

Reproduction and caste ratios under stress in trematode colonies with a division of labour

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SUMMARY

Trematodes form clonal colonies in their first intermediate host. Individuals are, depending on species, rediae or sporocysts (which asexually reproduce) and cercariae (which develop within rediae or sporocysts and infect the next host). Some species use a division of labour within colonies, with 2 distinct redial morphs: small rediae (non-reproducing) and large rediae (individuals which produce cercariae). The theory of optimal caste ratio predicts that the ratio of caste members (small to large rediae) responds to environmental variability. This was tested in *Philophthalmus* sp. colonies exposed to host starvation and competition with the trematode, *Maritrema novaezealandensis*. *Philophthalmus* sp. infected snails, with and without *M. novaezealandensis*, were subjected to food treatments. Reproductive output, number of rediae, and the ratio of small to large rediae were compared among treatments. *Philophthalmus* sp. colonies responded to host starvation and competition; reproductive output was higher in well-fed snails of both infection types compared with snails in lower food treatments and well-fed, single infected snails compared with well-fed double infected snails. Furthermore, the caste ratio in *Philophthalmus* sp. colonies was altered in response to competition. This is the first study showing caste ratio responses to environmental pressures in trematodes with a division of labour.

Key words: host diet, competition, colony reproductive success, *Philophthalmus* sp., *Zecumantus subcarinatus*, *Maritrema novaezealandensis*.

INTRODUCTION

Parasites live in an environment of limited resources (their host) which must be allocated efficiently to growth, reproduction and/or survival (Poulin, 1996). This allocation can be affected by host condition; any stress on the host may limit resources available to the parasite (Poulin, 1996, 2007). Selection should favour parasite individuals that adopt the most efficient allocation of limited host resources to different life-history demands (Poulin, 1996).

When hosts are under stress, especially food-deprived, one of two responses by parasites is expected: reduction in resources taken from the host to keep it alive, or, if the parasite cannot reduce its resource consumption and continues to exploit the host at the same rate, increased mortality in hosts under severe stress (Jokela *et al.* 2005). The latter, when combined with the immunosuppressive effect of stress (Latshaw, 1991; Pruett *et al.* 1993), can theoretically lead to disease outbreaks in stressed populations (Lloyd, 1995). In the past, this has been studied by measuring parasite effects on stressed hosts (susceptibility to infection, parasite-induced mortality or reduced development) (Krist *et al.* 2004; Jokela *et al.* 2005; Saarinen and Taskinen, 2005;

Fellous and Koella, 2010), and/or parasite reproductive output (Kendall, 1949; Shostak and Dick, 1986; Keas and Esch, 1997; Ebert *et al.* 2000; Sandland and Minchella, 2003; Bedhomme *et al.* 2004; Seppälä *et al.* 2008; Coors and De Meester, 2011).

In the case of trematodes, which have complex life cycles and multiple life-cycle stages, measuring the reproductive output from the first intermediate host by counting the free-swimming, infective cercariae may not fully capture how the parasite is responding to host stress. Cercariae represent the immediate reproductive output only. This, in relation to numbers of rediae (the within-host stages which produce cercariae), can provide a clearer quantification of the parasite's response in resource allocation between reproduction, growth and survival.

Furthermore, some species of trematodes have a division of labour within their colonies of rediae (Hechinger *et al.* 2011; Leung and Poulin, 2011; Miura, 2012). Typically, trematode rediae live within the first intermediate host where they asexually reproduce, forming a clonal colony (Galaktionov and Dobrovolskij, 2003). Cercariae develop within rediae and leave the host to encyst in or on the second intermediate host (Galaktionov and Dobrovolskij, 2003). In colonies of species with a division of labour, there exist 2 distinct redial morphs: a large, reproducing morph and a small, non-reproducing morph. Morphological and behavioural differences between the 2 morphs indicate that the small, non-reproducing

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morph appears specialized for defence against hetero- or conspecific colonies trying to establish within the same host, similar to the organization of many social insect colonies (Hechinger *et al.* 2011).

