The ups and downs of life: population expansion and bottlenecks of helminth parasites through their complex life cycle

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SUMMARY

The fundamental assumption underpinning the evolution of numerous adaptations shown by parasites with complex life cycles is that huge losses are incurred by infective stages during certain transmission steps. However, the magnitude of transmission losses or changes in the standing crop of parasites passing from upstream (source) to downstream (target) hosts have never been quantified in nature. Here, using data from 100 pairs of successive upstream–downstream life stages, from distinct populations representing 10 parasite species, we calculated the total density per m^2 of successive life stages. We show that clonal amplification of trematodes in their first intermediate host leads to an average 4-fold expansion of numbers of individuals at the next life stage, when differences in the longevity of successive life stages are taken into account. In contrast, trophic transmission to the definitive host results in almost no numerical change for trematodes, but possibly in large decreases for acanthocephalans and nematodes, though a correction for longevity was not possible for the latter groups. Also, we only found a positive association between upstream and downstream stage densities for transmission involving free-swimming cercariae in trematodes, suggesting a simple output-recruitment process. For trophic transmission, there was no coupling between downstream and upstream parasite densities. These first quantitative estimates of ontogenetic rises and falls in numbers under natural conditions provide new insights into the selective pressures acting on parasites with complex cycles.

Key words: complex life cycles, transmission mode, parasite evolution, helminths, longevity, trematodes.

INTRODUCTION

The complex life cycles of parasitic helminths consist of series of challenging transmission events from one host to the next. Explanations for the multiple adaptations shown by these parasites are founded upon the reasonable assumption that transmission success at each stage is generally very low, with only a fraction of individuals making it to the next stage of the cycle. Adaptations such as high fecundity relative to body size in adult worms (Jennings and Calow, 1975; Poulin, 1996), elaborate host-finding behaviours in free-living infective stages (Combes *et al*. 1994; Haas *et al*. 1995; Lewis *et al*. 1995) and the manipulation of intermediate hosts to increase transmission success to the definitive host (Lafferty, 1999; Poulin, 2010) are all viewed as evolutionary responses to low probability of transmission. However, there are only very few (e.g. Amundsen *et al*. 2003) solid estimates of the magnitude of transmission losses incurred under natural conditions by parasites moving up through their life cycle.

Another adaptation commonly associated with poor transmission success is the ability of certain parasites to multiply via asexual reproduction within an intermediate host, thus replenishing their numbers before entering the next phase of the life cycle. This is best known in trematodes, which multiply asexually within their mollusc first intermediate host to generate huge numbers of mobile infective stages (cercariae) which go on to infect the next host in the cycle (Galaktionov and Dobrovolskij, 2003). A similar ability to multiply has also evolved independently in some cestode lineages (Moore and Brooks, 1987). Although clearly beneficial at an individual level, the quantitative impact of this numerical expansion at the population level has not yet been measured in natural systems.

The lack of quantitative data on the magnitude of changes in the standing crop of parasite populations from egg to adult is not only limiting our understanding of the selective pressures driving their evolution, but also represents a major gap in the parameterization of epidemiological models (McCallum, 2000). Here we provide the first quantitative assessment of these ontogenetic ups and downs in numbers from one life stage to the next under natural conditions, replicated across parasite species and geographic localities. By measuring the density (numbers of individuals per unit area) of different life stages of the same parasite species in aquatic systems, we can compare cohort sizes at successive stages along the parasite's life cycle. Different

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numbers of individuals at consecutive life stages are due to losses during transmission (including failure to establish due to host defences), but also to differential mortality rates or longevity between the two stages. For instance, twice as many individuals at one life stage than at the next one could indicate that only half of the individuals are successfully transmitted, or that they survive twice as long in the original host. Therefore, we include a correction for estimated parasite longevity at each life stage.

