population biology of *Pseudodiplorchis americanus*. Most notably, there is an estimated 97% mortality of post-invasion parasites before the first opportunity for onward transmission. The studies also identify points where

the life cycle is highly vulnerable to disruption, especially the indirect environmental control of gut migration and its effects on the future reproductive contribution of each year's invading cohort. The margin between the 'norm' for this life cycle and complete failure seems surprisingly narrow prompting the question whether natural circumstances could shift this apparently fragile balance towards extinction. The occurrence of a parasite in a given geographical area should provide unambiguous evidence that environmental conditions do not significantly threaten long-term survival. Population data for *P. americanus* at specific local field sites have been interpreted as demonstrating remarkable year-to-year stability of infection levels (Tinsley, 1995). This pattern has been maintained despite annual fluctuations in rainfall that should generate variation in larval recruitment. It may be concluded that parasite population biology is buffered against changes of this magnitude, particularly by the efficient transmission process that leads to massive invasion of target populations. Additionally, the 3 (very rarely 4) year life span of the parasite should provide a buffer against occasional recruitment failure. Analysis of weather records for the study area together with direct field observations from 1981 to 1992 indicate that relatively wetter and drier years have tended to alternate over this period (see below and Tinsley & Tocque, 1995) permitting the reversal of periodic environmental checks.

The second part of this account focuses on a 'test case' that reconstructs the effects of extreme environmental variation on the host-parasite interaction. The circumstances were provided by a period of severe summer drought in the study area, beginning in 1992 and intensifying in 1993 and 1994. The analysis considers the background to the environmental conditions and reviews data documenting the host and parasite populations from field studies conducted up to 1992. The after-effects of the drought conditions were recorded during summer fieldwork in 1995–1998 and the interpretation presented below provides a preliminary analysis of these results.

EFFECTS OF EXTREME ENVIRONMENTAL PERTURBATION ON THE HOST-PARASITE SYSTEM

The environment

Field study sites are located in the San Simon valley, Arizona, an area of desert scrub and desert grassland with mean annual rainfall of 223 mm/year (Tinsley & Earle, 1983). Long-term environmental records providing a general guide to conditions are available from two weather stations: Portal, covering the period 1914–1955, and the Southwestern Research Station, for 1965 to the present (http: www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?azport).
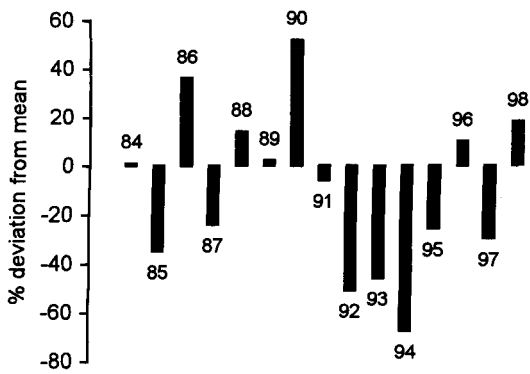
Fig. 4. Variations in July rainfall for the period 1984–1998. Zero represents the mean for this month based on the record of the Southwestern Research Station for 1965–1998. Bars for each year show the percentage deviation from this mean.

Both stations are at higher elevation than the desert field sites and have wetter and cooler conditions (mean for the 34 year record of the Research Station 541 mm/year, s.d. 120). Nevertheless, the annual profile of weather conditions is similar. Nearly half the annual rainfall occurs in July and August, facilitating a brief season of *Scaphiopus* activity until increasing aridity and declining temperatures bring surface activity to an end in September. Winter temperatures are cold and precipitation in November to April may fall as snow. Soil temperatures on the desert surface exhibit extreme variation: weekly minima are below freezing in November to March, but surface maxima are above 60 °C from April to September. In the buffered environment 15 cm beneath the soil surface, temperatures are below 10 °C for 4 months and below 15 °C for 6–7 months each year; at this depth, temperatures are above 25 °C for only 12 weeks each year (means of 3 years data from Tocque & Tinsley, 1991 *a*). An indication of rainfall variations influencing this study is provided by the records for July, normally the main period of surface activity for *Scaphiopus*. Fig. 4 compares July rainfall against the mean calculated from the 34 year data set (this is only a guide because conditions for host feeding and reproduction are also influenced by June and August rainfall). Other comparisons (below) employ 'percentage of the mean' using the average for the respective periods in the long-term weather record.

Assessment of environmental variation: rationale and interpretation

The seasonal restrictions imposed by the desert environment affect all aspects of *Scaphiopus* biology. Concentration of feeding into only a few weeks each year alternates with around 10 months of dormancy (and total starvation) and produces corresponding pulses of growth. In cross-section, bones show concentric growth rings which reflect age and annual

growth rates. An initial study was based on sectioned bones from an overall sample of 694 animals (femurs from toads dissected to record parasite infection or digits from mark-released live toads) collected in 1986–1992 (for methods and interpretation see Tinsley & Tocque, 1995). The present study, in 1995–1998, examined sectioned bones from 650 *S. couchii* from populations sampled at 13 sites along a 110 km transect (including all the sites from the earlier study). These field sites are given the labels A–M in the following account. Data for each dissected *S. couchii* included age, body weight and length, organ weights including fatbodies, haematocrit, together with records of total parasite infection. Detailed analysis is in progress (including an extension to 1999).

Age analysis of all individuals in the *P. americanus* populations was undertaken in 1996–98, using the criteria of body size and reproductive development outlined by Tocque & Tinsley (1991 *b*).

Bone growth rings provide sensitive information which correlates *Scaphiopus* ecology with environmental conditions (Tinsley & Tocque, 1995). The width of each growth ring reflects feeding success in the given period. Population analysis, covering growth rings laid down in 1978–1991, showed a strong positive correlation between ring width and summer rainfall: this determines the abundance of invertebrate prey populations, frequency of feeding opportunities (damp nights when toads can emerge to forage), and duration of the activity season (and hence the overall period to accumulate nutrients). The rainfall record shows that there was an alternation of relatively wet and dry years over the 14 year period (wetter in 1979, 1981, 1983, 1984, 1986, 1988, 1990, 1991 and drier in 1978, 1980, 1982, 1985, 1987, 1989). About 50% of the animals sampled showed a corresponding alternation of thick and thin growth rings. The age structure of toads in breeding populations showed a series of dominant cohorts (a high frequency of a particular age class); the years in which these were born correlate with the higher rainfall years, reflecting improved opportunities for spawning, increased survival to metamorphosis in temporary pools, and favourable conditions for recent metamorphs to feed.

The overall weather record shows relatively wide variations in rainfall with the total in the wetter years more than twice that in the drier. In some years, at some local sites, dry conditions may have prevented breeding. More commonly in harsh years, breeding did occur but recruitment failed, either because the ponds dried before metamorphosis or because the feeding opportunities after metamorphosis were insufficient to allow adequate growth and lipid storage. These are the years characterized by a very low frequency of the corresponding age cohorts of toads within the total population. However, the weather record from the early 1980s to the early

Table 1. *Resumé of rainfall patterns (hence general environmental conditions) and effects on host and parasite ecology, San Simon valley, Arizona 1993–98*

	Summer rainfall	Host spawning	Host recruitment	Host feeding	Parasite invasion
1993	+	+	0	+	+ +
1994	+	+	0	+	+ + + +
1995	+	0	0	+ +	0
1996	+ + + +	+ + + +	+ + + +	+ + + +	+
1997	+	+	+	+ +	+
1998	+ + +	+ + +	+ + +	+ +	+

Rainfall: below average in 1993, 1994, 1995 and 1997 (see Fig. 4), but extensive heavy July rains in 1996, 1998 with widespread flooding.