Division of labour has been best studied in social insects, where multiple castes have specialized functions. Their response to variations in environment and resource availability has been studied by looking at how the ratios of caste members change. Oster and Wilson (1978) predicted that with highly specialized castes, variation in ratios will be linked to overall colony success. Therefore, optimal ratios should vary and be adaptive for any given condition (Oster and Wilson, 1978). Such adaptation should occur if the system meets 4 assumptions: (1) non-reproducing castes have no other option but to help; (2) there is no constraint on the production of multiple castes; (3) the non-reproductive castes are fully sterile; and (4) the developing individuals have no control over which caste they join (Ratnieks *et al.* 2011).

In accordance with this theory of optimal caste ratio, it has been shown that caste ratios respond to environmental changes over time: proportionally more workers in response to changes in food resources (McGlynn and Owen, 2002), seasonal changes in caste ratios (Passera, 1977; Walker and Stamps, 1986), decreased caste ratio variability after a disturbance (Herbers, 1980) and increased production of non-reproductive caste members (Passera *et al.* 1996) or reproductive caste members (McGlynn, 2010) under competitive pressures. However, this response has not been seen in all systems (Calabi and Traniello, 1989). Across the social insects, there exists a wide diversity of reproductive strategies resulting in variation in relatedness among colony members, e.g. various types of parthenogenesis (aphids, bees and ants) and polyembryony (parasitoid wasps, bryozoans, trematodes) (Blackman, 1979; Seeley, 1995; Craig *et al.* 1997; Brusca and Brusca, 2003). In cases where colonies are not clonal, partial genetic relatedness make the quantification of colony fitness complicated. Trematode species with divisions of labour are an ideal model system to look at caste ratio theory because all colony members are clonal. Therefore the fitness of the colony is simply a sum of the fitness of all individuals.

A division of labour in *Philophthalmus* sp. colonies has recently been documented (Leung and Poulin, 2011). *Philophthalmus* sp. infects the common New Zealand mudsnail, *Zeacumantus subcarinatus*, as its first intermediate host. At least 3 other trematode species also infect this mudsnail, the most common being *Maritrema novaezealandensis* (Martorelli *et al.* 2004, 2008). Infections are common (50–80% prevalence by *M. novaezealandensis* and 3–8% by *Philophthalmus* sp.). Double species infections are seen in nature, and persist long-term when snails are kept in the laboratory (up to 2 years in some cases) (M. Lloyd, *personal observation*). Furthermore,

the reproductive output of colonies of both *M. novaezealandensis* and *Philophthalmus* sp. is reduced when they share the same host individual (Lloyd and Poulin, 2012).

Caste ratio (small rediae to large rediae, i.e. non-reproductives to reproductives) is variable in this system and ranges from 0.24 to 3.27 (Leung and Poulin, 2011 and the results of this study). According to the theory of optimal caste ratio, this ratio is hypothesized to change under different environmental conditions such as host stress or competition. As the small, non-reproductive rediae appear specialized for defence against co-infecting colonies (Hechinger *et al.* 2011), in the case of competitive interactions, it is hypothesized that there will be proportionally more small rediae in a *Philophthalmus* sp. colony. Since competition between trematodes in their first intermediate host is generally for space and food resources (Sousa, 1992; Poulin, 2001), we predict that colonies in competitive interactions subjected to further stress due to host starvation might intensify a change in caste ratio. Observational studies of *Philophthalmus* sp. infected snails with and without *M. novaezealandensis* indicate that the caste ratio is not responding to competition: the number of small rediae (relative to large rediae) in *Philophthalmus* sp. colonies shows some variation regardless of co-infection by *M. novaezealandensis* (Leung and Poulin, 2011). However, a change in caste ratios in response to competition may have been masked by the observational aspect of these studies and the relatively small sample sizes.

These species of trematodes provide a unique and interesting model in which to study resource allocation in parasite infections and caste ratio theory. Resources can be allocated to 3 different life-cycle stages that are specifically used for different functions: large rediae (growth and future reproductive output), small rediae (defence) or cercariae (reproductive output). Numbers of these life-cycle stages are variable (Leung and Poulin, 2011), and in the case of cercariae, respond to immediate environmental conditions (Lloyd and Poulin, 2012). The aims of this study were to use a long-term experiment to quantify changes in resource allocation and caste ratios in *Philophthalmus* sp. infections under the combined influence of host stress (induced by starvation) and competition by *M. novaezealandensis*. Evidence of changing caste ratios could highlight a new way in which parasites can alter the resources taken from the host in response to changes in availability.

