



## Small worms, big appetites: Ratios of different functional morphs in relation to interspecific competition in trematode parasites

Tommy L.F. Leung<sup>a,b,\*</sup>, Robert Poulin<sup>b</sup>

<sup>a</sup> Zoology, School of Environmental and Rural Science, University of New England, NSW 2351, Australia

<sup>b</sup> Department of Zoology, University of Otago, 340 Great King Street, P.O. Box 56, Dunedin 9054, New Zealand

### ARTICLE INFO

#### Article history:

Received 27 January 2011

Received in revised form 23 May 2011

Accepted 24 May 2011

Available online 31 May 2011

#### Keywords:

Eusociality  
Specialised morphs  
Co-infection  
Competition  
Life history  
Trematoda  
Philophthalmidae  
Rediae

### ABSTRACT

Animals living in colonies or collectives composed of highly-related individuals often produce morphs that are physically and behaviourally specialised to perform specific tasks. Because such morphs are often sterile, their production represents a fitness cost for the colony and there should be an optimal ratio of the numbers of sterile specialists and reproductive members that may be adjustable to environmental conditions. Trematode parasites undergo asexual multiplication within their snail intermediate host, resulting in large numbers of clonal stages known as rediae or sporocysts, depending on the trematode species. In areas with high prevalences of infection, the host can be infected with multiple species, which can lead to intense competition for limited resources. Here, we describe the existence of specialised 'mini-rediae' in the trematode *Philophthalmus* sp. that are morphologically and functionally specialised for interspecific competition. Mini-rediae were observed feeding on the sporocysts of a co-occurring trematode species – *Maritrema novaezealandensis*. In addition, in larger snails – which are less likely to have *M. novaezealandensis* infections – *Philophthalmus* sp. produces relatively fewer mini-rediae than expected. Our findings support results from a prior study which demonstrated the existence of morphs that perform specialised functions in antagonistic interspecific interactions in trematodes, and additionally shows that the number of these morphs in each host is associated with the likelihood of encountering other species within the same host. Trematodes may thus provide interesting models for studying morphological specialisation in colonial organisms.

© 2011 Australian Society for Parasitology Inc. Published by Elsevier Ltd. All rights reserved.

### 1. Introduction

Organisms living in colonies or similar collectives of highly-related individuals are often characterised by division of labour, whereby different members are specialised for performing different tasks for the colony as a whole. Division of labour can lead to extreme morphological and/or behavioural adaptations (e.g., Powell and Franks, 2005; Vieira-Neto et al., 2006; Evison et al., 2008). This is well-studied in eusocial insects such as ants (see Fjerdingstad and Crozier, 2006) and termites (see Korb and Hartfelder, 2008). Such specialised morphs are often sterile, and while Hamilton's (1964) theory of kin selection can explain why such individuals forgo their own reproductive self-interest to promote that of highly-related kin, investment into the production of sterile castes still represents a potential fitness cost for the colony as a whole. As such, instead of producing a fixed number of individuals for a certain role, it could be advantageous if the ratio of sterile

specialists to reproductive members was responsive to environmental variables. For example, colonies of the ant *Pheidole pallidula* increase investment in soldier production in the presence of foreign conspecific colonies (Passera et al., 1996).

Thus, there may be an optimal ratio of specialist and reproductive morphs within a given colony and this optimal ratio can be influenced by environmental variables such as resources and competition (e.g., McGlynn and Owen, 2002; Tyerman and Roitberg, 2004). Similar life-history challenges are also faced by other organisms that form multi-individual collectives, such as trematode parasites. As part of their multi-host complex life-cycle, trematodes undergo asexual multiplication within their gastropod first intermediate host, resulting in masses of clonal stages known as parthenitae that in many species can occupy the entire visceral mass of the snail (Galaktionov and Dobrovolskij, 2003; Lafferty and Kuris, 2009). The main role of the parthenitae in the trematode life cycle is the production of free-living cercariae which typically leave the snail host to infect the second host in the life-cycle. The infection within the snail grows in a modular manner through the continual clonal production of parthenitae by pre-existing individuals. The key aspect of this life history trait is that the parthenitae are clonal, therefore all individuals resulting from a single infection event in a

\* Corresponding author at: Zoology, School of Environmental and Rural Science, University of New England, NSW 2351, Australia. Tel.: +61 2 6773 4083; fax: +61 2 6773 3814.

E-mail address: [tommylfeung@gmail.com](mailto:tommylfeung@gmail.com) (T.L.F. Leung).

given host are genetically identical and thus share the same reproductive interest.

As found recently by Hechinger et al. (2011), while not widely recognised as such, trematode parthenitae share several characteristics with eusocial animals. They live in collectives composed of multiple kin (in this case clone-mates), and exploit a resource that is both limited and patchily distributed across their range (the gastropod host). Because trematode communities are often comprised of several different species that exploit a single species of snail as host, they can often encounter both unrelated conspecifics (Keeney et al., 2007) and other species of trematodes (Sousa, 1993; Kuris and Lafferty, 1994). Despite this, snails harbouring multiple infections are found less frequently than expected by chance alone, indicating that strong antagonistic interactions may be occurring between different species within the snail, and that the presence of certain trematodes species may exclude establishment by others (Sousa, 1993; Kuris and Lafferty, 1994).

