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Caste ratio adjustments in response to perceived and realised competition in parasites with division of labour

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Abstract

1. Colonial organisms with division of labour are assumed to achieve increased colony-level efficiency in task performance through functional specialisation of individuals into distinct castes. In social insects, ratios of individuals in different castes can adjust adaptively in response to external threats. However, whether flexibility in caste ratio also occurs in other social organisms with division of labour remains unclear. Some parasitic trematodes, in which clonal colonies within the snail intermediate host comprise a reproductive caste and a soldier caste, offer good systems to test the general nature of adaptive caste ratio adjustments.
2. Using the trematode *Philophthalmus* sp. as model, we test whether trematode colonies shift their composition towards more soldiers when exposed to a sustained risk of invasion by a competitor parasite species, and/or when experiencing sustained, active competition. We also quantify the colony-level fitness impact of caste ratio adjustments, measured as the colony's output of larval infective stages.
3. We conducted two long-term laboratory experiments on within-snail trematode colonies. First, snails harbouring *Philophthalmus* colonies were exposed to different levels of invasion risk by another trematode species, *Maritrema novaezealandense*. Second, the structure of *Philophthalmus* colonies was quantified after a year-long period of within-snail competition with the other trematode species.
4. When facing the risk of invasion by a competitor, independently of the level of risk, *Philophthalmus* colonies showed a significant shift towards producing more soldiers, resulting in altered caste ratio. Similarly, when experiencing actual competition by another trematode established in the same snail, *Philophthalmus* colonies also adjusted by producing significantly more soldiers. Greater investments in defense via more soldiers had negative impacts on the establishment and size of the competitor's colonies. Nevertheless, the presence of the competitor reduced the fitness (output of infective stages) of *Philophthalmus* colonies, although the production of more soldiers mitigated that effect.
5. Our findings demonstrate that trematode colonies with division of labour are capable of adaptive caste ratio adjustments in response to both the perceived threat of competition and actual competition, with trade-offs against reproductive success only apparent when soldier numbers are very high. Combined with results on social insects, our study suggests parallel adaptations of colonial organisms in phylogenetically disparate organisms.

KEYWORDS

castes, cercarial production, colony structure, Division of labour, *Maritrema novaezealandense*, *Philophthalmus* sp., social organisation, *Zeacumantus subcarinatus*

1 | INTRODUCTION

Social organisms with division of labour among specialised castes represent the outcome of a major evolutionary transition (Simpson, 2012; Szathmáry & Maynard Smith, 1995). Colonies of caste-forming species consist of a reproductive caste and various other morphologically distinct castes that generally do not reproduce but perform different functions for the colony's benefit (Duarte, Weissing, Pen, & Keller, 2011; Fjerdingstad & Crozier, 2006). Thus, colony success is enhanced by the increased efficiency of individuals in task performance through functional specialisation. Through selection acting at the colony level, ratios between different castes should not be fixed but instead capable of adjusting to changing conditions over the life span of the colony (Hasegawa, 1997; Herbers, 1980; Oster & Wilson, 1978). In particular, relative investments into different castes should vary in response to environmental factors such as competition, predation and resource availability (Gordon, 1996; Hasegawa, 1997; Oster & Wilson, 1978; Robinson, 1992). This prediction has been confirmed by classical experiments on social insects, in which adaptive changes in caste ratios were observed in the presence of increased competition (Harvey, Corley, & Strand, 2000; Passera, Roncin, Kaufmann, & Keller, 1996). Flexible caste ratios can therefore increase the colony's resilience in the face of immediate threats and longer term changes in external conditions.