MATERIALS AND METHODS

Study system

Zeacumantus subcarinatus is a common mudsnail inhabiting the intertidal zone of New Zealand mudflats. Macro-algae make up 80–90% of the snail's

diet, specifically the sea lettuce, *Ulva lactuca* (McClatchie, 1979). The snail is the first intermediate host to several trematode parthenitae (sporocysts or rediae, depending on the species), the 2 most common of which were used in this study: *M. novaezealandensis* (family Microphallidae) and *Philophthalmus* sp. (family Philophthalmidae) (Martorelli *et al.* 2004, 2008). As is the case with most trematode species, *Philophthalmus* sp. and *M. novaezealandensis* asexually reproduce within the snail, filling it with sporocysts (in the case of *M. novaezealandensis*) or rediae (in the case of *Philophthalmus* sp.). Free-swimming cercariae develop in either the sporocysts or rediae and are released into the water where they look to encyst in or on a second intermediate host (West, 1961; Martorelli *et al.* 2004). *Philophthalmus* sp. is somewhat unusual, in that the cercariae do not encyst inside the tissue of a second intermediate host, but on the outer shells of gastropods (Neal and Poulin, 2012). This makes the system ideal for laboratory studies; encysted metacercariae form on any hard substrate (glass, plastic, etc.), where they accumulate and are easily counted (Lei and Poulin, 2011). Sexual reproduction occurs after the encysted metacercariae are ingested by the adult host (a sea bird), and eggs are subsequently released back into the marine environment to re-start the cycle (Galaktionov and Dobrovolskij, 2003).

Snails were collected from Lower Portobello Bay, Otago Harbour, South Island, New Zealand (45°52'S, 170°42'E) between 28 February and 5 March 2012. Individual snails were placed in 5 mL wells of a 12-well culture plate with 4 mL of filtered seawater and incubated overnight at 27 °C under bright light to encourage emergence of cercariae. Snails identified as being infected by either *Philophthalmus* sp. or both *M. novaezealandensis* and *Philophthalmus* sp. were retained; those infected by *M. novaezealandensis* only and uninfected snails were not used in this study. All snails were kept in well-aerated plastic containers (17 × 17 cm) with *U. lactuca* for up to 2 weeks prior to the start of the experiment.

Experimental design

On day 0 of the experiment, snails were incubated overnight again to confirm their infection status and to release any prior accumulation of mature cercariae. On day 1 of the experiment, the maximum length of each snail was measured with callipers (to 0.01 mm) and snails within each infection type (*Philophthalmus* sp. only, or both *M. novaezealandensis* and *Philophthalmus* sp.) were separated into size classes to be assigned evenly across food treatments. This ensured that the average snail length (which indirectly corresponds to snail age) was equal across food treatments. Individual snails were placed into plastic cups (4.5 cm diameter × 5 cm height) filled with

65 mL of filtered seawater. Cups were numbered to allow for repeated counts of individual snails over the 10 weeks of the experiment.

Food stress was induced by controlling the amount of *U. lactuca* added to each plastic cup. The 3 treatments consisted of either a 5 cm² piece of *U. lactuca* (referred to as the well-fed treatment), a 1 cm² piece of *U. lactuca* (referred to as the intermediate food treatment) or no *U. lactuca* (referred to as the starved treatment). Food was replaced every 7 days throughout the experiment. At the time that food was replaced each week, some portion of all 5 cm² pieces remained and the majority of the 1 cm² portions were consumed, indicating that 1 cm² was just sufficient to meet the snail's diet. Sixteen *Philophthalmus* sp. infected snails were used per food treatment. Since snails infected with both *M. novaezealandensis* and *Philophthalmus* sp. are more rare, there were 7 or 8 snails per food treatment.