Trematode parthenitae come in two basic morphological types – rediae and sporocysts, depending on the trematode species (Galaktionov and Dobrovolskij, 2003). They show key structural and functional differences that have implications for interspecific interactions. While both rediae and sporocysts are filled with embryonic masses and developing cercariae, rediae also have a muscular pharynx and a digestive tract that allow them to consume host tissue or co-occurring trematodes, and some have appendages for locomotion within their host. In contrast, sporocysts are simple sacs that absorb nutrients through their tegument. Trematodes with rediae can feed directly on the sporocysts of other species (e.g., Basch and Diconza, 1975). Indeed, trematodes with rediae have been proposed as a form of biological control for trematodes with sporocysts such as schistosomes that cause human diseases (Lie, 1973; Combes, 1982). However, with a few exceptions (e.g., Sapp et al., 1998; Hechinger et al., 2011) the existence of individual rediae specialised for such antagonistic interactions is not well-documented.

The snail *Zeacumantus subcarinatus* is a common mollusc on the coastlines of New Zealand and serves as host for many different species of trematodes (Leung et al., 2009). The most common of these is *Maritrema novaezealandensis*, a sporocyst-producing trematode that can reach very high prevalence in some host populations and account for more than 60% of all infections (Fredensborg et al., 2005). The second most prevalent trematode is *Philophthalmus* sp. (for description and vouchers, see Martorelli et al., 2008; for genetic barcode, see Leung et al., 2009), a redia-producing species (Martorelli et al., 2008) which regularly co-occurs with *M. novaezealandensis* in the same host (Keeney et al., 2008). The occurrence of these species follows an interesting pattern in relation to host size. While snails of intermediate size often harbour double infections of both species, larger snails are almost exclusively infected by *Philophthalmus* sp. (hereafter referred to simply as *Philophthalmus*), a pattern that has been attributed to the possible elimination of *M. novaezealandensis* by *Philophthalmus* (Keeney et al., 2008).

As mentioned above, species with rediae have a competitive advantage over species with sporocysts due to their ability to feed directly on co-infections (Lim and Heyneman, 1972; Sousa, 1993). However, while most species produce rediae which follow a relatively uniform morphology for each particular species, with the immature rediae resembling smaller versions of the more mature rediae (see Ginetsinkaya, 1988), *Philophthalmus* differs in having rediae of two distinct morphotypes. In addition to having the cercaria-producing rediae such as those found in other species, *Philophthalmus* produces smaller rediae with a distinctive morphology (Martorelli et al., 2008; T. Leung, personal observation). These smaller individuals differ from the larger cercaria-producing rediae in a number of ways that can affect their functional

role within the host (T. Leung, personal observation). Apart from being much smaller than the cercaria-producing rediae, they have an elongated gut that runs the entire length of their body, do not produce cercariae and lack reproductive germinal masses (Martorelli et al., 2008; T. Leung, personal observation). The ‘mini-rediae’ are also very active and have posterior locomotor appendages which allow them to move around within the host. These mini-rediae are found in large numbers, usually equalling, if not exceeding, the number of larger cercaria-producing rediae, suggesting they may play a role beyond serving to replace larger rediae that become senescent (T. Leung, personal observation). While they may merely be juvenile versions of the larger rediae, it is possible that they are actually a separate morph with a specialised function.

There are earlier reports of alternative morphological types of rediae. Sapp et al. (1998) reported the presence of a specialised morph they called a “precocious mother redia” in *Echinostoma paraensei* which was behaviourally, developmentally and morphologically distinct from other redia stages. This distinct redia appeared to serve a protective function in preventing co-infection from establishing, although the mechanism providing protection was unclear (Sapp et al., 1998). More recently Hechinger et al. (2011) documented the presence of small “soldier” rediae that differ significantly in behaviour and morphology from larger cercaria-producing rediae. They were able to demonstrate that these morphs play a specialised role in interspecific competition, demonstrating the possibility of specialised individuals among trematode parthenitae (Hechinger et al., 2011).

While the allocation of resources to the production and maintenance of specialised individuals can be costly, it may be a plastic trait that is adjustable to circumstances. For instance, the number of mini-rediae within a given snail may increase in the presence of a competitor. Our study aims to test the hypothesis that mini-rediae serve a specialised function in interspecific interactions. We predict that the ratio of *Philophthalmus* mini-rediae to larger, cercaria-producing rediae (thereafter referred to as “producer rediae”) is affected by (i) the presence of co-infecting trematodes, (ii) the intensity of the co-infection and (iii) the size of the host which should dictate the carrying capacity of the host environment.

