Evol Ecol (2013) 27:1235–1247
DOI 10.1007/s10682-013-9646-y

RESEARCH ARTICLE

What determines the growth of individual castes in social trematodes?

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Received: 10 December 2012 / Accepted: 11 April 2013 / Published online: 18 April 2013

Abstract Phenotypic diversification among colony members often leads to formation of physical castes which are morphologically specialised for particular tasks within the colony. The relative abundance of these castes and their body sizes represent two key aspects of the demography of a colony that may reflect the colony's needs and conditions, and ultimately influence its survival and reproductive success. In a recently discovered social trematode, *Philophthalmus* sp., which exhibits a reproductive division of labour, the role of competition and colony composition in shaping reproductive success and behaviour of colony members has been documented. As body size variation within physical castes often influences colony efficiency, we investigated how the growth of reproductive and non-reproductive morphs of *Philophthalmus* sp. responds to competitive pressure, and to other attributes of colony demography such as colony size and composition. Our survey of a natural population and in vitro experiments demonstrate that the growth of reproductive colony members reflects the interaction between colony composition and the presence of a competitor, while the non-reproductive members simply grow larger in the presence of the intra-host competitor, *Maritrema novaezealandensis*. Furthermore, the close association between the volume and reproductive capacity of the reproductive members corroborates an adaptive value of colony member size in determining the fitness of the trematode colony as a whole. The present study is the first to demonstrate a fitness consequence, and identify the determinants, of the growth of colony members in social trematodes.

Keywords Division of labour · Social trematode · Colony growth · *Philophthalmus*

Introduction

In organisms with division of labour, phenotypic diversification of colony members often leads to formation of physical castes which are morphologically specialised for particular tasks within the colony (Oster and Wilson 1978). With notable ecological similarities,

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physical castes have evolved independently in a number of insects (Oster and Wilson 1978), snapping shrimps (Duffy 1996), sea anemones (Ayre and Grosberg 1996), mole rats (Jarvis 1981) and parasitic trematodes (Hechinger et al. 2011; Leung and Poulin 2011). The relative abundance of these castes and their body size are two key aspects of the demography of a colony that is expected to reflect the colony's strategy in a given environment (Wilson 1971). The optimal theory predicts that colony fitness should be a function of the efficiency of a particular adaptive demography under a given set of environmental conditions (Oster and Wilson 1978). Thus with changing environmental conditions, the adaptive demography of a colony is predicted to shift in such a way that ergonomic efficiency of the colony as a whole is optimally maintained (Hasegawa 1997). Given limited resources available to a colony, the optimal resource allocation must be achieved through a trade-off between various colony functions. In many insect colonies these include alteration of the number and size of offspring produced (Passera et al. 1996; Lecoutey et al. 2011; Schmidt et al. 2011), or morphological and behavioural plasticity (Wilson 1971; Ishikawa and Miura 2012).

In social insects, size polymorphism may be determined genetically with little flexibility with respect to the environment (Mitchell et al. 2012), or may alternatively respond to changes in habitat (Davidson 1978; Yang et al. 2004), seasonal cycles (Rissing 1987), competitive pressure (Davidson 1978; Yang et al. 2004), colony size (Porter and Tschinkel 1985) and colony composition (Porter and Tschinkel 1985). While these changes may be adaptively achieved over several generations, the average size of existing colony members in a given generation may also fluctuate depending on the amount of resources available before the animals' terminal size is reached. Understanding the extrinsic factors that influence individual caste growth is important as body size often affects efficiency of the specialised tasks in the colony (Kaspari 1996). Therefore, we investigate factors that may influence the growth of existing colony members using the clonal intermediate stage of social trematodes as a model system.