Host: general recruitment failure in 1993, 1994, 1995 producing a gap in maturing cohorts in 1995–97; first appearance of newly mature toads in mating assemblies in 1998.

Parasite: transmission successful in the 'drought years' 1993, 1994; invasion interrupted/poor in 1995 (local rainfall, little host spawning), 1996 (flooding), 1997 (local rainfall, little spawning) and 1998 (flooding); weak recruitment in some or all these years leading to reduced output of infective stages 2–3 years later; decline in infection levels in most populations but this was avoided at some local sites where transmission/recruitment was successful in 1996; otherwise some local populations reached point of extinction by 1998.

(negligible occurrence, 0; increasing extent of occurrence + → + + + +)

1990s, together with direct field observations, showed that conditions were sufficiently favourable, even in the worst years, for some breeding and foraging activity. The alternation of wet and dry summers led to the situation that, even if local conditions were exceptionally difficult in one year, better conditions in the next year would allow recovery (Fig. 4). Indeed, the evidence of this is provided in the alternation of wide and narrow growth rings in individuals and the regular succession of dominant age cohorts in the population age structure.

The adaptations of the parasite life cycle would predict a different scenario for the population biology of *P. americanus*. Recruitment into the parasite populations is likely to occur in most years as long as the toads assemble to spawn, regardless of the subsequent conditions determining host population recruitment. Studies at specific field sites during the period 1983–1990, when the rainfall pattern showed regular periodicity but never extreme fluctuations, showed that the parasite populations exhibited remarkable stability confirming relatively consistent recruitment (Tinsley, 1995).

A period of drier summers began in 1992 and continued through 1993 and 1994; effects were intensified by major reductions in spring rainfall, generally less than half of the mean, followed in each year by an exceptionally dry July (drier than all other years except one in the 34 year record of the Southwestern Research Station). August in 1993 was very wet but was followed by drought in September and October (combined rainfall only 16% of the mean), and any late breeding of *S. couchii* is unlikely

to have led to successful recruitment. The 12 months influencing the 1994 activity season (September 1993–August 1994) received about half the mean annual rainfall, and the July 1994 rainfall was only 32% of the mean for this month. This was focused into one storm which stimulated emergence and breeding of *S. couchii* but the ponds quickly dried up (Fig. 4).

This perturbation provides the background for this analysis of environmental effects on the host-parasite system: a major impact on population biology would be predicted. Following the 3 dry years, including severe drought in 1993 and 1994, the 4 fieldwork seasons of this study comprised two relatively dry summers (1995, 1997) and two relatively wet (1996, 1998) (Fig. 4). The disruption of *S. couchii* recruitment continued in 1995 when spring rainfall was 40% below average and summer rainfall was 30% below average. The July and August rains failed to produce torrential downpours necessary to create successful breeding sites. Initially, 1996 continued this trend with rainfall in December 1995–May 1996 only 22% of the average. However, June was exceptionally wet – over 6 times the average – followed by a very wet July, causing extensive flooding of the desert habitats. This led to repeated spawning assemblies across the range of field sites and the prolonged damp conditions are likely to have favoured survival and development of the cohort of metamorphosed toads. Total summer rainfall in 1997 was close to average but storms were localized and there was little *S. couchii* breeding in many of the study sites. The final field season, in 1998, included torrential downpours in July, with
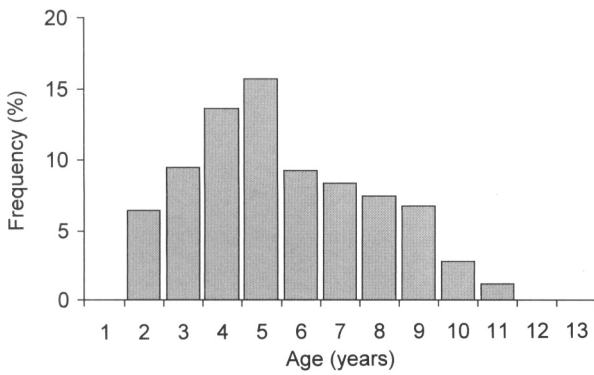
Fig. 5. Frequency distribution of age (numbers of growth rings) in male *Scaphiopus couchii* ($n = 359$) collected from breeding assemblies (data from Tinsley & Tocque, 1995). Combined sample of 7 years' records (1986–1992) from 4 study sites within a 7 km radius in the San Simon valley, Arizona.
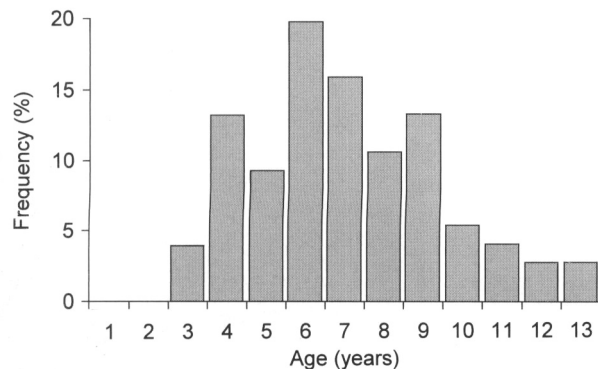

Fig. 6. *Scaphiopus couchii*: frequency distribution of age in the first post-drought year, 1995, based on a combined sample of 76 individuals from San Simon study sites, Arizona.

extensive flooding, and there was widespread *S. couchii* breeding. Key features are summarized in Fig. 4 and Table 1.

Host population ecology

Baseline information for the host populations at the main study sites is provided by Tinsley & Tocque (1995) derived from a combined sample of 7 years data, 1986–92 ($n = 518$). Counts of the annual growth rings in transverse sections of bone show that *S. couchii* is relatively long lived: most animals (65%) in breeding assemblies were at least 5 years old, about 33% were 7 years and older, and 5% were at least 10 years old. There are only minor differences between the survivorship of males and females: age at first appearance in breeding populations is 2 years for males and 3 years for females but greater numbers reach maturity in the following 1 or 2 years, contributing to an increasing frequency of representation of age classes up to 5 years; maximum longevity is slightly greater in females (13 years) than males (11 years) (Fig. 5 shows records for males).

However, these data do not constitute a general life table for *S. couchii* because the 7 years data set showed very different characteristics in each annual 'snap shot' influenced particularly by a succession of dominant cohorts. These reflect the outcome of successful recruitment originating in years particularly favourable for spawning and juvenile survival (relatively wetter years). Each dominant cohort formed a peak in the age distribution shifting by one year in each successive annual sample until it was replaced as the dominant cohort by the next successful age class (see Tinsley & Tocque, 1995). Because of this dynamic population structure, interpretation of life history characteristics is complicated. Nevertheless, the general patterns, including the record of recruitment (representation of the youngest age classes) and of longevity, provide important evidence of the effect of the period of severe drought on host ecology.