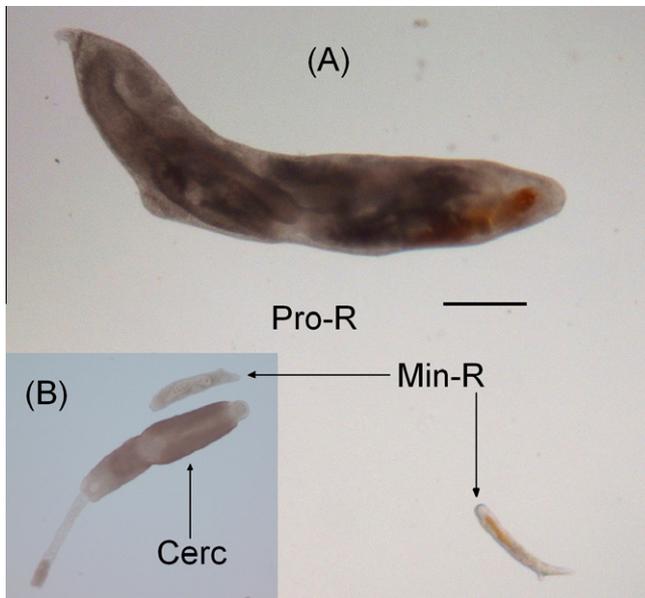
## 2. Methods and materials

### 2.1. Study organisms and quantification

Approximately 1,200 mud snails, *Z. subcarinatus*, were collected from Lower Portobello Bay, Otago Harbour (South Island, New Zealand) on 6th May 2009. The snails were first individually screened in 12-well plates incubated at 25 °C for 2 h. Snails found shedding *Philophthalmus* or both *Philophthalmus* and *M. novaezealandensis* cercariae were transferred to a separate plastic container (300 mm long × 130 mm wide × 150 mm high) filled with seawater and approximately 30 mm of fine sand, provided with strips of sea lettuce (*Ulva lactuca*) and aerated with an airstone prior to subsequent dissection.

Before dissection, the shell length of each infected snail was measured with Vernier callipers to the nearest 0.1 mm. The shell was carefully cracked with a hammer and the snail was removed from its shell as intact as possible. The visceral mass of the snail was then carefully teased apart to release the trematode parthenitae. Mini-rediae and producer rediae of *Philophthalmus* (Fig. 1) were counted separately. The mini-rediae were distinguished from the producer rediae by their much smaller size, pronounced gut sac and the lack of germinal masses (see Fig. 1).

*Maritrema novaezealandensis* sporocysts cannot be counted individually because they form sticky clumps that are difficult to separate without damaging them. Instead, infection intensity was



**Fig. 1.** Different morphs and stages of *Philophthalmus* sp. which are found in the snail host. (A) Producer redia (Pro-R) and mini-redia (Min-R) compared (scale bar = 150  $\mu\text{m}$ ), (B) Min-R next to a *Philophthalmus* sp. cercaria (Cerc).

scored based on how much of the snail's visceral mass they occupied. The scores ranged from 1 to 4, with 1 indicating *M. novaezealandensis* occupied a quarter or less of the visceral mass (which corresponds to up to 15 visible sporocysts), 2 indicating that it occupied from a quarter to half of the visceral space, 3 indicating that *M. novaezealandensis* occupied slightly more of the available space than *Philophthalmus*, and 4 indicating that *M. novaezealandensis* sporocysts were overwhelmingly more abundant than *Philophthalmus* rediae.

For two infected snails selected at random (one infected with *Philophthalmus* only, the other infected with both *Philophthalmus* and *M. novaezealandensis*), all rediae collected were heat-killed to avoid shrinkage and digital pictures of each redia were taken using an Olympus DP12 Microscope Digital Camera System connected to a stereomicroscope. The microscope was set to a standardised magnification and the sizes of rediae were measured to the nearest 0.1  $\mu\text{m}$  using ImageJ software (Wayne Rasband, National Institutes of Health, USA). Two measurements were taken for each redia, the total length and maximum width, which were used to calculate the volume of each redia as a cylinder.

A total of 12 rediae (consisting of rediae taken from three snails, with two mini-rediae and two producer rediae taken from each snail) were individually sequenced at the cytochrome oxidase subunit 1 (COI) region (see Leung et al. (2009) for primers used and PCR conditions) to confirm that the two morphs are of the same species.

## 2.2. Statistical analyses

None of our continuous variables required transformation to meet the assumptions of normality. Multiple regressions were used to, firstly, assess the effect of snail length and the number of producer rediae on the number of mini-rediae and, secondly, assess the effects of snail length and number of mini-rediae on the number of producer rediae.

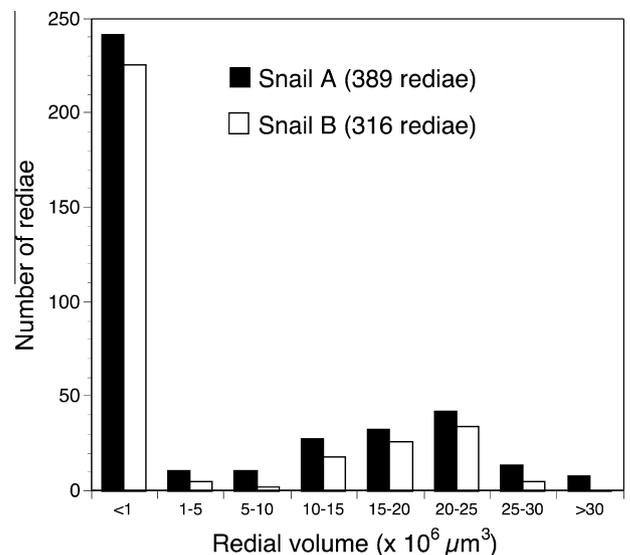
Generalised linear models (GLM) were used to determine what factors influence the ratio of mini-to-producer rediae within a snail; they were carried out with the statistical software JMP 7.0 (www.jmp.com). All models had a normal error structure and