In the clonal intermediate stage of two-caste trematode parasites, one morph produces cercariae, the next motile larval stage that leaves the gastropod host, while the second form is considerably smaller and reproductively inactive (Hechinger et al. 2011; Leung and Poulin 2011). Several lines of empirical evidence presented by Hechinger et al. (2011) point towards the prospect that the non-reproductive morphs of *Himasthla* sp. form a soldier caste: they have relatively larger mouthparts, are more active than reproductives, readily attack members of foreign colonies, and are particularly common at invasion fronts within the host's body. In addition, direct consumption of co-infecting species by the non-reproductives has been reported in *Philophthalmus* sp. (Leung and Poulin 2011). Furthermore, the prospect that non-reproductive members are soldiers is consistent with the widely-held view that interspecific competition for resources and space presents a potentially strong selection pressure for trematode parasites infecting gastropod intermediate hosts (Kuris and Lafferty 1994; Poulin 2001), which has been recently corroborated in our study system by Lloyd and Poulin (2012). Although *Philophthalmus* sp. appears to lack adaptive plasticity to adjust caste ratios in response to short-term environmental changes (Leung and Poulin 2011; Kamiya and Poulin 2013), growth and size variation among trematode colony members have implications for colony success as larger reproductives are expected to produce more cercariae while larger non-reproductives should be more effective as defensive specialists.

Kamiya and Poulin (2013) have demonstrated a clear fitness benefit of maintaining the non-reproductive castes in the presence of a competitor while illustrating the cost of such morphs in a naturally infected population when the competitor is absent, supporting the hypothesis that reproductive output of *Philophthalmus* sp. colonies is influenced by the interaction of caste ratios and intra-host competition. Here we aim to investigate whether the sizes of reproductive and non-reproductive morphs of *Philophthalmus* sp. reflect competitive pressure and other attributes of colony demography such as colony size, composition (i.e. absolute number of each caste's members) and caste ratios (relative number of each caste's members) in natural populations. The effects of those variables were also tested in manipulative in vitro experiments in an attempt to elucidate causal factors that affect the growth of individual castes. Considering the findings of Kamiya & Poulin (2013) we would expect a context dependent response in the size of reproductives with regard to colony composition and the presence of a competitor. In this system the presence of a competitor may provide extra food resources for certain colony members, facilitating their growth. If defensive morphs preferentially consume tissues of competing species, which they should if non-reproductives are soldiers as proposed by Hechinger et al. (2011), higher growth of non-reproductive morphs would be expected where the colony faces competition while the size of reproductives may decrease as a result of exploitation competition.

Methods

Study organisms, screening and maintenance

Philophthalmus sp. is one of many trematode parasites with complex life cycles that infect the mud snail, *Zeacumantus subcarinatus,* as their first intermediate host. It is the second most common trematode parasite at our study site with up to 8 % prevalence, second only to *Maritrema novaezealandensis* whose prevalence can be as high as 60 %, while up to 11 % of hosts can be infected by both species (Keeney et al. 2008; Martorelli et al. 2008). Both trematode species multiply asexually in the gonadal tissues of the snail, forming masses of clonal stages collectively known as parthenitae that produce free-swimming dispersal stages known as cercariae, which then leave the snail. Parthenitae of *Philophthalmus* sp. are called rediae and possess mouthparts, and occur as non-reproductive and reproductive morphs; those of *M. novaezealandensis* are of a different type known as sporocysts and lack mouthparts. *Philophthalmus* sp. provides an ideal system to study sociality of trematodes because its proximate ecological properties such as life cycle history (Martorelli et al. 2008), larval dispersion characteristics (Koprivnikar and Poulin 2009), thermal and salinity tolerance (Bates et al. 2011; Lei and Poulin 2011) and co-infecting species in the gastropod host (Martorelli et al. 2008) have been relatively well documented. Furthermore, in vitro culture techniques have been optimised for this particular species so that manipulative experiments may be conducted to infer causal mechanisms (Lloyd and Poulin 2011).

Mud snails, *Z. subcarinatus,* were collected in February and March 2012 from Lower Portobello Bay, Otago Harbor, South Island, New Zealand (45°520 S, 170°420 E). The snails were individually incubated in wells of a 12-well culture plate filled with filtered saltwater at 25 °C for 24 h in order to induce the trematode parasites to release cercariae, which were identified using a stereomicroscope. Incubation also served to 'empty' cercariae stored in the host, to standardise the cercarial content of each colony to approximately zero at the start of the experiments. Snails found shedding cercariae of *Philophthalmus* sp. or both *Philophthalmus* sp. and *M. novaezealandensis* were individually transferred to separate plastic containers (17 cm × 17 cm × 8 cm), filled with filtered saltwater and supplied with *Ulva lactuca*. One-off incubations of snails do not guarantee accurate assessments of infection status. Therefore, snails were re-examined following the same procedure at least 48 h after the initial incubation. Snails that showed signs of infection from other trematode species were excluded. The infection status of the hosts was re-confirmed upon dissection.