In 1995, the first "post-drought" field season, low intensity rainfall produced few waterbodies in which spawning was successful. However, conditions were ideal for feeding. Records of gut contents showed food intake equivalent to that documented by Tocque *et al.* (1995) which would have restored energy reserves and gonadal investment after the stress of the preceding drought years. Age analysis of population samples in 1995 suggests a slight shift to the right (to older age classes) in the sample of 76 *S. couchii* (Fig. 6). There were few animals in breeding assemblies with less than 4 annual growth rings: 2 year olds were missing and 3 year olds comprised only 4% of the population. This reflects the failure of recruitment in the preceding years, both from drying up of ponds before tadpole metamorphosis and high mortality of any post-metamorphs. For established adults, however, there is no evidence that the harsh conditions resulted in selective mortality. Indeed, modal age spans the 6th and 7th year classes in 1995 in comparison with the 4th and 5th years in the 1986–92 records and there is a greater representation of the oldest age classes (28% of toads are 10 years and older compared with 5% in the 7 year data set). Detailed comparison is complicated by cohort interpretation and the aggregation of data from discrete sites; however, there is no evidence from population age structure that adult toads experienced significant negative age-specific effects from the successive seasons of severe drought.

The following years' data demonstrate progressively ageing populations. At site H, one of the key sites followed since 1983, age structure showed a mean of 7 years in 1995, with 11% ($n = 19$) aged 10 years or older. In 1996 and 1997 ($n = 54$), over two-thirds (69%) were 7 and over, and in 1998 ($n = 31$), 42% of toads were aged 10 years and over (maximum 15 years). Nevertheless, this site received a trickle of young recruits (aged 2–4 years) (which depressed the mean age). At other sites, these younger age groups
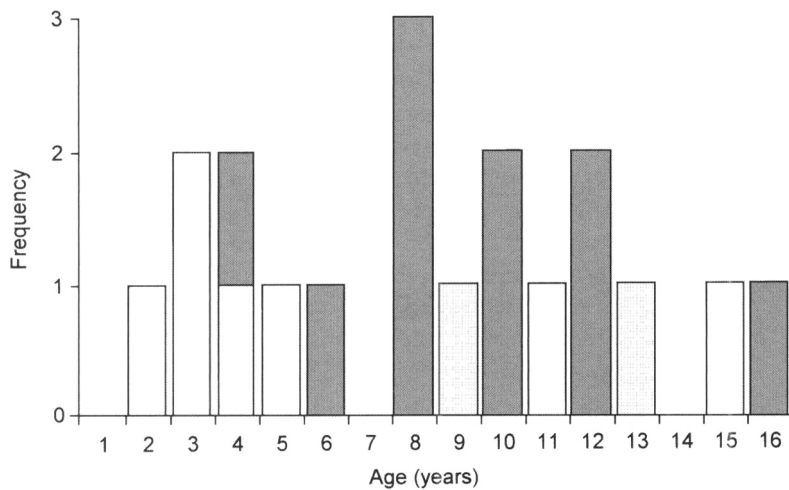
Fig. 7. Frequency distribution of age of *Scaphiopus couchii* at Site F, 1998 ($n = 19$). Shading represents *Pseudodiplorchis americanus* infection status: (□) uninfected (principally youngest individuals); (▨) infected by adults whose uterine larvae are retarded in development (not yet infective); (■) infected by gravid adult parasites. Wide host age range promotes transfer of infection between older and younger cohorts, especially to new recruits breeding for the first time and previously uninfected.
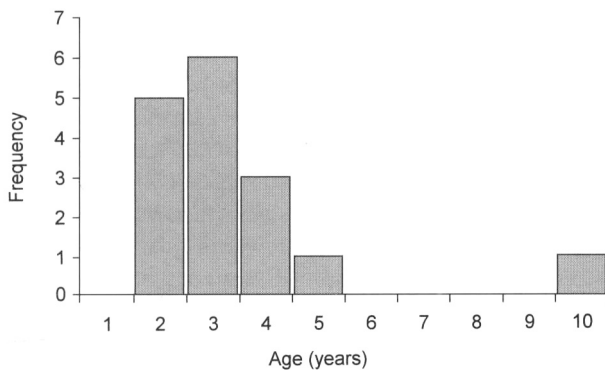
Fig. 8. Frequency distribution of age of *Scaphiopus couchii* at Site D, 1998 ($n = 16$), showing influx of young individuals breeding for the first time.

were lacking: thus, at site B in 1995, 1996 and 1997, mean age increased from 6·3 to 7·8 and 8·2 years respectively, and no toads in any year were aged < 6 years ($n = 29$). This confirms the repeated failure of recruitment. At site F, the 1995 sample indicated a relatively young population: mean 4·9 years, max 7·0 years, 78% aged 5 years or less. The next years illustrate negligible recruitment to balance increasing age: in 1996, mean 7·0 years, 36% aged 5 years or less ($n = 22$); in 1997, mean 7·6 years, 8% aged 5 years or less ($n = 13$).

In 1998, significant recruitment into breeding populations was recorded at some sites. At site F, an influx of younger toads occurred alongside the ageing individuals referred to above: in a sample of 19 toads virtually all year classes between 2 and 16 were represented, with 42% aged 10 years and older (exactly as at site H), but 32% aged 5 years and younger, and an overall mean of 8·4 years (Fig. 7). This appearance in the breeding assemblies of new recruits, 2 year olds from the wet summer of 1996,

provides evidence of the first successful breeding since the 1993–1994 drought years. Confirmation is given by successive samples from site D that show a major reversal in age structure. In 1997, the sample showed a mean age of 7·1 years with no individuals under 5 years; in 1998, mean age was 3·4 years: one toad had 10 annual growth rings, one had 5 rings, and all others (88%) had 2–4 growth rings (Fig. 8). The 2 ring animals were attributable unambiguously to the good spawning season in 1996 and probably represent the start of a new dominant cohort. The animals with 3–5 rings were atypical of their presumed age classes: comparison with Tinsley & Tocque's (1995) data set indicates that these were around 10 mm (up to 20%) shorter and 10–15 g (up to 50%) lighter than expected at age 3–5 years, falling instead within the size range of the 2 year olds. No toads with less than 5 annual rings occurred in samples from spawning aggregations at this site in the preceding years and it is likely that 15 of the 16 toads in the 1998 sample were first-time breeders. This provides evidence that, where *Scaphiopus* breeding and metamorphosis did occur in the drought years, 1993–95, the surviving recruits carry a signal of both stunted growth and delayed maturation.