Our focus is on relative changes in numbers associated with two key transmission events from an upstream (source) host to a downstream (target) host, in the life cycles of many helminths (Fig. 1). First, we test the hypothesis that the asexual amplification of trematodes in their first intermediate host leads to a numerical expansion, i.e. that the production of large numbers of cercariae more than compensates for the losses they incur during transmission. Cercariae are free-swimming and infect the downstream host by contact followed by penetration and encystment as metacercariae (Galaktionov and Dobrovolskij, 2003). They are short-lived and sensitive to abiotic conditions in the water (Pietrock and Marcogliese, 2003; Koprivnikar *et al*. 2010) as well as exposed to a range of non-host predators (Thieltges *et al*. 2008; Johnson and Thieltges, 2010) during their search for the downstream host. In spite of these losses, we expect the cohort size of metacercariae to exceed that of the preceding life stage in their first intermediate host.

Second, we test the hypothesis that trophic transmission of helminths to their definitive host results in a decrease in their numbers (Fig. 1). Juvenile stages of numerous helminths (metacercariae in trematodes, cystacanths in acanthocephalans, etc.) inside the upstream host await ingestion by the downstream host. Many will either fall victim to non-host predators (Mouritsen and Poulin, 2003; Kaldonski *et al*. 2008; Seppälä and Jokela, 2008; Thieltges *et al*. 2013) or simply die within the upstream intermediate host if the latter is never captured by a downstream definitive host. Therefore, we expect that not all upstream stages will successfully reach the downstream host and mature into adult worms.

We test these two hypotheses with a large dataset involving many parasite taxa and four different lake ecosystems. We therefore provide a general assessment of some basic assumptions in parasitology. Our specific objectives were to (i) test whether the density of parasites in the upstream host regulates the density of the next life stage within the downstream host, and (ii) quantify the relative cohort sizes of successive parasite stages in the upstream *vs* downstream hosts, both with and without a correction for estimated parasite longevity. In both cases, we assessed the influence of mode of transmission, i.e. trophic transmission *vs* cercarial transmission, to quantify the extent to which the former is associated with a bottleneck and the latter with an expansion of the parasite population.

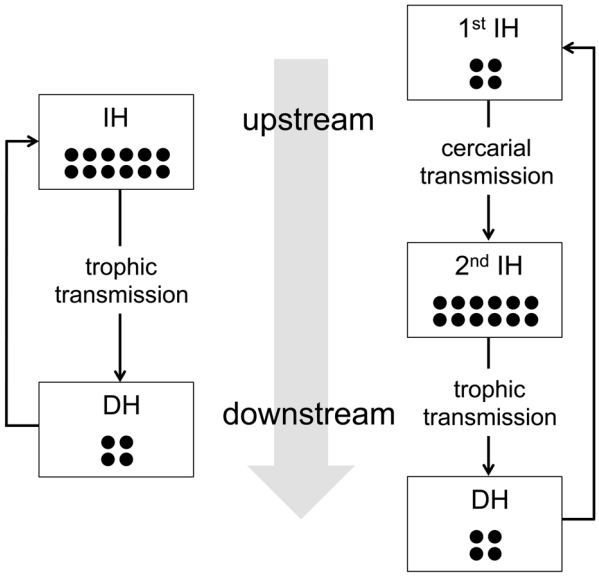

Fig. 1. Schematic summary of expected changes in relative cohort sizes of helminths through their complex life cycle. Black circles represent parasite individuals, and changes in their numbers reflect expansions or bottlenecks in a downstream direction, i.e. from source to target host. Differences in longevity between life stages are not considered here. The two-host life cycle on the left is typical of many acanthocephalans and nematodes, whereas the three-host cycle on the right is that of many trematodes. IH, intermediate host; DH, definitive host.

MATERIALS AND METHODS

Field sampling and laboratory processing

We investigated changes numbers through the life cycle of several helminth parasites from the littoral zone of 4 lakes on the South Island of New Zealand. We chose small-to-medium sized lakes, mostly shallow, and at different altitudes and distances from the coast (for name, location and characteristics of each lake, see online Fig. S1 and Table S1 in Supplementary material). In each lake, we sampled 4 square areas (15 m × 15 m) with one side of the square along the shore, distant by 123 to 2250 m from each other and selected to represent all habitat types (substrate, macrophytes, riparian vegetation, etc.) present within each lake. This gave us 16 study sites (4 lakes ×4 sampling sites per lake). Each site was sampled in three seasons (September 2012, January and May 2013), and on each occasion we sampled fish, benthic and demersal invertebrates, and all helminth parasites within these organisms.