In-vivo trematode census

Following the infection checks, the snails were maintained individually in wells (16.8 cm^3) of 6-well culture plates filled with filtered saltwater with a standard-sized piece of *U. lactuca* for 14 days at room temperature in order to measure reproductive output (see Kamiya and Poulin 2013 for results). Prior to dissection, the maximum shell length of each snail was measured to the nearest 0.1 mm using a set of callipers. In order to minimise differences between infection groups, the size of individual snails were matched as closely as possible between *Philophthalmus*-only (mean shell length = 13.20 mm) and *Philophthalmus*-*Maritrema* double infected snails (12.95 mm) (Welch Two Sample *t* test: T-value = 0.749, *P* value = 0.458). In total 28 *Philophthalmus*-only snails and 30 *Philophthalmus*-*Maritrema* double infected snails were included in this study. After cracking shells and removing the host tissue, the parasite tissues were dyed with Neutral Red, pressed between two glass plates and examined under a stereomicroscope. First the numbers of *Philophthalmus* sp. reproductive and non-reproductive rediae were counted. Then the volume of a subset of reproductive (N = 10) and non-reproductive rediae (N = 3 to 10) in each colony was estimated by calculating the circumference from the width of flattened rediae in micrographs and then deducing their radius. Redial volume was then calculated by equating the shape of a redia to that of a cylinder. The variation in the sample size of non-reproductive rediae across colonies arose due to overlap with other structures in the micrographs or unreliable staining which resulted in unclear images. The images were analysed using ImageJ 1.45 s. We were unable to count the number of *M. novaezealandensis* sporocysts as they cluster together in tight clumps, hence they were recorded as either present or absent.

Three separate linear mixed models (*lme4* package) were employed to investigate the relationship between redial volume (response variable) and (a) the colony size (i.e. the total number of colony members), (b) composition (i.e. the number of reproductives and non-reproductives) and (c) caste ratio (i.e. ratio of non-reproductives to reproductives) of *Philophthalmus* sp. colonies in the presence and absence of the co-infecting competitor, *M. novaezealandensis*. Since the demographic traits, namely colony size, composition and caste ratio, are not independent of each other, we employed three separate models to avoid multicollinearity. Interactions between competition and those demographic properties in determining the redial volume were also included in each model. Host shell length was excluded from the models as the variable was strongly inter-correlated with the total number of colony members (N = 58, R = 0.466) and exclusion resulted in considerably lower AIC values in all models ($\Delta AIC > 6$). The potential influence of differences in the genotype of the rediae was taken into account by including host identity as a random effect. All statistical analyses were conducted in R 2.14.1 (R Development Core Team 2011).

In-vitro experiment

Snails (different individuals from those used in the in vivo study) were carefully cracked using a hammer and *Philophthalmus* sp. rediae and *M. novaezealandensis* sporocysts were dissected out of separate singly-infected snails. Parthenitae were cultured in a 12-well culture plate filled with 1 ml culture medium which has been previously developed and optimised for use with both species of trematode (see Lloyd and Poulin (2011)). The culture medium consisted of 400 mg L-15 powder (Sigma L4386) dissolved in 50 ml sterile water, 1.56 g Instant Ocean powder (Aquarium Systems), 10 ml chicken serum (Invitrogen) and 2 ml of a penicillin–streptomycin-neomycin solution (Sigma P4083). The dissected rediae were allocated to 6 different treatments all with a fixed number of reproductives (6 rediae), but differing in the number of non-reproductive rediae (i.e. 2, 6, 18) and in the presence of *M. novaezealandensis* sporocysts (i.e. approximately 30 sporocysts, or 0 sporocysts). Rediae from a single snail were used for one replicate of each treatment, and *M. novaezealandensis* sporocysts were also taken from a separate single snail for each replicate, to homogenise genotypes across treatments. Although we cannot preclude the possibility of multiple-clone infections of *Philophthalmus* sp. within a single snail, most colonies genotyped in preliminary assessments in our laboratory were of single clones (36/38, 95 % using 8 polymorphic microsatellite markers; M. Lloyd, personal communication). The cultures were maintained for 28 days at 18 °C in an incubator under ambient light and replenished with new media every 3–4 days. At the end, redial volumes were calculated as described earlier. The experiment was replicated 14 times using 14 independent *Philophthalmus* sp. colonies.