In summary, this end-point in the series of field samples demonstrates that the *S. couchii* populations did experience a major check in recruitment for up to 3 successive years (from 1992), but the populations were sustained by ageing individuals whose longevity represents a very important survival characteristic. However, the eventual success of recruitment (from spawning in 1996) produced a major shift in population age structure and the new cohorts of younger age classes set in place the future survival of the breeding populations.
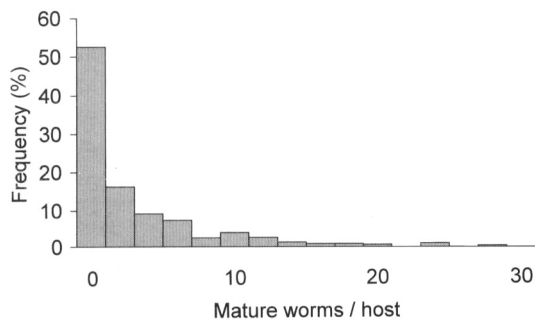
Fig. 9. *Pseudodiplorchis americanus*: frequency distribution of infection levels of gravid adult parasites in male *Scaphiopus couchii* ($n = 297$) preceding the period of extreme drought (data from Tinsley, 1993).
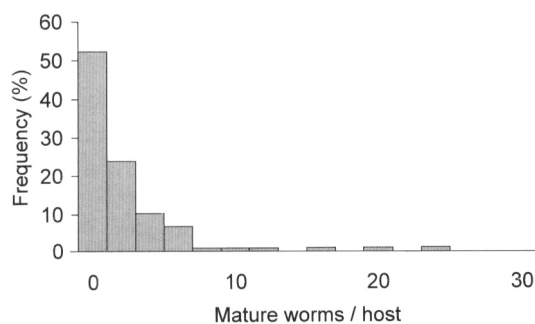

Fig. 10. *Pseudodiplorchis americanus*: frequency distribution of infection levels of gravid adult parasites in 1995, following the drought period ($n = 88$ *S. couchii*) (based on the same samples contributing to host age distribution in Fig. 6).

Parasite population ecology

Baseline data for the parasite suprapopulations is provided by the records of Tinsley (1993, 1995). Amongst male *S. couchii* sampled over several years in the 1980s ($n = 297$), the prevalence of adult (gravid) parasites was 48 % and mean intensity was 5·9 worms/host. The distribution of infection levels was over-dispersed: 25 % of toads carried small burdens of 1–4 worms/host and 9 % carried over 9 worms each up to a maximum of 27 (although this minority of the host population actually contains nearly half (48 %) of the total parasite population) (Fig. 9). This pattern of infection has been found to be remarkably stable in records from the same field area between 1983 and 1990, with prevalence consistently close to 50 % and an overall mean abundance across the 8 year data set of 3·1 worms/host (based on adult parasites with infective larvae *in utero*) (Tinsley, 1995).

At the end of the 'drought years', the records of gravid parasites in the same field area in 1995 provide a guide to effects on overall infection levels. In a combined sample of 88 toads, prevalence was 48 %, mean intensity 4·5 worms/host and mean abundance 2·4 worms/toad. Figure 10 shows a slightly higher representation of the lowest worm burdens; nevertheless, the pattern shown by this smaller sample is comparable with the 'pre-drought'

data, with 34 % of toads with burdens of 1–4 worms and 6 % with over 9 worms/host, maximum 23. Detailed comparison is influenced by the aggregation of data from various sites and the differences in sample size. Nevertheless, there is no suggestion of truncation of the distribution pattern attributable to density-dependent parasite-induced host mortality. Correlation with data on host population age structure for the same samples (Figs 5 and 6) also provides no indication of selective host mortality: as noted above, the age profile shows a slight shift towards older age classes and a decrease in representation of the youngest ages probably reflecting reduced recruitment. This youngest fraction of the host population is not infected until first entry into the breeding assemblies so its poorer representation cannot reflect potential negative effects of infection. Mean and maximum ages are slightly increased and there is no reduction in the 'middle' year classes (6–8 years) where worm burdens tend to be heaviest (Tinsley, 1995).

More precise interpretation of the outcome of parasite transmission was undertaken in 1996–98 with age analysis of all parasite individuals using the criteria defined by Tocque & Tinsley (1991*b*). At site E in 1996 (Table 2), the overall reproducing parasite population (prevalence 58 %, mean abundance 2·0 worms/toad) is attributable almost entirely to a single large cohort of worms, those invading in 1994 (prevalence 54 %, mean abundance 1·9). There are no parasites representing the 1993 transmission season and input in 1995 was very weak (prevalence 13 %, mean abundance 0·2). This age distribution is likely to have had a negative effect on transmission in 1996: according to Tocque & Tinsley (1991*b*), the output of oncomiracidia from 3 year old worms normally contributes 40 % of total output from the suprapopulation (because of the much greater *per capita* production of offspring by these older worms). This contribution was entirely lacking in 1996. The second important feature of these data concerns future transmission. The large cohort of 2nd year worms present in 1996 is likely to be much reduced in 1997: Tocque & Tinsley's analysis indicated a 50 % decrease in worm numbers between years 2 and 3. Moreover, the very small 1995 cohort would have made a limited contribution to transmission in 1997 and 1998 when the age-specific contribution to larval output should be greatest. This age analysis shows that, although overall infections appear relatively robust, the strength lies in a single age class and this identifies a future weakness in transmission potential regardless of invasion success in 1996.

The same effects are evident at other breeding/transmission sites in the 1996 season. At site I (20 km south of site E), overall infection levels appear moderately high in comparison with the long-term, baseline data (prevalence 41 %, mean abundance 3·1) but, as at site E, a single year class

Table 2. Characteristics of *Pseudodiplorchis americanus* infection in *Scaphiopus couchii* (*n* = 24) at Site E, 1996

Parasite age/state	Cohort origin	Prevalence	Intensity	Mean abundance
Delayed/ non-gravid	1995	0	0	0
1st year gravid	1995	12·5	1·3	0·17
2nd year gravid	1994	54·2	3·5	1·88
3rd year gravid	1993	0	0	0
Total gravid	1993/5	58·3	3·43	2·00

Table 3. Characteristics of *Pseudodiplorchis americanus* infection in *Scaphiopus couchii* (*n* = 17) at Site I, 1996

Parasite age/state	Cohort origin	Prevalence	Intensity	Mean abundance
Overall 1st year	1995	11·8	3·0	0·35
Delayed/ non-gravid	1995	5·9	3·0	0·18
1st year gravid	1995	5·9	3·0	0·18
2nd year gravid	1994	35·3	6·5	2·29
3rd year gravid	1993	5·9	11·0	0·65
Total gravid	1993/5	41·2	7·57	3·12

Table 4. Characteristics of *Pseudodiplorchis americanus* infection in *Scaphiopus couchii* (*n* = 21) at Site J, 1996

Parasite age/state	Cohort origin	Prevalence	Intensity	Mean abundance
Overall 1st year	1995	47·6	4·9	2·33
Delayed/ non-gravid	1995	28·6	4·5	1·29
1st year gravid	1995	19·0	5·5	1·05
2nd year gravid	1994	66·7	5·7	3·81
3rd year gravid	1993	4·7	2·0	0·10
Total gravid	1993/5	66·7	7·21	4·81

(1994) is responsible for most of the suprapopulation (Table 3). Poor invasion success in 1995 is reflected by infections in only 2 out of 17 toads, carrying only 15 % of the total worm burden present in the 1994 cohort; only a single animal carried infection from 1993. Again, the very small 1st year class has