Fish were sampled using a combination of gear types following a standardized protocol so that samples accurately represented fish diversity and

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density (see online Supplementary material for full details). Two fyke nets were set overnight along the edges of the sampling area, perpendicular to the shore, and two 15 m long multi-mesh gillnets were deployed in the same place during the day. These were used to capture all fish swimming in and out of the area, i.e. both residents and visitors to the area. In addition, a standard, fine-mesh purse seine net was dragged across the whole area to capture small and/or sedentary resident fish not captured by passive gear like fyke nets or gillnets. All fish caught were identified to species, counted, measured and a subsample was returned to the laboratory for dissection. In each site and in each season, 6 samples of benthic invertebrates, distributed haphazardly across the sampling area, were taken using a standard Surber sampler net with a 0·1 m^2 horizontal metal frame fitted with a 250 μM mesh collecting net. In addition, 6 samples of demersal invertebrates, living on or near the substrate but not captured in Surber nets, were sampled using a rectangular dip net (30 cm wide and 22 cm high opening) with a 250 μM mesh net; each sample consisted of a fast, 2 m-long sweep of the net along the lake bottom without dredging the substrate. All invertebrate samples were preserved in ethanol for later identification, counting and dissection.

In the laboratory, all individuals were identified to species and counted, after which large subsamples of each fish (>600 individuals total) and invertebrate (>40 000 individuals total) species were dissected carefully following a standardized protocol for parasite recovery and identification (see online Supplementary material for full details).

Host and parasite variables

We defined a parasite 'cohort' as all parasites of a given species at a particular life stage occurring in all suitable hosts at one locality. For each cohort of particular life stages of different parasite species, we calculated density (individuals per m^2) as a measure of local cohort size. Although parasite populations are usually quantified as individuals per host rather than per surface area, density provides a common metric to compare different life stages and a better reflection of cohort size. Parasite densities were calculated as the product of abundance of infection (mean number of parasites per individual host, including uninfected ones) and host density (see online Supplementary material for details). In most cases, a parasite cohort exploited a single host species; in other cases, we still used total density per m^2 of that life stage summed across all its host species. Density was first averaged across samples, then across seasons, to obtain a single density per site for each life stage of each species. Each entry in the dataset corresponds to a pair of successive upstream–downstream life stages of the same parasite species found in the same sampling site (i.e. some upstream–downstream pairs of the same species appear more than once in the dataset, but each comes from a different locality).

In the case of trematodes in their snail first intermediate host, we did not count each individual redia or sporocyst as separate individual parasites, since these are the product of within-host clonal multiplication and not the outcome of independent infection events. Except for rare cases of multiple infections, all rediae or sporocysts in one snail have the same genotype and are issued from the same larva hatched from a single egg. Their numbers do not reflect transmission processes, and therefore the densities of these life stages were measured as the number of infected snails per m^2 (but see the section Discussion). We only considered clonal individuals as 'separate' individuals once they left the snail host as cercarial transmission stages.

Each pair of successive upstream–downstream life stages was classified based on its transmission mode between hosts. The two transmission modes were: (i) direct cercarial transmission, applying to trematodes only, and involving free-swimming infective stages mass-produced asexually in the upstream host before leaving to seek the downstream host; and (ii) trophic transmission, in which a definitive downstream host acquires packets of juvenile parasites each time it eats an infected upstream host.

The cohort densities quantified as described above represent raw measures of the standing stock of conspecific parasites at a given life stage in one locality. For proper comparisons between cohorts at successive life stages to assess actual demographic expansion or reduction, we need to correct these cohort densities for estimated parasite longevity. Longevity-corrected cohort sizes could only be calculated for trematodes. Sporocysts and rediae can generally survive in a snail first intermediate host until host death (e.g. Curtis, 2003); therefore, we calculated maximum longevity of these stages as the time between the age of the smallest snails seen with infections, and the maximum lifespan of the only snail species involved in our study, *Potamopyrgus antipodarum* (Winterbourn, 1970; Levri and Lively, 1996). Metacercariae were also assumed to survive as long as the second intermediate host, because these are encysted stages with low metabolic activity (Galaktionov and Dobrovolskij, 2003) and because we never found dead metacercariae in the crustacean and fish second intermediate hosts dissected (Herrmann and Poulin, 2011). We calculated maximum longevity of metacercariae as the time between the approximate age of the smallest host seen with infections, and the maximum lifespan of the crustacean or fish species involved (Rowe, 1999; Jellyman *et al*. 2000; Lagrue and Poulin, 2008*a*, *b*). For adult worms, which senesce in the definitive host and die well before their host, we