Linear mixed models were constructed with the redial volume as the response variable (log-transformed) comparing cultures with and without the presence of *M. novaezealandensis* sporocysts using the package *lme4*. The number of non-reproductive rediae per culture and its interaction with competition were also added as predictors after centring. Since colony composition exhibited no association with the volume of non-reproductives in the in vivo census, only those from the 18:6 treatments were used for analysing the non-reproductive redial volume. The potential influence of differences in the genotype of the rediae and repeated measurements from the same cultures was taken into account by including clone identity as a random effect. Inactive rediae, i.e. individuals showing no movement for 30 s at the end of the experiment were excluded from the analyses.

Redial volume and reproductive potential

Using freshly caught snails (less than 5 days between capture and dissection), 20 mature rediae each (containing at least one cercaria) from hosts infected with *Philophthalmus* sp. (N = 6) and those with both *Philophthalmus* sp. and *M. novaezealandensis* (N = 6) were examined for the relationship between their volume and the number of mature cercariae they contained. The redial volume was calculated as before, by measuring rediae as cylinders from micrographs, while the number of mature cercariae was counted by dissecting individual reproductives following photographing. A linear mixed model was employed to analyse the relationship between the volume of reproductive rediae and their reproductive capacity, measured as the number of mature cercariae they contained, as the response (log-transformed). The presence of *M. novaezealandensis* and the maximum host shell length (i.e. a proxy for space available for parasites) were also added as predictors and the potential influence of differences in the genotype of the rediae was taken into account by including host identity as a random effect.

Results

Reproductive morphs

In-vivo trematode census

Regardless of the presence of *M. novaezealandensis,* the average size of reproductives from each colony did not correlate with that of non-reproductives (*Philophthalmus*-only: N = 28, adjusted $R^2 = 0.018$, *P* value = 0.475; *Philophthalmus-Maritrema*: N = 30, adjusted $R^2 = 0.034$, *P* value = 0.879). The colony size, measured as the total number of individuals in the colony, exhibited no association with the volume of reproductives in colonies, either with or without the intra-host competitor, *M. novaezealandensis* (Table 1a). However, the volume of reproductives was positively related to the absolute number of reproductives in the absence of the competitor while the association was negative in colonies facing competition. Conversely, the volume of reproductives exhibited a negative association with the absolute number of non-reproductives in the absence of competition while a positive link was observed in the presence of *M. novaezealandensis* (Table 1b), indicating a benefit of non-reproductives in the presence of the competitor. An identical pattern was observed when the relative numbers of the two functional morphs was used as the predictor, in the analysis of caste ratios (Fig. 1; Table 1c). The volume of reproductive morphs was not associated with the presence of *M. novaezealandensis* per se in any of the models tested, suggesting that the effect of intra-host competition is likely to be context-dependent in the natural population (Table 1).

Table 1 Results of linear mixed models evaluating the relationship between the log-transformed volume of the reproductive morphs and **a** the colony size (i.e. the total number of colony members), **b** composition (i.e. the number of reproductives and non-reproductives) and **c** caste ratio (i.e. ratio of non-reproductives to reproductives) and the presence of the co-infecting competitor, *Maritrema novaezealandensis,* in colonies of the trematode *Philophthalmus* sp.

	Estimate	SE	T value	*P* value
(a)				
(Intercept)	−3.273	0.093	−35.30	**<0.001**
Colony size	−0.001	0.084	−0.02	0.987
Competitor	0.016	0.129	0.13	0.901
Colony size × competitor	−0.097	0.132	−0.74	0.462
(b)				
(Intercept)	−3.286	0.082	−40.07	**<0.001**
Reproductives	0.207	0.090	2.31	**0.022**
Non-reproductives	−0.222	0.103	−2.17	**0.032**
Competitor	0.001	0.114	0.01	0.991
No. repro. × competitor	−0.576	0.138	−4.17	**<0.001**
No. non-repro. × competitor	0.353	0.134	2.64	**0.009**
(c)				
(Intercept)	−3.270	0.084	−38.88	**<0.001**
Caste ratio	−0.203	0.071	−2.88	**0.004**
Competitor	0.026	0.117	0.23	0.822
Caste ratio × competitor	0.454	0.113	4.00	**<0.001**