To measure cercarial output of individual colonies within snails, a standard glass microscope slide was placed in each cup. Once weekly, encysted metacercariae on the glass slides were counted under a dissecting microscope. This method has been used in the past to measure relative, if not absolute, cercarial output from *Philophthalmus* sp. infected snails kept in laboratory conditions (Lei and Poulin, 2011). Since the plastic cups were small, encysted metacercariae on the bottom of the cups were also easily counted. The slides were replaced, water was changed and plastic cups were cleaned once weekly. Cercarial counts were completed just prior to cleaning to ensure that no cercariae were lost during the cleaning process.

Caste ratio

After 10 weeks of exposure to the above food treatment, snails were dissected and small and large rediae in each snail were counted. The snail visceral mass was dissected out of the snail shell and teased apart to release *Philophthalmus* sp. rediae and *M. novaezealandensis* sporocysts. Parasite parthenitae were dyed with neutral red, and pressed between 2 glass slides. *Philophthalmus* sp. small and large rediae were counted separately. The caste ratio was calculated as the number of small rediae divided by the number of large ones.

Statistical analysis

Differences in numbers of *Philophthalmus* sp. encysted metacercariae from single and double infected snails in different treatments were tested using a Linear Mixed Model (LMM) performed in R version 2.14.0 (R Development Core Team, 2011) using the package LME4 (Bates *et al.* 2011). Fixed effects included food treatment, infection type (either *Philophthalmus* sp. only or *Philophthalmus* sp. and

M. novaezealandensis), the interaction between food treatment and infection type, and host size (as measured at the start of the experiment). Host size was included as a factor because the average size between snails of different infection types was slightly different (single infection $17.47 \text{ mm} \pm 2.90 \text{ mm}$, double infection $14.91 \text{ mm} \pm 1.90 \text{ mm}$). To account for temporal pseudo-replication, snail identity was included as a random effect. The number of encysted metacercariae was $\log(x+0.5)$ transformed to meet assumptions of normality.

The small-to-large rediae caste ratio, total number of rediae, and number of large and small rediae per colony (i.e. per snail) were all compared between treatments using separate Linear Models (LMs). When comparing caste ratios between treatment groups, main factors included food treatment, infection type (either *Philophthalmus* sp. only or *Philophthalmus* sp. and *M. novaezealandensis*), the interaction between food and infection, and total number of rediae. Host size was considered as a factor, but was not significant, and removing it increased the fit of the model (according to Akaike Information Criterion (AIC) values). Differences in total number of rediae between treatments were also analysed using a LM. In this case, the main factors were infection, food treatment, the interaction between food and infection, host size and caste ratio.

Additional LMs were performed to verify whether the treatment levels affected only one caste independently of the other. When analysing numbers of small rediae, factors included food treatment, infection type (either *Philophthalmus* sp. only or *Philophthalmus* sp. and *M. novaezealandensis*), the interaction between the two, host size and the number of large rediae. The number of small rediae was $\log(x+0.5)$ transformed to meet assumptions of normality. Similarly, when analysing numbers of large rediae, factors included food treatment, infection type, the interaction between the two, host size and number of small rediae. The number of large rediae was $\log(x+0.5)$ transformed to meet assumptions of normality.

RESULTS

Over the 10 weeks of the experiment, 8 *Philophthalmus* sp. infected snails died, 1 from the well-fed treatment, 3 from the intermediate food treatment and 4 from the starved treatment. One double infected snail from the intermediate food treatment group died.

Cercarial output

The numbers of cercariae (counted as encysted metacercariae) that emerged from well-fed snails of both infection types were significantly higher than those of snails in the intermediate and starved food

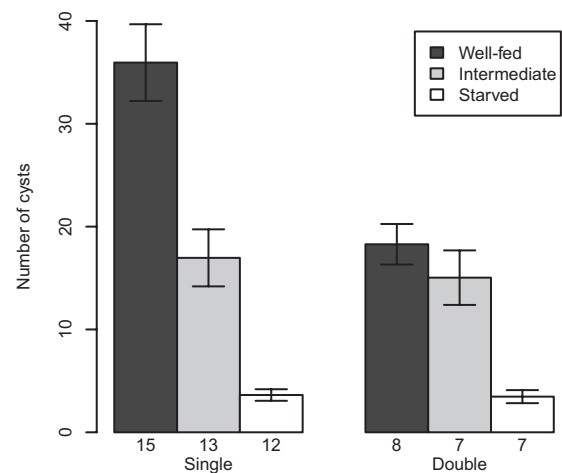


Fig. 1. Average (\pm s.e.) total number of *Philophthalmus* sp. encysted metacercariae released over the ten week period from colonies without or with competition (i.e. single or double infections) by *Maritrema novaezealandensis* in three food treatments. Sample size is indicated at the bottom of each bar.