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used data on adult body length and maximum longevity compiled by Trouvé *et al*. (1998) on 15 trematode species to calculate the following regression ($R^2 = 0{\cdot}387$, $P = 0{\cdot}0133$):

$$\text{Log longevity (days)} = 1 \cdot 2096 + (1 \cdot 5085) \times (\text{Log adult length (mm)})$$

We used data on body length of adult worms of our study species, obtained from original species descriptions, to estimate their maximum longevity using the above regression equation. We then corrected the density of each cohort, i.e. all parasites of a given species at a particular life stage per m^2, by dividing it by its estimated maximum longevity, separately for each sampling locality.

Statistical analysis

Densities of downstream parasite stages per sampling site were log-transformed and then analysed using a mixed-effects model with Gaussian error structure implemented in JMP version 11.0 (SAS Institute Inc., Cary, NC, USA). Our main goal was to test the effect of the density of the upstream cohort on that of the downstream stage, and to test whether this effect differed among different transmission modes. Therefore, density of the upstream parasite stage (log-transformed) and transmission mode (cercarial transmission or trophic transmission) were included as fixed factors in the model, as well as the two-way interaction between them.

The identity of the lake sampled was included as a random factor in the model. This accounts for idiosyncrasies of particular lakes and for the non-independence and correlated structures in the data arising from the fact that multiple data points come from the same lake. In addition, parasite species nested within their higher taxon (trematodes, nematodes or acanthocephalans) were also included as random factors to account for any phylogenetic influences. We calculated the proportion of the total variance unexplained by the fixed effects that could be accounted for by each random effect (Nakagawa and Schielzeth, 2013).

Finally, the ratio of downstream cohort density to that of the upstream cohort was calculated for each upstream–downstream life stage pair. This was done separately for the 'raw' cohort densities and (for trematodes only) for those corrected for longevity of each life stage. The uncorrected ratios indicate which life stage includes the most individuals, whereas the ratios corrected for longevity indicate whether the parasite population undergoes a numerical bottleneck (reduction, log-transformed ratio <1) or expansion (increase, log-transformed ratio >1) during the transition from one stage to the next. The ratios were compared between the two modes of transmission using a two-sample *t*-test.

RESULTS

The dataset comprised 100 pairs of successive upstream–downstream life stages, representing 10 different parasite species, most of which are trematodes (Table 1). Average densities of particular parasite life stages ranged from less than 1 to over 14 000 individuals m^{-2}.

Densities of downstream cohorts were significantly affected by the density of the upstream parasite stage and by transmission mode, as well as by the interaction of these two factors (Table 2). Parasite stages generally achieved higher densities in downstream hosts reached by cercarial transmission than they did in downstream hosts reached by trophic transmission. More importantly, the positive association between upstream and downstream cohort densities only existed in the case of cercarial transmission (Fig. 2); for adult parasites having reached their downstream host via trophic transmission, there was no effect of the density of the preceding life stage. Finally, the identity of the parasite species and its higher taxon were the only random factors to explain a substantial portion of the remaining variance in downstream cohort densities. In contrast, lake identity explained only about 8% of the variance (Table 2).

The uncorrected downstream–upstream stage density ratio, used to quantify which life stage comprises the most individuals in a parasite population, differed significantly between cases of trophic transmission and those involving transmission by cercariae (log-transformed data: $t = 6{\cdot}525$, D.F. = 98, $P < 0{\cdot}0001$). In trematodes, the cohort in the second intermediate hosts, originating from free-swimming cercariae, was larger than that at the previous stage of the life cycle, in the snail first intermediate host (Fig. 3). The most extreme values we observed indicate a 2000-fold difference; however, the back-transformed mean value indicates a more modest 6-fold difference on average. In contrast, adult cohorts in the definitive host, reached via trophic transmission, were generally smaller in size than those at the last juvenile stage, in all parasite taxa (Fig. 3). For trematodes, the back-transformed mean ratio suggests a 7-fold difference on average. The contrast appears greater for acanthocephalans and nematodes, with approximately 100-fold and 36-fold differences on average, though the latter two estimates are each based on few observations from a single species.