Bold type indicates significant results (*P* value < 0.05)

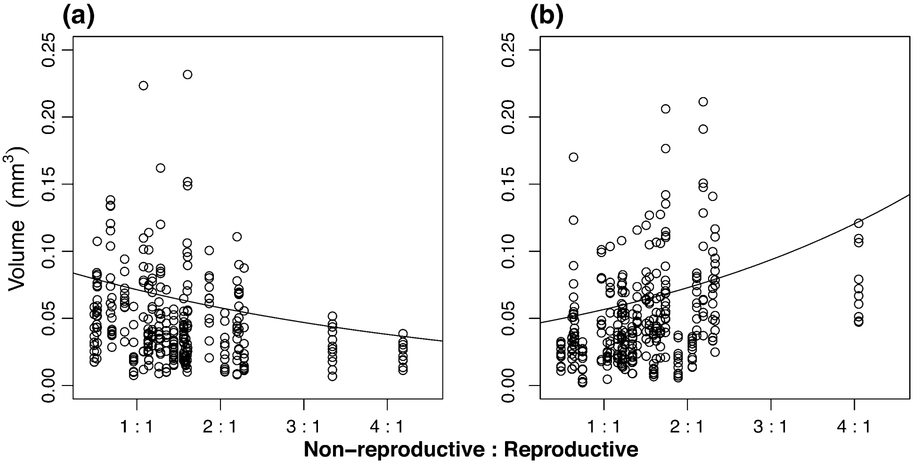

Fig. 1 The volume of *Philophthalmus* sp. reproductive morphs in live snail hosts infected with **a** only *Philophthalmus* sp. and **b** both *Philophthalmus* sp. and *Maritrema novaezealandensis,* against the ratio of non-reproductive to reproductive morphs. Random samples of ten reproductives from each colony (i.e. *Philophthalmus*-only: N = 28, *Philophthalmus*-*Maritrema*: N = 30) are plotted. The regression lines were exponentially converted from log-transformed data used in a linear model (refer to Table 1c for results)

In-vitro experiment

The volume of the reproductives of *Philophthalmus* sp. was significantly smaller in the presence of *M. novaezealandensis* (Table 2), indicating clear-cut competitive interactions between the two species which was undetected in the natural parasite census above. While the number of non-reproductives did not affect the volume of the reproductives in sole cultures, their volume increased as a function of the ratio of non-reproductives to reproductives when the intra-host competitor was co-cultured, indicating a benefit of non-reproductives in the presence of competition (Fig. 2; Table 2).

Non-reproductive morphs

In-vivo trematode census

The presence of the intra-host competitor was positively associated with the volume of non-reproductive morphs in natural colonies of *Philophthalmus* sp. while none of the

Table 2 Results of a linear mixed model evaluating the relationship between the log-transformed volume of the reproductive morphs and the interaction between the presence of the competitor, *Maritrema novaezealandensis,* and the number of non-reproductives in in vitro cultures of the trematode *Philophthalmus* sp.

	Estimate	SE	T value	*P* value
(Intercept)	−3.113	0.143	−21.76	**<0.001**
Competitor	−0.205	0.091	−2.26	**0.025**
Non-reproductives	−0.008	0.006	−1.39	0.165
Competitor × non-reproductives	0.023	0.008	2.77	**0.006**

Bold type indicates significant results (P value < 0.05)