identity function, as these provided the best match to the data. After starting with a full model, significance levels were based on the deviance explained by each factor, based on  $\chi^2$  statistics, following backward stepwise elimination of non-significant ( $P > 0.05$ ) terms. Only the final models are presented in the results; two-way interactions were initially included but none were retained as significant.

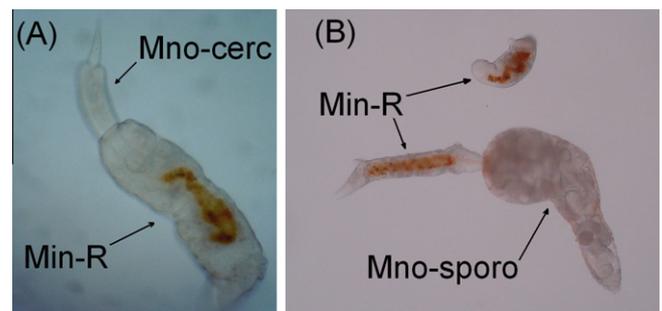
The ratio of mini-to-producer rediae within a snail was used as the dependent variable in each of two GLMs. In the first analysis including all snails, three explanatory variables were included in the initial GLM: the total number of rediae per snail, snail shell length and the presence or absence of *M. novaezealandensis* infections (treated as a binary variable). The second analysis included only snails harbouring both *Philophthalmus* and *M. novaezealandensis*. It also initially included three explanatory variables: the total number of rediae per snail, snail shell length and the intensity of *M. novaezealandensis* infections (scored 1–4, treated as an ordinal variable).

## 3. Results

Thirty snails harbouring *Philophthalmus* were dissected, of which 14 also harboured *M. novaezealandensis*. The snails ranged in shell length from 12.2 to 19.9 mm; there was no difference in



**Fig. 2.** Size distribution of the *Philophthalmus* sp. rediae from two infected snails used in the study.



**Fig. 3.** *Philophthalmus* sp. mini-rediae attacking and consuming co-occurring heterospecifics. (A) A *Philophthalmus* sp. mini-redia (Min-R) in the process of swallowing a *Maritrema novaezealandensis* cercaria (Mno-cerc), (B) *Philophthalmus* sp. Min-R attacking and eating a *M. novaezealandensis* sporocyst (Mno-sporo).

snail length between those infected by *M. novaezealandensis* and those not infected by that trematode (mean  $\pm$  S.E.;  $15.4 \pm 0.60$  mm versus  $16.1 \pm 0.56$  mm,  $t = 0.835$ ,  $df = 28$ ,  $P = 0.411$ ). The total number of *Philophthalmus* rediae per snail ranged from 48 to 1,128, with no difference between those with and without *M. novaezealandensis* ( $502 \pm 72$  versus  $559 \pm 68$ ,  $t = 0.569$ ,  $df = 28$ ,  $P = 0.574$ ).

Since one of the original 12 specimens did not amplify, 11 rediae from three snails consisting of five mini-rediae and six producer rediae were sequenced at the COI region, resulting in 650 bp of readable sequences. The sequences of mini-rediae and producer-rediae from the same snail were identical. Rediae from different snails diverged by a maximum of 3 bp, indicating the *Philophthalmus* mini-rediae and producer-rediae observed in this study belonged to the same species.

The ratio of mini-to-producer *Philophthalmus* rediae within a snail ranged from 0.76 to 3.27, with a mean ( $\pm$ S.E.) of  $1.78 \pm 0.13$ ; therefore, small rediae typically outnumbered large ones within a snail. The contrast in size between mini-rediae and producer rediae is clear-cut: in two snails for which all rediae were measured, the distribution of redial sizes is markedly bimodal (Fig. 2). During dissection, mini-rediae of *Philophthalmus* were frequently seen feeding on either sporocysts or cercariae of *M. novaezealandensis* (Fig. 3). Cercariae were often ingested whole whereas sporocysts

were emptied of their contents by suction (see Supplementary Movies S1–S3). The cercariae-producing rediae were never seen feeding on either cercariae or sporocysts of *M. novaezealandensis*.