However, the longevity-corrected ratios (Table 3) of downstream–upstream stage density, used to quantify ontogenetic bottlenecks and expansions in trematode populations, only differed slightly between cases of trophic transmission and those involving transmission by cercariae (log-transformed data,

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Table 1. Parasite species included in the present analysis, and number of populations (i.e. number of sites, out of 16 from 4 lakes) where an upstream–downstream cohort contrast in life stage density was possible

Parasite species	Upstream stage	Upstream host	Transmission mode	Downstream stage	Downstream host	Number of populations
Acanthocephala						
Acanthocephalus galaxii	Cystacanth	Amphipod[1]	Trophic	Adult	Fish[7,8,9,10,11]	3
Nematoda						
Hedruris spinigera	Juvenile	Amphipod[2]	Trophic	Adult	Fish[8,9,11,12,13]	3
Trematoda						
Apatemon sp.	Sporocyst	Snail[5]	Contact	Metacercaria	Fish[6]	8
Coitocaecum parvum	Sporocyst	Snail[5]	Contact	Metacercaria	Amphipod[1,2]	11
	Metacercaria	Amphipod[1,2]	Trophic	Adult	Fish[6,8,9,10,12,13]	11
Maritrema poulini	Sporocyst	Snail[5]	Contact	Metacercaria	Amphipod[1,2,3], isopod[4]	7
Notocotylus sp.	Redia	Snail[5]	Contact	Metacercaria	Snail[5]	2
Pronocephaloid sp. I	Redia	Snail[5]	Contact	Metacercaria	Snail[5]	11
Pronocephaloid sp. IV	Redia	Snail[5]	Contact	Metacercaria	Snail[5]	7
Stegodexamene anguillae	Redia	Snail[5]	Contact	Metacercaria	Fish[6,7]	2
	Metacercaria	Fish[6,7]	Trophic	Adult	Fish[13,14]	16
Telogaster opisthorchis	Redia	Snail[5]	Contact	Metacercaria	Fish[6,7]	5
	Metacercaria	Fish[6,7]	Trophic	Adult	Fish[13,14]	14

Host species: 1, *Paracalliope fluviatilis*; 2, *Paracorophium excavatum*; 3, *Orchestia* sp.; 4, *Austridotea annectens*; 5, *Potamopyrgus antipodarum*; 6, *Gobiomorphus cotidianus*; 7, *Galaxias maculatus*; 8, *Perca fluviatilis*; 9, *Rhombosolea retiaria*; 10, *Salmo trutta*; 11, *Aldrichetta forsteri*; 12, *Retropinna retropinna*; 13, *Anguilla dieffenbachii*; 14, *Anguilla australis*.

Table 2. Results of the mixed-effects model with density of the downstream parasite stage as the response variable, showing the effects of the main predictors and the proportion of the remaining variance accounted for by the random factors

Fixed factors	Estimate	S.E.	*t*-value	*P*	Random factors	% variance
Intercept[a]	0·2657	0·4673	0·57	0·5831	Lake	8·37
Log density of upstream stage	0·2510	0·0994	2·52	0·0134	Parasite species (higher taxon)	68·63
Transmission mode (cercarial)	0·6164	0·1088	5·67	<0·0001		
Transmission × upstream stage density	0·4346	0·0908	4·78	<0·0001		

[a] Trophic transmission is included in the intercept.

mean ± S.E.: cercarial transmission: 0·603 ± 0·182; trophic transmission: 0·133 ± 0·211; $t = 1·67$, D.F. = 92, $P = 0·0488$). In trematodes, the transmission from first to second intermediate hosts via free-swimming cercariae resulted in a numerical increase at the next stage of the life cycle. The most extreme values we observed indicate 100- to 2000-fold increases; however, the back-transformed mean value indicates a more modest 4-fold expansion on average. In contrast, trophic transmission generally resulted in almost no change in density during the transition from the metacercarial stage to the adult stage; the back-transformed mean ratio suggests a 1·3-fold rise in density on average.