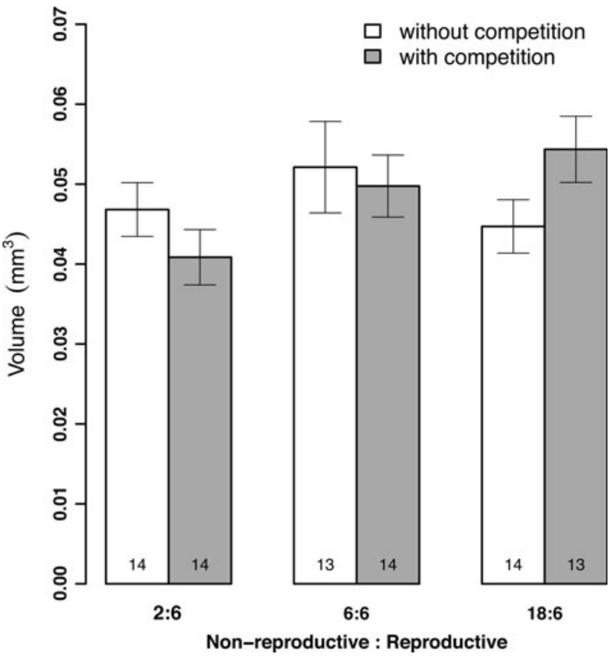

Fig. 2 The volume of *Philophthalmus* sp. reproductive morphs after 28 days of in vitro culture which contained six *Philophthalmus* sp. reproductive morphs with different ratios of non-reproductive to reproductive morphs, with and without the presence of the intra-host competitor *Maritrema novaezealandensis*. Sample sizes shown inside *bars* indicate the number of replicates where each replicate consisted of six reproductive rediae. *Standard error bars* are also shown

factors regarding their colony size and composition exhibited a significant relationship (Fig. 3; Table 3).

In-vitro experiment

The volume of the non-reproductive rediae of *Philophthalmus* sp. was 37 % higher on average in the presence of *M. novaezealandensis* after 28 days than in its absence (Estimate = 0.345, SE = 0.047, T-value = 7.34, P value < 0.001) (Fig. 3).

Redial volume and reproductive potential

A strong positive relationship was found between the volume of reproductive rediae and the number of mature cercariae they contained after controlling for the potential confounding effect of different genotypes ($R^2 = 0.465$, $N = 240$), indicating that the capacity of the rediae to produce cercariae is predicted by their volume (Fig. 4). Host shell length and the presence of the competing *M. novaezealandensis* showed little effects on the association (Table 4).

Discussion

Our findings demonstrate that factors determining the growth of *Philophthalmus* sp. colony members differ between the reproductive and non-reproductive morphs. While the

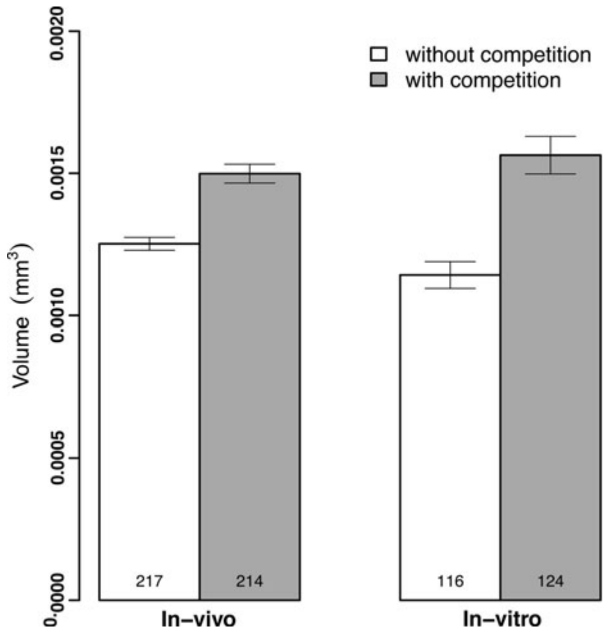

Fig. 3 The volume of *Philophthalmus* sp. non-reproductive morphs in live snail hosts infected with only *Philophthalmus* sp. and both *Philophthalmus* sp. and *Maritrema novaezealandensis* after 14 days of the in vivo experiment (*left*) and after 28 days of in vitro culture which contained 18 *Philophthalmus* sp. non-reproductive morphs with 6 reproductive morphs (*right*), with and without the presence of the competitor. Sample sizes are shown inside each *bar*. *Standard error bars* are also shown

reproductive morph size was determined by the interaction between colony composition and the presence of a competitor, the non-reproductives simply grew larger when co-occurring with the inter-specific competitor. Furthermore, the close association between the redial volume and reproductive capacity corroborates an adaptive value of a central aspect of colony demography, namely colony member size, in determining the fitness of the colony as a whole.