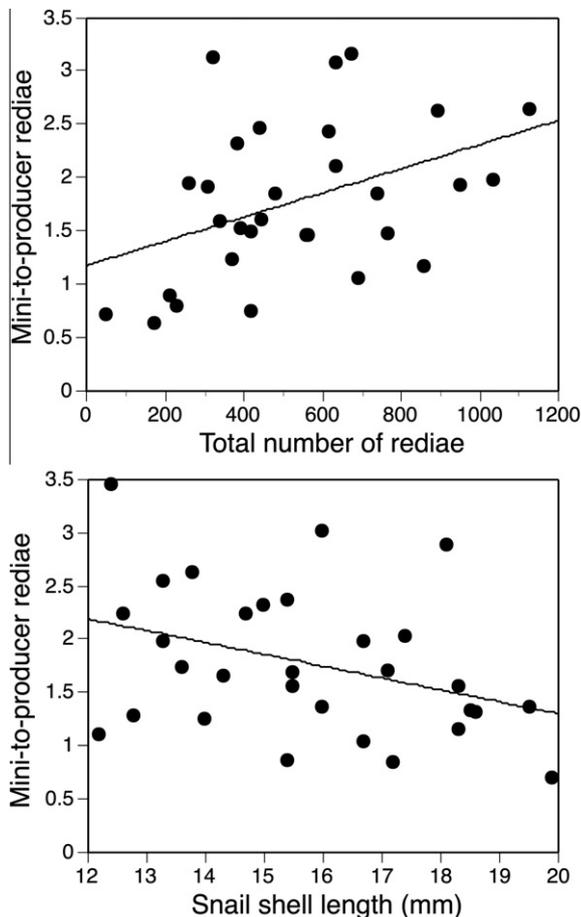
Multiple regression with the number of mini-rediae as the response variable and the number of producers and snail shell length as predictor variables revealed that the number of mini-rediae correlated with the number of producers ( $r^2 = 0.500$ ,  $P = 0.0018$ ) but not with snail size ( $P = 0.8652$ ). In contrast, the multiple regression with the number of producers as the response found that the number of producers is strongly correlated only with snail size ( $r^2 = 0.715$ ,  $P = 0.0001$ ). The net effect of these trends is that the number of mini-rediae relative to the number of producers, or the mini-to-producer ratio, decreases with increasing snail size (Fig. 4).

In the analysis including all snails, the presence of *M. novaezealandensis* infections was not retained in the final model. However, the ratio of mini-to-producer *Philophthalmus* rediae within a snail covaried with both the total number of rediae per snail ( $\chi^2 = 10.88$ ,  $df = 1$ ,  $P = 0.0010$ ) and snail shell length ( $\chi^2 = 7.75$ ,  $df = 1$ ,  $P = 0.0054$ ). The two predictor variables retained in the model, the total number of rediae and shell length, are strongly positively correlated to each other ( $r = 0.653$ ,  $P = 0.0001$ ). However, collinearity between these variables is unlikely to affect the results of the GLM, since the two predictors had opposite effects on the ratio of mini-to-producer *Philophthalmus* rediae within a snail. The ratio tended to be higher in snails harbouring high numbers of rediae and to decrease with increasing shell length (Fig. 4). In the analysis including only the 14 snails harbouring both *Philophthalmus* and *M. novaezealandensis*, only the total number of rediae was retained in the final model ( $\chi^2 = 6.94$ ,  $df = 1$ ,  $P = 0.0084$ ), the GLM again showing that the ratio of mini-to-producer rediae tended to be higher in snails harbouring high numbers of rediae; snail shell length and the intensity of *M. novaezealandensis* infections had no significant effect on the dependent variable.

#### 4. Discussion

The discrepancy in size and overall morphology between the mini-rediae and the cercaria-producing rediae marks the former as being a specialised morph. Specialisation of individuals for a particular task has been observed in other modular, clonally-reproducing animals such as sea anemones that can also produce specialised “warrior-scout” polyps in response to competition (Ayre and Grosberg, 2005). In addition to being smaller and armed with larger numbers of stinging batteries, these specialised polyps display behaviours associated with searching and attacking rival colonies (Ayre and Grosberg, 2005). The morphology of the mini-rediae of *Philophthalmus* is well-suited for its presumed role in preying upon co-infecting parasites. In addition to its muscular mouthpart and elongated gut, its smaller size enables it to gain access to parts of the host that cannot accommodate the larger producer rediae. While it is difficult to observe the behaviour of rediae in situ, the mini-rediae are noticeably more mobile than larger cercaria-producing individuals when dissected from their host (T. Leung, personal observation), and the presence of prominent posterior locomotor appendages should enable them to navigate within the host visceral mass. Finally, the observation that they actively attack the sporocysts and cercariae of co-infecting *M. novaezealandensis* certainly indicates that these mini-rediae play an active role in interspecific antagonism. This is in accordance with the findings of Hechinger et al. (2011).

The manner in which the *Philophthalmus* mini-rediae were seen attacking the *M. novaezealandensis* co-infection is very similar to what has been described by Lie (1967), where the rediae of *Paryphostomum segregatum* attacked the sporocysts of co-infecting



**Fig. 4.** Relationship between the ratio of mini-to-producer *Philophthalmus* sp. rediae within a snail and both the total number of rediae per snail and the shell length of the snail host. The ratios shown are partial residuals derived from the model, used here to illustrate the individual effect of each predictor variable. Thus, in the graph with the X axis labelled “Total number of rediae”, the ratios are those expected for shell sizes standardized at 16 mm, and in the graph with the X axis labelled “Snail shell length (mm)”, the ratios are those for a standardized total of 530 rediae per snail.