DISCUSSION

Our study provides the first quantitative assessment performed under natural conditions of the ontogenetic fluctuations in relative cohort sizes experienced by helminths through their complex life cycle. The results support one of our two hypotheses: asexual multiplication of trematodes in their first intermediate host leads to a measurable expansion of their numbers at the next life stage. Intriguingly, whereas trophic transmission to their definitive host leads to a drop in cohort density for all helminths, for trematodes it results in practically no numerical change once the relative longevity of the different life stages is taken into account. Notably,

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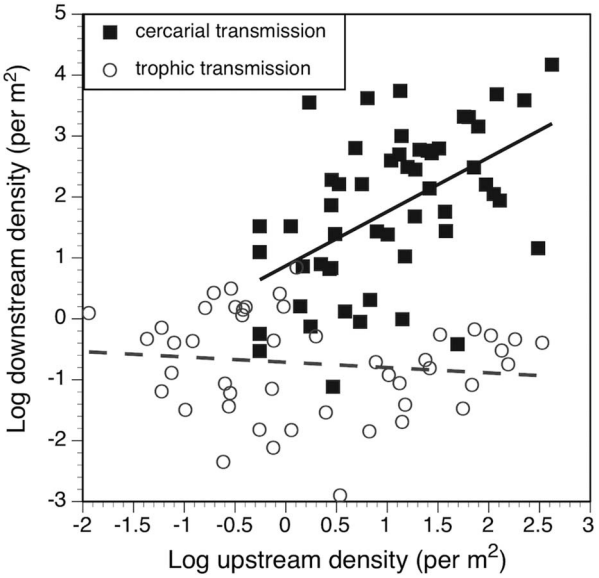

Fig. 2. Relationship between the density of individuals at a downstream stage in their complex life cycle and that at the preceding (upstream) stage in the cycle, for 100 populations of helminth parasites with complex life cycles. Data are shown separately for life cycle transitions involving transmission by free-living cercarial stages (simple linear regression: $N = 53$, $R^2 = 0{\cdot}266$, $P < 0{\cdot}0001$) and those involving trophic transmission ($N = 47$, $R^2 = 0{\cdot}015$, $P = 0{\cdot}4153$).

a positive association between upstream and downstream stage densities was only found in the case of cercarial transmission of trematodes. This reflects a simple output-recruitment process: the greater the density of cercariae-emitting snails per area, the greater the number of metacercariae occurring in second intermediate hosts in the same area. For trophic transmission, however, the dynamics of predation may uncouple downstream parasite densities from upstream ones. Prey selection by the definitive host, local availability of alternative prey species, efficiency of intermediate host manipulation by the parasites and other factors probably combine to generate variability in the proportion of upstream stages successfully reaching the downstream host.

Our findings are robust to idiosyncrasies of particular localities, since very little of the unexplained variance in downstream life stage densities was associated with lake identity. In contrast, differences among parasite taxa (included as a random factor in the main model) accounted for a substantial proportion of the unexplained variance in downstream stages. This is not surprising, however, as many aspects of the transmission ecology of parasites, such as cercarial output and cercarial behaviour, are species-specific traits (McCarthy *et al*. 2002; Koehler *et al*. 2012). Therefore, while the general trends we report apply broadly, precise downstream–upstream ratios in density will vary among parasite taxa.