There was a strong parallel between the determinants of the size of reproductive morphs and the reproductive output of colonies reported by Kamiya and Poulin (2013). Thus the benefit of maintaining a high proportion of non-reproductive morphs was evident as reproductive morph size increased with the ratio of non-reproductives to reproductives when the intra-host competitor was present, in both live hosts and in the in vitro experiment.

The reduced volume of reproductives seen with a higher proportion of non-reproductives in the absence of competition in the naturally infected population indicates a fitness cost associated with such conditions, probably due to necessary sharing of limited space and/or resources with the non-reproductives. The discrepancy between the field data and the in vitro culture experiment is likely to be due to logistical constraints of the culture system; resource and space limitations necessary for demonstrating the cost of non-reproductive individuals to the colony cannot be re-created in vitro. It is also noteworthy that the presence of the competitor was a significant predictor of reproductive morph size only in the in vitro manipulative experiment. This was probably due to confounding factors in natural populations that masked the effect of competition on the size of reproductives

Table 3 Results of linear mixed models evaluating the relationship between the log-transformed volume of the non-reproductive morphs and (a) the colony size (i.e. the total number of colony members), (b) composition (i.e. the number of reproductives and non-reproductives) and (c) caste ratio (i.e. ratio of non-reproductives to reproductives) and the presence of the co-infecting competitor, *Maritrema novaezealandensis,* in colonies of the trematode *Philophthalmus* sp.

	Estimate	SE	T value	*P* value
(a)				
(Intercept)	−6.706	0.029	−228.88	**<0.001**
Colony size	0.032	0.026	1.21	0.228
Competitor	0.143	0.041	3.49	**<0.001**
Colony size × competitor	−0.034	0.040	−0.84	0.400
(b)				
(Intercept)	−6.708	0.030	−224.79	**<0.001**
Reproductives	0.030	0.032	0.95	0.341
Non-reproductives	0.004	0.038	0.12	0.909
Competitor	0.146	0.042	3.49	**<0.001**
No. repro. × competitor	−0.007	0.049	−0.14	0.889
No. non-repro. × competitor	−0.021	0.048	−0.43	0.667
(c)				
(Intercept)	−6.705	0.030	−226.59	**<0.001**
Caste ratio	−0.015	0.028	−0.54	0.588
Competitor	0.141	0.041	3.41	**<0.001**
Caste ratio × competitor	0.004	0.043	0.09	0.931

Bold type indicates significant results (*P* value < 0.05)

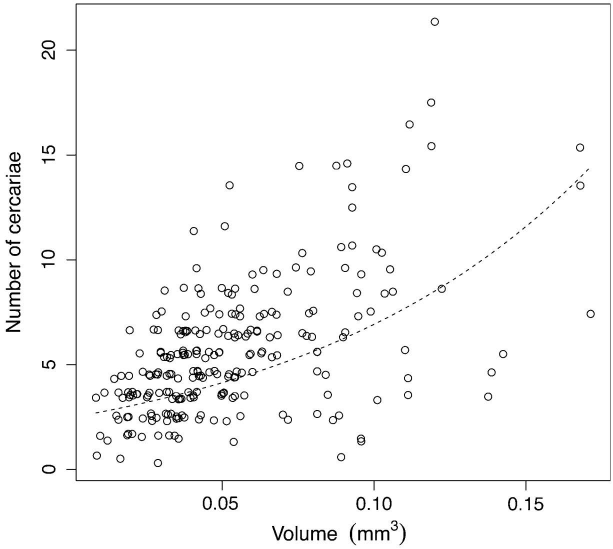

Fig. 4 The reproductive capacity, measured as the number of cercariae contained within *Philophthalmus* sp. reproductive morphs, regressed against the volume of those reproductives (N = 240). The regression line was exponentially converted from log-transformed data used in a linear model (refer to Table 4 for results)

Table 4 Results of a linear mixed model evaluating the relationship between the log-transformed number of mature cercariae contained in a reproductive morph and the volume of that morph, with or without the co-infecting competitor, *Maritrema novaezealandensis,* and with host identity as a random effect (N = 240)

	Estimate	SE	T value	*P* value
(Intercept)	−1.456	0.133	10.97	**<0.001**
Volume of reproductives	0.335	0.036	9.33	**<0.001**
Competitor	0.224	0.190	1.18	0.238
Host shell length	0.121	0.095	1.27	0.204

Continuous independent variables were centred. Bold type indicates significant results (*P* value < 0.05)

alone. In contrast, the in vitro approach standardised a range of confounding variables, such as nutrient availability, which vary from host to host in nature.