*Schistosoma mansoni* by applying vigorous suction with their mouthpart. It is unknown whether *Philophthalmus* also secretes some kind of growth-inhibition chemicals that could suppress the development of co-infecting species, although this may be unnecessary with the mini-rediae already actively attacking co-infecting sporocysts.

Host body mass can limit the total biomass of parasite that can be supported (Poulin and George-Nascimento, 2007), and this also applies to trematodes in snails. As expected, larger snails were found to harbour higher numbers of *Philophthalmus* rediae overall, which corroborates what has been reported in other trematode–mollusc systems (Zischke, 1967; Vignoles et al., 2006; Hechinger et al., 2009). Also as expected, the overall number of mini-rediae increased with the number of producer rediae. However, more interesting is that relative to that of producer rediae, the number of mini-rediae actually decreased proportionally in larger hosts (Fig. 4), indicating a decrease in relative investment in mini-rediae associated with increasing host size. This stands in contrast to the findings of Hechinger et al. (2011) as they found a disproportionate increase in the number of “soldier” rediae (equivalent to our “mini-rediae” in the species they studied) with increasing colony size (which relates to host size). This may reflect differential competitive pressure and dynamics of antagonistic interspecific interactions within the trematode communities of the respective ecosystems we examined.

In our system, field data indicate that snails of smaller size classes are usually only infected with *M. novaezealandensis*, while snails of intermediate sizes are more likely to have double infections of *Philophthalmus* and *M. novaezealandensis*, and larger snails harbour only *Philophthalmus* infections (Keeney et al., 2008). It is unknown whether this shift is due to (i) eventual displacement of *M. novaezealandensis* from the host due to subsequent infection by *Philophthalmus*, (ii) the latter intrinsically preferring larger snails, or (iii) parasite-induced mortality due to *M. novaezealandensis* killing the host before it can reach larger size classes (Fredensborg et al., 2005).

The lower ratio of mini-to-producer rediae seen in larger snails may represent a demographic shift, with the relative decrease in the number of mini-rediae in larger snails resulting from a lower probability or intensity of interspecific competition. While greater numbers of mini-rediae relative to producers may be necessary to eliminate the hundreds of sporocysts that are present in an established *M. novaezealandensis* infection, their role in interspecific competition becomes less important in larger snails. While these mini-rediae are still present in larger snails, their main function may shift to preventing new infections from establishing. With the decreased surface area to volume ratio of larger snails, fewer mini-rediae are necessary to patrol the peripheral site where new invaders may be found. As such, the mini-rediae may function in a density-dependent manner.

The presence of such morphs in *Philophthalmus* is somewhat reminiscent of the soldiers of polyembryonic parasitoid wasp larvae (Giron et al., 2007; Segoli et al., 2009). Much like trematode rediae, the parasitoid larvae live inside their host as collectives composed of multiple clonal individuals that face antagonism from co-occurring rivals. While the soldier larvae of parasitoid wasps never mature and reproduce (Gardner et al., 2007), the cost of producing sterile specialised morphs can be alleviated if it is simply a stage in the organism’s ontogeny, with these individuals eventually contributing to reproduction. This occurs in the asexual stage of the social gall aphid, *Pemphigus spyrothecae*, which produces defensive “soldier morphs” that eventually mature into reproductive adults (Pike and Manica, 2006). In the aphid system, context-dependent increased investment in defensive morphs involves delaying maturation of the younger instars, extending the period during which individuals remain as “soldiers” (Pike et al., 2004).

It is currently unknown which of the above systems most closely resembles that of *Philophthalmus*, as it depends upon whether (i) the mini-rediae of *Philophthalmus* sp. eventually contribute to reproduction by maturing into cercaria-producers or (ii) they persist as mini-rediae for their whole life. Hechinger et al. (2011) showed that most of the “soldiers” in the species they examined never grow up to be cercaria-producing rediae. It is currently unknown if that is also the case for *Philophthalmus*. Given that the ratio shifts in favour of relatively more cercariae-producing rediae without a corresponding increase in mini-rediae in larger snails, we predict that larger snails release relatively more cercariae than would be expected if cercarial production increased linearly with host size. If the mini-rediae do eventually mature into cercaria-producers (a possibility mentioned above), then the shift in ratio may be due to mini-rediae maturing into cercaria-producers at a higher rate than they are being replaced in larger hosts. With respect to the evolution of distinct morphs in *Philophthalmus*, perhaps the mini-rediae was originally an active feeding stage to fuel rapid growth – loosely comparable to the caterpillar stage in lepidopterans. A large proportion of its body is devoted to the feeding and digestive apparatus (Figs. 1 and 3). Rediae not only become less mobile as they mature, their digestive system also becomes reduced and the function of nutrient acquisition passes primarily to absorption through the tegument (Galaktionov and Dobrovolskij, 2003). If the mini-rediae of *Philophthalmus* represent a juvenile form which does not mature, this can be an extreme case of arrested development.