The present results have important implications for our growing understanding of the evolution of complex life cycles. A simple one-host life cycle can evolve into a two-host cycle by the addition of either an upstream host or a downstream host (Parker *et al*. 2003). Addition of a downstream predatory host can increase parasite fitness if the greater outcrossing made possible by the concentration of adult worms in the new definitive host, and the increased reproductive output allowed by the exploitation of a larger host, offset the need for a new trophic transmission step (Brown *et al*. 2001; Parker *et al*. 2003). Acanthocephalan life cycles have taken this route, with the vertebrate predatory host added to the ancestral life cycle that consisted of a single arthropod host (Herlyn *et al*. 2003). Alternatively, addition of an upstream host can increase parasite fitness if it provides a better conduit for the parasite's larvae to return to the original host by first being gathered by a new small-bodied intermediate host (Choisy *et al*. 2003; Parker *et al*. 2003). This seems to have been the path followed by some lineages of parasitic nematodes, starting out as parasites of vertebrates and later adding an intermediate host (Adamson, 1986; Blaxter and Koutsovoulos, 2014). Our findings indicate that in nematodes, acanthocephalans and trematodes, much more individuals in a population are at the pre-trophic transmission stage than at the adult stage, at any point in time. These numerical differences provide a quantitative perspective on the demographic cost of adding an extra host to the life cycle. However, at least for trematodes, the parasites do not appear to incur any reductions in their numbers during trophic transmission, once we take into account the fact that egg-producing adults do not live as long as juvenile stages. One possible explanation is that the strength of selective pressures acting at this stage of the life cycle has resulted in efficient strategies to maintain high transmission rates and prevent transmission bottlenecks. Another, more likely explanation is that the longevity estimates we obtained for adult worms were inaccurate. They were based on a size-longevity regression derived from data in Trouvé *et al*. (1998) on a small number of trematode species, most parasitic in endothermic vertebrates, which may not be representative of trematodes in general.

In trematodes, life cycle evolution followed a somewhat more complicated route. Once the ancestral mollusc-to-vertebrate two-host life cycle was established, asexual multiplication within the mollusc host evolved as a new parasite strategy (Cribb *et al*. 2003). Complexity was further increased when a second intermediate host was inserted between the mollusc and the vertebrate, independently in separate branches of the trematode phylogenetic tree (Cribb *et al*. 2003). This allowed the prolongation of the infective life of the cercarial stage, and may also promote the mixture of different clones produced asexually in the mollusc

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Table 3. Estimated maximum longevity of all trematode life stages included in the present analysis

Parasite species	Redia/sporocyst (days)	Metacercaria (days)	Adult (days)
Apatemon sp.	270	990	–
Coitocaecum parvum	270	255	46
Maritrema poulini	270	255	–
Notocotylus sp.	270	270	–
Pronocephaloid sp. I	270	270	–
Pronocephaloid sp. IV	270	270	–
Stegodexamene anguillae	270	990	136
Telogaster opisthorchis	270	990	44

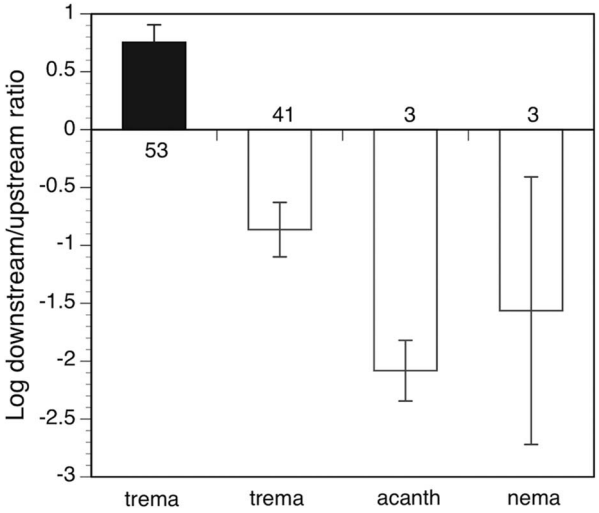

Fig. 3. Mean (+S.E.) log-transformed ratios of the density of the downstream stage to that of the upstream stage, for life cycle transitions involving transmission by free-living cercarial stages (filled bar) and trophic transmission (open bars). Densities are calculated as total number of individuals of a species at a given life stage per m^2, and are not corrected for differential longevity at different life stages. Data are shown separately for trematodes (trema), acanthocephalans (acanth) and nematodes (nema); numbers at the base of bars indicate the number of populations in each group.

host and outbreeding following their passage to the definitive vertebrate host (Rauch *et al*. 2005). Our data quantify the extent to which asexual multiplication within the mollusc host amplifies the numbers of individuals reaching the next host, even when accounting for differences in longevity between stages; this may by itself offset further reductions experienced elsewhere in the life cycle.