treatments ($P < 0.0001$). Also significant was the interaction between infection type and the intermediate food treatment ($P = 0.039$), but not the effect of infection itself (Fig. 1, Table 1). Well-fed snails with only *Philophthalmus* sp. infections released approximately twice as many cercariae as well-fed snails with both *Philophthalmus* sp. and *M. novaezealandensis*. However, this difference between infection types was only seen in the well-fed treatment. In the intermediate food treatment, the numbers of cercariae that emerged from single infected snails were only slightly higher than those of double infected snails; and in the starved treatment, the numbers of cercariae emerged from single and double infected snails were similar (Fig. 1, Table 1).

Caste ratio and rediae

At the end of the experiment, 43 single infected snails and 22 double infected snails were dissected and their rediae counted. Snails were not included if they died before they could be dissected and the rediae inside had started to decompose. The caste ratio of small-to-large rediae ranged from 0.25 to 1.75 and was significantly higher in snails with both *Philophthalmus* sp. and *M. novaezealandensis* than in snails with only *Philophthalmus* sp. ($P = 0.004$). In addition, there was a significant interaction between the intermediate food treatment and infection type, although no effect of food treatment was detected (Fig. 2, Table 2). The double infected snails in the intermediate food treatment group hosted colonies with a lower ratio of small-to-large rediae than either the well-fed treatment or the starved treatment group. The total number of rediae per colony ranged from 66 to 510. The only significant factors affecting

Table 1. Factors affecting number of encysted metacercariae from *Philophthalmus* sp. colonies with or without competition in three food treatments

(Results of a Linear Mixed Model comparing numbers of encysted metacercariae from *Philophthalmus* sp. colonies with and without co-infection by *M. novaezealandensis* ('Infection') in 3 food treatments (well-fed, intermediate or starved) over 10 weeks.)

Factor	Estimate	S.E.	t value	P value
Intercept*	0.445	0.202	2.200	0.028
Infection	-0.046	0.099	-0.468	0.640
Intermediate food treatment	-0.468	-0.473	-5.729	<0.0001
Starved food treatment	-0.814	0.0855	-9.633	<0.0001
Host size	0.041	0.011	3.602	0.0003
Infection*Intermediate food treatment	0.284	0.138	2.062	0.039
Infection*Starved food treatment	0.055	0.137	0.404	0.686

* The effect of the well-fed treatment is included in the intercept.

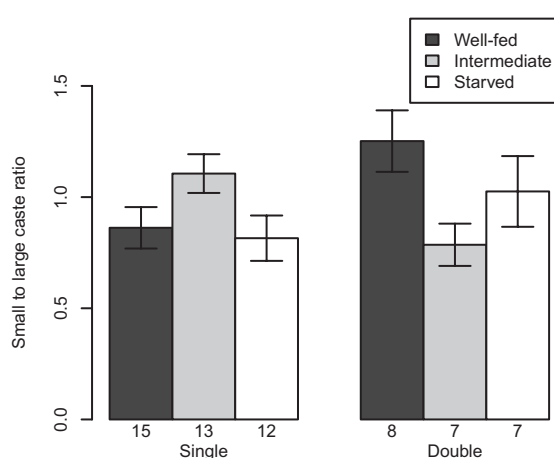


Fig. 2. Average (\pm S.E.) caste ratio of small-to-large rediae in *Philophthalmus* sp. colonies without or with competition (i.e. single or double infections) by *Maritrema novaezealandensis* in three food treatments after 10 weeks. Sample size is indicated at the bottom of each bar.

total number of rediae were host size and the caste ratio (Table 3).