Table 5. Characteristics of *Pseudodiplorchis americanus* infection in *Scaphiopus couchii* (*n* = 13) at Site F, 1997

Parasite age/state	Cohort origin	Prevalence	Intensity	Mean abundance
Overall 1st year	1996	61·5	4·4	2·69
Delayed/ non-gravid	1996	30·8	3·8	1·15
1st year gravid	1996	46·2	3·3	1·54
2nd year gravid	1995	23·1	2·3	0·54
3rd year gravid	1994	7·7	2·0	0·15
Total gravid	1994/6	69·2	3·22	2·23

important future significance for reduced larval output over the following two transmission seasons. The 1995 cohort also shows the retarded development of some parasites and their failure to produce infective stages in 1996: this probably reflects invasion occurring relatively late in the host's activity season (see above). At site J (18 km south of site I), there was effective transmission in 1995 (prevalence 48 %, mean abundance 2·3) but over half of the surviving worms failed to complete development within the year, probably because of late transmission and delayed migration (Table 4). This site confirms the other indications that 1994, a year characterized by severe drought, was highly successful in terms of parasite invasion (prevalence 67 %, mean abundance 3·8 of two year old worms) with about 40 % more worms than in the 1st year class. Again, the 1993 year class is very weakly represented, contributing little to overall larval output.

It is striking how consistent are the general trends in population dynamics from these and other widely separated sites across the desert. In summary, the age analysis revealed that production of infective stages by the parasite suprapopulation in 1996 depended disproportionately on only 1 of the 3 parasite year classes (invading in 1994). A significant impact on transmission success would be predicted. In subsequent years, the negative influence of the weak 1993 cohort on total larval output would be displaced, but the small 1995 cohort could cause a ripple effect on transmission for 3 years with the potential to prejudice future survival. Two alternative outcomes became evident over these subsequent years: recovery at some sites and extinction at others.

The outcome of environmental perturbation: recovery

In 1997, at site F (Table 5), the parasite suprapopulation shows the 'tail-end' of the widely successful 1994 cohort, now reduced to 3rd year

Table 6. Characteristics of *Pseudodiplorchis americanus* infection in *Scaphiopus couchii* (*n* = 19) at Site F, 1998

Parasite age/state	Cohort origin	Prevalence	Intensity	Mean abundance
Overall 1st year	1997	36·8	2·3	0·84
Delayed/ non-gravid	1997	10·5	4·0	0·42
1st year gravid	1997	26·3	1·6	0·42
2nd year gravid	1996	36·8	4·4	1·63
3rd year gravid	1995	21·1	2·3	0·47
Total gravid	1995/7	53·0	4·80	2·53

worms (prevalence 8 %, mean abundance 0·15). This is accompanied by a weak representation of 2nd year worms (from the 1995 season) (prevalence 23 %, mean abundance 0·54). Thus, the two year classes that should, according to the analysis of Tocque & Tinsley (1991 *b*), make a combined contribution of over 80 % of the infective stages released into transmission sites, are represented by a tiny population (combined mean abundance only 0·7 worms/toad). This confirms the predicted effect on future transmission. However, the 1997 sample demonstrates that, at this site, the 1996 invasion was relatively successful. Overall prevalence of 1st year parasites in the 1997 host mating assembly was 62 %, mean abundance 2·7. Their potential contribution to transmission was greatly reduced because 43 % of the worms had failed to complete reproductive development. Nevertheless, this 1996 cohort provides a basis for the recovery of the parasite suprapopulation in future years.

Confirmation of this recovery is provided by data for this same site in 1998 (Table 6). The "good" 1994 age class has now disappeared and the 1996 cohort has a dominant role. In this sample, the numbers of surviving 2 year old worms represent only 60 % of the total recorded in the previous year originating from the same invasion. Nevertheless, this cohort contains more parasites than the 1st and 3rd year classes combined. The 1995 cohort is represented by only 9 parasites amongst the 19 toads. The 1997 cohort reflects poor transmission and development was also disrupted since 50 % of the parasites failed to produce infective offspring during the year. However, the contribution from all 3 years combines to represent a transmission potential (prevalence 53 %, mean abundance 2·5 gravid worms/toad) close to the original baseline levels (prevalence 50 %, mean abundance 3).

Despite this superficial similarity to 1983–1990 infection levels, the 1998 sample at this site is too

heterogeneous for detailed comparison. The age profile of the host population comprises 2 distinct subsets (Fig. 7). Sixty three per cent of the sample is aged 8 years and older (up to 16 years), and 83 % of these carry *P. americanus* infections (gravid and non-gravid) from infections in 1995–1997. These older toads represent a major influence on transmission. The remainder of the sample comprises young toads, including a majority of 2–4 year olds (probably first-time breeders and all uninfected). This influx of naïve toads reduces prevalence and abundance. However, the overlap in time and space between these older and younger host generations would allow the transfer and establishment of a subset of reproducing parasites in the toads that will contribute to future breeding (and transmission) assemblies.

The outcome of environmental perturbation: extinction

An alternative outcome of the ripple-effect, attributable to poor transmission success beginning in 1995, is evident in other 1998 field samples. At a series of sites where, in the 1983–90 record, infection levels had been consistently relatively high, the 1995–97 samples showed a year-on-year decline in worm burdens. These were sites where the 1996 season, characterized by extensive flooding, did not result in the recruitment required to reverse the downward trend. In the 4th year of the series, this led finally to the disappearance of *P. americanus*. Thus, at site D, a sample of 16 male *S. couchii* carried no established parasite infections in 1998. The age structure data for the host population sample makes an important contribution to interpretation of this outcome (Fig. 8). Since *Scaphiopus* is not infected before breeding, the mass appearance in the mating assembly of toads that had not previously bred has the direct effect that *none* of these animals (94 % of the sample, 2 year olds together with stunted 3–5 year olds) would have had pre-existing (reproducing) *P. americanus* infection. As outlined above, this major shift in host age structure – to young age classes – is important for the future survival of the *Scaphiopus* populations. However, it has a major negative effect on parasite prevalence. Indeed, since the single older toad in the sample (10 years old) was also uninfected, all contribution to transmission by this fraction of the overall host population was abolished (Fig. 8).

Since parasite transmission is almost instantaneous, samples of the hosts aggregated in breeding assemblies provide two distinct assessments of the parasite suprapopulation. First, the animals examined provide a direct representation of pre-existing infection in this subset of the host population, i.e. parasites established for at least one year since the last exposure to infection. Second, these

same animals contain evidence of larval invasion, derived in the few hours before collection, that provides an indirect reflection of adult parasite occurrence in the wider host population. Amongst the sample of 16 toads at Site D, one carried a single post-oncomiracidium in the nostrils. This could not have been derived from any individuals in the population sample; instead, it indicates that the breeding assembly (of about 50 toads at the time of sampling, at 02·00 h) did contain at least one gravid *P. americanus* that contributed to transmission. However, from this measure of wider invasion success within the host population (mean abundance 0·06 worms/toad), and the estimates that fewer than 3% of invading larvae survive to maturity (see above), it may be predicted that successful recruitment into the reproducing adult parasite population in 1999 is highly unlikely. At this site, therefore, the 1998 data suggest that the parasite population is at the point of extinction.