Two issues relating to trematodes need to be addressed here. Firstly, for sporocysts or rediae in their snail first intermediate host, which result from within-host clonal multiplication, we adopted the principle that one individual corresponds to one genotype. We assumed a single genotype per infected snail, and the densities of these life stages were measured as the number of infected snails per m^2. However, the assumption does not hold. In the only trematode species in our dataset which has been studied using microsatellite genotyping, *Coitocaecum parvum*, the majority of snails harbour a single genotype but some possess more than one, for an average of 1·4 genotypes per infected snail (Lagrue *et al*. 2007). In *Maritrema novaezealandensis*, a marine relative of the freshwater trematode *Maritrema poulini* included in our study, this average was 1·9 genotypes per infected snail (Keeney *et al*. 2007). Using these numbers as a correction for multiple genotypes per snail reduces the estimated expansion experienced by trematodes during cercarial transmission from first to second intermediate hosts from 4-fold (longevity-corrected ratios) down to 2- to 3-fold. This still indicates that asexual multiplication within the first intermediate host and the associated mass production of cercariae more than compensates for losses during transmission that may result from unfavourable abiotic conditions, predation or penetration of unsuitable hosts (Pietrock and Marcogliese, 2003; Thieltges *et al*. 2008; Johnson and Thieltges, 2010; Koprivnikar *et al*. 2010).

Secondly, two of the trematode species included in our analysis, *C. parvum* and *Stegodexamene anguillae*, are capable of abbreviating their three-host life cycle by attaining precocious maturation (progenesis) while inside the second intermediate host (Holton, 1984; Lagrue and Poulin, 2008*a*; Herrmann and Poulin, 2011). This facultative strategy allows an individual to bypass the definitive host by producing viable eggs at the metacercarial stage, while still inside the intermediate host, thereby truncating the life cycle to just two hosts. Although anywhere between 10 and 50% of individuals of these species can be progenetic in samples from the field (Lagrue and Poulin, 2008*a*; Herrmann and Poulin, 2011), they can nonetheless continue their life and possibly achieve greater egg production if ingested by their definitive host. Thus their inclusion in estimates of potential trophic transmission bottlenecks is justified.

Besides cercarial transmission and trophic transmission, there is another transmission event necessary to complete the life cycle: release of eggs by adult worms in the definitive host and the subsequent infection of the first (or only) intermediate host. We did not include it in our main analysis to avoid

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issues of circularity, i.e. the same values for density of a particular life stage used as both predictor and response variables. This logical difficulty is most acute for parasites with two-host life cycles such as nematodes and acanthocephalans. However, for illustrative purposes, we calculated the ratio of the density of infected snails (each corresponding to at least one successful infection) to that of the adult worm stage, for the 18 populations of 3 trematode species for which this was possible. The average ratio, once back-calculated from log-transformed data, is about 30. This may suggest that an adult trematode in our system gets about 30 eggs to successfully infect the first intermediate host. Here too, a correction for maximum longevity at different stages is necessary. Indeed, the juvenile stages inside snails survive much longer than the lifespan of adult worms; therefore, the larger standing crop of juvenile trematodes inside snail hosts compared to the adult worm population probably reflects the egg contributions of a few to several adult generations.

Overall, our results suggest that, for trematodes, the expansion resulting from asexual multiplication in the first intermediate host more than compensates for any attrition occurring downstream during trophic transmission or elsewhere in the life cycle. For nematodes, data on adult worm longevity are unavailable, and we therefore cannot correct ratios of downstream–upstream cohort densities. For acanthocephalans, Kennedy (2006) gives an adult lifespan of 6·1 months for *Acanthocephalus lucii*, a congener of *Acanthocephalus galaxii* from our study; using this longevity value and assuming cystacanths live as long as their amphipod intermediate hosts, we get a corrected ratio of downstream–upstream cohort densities of about 70 instead of the uncorrected value of 100. Both these ratios suggest large losses incurred during trophic transmission (at least for the species included in our analysis). If these were accurate, there would be strong pressures for adaptations such as high adult egg output or manipulation of the intermediate host, a phenomenon particularly common among acanthocephalans (Moore, 1984). The estimates we obtained here of ontogenetic rises and falls in numbers under natural conditions provide the first quantitative assessment of the intra-generational dynamics driving the evolution of parasite life cycles.

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit http://dx.doi.org/doi:10.1017S0031182014001917.

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