The number of small rediae per colony ranged from 20 to 265; the significant factors affecting the number of small rediae were the number of large rediae and the interaction between infection type and the intermediate food treatment (Supplementary Table 1, in Online version only). In a close parallel, the number of large rediae per colony ranged from 35 to 235; the significant factors affecting the number of large rediae were host size, the number of small rediae, and the interaction between infection type and the intermediate food treatment (Supplementary Table 2, in Online version only).

DISCUSSION

Caste ratio

This study presents the first evidence that caste ratios in trematodes with a division of labour are influenced

by their environment. *Philophthalmus* sp. colonies which competed with *M. novaezealandensis* had a higher caste ratio of non-reproducing rediae to reproducing rediae than *Philophthalmus* sp. colonies without a competitor. The caste ratio of colonies in ideal conditions (those in well-fed snails) increased from a mean of 0.86 small rediae per large rediae in single infected snails to a mean of 1.25 small rediae per large rediae in double infected snails (Fig. 2). In previous observational studies, the caste ratio of small, non-reproducing to large, reproducing rediae in double infected snails was not higher than that in single infected snails (Leung and Poulin, 2011). However, these studies used field-collected snails and/or snails maintained in the laboratory for only a short period of time, thus ignoring long-lasting effects of what resources had been available to the snail host in nature. If non-reproducing rediae are specialized for defence, as was previously suggested (Hechinger *et al.* 2011), one would expect the caste ratio in *Philophthalmus* sp. colonies to respond to co-infection by *M. novaezealandensis* with an increase in the relative numbers of non-reproductive rediae. Similar to our results, caste ratios in social insects did not vary due to ecological factors (one being competition) in a field observational study (Calabi and Traniello, 1989), but caste ratios did respond to competition by increasing soldier production in a 7-week laboratory experiment (Passera *et al.* 1996). This may indicate that there are many confounding environmental factors that determine caste ratio which can best be standardized by using experimental treatments either in the field or in the laboratory.

These results could also be interpreted within the traditional understanding of redial generations occurring in most trematode species, in which young, immature rediae with higher mobility grow into larger rediae capable of producing cercariae (Galaktionov and Dobrovolskij, 2003; Rondelaud *et al.* 2009). In this case, the division of labour would be sequential and follow the ontogeny of rediae, as opposed to involving distinct morphs. If this were the

Table 2. Factors affecting caste ratio of small : large rediae

(Results of a Linear Model comparing caste ratio (small rediae per large rediae) of *Philophthalmus* sp. colonies with and without competition by *Maritrema novaezealandensis* ('Infection') in 3 food treatments (well-fed, intermediate or starved) after 10 weeks.)

Factor	Estimate	S.E.	<i>t</i> value	<i>P</i> value
Intercept*	0.625	0.153	4.073	<0.0001
Infection	0.463	0.158	2.920	0.005
Intermediate food treatment	0.239	0.131	1.827	0.073
Starved food treatment	-0.018	0.131	-0.138	0.890
Total rediae	0.001	0.0005	1.917	0.060
Infection*Intermediate food	-0.700	0.224	-3.127	0.003
Infection*Starved	-0.243	0.226	-1.074	0.287

* The effect of the well-fed treatment is included in the intercept.

Table 3. Factors affecting the total number of rediae

(Results of a Linear Model comparing total rediae in *Philophthalmus* sp. colonies with and without competition by *Maritrema novaezealandensis* ('Infection') in 3 food treatments (well-fed, intermediate or starved) after 10 weeks.)

Factor	Estimate	S.E.	<i>t</i> value	<i>P</i> value
Intercept*	-218.916	74.232	-2.949	0.005
Infection	-44.460	35.402	-1.256	0.214
Intermediate food treatment	-30.227	28.402	-1.064	0.292
Starved food treatment	-22.831	27.498	-0.830	0.410
Host size	23.686	3.786	6.257	<0.0001
Caste ratio	87.089	26.988	3.227	0.002
Infection*Intermediate food	52.720	50.840	1.037	0.304
Infection*Starved	56.345	47.496	1.186	0.240

* The effect of the well-fed treatment is included in the intercept.

case, the changes in caste ratios we observed here among treatments would represent demographic processes, i.e. adjustments to the developmental schedule of growing rediae, and not the differential production of distinct morphs. These could also be interpreted as adaptive responses as we explain above and below, following the same rationale driven by what is the optimal strategy for the whole colony. However, given that the small rediae of our study species, when left in a suitable culture medium for 2 months, never develop, even partially, toward becoming large reproductive rediae (M. Lloyd, *personal observation*), we believe that distinct castes do exist in this species as in other trematodes (Hechinger *et al.* 2011; Miura, 2012).