DISCUSSION

Extreme environmental perturbation

This study provides new insight into parasite population dynamics based on the application of techniques to determine age in both parasite and host individuals. Specifically, the fieldwork area in Arizona experienced a period of exceptional drought, with 3 successive years of the lowest summer rainfall in a 34 year weather record. The data, in combination with long-term records of the parasite and host populations at specific sites in the desert, have revealed effects operating over a time-course of several years after this major environmental perturbation.

Effects on the host populations

Analysis of age structure of the *S. couchii* populations provided no evidence for major mortality of already established adult populations; instead, these were found to have aged progressively until, in 1997, nearly half of some populations were aged 10 years or more (maximum 17 years). Survival can be attributed to an exceptional ability to feed intensively, even with limited opportunities, and to store lipid reserves for prolonged hibernation. Field studies show that some *S. couchii* contain sufficient fat to survive two years of dormancy and total starvation (Seymour, 1973; Tocque, 1993; Tinsley, 1995). Whilst the population age structure data provided no indication of unusual adult mortality, the effect of several successive years of failed recruitment at some sites was clearly demonstrated by a complete absence of the youngest cohorts in the age profile. Baseline data for the same *S. couchii* populations sampled in

1986–92 indicated that, on average, 35% of individuals in breeding assemblies were aged 2–4 years representing recent recruits, including first-time breeders. By 1997, when the succession of recruitment failures had gained maximum impact, there were no individuals aged under 7 years at some breeding sites. Most of the offspring born in the drought years had probably died, although the 1998 data demonstrated that there were some survivors characterized by stunted growth and delayed maturation. The ability of *S. couchii* populations to tolerate a succession of years when recruitment is severely reduced can be attributed to the relatively long life span of this species. Toads aged up to 16 years (female) and 17 years (male) spawned successfully in mating assemblies. Host longevity enables the populations to 'ride out' an environmental check until recruitment can be resumed in favourable years. In this study, 1996 provided exactly these conditions for recovery with twice the mean June and July rainfall, the highest in the 34 year Southwestern Research Station record. This enabled widespread successful breeding across flooded desert habitats with metamorphosis sufficiently early in the season to allow good feeding in preparation for hibernation. The outcome was reflected in the mass appearance of 2 year old adults entering breeding assemblies for the first time in 1998 and likely to represent a dominant cohort in succeeding years.

At the start of this study on the effects of the severe drought, it was not known that adult toads could survive such restriction in their activity season (limiting opportunities to feed and accumulate lipid reserves for hibernation), nor that age structure would shift into such old age classes. Now, given this knowledge, the effects of the successive drought years appear relatively straightforward: a failure to recruit for 3 years in succession led to a progressively ageing population which subsequently reproduced very successfully with the return of favourable conditions. For the parasite populations, the effects were not as straightforward.

Effects on the parasite populations

The parasite population data provide conclusive evidence that the 1994 season, characterized by exceptionally low rainfall and almost complete failure of host recruitment, resulted in highly effective transmission. This outcome created a signal recognizable in widespread high prevalence and intensity of *P. americanus* infection, specifically of 2 year old parasites in 1996 and 3 year-olds in 1997. A single major storm occurred in July 1994 which prompted mass spawning, but the newly-created ponds quickly dried up and most *S. couchii* progeny died. In contrast, parasite recruitment is geared to short-term episodes of transmission (each a maxi-

mum of 7 h), so the single period of spawning – although subsequently unsuccessful for the host – will have permitted mass parasite invasion.

The link between parasite transmission and host spawning guaranteed successful invasion in 1994 despite the brief exposure. However, the same principle was responsible for very weak transmission in 1995: here, limited rainfall in the form of low intensity showers prompted *S. couchii* emergence and provided good feeding but there was insufficient runoff to create breeding ponds. In this year, therefore, there was little host spawning and, significantly, little parasite invasion. At widely separated sites across the desert, this outcome was recorded in very low infection levels of 1st year worms in 1996, 2nd years in 1997 and 3rd years (at some sites) in 1998.

Paradoxically, the very heavy rainfall of 1996 that was responsible for major host breeding had a variable effect on parasite transmission. In many areas, discrete breeding ponds that usually form in shallow depressions, including ditches, were transformed into large expanses of moving floodwater. Spawning toads, that typically occur in high-density aggregations, were dispersed, and parasite infective stages, usually released into confined bodies of standing water, would have been washed away. At some study sites, a cohort of parasites confirmed that transmission did occur; however, at most others, this year class was missing.

The summer of 1997 rainfall pattern closely resembled that in 1995 (almost identical June and July combined rainfall in these 2 years) and produced a third successive year in which invasion levels were much reduced.

Local variations in rainfall intensity are likely to have produced conditions at some sites favourable for parasite transmission, irrespective of the dominant environmental influence recorded at a majority of other sites. Thus, in rare cases there was a good 1995 year class recorded in 1996 (e.g. site J, see above), and, as mentioned, a good 1996 year class in 1997 (e.g. site F). Nevertheless, despite these local exceptions, the rainfall patterns during the period 1995–1997 were sufficiently pronounced to produce a strong negative effect on the annual succession of parasite age cohorts. At some sites, 3 successive years of poor recruitment could no longer be tolerated and the parasite suprapopulations were virtually extinct in 1998.

Factors contributing to extinction

The present analysis provides detailed evidence for the sequence of events leading to local extinction. Three factors contributed. First, the succession of years (1993, 1994 and 1995) in which *S. couchii* recruitment was precluded led to a shift in host population age structure that had a knock-on effect on the parasite populations. The lack of input of the youngest age classes into host mating aggregations, especially those breeding for the first time, removed the normal 'diluting effect' of uninfected (previously unexposed) immigrants leading to an increase in parasite prevalence. The increase in the proportion of older toads increased the representation of age classes that typically have lower infection levels, particularly the absence of the highest worm burdens. This effect would, to some extent, counteract the increase in prevalence caused by the lack of the diluting influence of the youngest cohorts but, more significantly, it would be predicted to result in a decrease in intensity.

Second, the parasite populations also experienced reduced recruitment over several years (1995, 1996 and 1997). Because parasite transmission depends heavily on worms that are 2 and 3 years old, missed invasion has a delayed effect on future reproduction: it produces a ripple in the succession of parasite age classes so that there is greatly reduced output of infective stages 2 and 3 years after the poor recruitment. In some of the present study sites, the trough in transmission potential, attributable to reduced invasion beginning in 1995, exerted its maximum effect in 1997 and 1998.

Third, successful host breeding in 1996 led to the mass appearance of newly matured recruits in the mating sites in 1998. At the same time, older toads (with 3–5 growth rings) that represented the stunted survivors of recruitment in the drought years also entered the mating sites as delayed first-time breeders. The effect on host population age structure was a sudden massive switch from older age classes (at some sites 40% of toads were > 10 years and none were < 7 years) to the youngest (70% or more of toads aged 2–3 years). Significantly, the overall population was swamped with first-time breeders that had never previously been exposed to *P. americanus* invasion (94% of the sample collected at Site D) coinciding with minimum population size of reproducing parasites in the remainder of the host population. Thus, parasite extinction was determined by the combination of three independent factors involving the delayed effects of events affecting the host populations 2–5 years previously and the parasite populations 1–3 years previously.