Much less is known regarding caste ratio adjustments in social organisms other than insects. Recently, division of labour and distinct castes have been reported in clonal colonies of trematode parasites (Hechinger, Wood, & Kuris, 2011; Leung & Poulin, 2011a). Trematodes have multihost life cycles, with adult worms living in vertebrate definitive hosts where they reproduce sexually and release eggs which pass out, usually in the host faeces (Galaktionov & Dobrovolskij, 2003). Almost invariably, larvae from these eggs infect a snail first intermediate host. Within the snail, the parasite multiplies asexually, resulting in a colony of clones, which grow to produce dispersal larval stages known as cercariae. These cercariae leave the snail to seek the parasite's next host and continue the life cycle. In some species, the colonial stages within the snail host, called rediae, consist of two distinct morphs which display a clear division of labour (Hechinger et al., 2011; Leung & Poulin, 2011a). The reproductive morph produces cercariae, whereas the smaller and much more mobile nonreproductive morph is equipped with relatively large mouthparts and appears specialised for colony defense, that is, aggressive interactions against intra- or interspecific competitors (Hechinger et al., 2011; Leung & Poulin, 2011a). The nonreproductive rediae, hereafter called soldiers, latch on to competitors with their mouthparts to physically damage and kill them (Hechinger et al., 2011; Leung & Poulin, 2011a; Mouritsen & Halvorsen, 2015). This social organisation into two distinct castes has now been reported in multiple trematode species (Garcia-Vedrenne et al., 2016, 2017; Hechinger et al., 2011; Leung & Poulin, 2011a; Miura, 2012; Nielsen, Johansen, & Mouritsen, 2014). In some trematodes, however, the division of labour is age-based, with young rediae taking on a defensive role and older ones a reproductive function (Galaktionov, Podvyaznaya, Nikolaev, & Levakin, 2015).

Theoretical arguments developed to explain the relative investment into reproduction and defense in social insects (Oster & Wilson, 1978) should also apply to social trematodes. Excess production of soldiers when the colony is not under threat of competition may come at the expense of reproduction; with total colony size constrained by host resources, this trade-off suggests that there must be a threat-dependent optimal caste ratio. Current evidence suggesting that the presence of competitors may shift caste ratios in favour of more soldier rediae is weak, correlational and/or inconclusive (Leung & Poulin, 2011a; Lloyd & Poulin, 2012, 2013; Mouritsen & Andersen, 2017). However, previous studies have only allowed a few weeks for caste ratios to shift in colonies experiencing competition relative to those not facing competition but under identical resource conditions; it is likely that the turnover of individuals in a colony requires months instead of weeks. In addition, the defensive function of soldiers can benefit the colony both pre- and postinfection by a competitor. Soldier rediae may be capable of intercepting and killing any larval trematode entering the snail host before this new arrival establishes a competing colony. Indeed, soldier rediae are often located mostly at invasion fronts within a snail, that is, the points of entry for other parasites attempting to colonise the host (Hechinger et al., 2011). The presence of one trematode species in a snail can sometimes facilitate establishment of further trematode species in the same snail, through immunosuppression of the host (Bayne, 1983; van der Knaap & Loker, 1990); however, soldier rediae can perhaps mitigate this risk of secondary infection. If this fails and the new invader successfully establishes a colony, soldiers can then proceed to attack members of the competing colony. Therefore, we expect a role for soldier rediae in both prevention of infection by competitors and elimination of competing colonies. As a consequence, caste ratios are predicted to shift towards more soldiers both when a colony is exposed to frequent invasion attempts and when it is sharing the host with a competing colony.

Here, we explore these predictions using the trematode *Philophthalmus* sp. as a model system and over a more realistic timeframe for colony turnover. *Philophthalmus* colonies comprise both reproductive and soldier rediae within their snail intermediate host, the mudsnail *Zeacumantus subcarinatus* (Leung & Poulin, 2011a; Lloyd & Poulin, 2012). Ratios of reproductive to soldier rediae vary substantially among *Philophthalmus* colonies from the same locality and among geographical localities (Leung & Poulin, 2011a; Lloyd & Poulin, 2012, 2014a). *Philophthalmus* colonies are likely to experience strong competition from other trematodes that use the same snail host, especially *Maritrema novaezealandense* (hereafter referred simply as *Maritrema*). In some localities, including our study site, the prevalence of *Maritrema* exceeds 50% in the snail population (Fredensborg, Mouritsen, & Poulin, 2006), and it is the most prevalent parasite competitor of *Philophthalmus* in all populations sampled across the snail's geographical range (Lloyd & Poulin, 2014b).