Analyses of caste ratios of colonies in snails within different food treatments indicate that the ratios may be altered in response to available food treatments, although the mechanism for this is not clear. In well-fed snails, the caste ratio of *Philophthalmus* sp. colonies in double infected snails was higher than single infected snails. In contrast, the caste ratio of *Philophthalmus* sp. colonies in snails in the intermediate food group decreased when co-infected with *M. novaezealandensis* (Fig. 2). This interaction effect between infection type and the intermediate food

treatment was seen in the LMs for caste ratio, large rediae and small rediae (Table 2, Supplementary Table S1, Supplementary Table S2, in Online version only). In social insects, there is a temporal constraint on caste ratio responses to changes in environmental condition (Herbers, 1980; Walker and Stamps, 1986). Perhaps the caste ratio in the trematode system responds over a long time period and had not had enough time to fully respond to the food stress treatment over 10 weeks. For instance, caste ratios of social ants respond to changes in food availability over 4 months (McGlynn and Owen, 2002). Another explanation for this interaction effect may be that only colonies in unstressed hosts are able to increase production of small per large rediae: the caste ratio of colonies in well-fed hosts increased in response to competition while the caste ratio of colonies in stressed hosts either decreased or stayed the same in response to competition (Fig. 2).

Previous studies show that non-reproducing rediae provide some benefit to *Philophthalmus* sp. colonies resulting in a higher reproductive output (Lloyd and Poulin, 2012). While the mechanisms of this benefit remain unclear, the present experiment shows that colonies may be able to alter caste ratios in response to changes in environmental conditions, further

indicating that small rediae play an important functional role.

Cercarial output strategy

Results also show *Philophthalmus* sp. colonies alter the way in which they use resources taken from the host in response to both starvation and competition. Colonies in well-fed snails had a higher reproductive output (up to 2-fold difference) than colonies in snails in the intermediate or starved food treatments (Fig. 1). When faced with a competitor, *Philophthalmus* sp. colonies had a lower reproductive output, but this difference was seen only between snails in the well-fed treatment group. Compared with the least stressed colonies (i.e. those in well-fed, single infection snails), those in the intermediate food treatment group showed a 2-fold decrease in reproductive output regardless of infection type. Colonies in snails in the starved treatment group showed a dramatic reduction in reproductive output regardless of infection type. The generally negative impact of food availability for the host on parasite reproductive output is not surprising as this trend has been recorded multiple times (Kendall, 1949; Keas and Esch, 1997; Seppälä *et al.* 2008). The interaction between food treatment and infection type provides more information on the effect of both: the fact that competition was only seen between the well-fed treatments groups indicates that the effects of host nutrition might outweigh those of competition, and thus that competition has resource-dependent effects on trematode colonies.

The total number of small and large rediae per colony did not respond to the infection type of its host. This provides evidence that *Philophthalmus* sp. colonies do not change their investment into growth or future reproductive output (large rediae) in response to competition. The same result has been seen in *Philophthalmus* sp. colonies in response to co-infection by *M. novaezealandensis* (Keeney *et al.* 2008), as well as in another similar trematode species pair (Hendrickson and Curtis, 2002). The difference in the numbers of cercariae produced by *Philophthalmus* sp. colonies in unstressed hosts (well-fed, single infections) and stressed hosts (starved, double infections) indicates that rediae appear able to increase their *per capita* cercarial output when not under stress.

Taken together, the results from this study indicate that *Philophthalmus* sp. colony organization may be altered in response to environmental conditions. In addition, there was no difference in host survival between any treatment groups; only 8 out of 71 snails died over the course of the experiment. This provides further support for the hypothesis put forth by Jokela *et al.* (2005) that parasites can alter the amount of resources taken from a host under stress

to avoid over-exploitation and parasite-induced mortality.

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