As previously seen for these trematodes, the presence of a competitor presents an ‘opportunity’, as opposed to a ‘threat’ when colonies consist of a high proportion of non-reproductive soldiers (e.g. colonies with a high proportion of soldiers enjoy higher success in the presence of competition; Kamiya and Poulin 2013). Although the size difference observed in the natural population could be explained through an adaptive shift in the size of newly produced castes, our in vitro experiments strongly indicate that the presence of competitors lead to changes in the size of existing members, not of next generations because the experimental colonies did not produce new members. Therefore the larger size of non-reproductive morphs found in co-infected hosts is likely to be due simply to increased food availability resulting from the presence of the competitor on which the non-reproductives feed upon as opposed to an adaptive shift of resource allocation as seen in insect colonies (e.g. Yang et al. 2004). In fact, in our preliminary observations made at various intervals to quantify contacts between the two types of rediae and the competitor, a higher proportion of non-reproductives (95 % CI 0.802–0.897) were in physical contact with the competitor in comparison to reproductives (95 % CI 0.215–0.386) in the in vitro experiment. In addition, the size of non-reproductive morphs was greater in the presence of the competitor both in vivo and in vitro, while a competitor-induced volume reduction was evident in vitro for the reproductives. These lines of evidence indicate that the non-reproductive morphs are likely to feed preferentially on the competing species when they are present whereas the reproductives experience a size reduction likely as a result of resource limitation due to direct competition.

Prior to the present study, factors that affect redial growth in trematodes had not been documented in the literature, to the best of our knowledge. Nonetheless, nutrient quality and water chemistry (namely, calcium ions are a limiting factor for host growth) have been recognised as key determinants of development of redial generations (Belfaiza et al. 2004b; Rondelaud et al. 2004), suggesting that they may have a role in the growth and development of individual rediae. Thus, while nutrient quality inevitably exerts a direct effect on colony survival and reproductive success in trematodes (Belfaiza et al. 2004a), the present study hints that changes in such environmental factors may also indirectly affect the reproductive success of social trematode colonies through changes in colony demography.

Although morphological differences within the worker caste are mediated through nutritional differences at the larval stage or fixed at eclosion in social insects (Couvillon and Dornhaus 2009), the pattern observed here suggests that the clonal intermediate stage of *Philophthalmus* sp. is capable of size increases in a short time period regardless of maturity. Thus ergonomic efficiency of the colony is likely to be sustained through demographic shifts in the size of colony members as a consequence of altered resource availability due to the presence of a competitor. This seems to trigger a feedback loop where the presence of a competitor promotes the growth of non-reproductives which then become more efficient at reducing the competitor biomass. Thus, the size and hence, the efficiency of the non-reproductives should fluctuate with the abundance of the competitor, thereby allowing the colony to maintain ergonomic efficiency without adaptive demographic shifts over several generations.

Finally, we found a strong association between the volume and reproductive capacity of reproductive morphs; as predicted, large reproductives harboured more mature cercariae. This relationship is consistent with the well-established gamete or offspring production pattern of invertebrates that is directly proportional to body size (Paris and Petelka 1962; Rinkevich and Loya 1979; Suchanek 1981). The pattern also serves to elucidate the close parallel observed between the determinants of reproductive output (Kamiya and Poulin 2013) and the growth of reproductives (the present study); the interaction between competition and the relative abundance of non-reproductives similarly affected the two variables. Furthermore, the tight coupling of the reproductives' volume and their reproductive capacity indicates that the interactive effect of competition and colony composition on the size of reproductives has direct downstream repercussions upon colony success.

In summary, the present study illustrates a fitness consequence of colony demography in social trematodes, in which the interaction between competition and colony composition was identified as the determinant of growth of individual castes.

Acknowledgments We are grateful to Manami Iwakura for field assistance; Melanie Lloyd for lab assistance, and to two anonymous reviewers for useful comments.

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