Philophthalmus colonies face a trade-off between reproduction and defense. Using production of cercariae as a measure of a colony's reproductive success, sharing a snail host with *Maritrema* decreases the fitness of *Philophthalmus* colonies (Lloyd & Poulin, 2012). Increasing proportions of soldiers per colony can mitigate this decrease, though in the absence of competition, large numbers of soldiers negatively affect cercarial output (Kamiya & Poulin, 2013). Therefore, maintaining a large soldier force in the absence of competition is disadvantageous to the colony, unless they allow a rapid response when defense is required (see Charbonneau, Sasaki, & Dornhaus, 2017 for social insects). Establishing whether colonies adjust their caste ratio as a function of current or the threat of future competition is thus crucial to understand the evolution of social structure in trematodes.

We use two long-term laboratory experiments to address the following questions regarding the social structure of *Philophthalmus* colonies: (a) Are caste ratios shifting towards more soldiers in colonies exposed to a sustained high risk of invasion by the competitor *Maritrema*? (b) Are caste ratios shifting towards more soldiers in colonies experiencing sustained, active competition from an established *Maritrema* colony? (c) Are shifts towards relatively more soldiers effective at either, or both, preventing infection by the competitor or eliminating competing colonies? and (d) What are the fitness costs, measured as reduced cercarial output, of these caste ratio adjustments? We provide the most rigorous test of the effect of competition on social organisation in trematodes, extending our general understanding of factors shaping organisms with division of labour.

2 | MATERIALS AND METHODS

2.1 | Field collection, snail screening and parasite identification

Snails (*Zeacumantus subcarinatus*) were collected in Lower Portobello Bay (Otago Harbour, South Island, New Zealand; 45°52′ S, 170°42′ E): around 5,000 individuals in November 2015 and another 10,000 in October 2016. Snails were screened for infection by *Philophthalmus*, *Maritrema* or both parasite species together (double infection) by incubating individuals overnight at 26°C and under constant light, conditions known to trigger the emergence of the parasites' cercariae. During incubation, snails were kept individually in wells of 12-well culture plates filled with natural sea water (Lloyd & Poulin, 2011; MacLeod & Poulin, 2015). Parasite species were identified morphologically by inspecting each well under a dissecting microscope. Snails identified as infected (i.e., shedding cercariae) were separated by infection status (*Philophthalmus*, *Maritrema* or double infection), maintained at room temperature (16 ± 1°C) for 2 weeks before being screened a second time, and then a third time 2 weeks later, to confirm their infection status. Uninfected snails were also kept separately and screened the same way as infected individuals to provide an uninfected control group when required. Snails infected with other parasite species were discarded.

2.2 | Prevention of competitor establishment

An equal number of uninfected and *Philophthalmus*-infected snails (440 individuals per infection status) were identified and used in the following experiment. In December 2016, snails were haphazardly separated in 80 groups of eleven individuals: 40 groups of uninfected and 40 groups of *Philophthalmus*-infected individuals. These were used to test whether the presence of a *Philophthalmus* colony in a snail affected the likelihood of infection by the competitor *Maritrema*. Each group of snails was maintained in a one-litre container at room temperature (16 ± 1°C) in aerated sea water and fed sea lettuce (*Ulva* spp.) *ad libitum*. This density (11 individuals per litre) is one order of magnitude lower than natural densities (>15,000 individuals/m^2 at low tide; Jones & Marsden, 2005); therefore, even the death of one or two snails will not cause biologically meaningful changes in host density. Two empty shells of New Zealand clams *Austrovenus stutchburyi* were also added in each container as grazing substrate and a source of calcium carbonate for shell production. Containers were cleaned, water replaced and fresh sea lettuce added every month for the duration of the experiment.