The longer-term data set reveals that each of these events may be intermittent features of the host-parasite interaction. Records of *S. couchii* population dynamics by Tinsley & Tocque (1995) showed a series of years – 1986, 1987 and 1988 – in which modal age was influenced by a dominant cohort of toads aged 5, 6 and 7 years in the successive annual samples (originating from a very wet summer, favourable for breeding and juvenile survival, in 1981). These host age-classes would be expected to have maximum infection levels with a succession of parasite age classes contributing to transmission. In

contrast, in 1989, modal age was 3 years (comprising about 30% of the total population) originating from a wet summer in 1986 and including a majority of first-time breeders (uninfected by *P. americanus*) that would have greatly reduced prevalence and transmission. Similarly, in 1990 there was a major influx of 2 year-old toads (originating in 1988) that would also have made no contribution to transmission. However, in each of these data sets there was a spectrum of other age classes which would have maintained the reproducing parasite population and served to infect each annual influx of new recruits. In the present study, this reservoir of older infected toads was at a minimum and the continuity between older and younger host generations, important for parasite population stability, was broken.

Factors involved in recovery

Although local parasite extinction was documented at a series of sites, parasite population recovery occurred at others. Two key features distinguish the sites, D and F described above, where these contrasting outcomes were recorded. First, recovery was attributable to local conditions that promoted successful invasion in 1996 and interrupted the succession of poor recruitment seasons in 1995–97. Second, where recovery occurred, there was a spatial and temporal overlap in the two discrete subsets of the host population comprising older toads with reproducing parasite infections and young, naïve, recruits without pre-existing infections. These circumstances promoted transfer of infection between the host cohorts at Site F (Fig. 7). At this and other sites analysed in 1998, there is an apparent trend towards lower worm burdens. Interpretation is affected by the statistical chance of locating relatively rare high intensities in small samples (combined total $n = 108$ in 1998). If the worm burdens encountered are representative of the meta-populations of the wider study area, then loss of the highest burdens could have important effects on the host-parasite interaction. It would remove the infections in which the greatest pathogenic effects are evident. Tocque & Tinsley (1992) quantified the parasite-induced removal of host blood, and Tocque (1993) and Tocque & Tinsley (1994b) demonstrated a density-dependent effect of infection on host blood composition and fat reserves. A trend towards lower worm burdens could produce the outcome, at a population level, that *P. americanus* becomes more 'benign'. It would also remove those infra-populations that make the greatest contribution to reproduction: Tinsley (1993) showed that 9% of toads, those with the highest burdens, carry 48% of the total parasite population. Their elimination would reduce the intensity of transmission and hence future recruitment into the reproducing parasite population. Coincidentally, a reduction in the

highest worm burdens would also reduce the competitive interactions demonstrated by Tocque & Tinsley (1991b) and hence intraspecific negative effects on transmission. These trends would be predicted from the experimental studies carried out on this system but confirmation awaits further data analysis and examination of samples taken in 1999 (in progress).

Loss of the heaviest worm burdens could be attributed to density-dependent, parasite-induced host mortality. However, the present comprehensive data provide no evidence for this. The host population age profile shows no indication of selective mortality of age-classes that have the highest worm burdens (typically 6–8 year olds) nor of the oldest ages that might have accumulated the effects of repeated challenge. Indeed, in the absence of effective recruitment, mean host age increased smoothly across the years of this study. Instead, a loss of high burdens would be better attributed to an increase in host resistance (larger numbers of older hosts that typically have lower infection intensity) and a reduction in invasion success.

Life cycle characteristics

Given the superb adaptations of *P. americanus* to prepare during the long period of host inactivity for the moment of transmission, the very brief episode of host-to-host transfer seems to be accomplished with great ease. Indeed, this may be quantified by the calculation that the probability of individual invasion success is about 0·3. Where equivalent data exist for helminths that produce hundreds, thousands or even hundreds of thousands of offspring/parasite/day (see for instance Tinsley, 1990a), comparison suggests that *P. americanus* has one of the highest transmission efficiencies amongst helminth parasites. For the few hours each year of host-to-host transfer, *P. americanus* operates in "ancestral mode", equivalent to a fish parasite; significantly, however, this efficiency is maximized because all the difficulties of host availability and host location, inherent in most parasite life cycles, are reduced to a minimum.

Insight into the optimum circumstances for transmission is provided by the contrasting outcomes of the 1994 and 1996 seasons. Severe drought in 1994 (July rainfall only one-third of the mean) produced intense transmission because this was focused into the few, restricted host breeding sites. The very wet conditions in 1996 (beginning early with June rainfall 6 times the mean) produced poor parasite recruitment because of extensive flooding. Thus, the life cycle is most efficient when conditions for host-to-host transfer are, by comparison with other parasites employing aquatic invasion, most extreme.

For this parasite, adapted for transmission in a desert, the most hostile environmental conditions

begin once it is within the host. No other mono-geneans experience such diverse physiological conditions as *P. americanus*. Developing stages in the respiratory tract live at the air/water interface and, whilst in the lungs, are exposed to surfactants. Migration through the digestive tract involves exposure to extremes of pH, digestive enzymes, bile and to anaerobic conditions. Within the urinary bladder, the worms experience wide variations in urine composition: little or no urination occurs during the 10 months of host hibernation each year and there is a progressive increase in the osmolarity of the urine, up to 200 osmol/l, as urea accumulates and water is withdrawn by the host (Ruibal *et al.* 1969) (Fig. 1).

Migration of *P. americanus* through the digestive tract involves exposure to environmental conditions that are, by all criteria, extreme. Of course, the other major helminth groups have all adapted to survive conditions in the gut but the challenge to *P. americanus* must be assessed alongside the capabilities of the class Monogenea in which the overwhelming majority of the c. 1500 species occur on the external surfaces (skin and gills) of fishes and therefore experience an aquatic environment. The severity of the conditions within the digestive tract for *P. americanus* is clearly demonstrated by the rapid death (in 1–2 min) of pre-migrants transferred experimentally to stomach or intestinal contents (Tinsley & Jackson, 1986). That migrating worms can tolerate these conditions for 4–8 h is a measure of the effectiveness of their tegumental adaptations.

Whilst these adaptations for gut migration represent a unique response to hostile conditions, this component of the life cycle also creates a weak link. Because migration requires a host cue that is linked to external environmental conditions (promoting host activity), the course of parasite development is prone to disruption by chance variations in rainfall and temperature. The present age-specific data highlight the population consequences of this life cycle requirement. The disturbed weather patterns occurring during this study were probably responsible for the outcome, noted in many local populations cited above, that up to half of each invading cohort failed to complete reproductive development, precluding a contribution to transmission one year p.i. This vulnerability of the life cycle design can result in a loss of one year of reproductive output out of a normal maximum of three.