We present both direct and indirect evidence for *Philophthalmus* mini-rediae playing a specialised role in interspecific competition. Redial stages similar to those that we found have been described in some other trematode species (e.g., Beaver, 1939; Bearup, 1960; Martin and Adams, 1961; Stunkard, 1966), but previous authors never suggested that they might play a specific function in interspecific interactions, which was demonstrated to be the case by Hechinger et al. (2011). In our study, we found evidence to support the findings by Hechinger et al. (2011). Furthermore, we examined how the presence of a competing species might influence the level of resources allocated to the production of such specialised morphs. From our findings, we can make a prediction that can guide future research. We predict that species with such specialised morphs occur in areas with high prevalence and/or species-rich trematode communities. Experimental infections should be used next to determine whether *Philophthalmus* infection can prevent subsequent infection by *M. novaezealandensis* or other species.

Of particular interest is the role that mini-rediae play in intraspecific competition – are the mini-rediae capable of distinguishing between kin and non-kin within the host? And if so, does some priority effect favour the original occupant or are there other mechanisms regulating intraspecific interactions? Such investigations would require techniques that allow fine-scale genotyping such as the use of microsatellites or other polymorphic markers to distinguish the presence of multiple clones within the same host. We encourage future researchers to apply concepts and knowledge gained from studying eusocial or gregarious clonal animals to the investigation of trematode communities. Conversely, the adaptations of trematode parthenitae within their molluscan host may offer interesting models for studying functional specialisation in modular organisms (e.g., siphonophores: Dunn and Wagner, 2006) and the evolution of eusociality.

## Acknowledgements

We would like to thank Anson Koehler and Sabine Melzer for assistance in collecting snails, and the University of Otago’s Ecological Parasitology Research, New Zealand group for providing

comments on an earlier draft. This research was supported by a grant from the Marsden Fund (New Zealand).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpara.2011.05.001.