Twenty groups (ten of each infection status) were then assigned to four different treatments [zero (control), low, medium and high] according to *Maritrema* infection risk. The range of exposure level to *Maritrema* (i.e., infection risk) was created as follows. Every second week, fresh faeces of red-billed gull (*Larus novaehollandiae scopulinus*), the definitive host of *Maritrema*, were collected from a roosting site in Lower Portobello Bay (Martorelli, Fredensborg, Mouritsen, & Poulin, 2004). Fifty grams was weighed, and half was frozen overnight (−20°C) to kill parasite eggs while the other half was kept fresh. The next day, each batch of bird faeces was diluted in 250 ml (0.1 g of faeces per ml) of sea water and filtered through a sieve (500 μm mesh) to remove large debris. We thus obtain two solutions of suspended faeces: one with frozen faeces (i.e., dead parasite eggs) and one with fresh (live) faeces. To create a range of exposure (four levels), aliquots of each solution were separated and mixed as required. Zero (control) exposure treatments were made of 100 ml aliquots of solutions of frozen faeces, low exposure treatments of 25 ml fresh faeces solution and 75 ml frozen faeces solutions, medium exposure treatment of 50 ml fresh faeces solution and 50 ml frozen faeces solutions, and high exposure treatments of 100 ml fresh faeces solution. Five millilitres of solution was added to each snail container according to the treatment they were assigned to. All snail groups were therefore exposed to the same amount of bird faeces but varying levels of exposure to *Maritrema* infections. We thus controlled for the potential effects of bird faeces itself on *Philophthalmus* colonies and caste ratio within snail hosts while modulating actual exposure risk to infection by *Maritrema*.

After 7 months of biweekly exposure to bird faeces and potential infection by a competitor, snail survival was high and generally similar among the eight experimental combinations (i.e., *Philophthalmus*-infected vs. *Philophthalmus*-free snails; zero, low, medium and high exposure levels to *Maritrema*), ranging between 81% and 96%. As observed in previous studies, survival was slightly higher in *Philophthalmus*-infected (93%) than *Philophthalmus*-free snails (85%; Fisher's exact test, $\chi^2 = 16.15$, $p = 0.0001$; see Fredensborg, Mouritsen, & Poulin, 2005; Lloyd & Poulin, 2013; MacLeod, Poulin, & Lagrue, 2017). However, no difference was detected among the three exposure levels (Fisher's exact tests, all $p > 0.05$). Surviving snails were incubated individually overnight at 26°C and under constant light in wells of 12-well culture plates as described above. The next day, cercariae shed by each snail were identified and counted. Snails were then dissected. During dissection, infection status was confirmed, snails that acquired *Maritrema* infection were recorded and numbers of parasite individuals were counted. Caste ratio in snails carrying *Philophthalmus* was also estimated. The first, largest whorl of each snail shell was carefully cracked with a hammer, and the snail was removed from its shell as intact as possible (Leung & Poulin, 2011a). The snail was transferred into a Petri dish filled with filtered sea water and examined under a dissecting microscope. The visceral mass was then carefully teased apart to release parasites and confirm infection status (Lloyd & Poulin, 2014b). When present, *Philophthalmus* rediae were separated from snail tissue using fine tweezers. The number of reproductive (i.e., large, cercariae-producing morph) and nonreproductive rediae (i.e., small, soldier morph) were also counted and used to calculate caste ratio (number of soldiers divided by the number of reproductives) for each *Philophthalmus*-infected snail (Kamiya & Poulin, 2013; Lloyd & Poulin, 2013, 2014a). In snails that acquired *Maritrema* infection during the experiment, those parasites were also counted. As *Maritrema* individuals cluster together and adhere in tight masses (Kamiya & Poulin, 2013), clumps of individuals were gently pressed between two microscope slides to allow accurate counts.

2.3 | Elimination of competitor

A total of 50 snails per infection status were identified and used in the following experiment. In January 2016, all snails selected for this experiment were marked with individual identification labels (numbered and coloured plastic tags [Queen Marking Kit, The Bee Works©, Orillia, ON, Canada] fixed with cyanoacrylate glue; Lloyd & Poulin, 2012). Snails of different infection status were collectively identified through colour-coded tags and individually through a unique alphanumeric number (from 1 to 50 in each colour code). Individually tagged snails were maintained together in a large 50-L tank at room temperature (16 ± 1°C) in aerated sea water and fed sea lettuce (*Ulva* spp.) *ad libitum*. Ten empty shells of the clam *A. stutchburyi* were also provided as grazing substrate and a source of calcium carbonate for shell production. The tank was cleaned, water replaced and fresh sea lettuce added every month for the duration of the experiment.