CONCLUSIONS

The environmental perturbations recorded in this analysis proved to have more serious effects on the parasite than its host. In both cases, recruitment was interrupted (to a greater or lesser extent) for 3 successive years: 1993–95 for the host and 1995–97

for the parasite. In both, the effect was recognizable in terms of missing year classes in the succession of annual age cohorts. The different outcomes are attributable to differences in longevity. The relatively long-lived host can tolerate a succession of years when recruitment fails until population numbers are restored in years favourable for offspring survival. For the parasite, however, which lives for up to 3 (very rarely 4) years, interruption to the succession of annual cohorts has serious consequences for future transmission. This study emphasizes the relatively long time course of these perturbations, particularly that the major effects of failed parasite recruitment occur 2 and 3 years afterwards when these year classes would normally make their greatest contribution to further transmission.

The overview of the population biology of *P. americanus* has implications at three levels for assessing environmental constraints on parasite survival. First, the desert environment, characterized by extremes of physical conditions that are hostile to organisms without specific adaptations, normally has only relatively minor effects on the survival of *P. americanus*. Clearly there are major constraints on both the host and its parasite which restrict 'external' activity to relatively brief episodes: feeding, breeding and other desert surface activities for the toad, and host-to-host transmission for the parasite. For both partners, the major part of the year is spent protected from the harsh external environment: the host buried in deep burrows beneath the soil surface, and the parasite buried within the host. A major part of the adaptation to the desert environment involves preparation to exploit the briefly-favourable conditions and, particularly, the precise timing of the response. Clearly, the remarkable and in many cases unique specializations of *P. americanus* are absolutely essential to allow a member of a parasite group so apparently ill-suited to desert conditions to exploit its elusive host. Thus, it would be difficult to envisage how a parasite could cope with the unpredictable period between transmission episodes and yet release infective larvae instantly, when the opportunity arises, without a very special mechanism of *in utero* nutrition of embryos and, then, an immediate response to a precise oviposition trigger (see above).

Counterintuitively, the most important constraint imposed on this system by the desert environment concerns low temperatures rather than high. The winter temperatures in the Sonoran Desert create a powerful negative effect on the rates of parasite development and reproduction. The temperature threshold – around 15 °C – determines that development and reproduction can normally proceed only between May and October. This regulates the ability of the parasite to complete reproductive development between transmission seasons and the numbers of

infective stages available for infection. From a critical viewpoint therefore, the external environment in the Sonoran Desert does represent a significant constraint on the life cycle of *P. americanus*, but this is not a function of any its *extreme* characteristics. In comparison with this specialized desert environment, exactly the same environmental check on parasite population biology occurs in the equable temperate habitats of *Discocotyle sagittata*, *Fasciola hepatica* and *Ascaris lumbricoides*. In these unrelated helminths, development of stages in the external environment (especially eggs) is halted below 10 °C for the same period, over half of each year, between October/November and May (see Gannicott & Tinsley, 1998 *a*, *b*). On the other hand, paradoxically, the low temperature check in the *P. americanus* life cycle has its most important effect to the benefit of the parasite, increasing parasite survival (see below).

Second, the greatest attrition of the parasite populations occurs within the host. Whilst the probability of successful host-to-host transfer is at least 0·3, the probability of subsequent survival until the first opportunity for onward transmission is < 0·03. A series of factors are involved, including the hazards of migration along the alimentary tract. However, the major fraction of parasite mortality appears best interpreted as a function of a temperature-dependent host immune response (Tinsley, 1995 and references therein). The conclusion, that the environment created by host defences represents the most hostile part of the parasite life cycle, is not novel. However, this *Scaphiopus–Pseudodiplorchis* system provides comprehensive data for quantitative comparisons. Annual infections disappear in 50 % of the host population (perhaps the resistant fraction of the population) but worm burdens are reduced in all toads. Field observations are mirrored by laboratory experimental findings confirming a progressive decline in worm burdens with time. The margin between parasite survival and elimination is remarkably narrow: 'normally' only about 3 % survive from invasion to the first contribution to reproduction.

For a host-parasite system involving an ectothermic vertebrate host, this second environmental constraint on parasite biology – immunity – clearly interacts with the first, the seasonal variations in temperature. Indeed, laboratory experiments show that if environmental temperatures remain high throughout the year then *P. americanus* would not survive from one transmission season to the next. Temperature therefore has a dual effect on parasite population dynamics: first, direct temperature-dependent effects influence the rates of parasite life history processes, and second, indirect effects operating through host physiology influence the ability of the immune system to control the parasite. As outlined by Tinsley (1995), significant departures

from the temperature cycles normally experienced by the *Scaphiopus–Pseudodiplorchis* system could result in parasite extinction and hence may exert a major influence on geographical distribution (unless there are geographical variants with different temperature thresholds).

The third level of environmental constraint documented in this study is distinguished by its ability to cause local parasite extinction. The severe drought that triggered this effect constitutes a rare event (by the evidence of the lowest summer rainfall in the existing records for the area); the fundamental challenge to parasite survival was created by the series of consecutive years of recruitment failure attributable both to summers that were too dry and too wet. A 3 year parasite life span actually provides considerable buffering against occasional episodes precluding transmission. However, the inability of *P. americanus* to cope with a succession of such constraints could be predicted from knowledge of this life span. Tocque & Tinsley (1991 *b*) noted the rare occurrence of a parasite (1 out of 178 worms in their study) that survived to 4 years. The relevance of this trait includes both an ability to bridge a more extended interruption to recruitment and a marked age-dependent increase in reproductive output. Thus, this single 4 year old worm contained a complement of infective stages equal to that in the total of 73 first year worms in the same suprapopulation sample. In areas where repeated recruitment failures are frequent, such differences in parasite longevity, if heritable, would have important selective advantage and would provide the basis for future evolution by *P. americanus* in its adaptation to extreme environmental constraints. The present data emphasize that the environmental effects leading to parasite extinction were also intimately linked with the succession of drought-induced recruitment failures of the host: this broke the overlap between older infected and younger naïve cohorts within the *S. couchii* mating populations.

Each of the environmental constraints encountered in the life cycle of *P. americanus* is met by superb adaptations. Many of the specializations are unique and their combined effect is that the external environment normally exerts only a moderating influence on parasite population biology. In contrast, the severity of the conditions imposed within the internal host environment creates an almost overwhelming constraint. The attrition of parasite stages in the lungs and bladder has the hallmarks of an immune response, although evidence is so far only circumstantial (see Tinsley, 1995): it may make a major contribution to the pre-reproductive mortality of at least 97 % of each invading parasite cohort. The value of this assessment of parasite survival in extreme desert conditions is that it puts into perspective the relative difficulties of the external and internal environments. With appropriate

adaptations, this monogenean parasite experiences rate-limiting regulation by the external factors, but these are principally temperature-dependent effects common to most environments. Many aspects of the life cycle require special adaptation to other constraints occurring within the host (such as the extreme conditions that are a natural feature of the gut). However, the conclusion emerges that the interaction with the living reactive host environment – and its response directed specifically at the parasite – creates a far greater constraint on parasite survival. Thus, whilst this paper has focused on the desert environment to illustrate the evolution of remarkable parasitic adaptations, the analysis serves additionally to reinforce the conclusion that the most severe conditions in this case study may actually be found in every parasite life cycle – those created by the host.

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