## References

- Ayre, D.J., Grosberg, R.K., 2005. Behind anemone lines: factors affecting division of labour in the social cnidarians *Anthopleura elegantissima*. *Anim. Behav.* 70, 97–110.
- Basch, P.F., DiConza, J.J., 1975. Predation by echinostome rediae upon schistosome sporocyst in vitro. *J. Parasitol.* 61, 1044–1047.
- Bearup, A.J., 1960. Life history of *Acanthoparyphium spinulosum* Johnston, 1917 (Trematoda: Echinostomatidae). *Aust. J. Zool.* 8, 217–225.
- Beaver, P.C., 1939. The morphology and life history of *Psilostomum ondatrae* Price, 1931 (Trematoda: Psilostomidae). *J. Parasitol.* 25, 383–393.
- Combes, C., 1982. Trematodes: antagonism between species and sterilizing effects on snails in biological control. *Parasitology* 84, 151–175.
- Dunn, C.W., Wagner, G.P., 2006. The evolution of colony-level development in the Siphonophora (Cnidaria: Hydrozoa). *Dev. Genes. Evol.* 216, 743–754.
- Evison, S.E.F., Hart, A.G., Jackson, D.E., 2008. Minor workers have a major role in the maintenance of leafcutter ant pheromone trails. *Anim. Behav.* 75, 963–969.
- Fjerdingstad, E.J., Crozier, R.H., 2006. The evolution of worker caste diversity in social insects. *Am. Nat.* 167, 390–400.
- Fredensborg, B.L., Mouritsen, K.N., Poulin, R., 2005. Impact of trematodes on host survival and population density in the intertidal gastropod *Zeacumantus subcarinatus*. *Mar. Ecol. Prog. Ser.* 290, 109–117.
- Galaktionov, K.V., Dobrovolskij, A.A., 2003. The Biology and Evolution of Trematodes. Kluwer Academic Publishers, London.
- Gardner, A., Hardy, I.C.W., Taylor, P.D., West, S.A., 2007. Spiteful soldiers and sex ratio conflict in polyembryonic parasitoid wasps. *Am. Nat.* 169, 519–533.
- Ginetsinkaya, T.A., 1988. Trematodes, Their Life Cycles, Biology and Evolution. Amerind Publishing, New York.
- Giron, D., Ross, K.G., Strand, M.R., 2007. Presence of soldier larvae determines the outcome of competition in a polyembryonic wasp. *J. Evol. Biol.* 20, 165–172.
- Hamilton, W.D., 1964. The genetical evolution of social behaviour. I and II. *J. Theor. Biol.* 7, 1–52.
- Hechinger, R.F., Lafferty, K.D., Mancini III, F.T., Warner, R.R., Kuris, A.M., 2009. How large is the hand in the puppeteer? Ecological and evolutionary factors affecting body mass of 15 trematode parasitic castrators in their snail host. *Evol. Ecol.* 23, 651–667.
- Hechinger, R.F., Wood, A.C., Kuris, A.M., 2011. Social organization in a flatworm: trematode parasites form soldier and reproductive castes. *Proc. R. Soc. B-Biol. Sci.* 278, 656–665.
- Keeney, D.B., Boessenkool, S., King, T.M., Leung, T.L.F., Poulin, R., 2008. Effects of interspecific competition on asexual proliferation and clonal genetic diversity in larval trematode infections of snails. *Parasitology* 135, 741–747.
- Keeney, D.B., Waters, J.M., Poulin, R., 2007. Clonal diversity of the marine trematode *Maritrema novaezealandensis* within intermediate hosts: the molecular ecology of parasite life cycles. *Mol. Ecol.* 16, 431–439.
- Korb, J., Hartfelder, K., 2008. Life history and development – a framework for understanding developmental plasticity in lower termites. *Biol. Rev.* 83, 295–313.
- Kuris, A.M., Lafferty, K.D., 1994. Community structure: larval trematode in snail hosts. *Annu. Rev. Ecol. Syst.* 25, 189–217.
- Lafferty, K.D., Kuris, A.M., 2009. Parasitic castration: the evolution and ecology of body snatchers. *Trends Parasitol.* 25, 564–572.
- Leung, T.L.F., Donald, K.M., Keeney, D.B., Koehler, A.V., Peoples, R.C., Poulin, R., 2009. Trematode parasites of Otago Harbour (New Zealand) soft-sediment intertidal ecosystems: life cycles, ecological roles and DNA barcodes. *N.Z. J. Mar. Freshw. Res.* 43, 857–865.
- Lie, K.J., 1967. Antagonism of *Paryphostomum segregatum* rediae to *Schistosoma mansoni* sporocysts in the snail *Biomphalaria glabrata*. *J. Parasitol.* 53, 969–976.
- Lie, K.J., 1973. Larval trematode antagonism: principles and possible application as a control method. *Exp. Parasitol.* 33, 343–349.
- Lim, H.-K., Heyneman, D., 1972. Intramolluscan inter-trematode antagonism: a review of factors influencing the host–parasite system and its possible role in biological control. *Adv. Parasitol.* 10, 191–268.
- Martin, W.E., Adams, J.E., 1961. Life cycle of *Acanthoparyphium spinulosum* Johnston, 1917 (Echinostomatidae: Trematoda). *J. Parasitol.* 47, 777–782.
- Martorelli, S.R., Fredensborg, B.L., Leung, T.L.F., Poulin, R., 2008. Four trematode cercariae from the New Zealand intertidal snail *Zeacumantus subcarinatus* (Batillariidae). *N.Z. J. Zool.* 35, 73–84.
- McGlynn, T.P., Owen, J.P., 2002. Food supplementation alters caste allocation in a natural population of *Pheidole flavens*, a dimorphic leaf-litter dwelling ant. *Insect. Soc.* 49, 8–14.
- Passera, L., Roncin, E., Kaufmann, B., Keller, L., 1996. Increased soldier production in ant colonies exposed to intraspecific competition. *Nature* 379, 630–631.
- Pike, N., Braendle, C., Foster, W.A., 2004. Seasonal extension of the soldier instar as a route to increased defence investment in the social aphid *Pemphigus spyrothecae*. *Ecol. Entomol.* 29, 89–95.
- Pike, N., Manica, A., 2006. The optimal balance of defence investment strategies in clonal colonies of social aphids. *Behav. Ecol. Sociobiol.* 60, 803–814.
- Poulin, R., George-Nascimento, M., 2007. The scaling of total parasite biomass with host body mass. *Int. J. Parasitol.* 37, 359–364.
- Powell, S., Franks, N.R., 2005. Caste evolution and ecology: a special worker for novel prey. *Proc. R. Soc. B-Biol. Sci.* 272, 2173–2180.
- Sapp, K.K., Meyer, K.A., Loker, E.S., 1998. Intramolluscan development of the digenean *Echinostoma paraensei*: rapid production of a unique mother redia that adversely affects development of conspecific parasites. *Invertebr. Biol.* 117, 20–28.
- Segoli, M., Harari, A.R., Bouskila, A., Keasar, T., 2009. Brood size in a polyembryonic parasitoid wasp is affected by relatedness among competing larvae. *Behav. Ecol.* 20, 761–767.
- Sousa, W.P., 1993. Interspecific antagonism and species coexistence in a diverse guild of larval trematode parasites. *Ecol. Monogr.* 63, 103–128.
- Stunkard, H.W., 1966. The morphology and life history of the digenetic trematode, *Himastha littorinae* sp. n. (Echinostomatidae). *J. Parasitol.* 52, 367–372.
- Tyerman, J.G., Roitberg, B.D., 2004. Factors affecting soldier allocation in clonal aphids: a life-history model and test. *Behav. Ecol.* 15, 94–101.
- Vieira-Neto, E.H.M., Mundim, F.M., Vasconcelos, H.L., 2006. Hitchhiking behaviour in leaf-cutter ants: an experimental evaluation of three hypotheses. *Insect. Soc.* 53, 326–332.
- Vignoles, P., Novobilský, A., Rondelaud, D., Bellet, V., Treuil, P., Koudela, B., Dreyfuss, G., 2006. Cercarial production of *Fascioloides magna* in the snail *Galba truncatula* (Gastropoda: Lymnaeidae). *Parasitol. Res.* 98, 462–467.
- Zischke, J.A., 1967. Redial populations of *Echinostoma revolutum* developing in snails of different sizes. *J. Parasitol.* 53, 1200–1204.