After 2 weeks of acclimation, snails were incubated individually overnight at 26°C and under constant light in wells of 12-well culture plates as described above. Because cercariae accumulate inside a snail and are only released when the temperature exceeds approximately 20°C (Studer, Thieltges, & Poulin, 2010), this procedure reliably captured total cercarial production since the last incubation. The next day, cercariae shed by each snail were identified and counted, and snails returned to their fifty-litre tank. Snails were subsequently incubated and cercariae counted once monthly for a year to assess temporal variations in parasite larval production in snails containing *Philophthalmus*, *Maritrema* or coinfections. Temporal variation in cercarial production was used as a proxy for parasite colony growth or decline in snail hosts and to test whether *Philophthalmus* colonies could eliminate the *Maritrema* competitor, and if so, at what rate.

After a year, snail survival was high in all three infection classes (94% in snails with *Philophthalmus* only, 78% in snails with *Maritrema* only and 86% in snails with both *Philophthalmus* and *Maritrema*). Survival was slightly higher in snails harbouring *Philophthalmus* only than those with *Maritrema* only (Fisher's exact test, $\chi^2 = 5.32$, $p = 0.041$) but no other difference could be detected in pair-wise comparisons (Fisher's exact tests, all $p > 0.05$). Surviving snails were dissected as described above. During dissection, infection status was confirmed, numbers of parasite individuals were counted and caste ratio in snails carrying *Philophthalmus* and double infections was also estimated. Snails that died during the experiment could not be used to estimate caste ratio in *Philophthalmus* as both host and parasite tissues decompose very quickly after snail death; they were excluded from the dataset. During dissections, two snails in the *Maritrema* only infection class were found to also harbour *Acanthoparyphium* sp. (Trematoda; Leung et al., 2009) and discarded from the dataset. A further three snails in the *Maritrema* only group and three in the *Philophthalmus*-only group (six in total) were found to harbour both parasites and were reclassified as such *a posteriori*. Sample sizes for the statistical analyses were thus 43, 34 and 49 for snails with *Philophthalmus* only, *Maritrema* only, and both *Philophthalmus* and *Maritrema*, respectively.

2.4 | Statistical analyses

2.4.1 | Prevention of competitor establishment

We used a generalised linear mixed effect model (GLMM) to test the factors contributing to the ability of *Philophthalmus* to prevent invasion by the competitor *Maritrema*. The model had a binomial error structure as the response variable, that is, whether or not a snail becomes infected by *Maritrema*, had a success/failure binary format. This model was constructed using the *glmer* function in the R package *lme4* (Bates, Maechler, Bolker, & Walker, 2015). Fixed effects were *Maritrema* exposure level (control, low, medium and high) and presence/absence of a *Philophthalmus* colony in the snail. The interaction between exposure level and *Philophthalmus* colony presence/absence was removed during model construction based on model diagnostics and relative AIC values. Container number was also included as a random effect to control for potential differences among snail groups. *p*-values were calculated using the *F*-statistic and *F*-numerator generated by the GLMM described above and the *F*-denominator value from a general linear model (function *glm* in the R package stats) of the same structure as the GLMM, but without the random effect of tank number. *Post hoc* analysis was conducted using the *ghlt* function in the R package *multcomp* (Hothorn, Bretz, & Westfall, 2008).

A second linear model was constructed using only data from snails with *Philophthalmus* colonies. This model tested the effect of exposure level (to invasion by *Maritrema*) on the number of reproductive and soldier rediae, the total number of rediae, caste ratio and the number of cercariae shed by snails at the end of the experiment. Here, a linear model was modified using the *cbind* function in the R package base to simultaneously analyse multiple response variables (listed above) against a single predictor variable (exposure level), with the random effect container number. All response variables except number of reproductive rediae were transformed using the function *powerTransform* to meet the assumptions of normality, and *post hoc* analysis was conducted using the *ghlt* function.

A third linear mixed effect model was used to analyse differences in rediae number, caste ratio and cercarial production between *Philophthalmus* colonies that were in direct competition with *Maritrema*, that is, those whose hosts were invaded by the competitor during the experiment, and those that did not experience competition. In this model, response variables were the number of reproductive and soldier rediae, caste ratio and cercariae shed at the end of the experiment, and the fixed effect was level of exposure to invading competitors; container number was again included as a random effect. All response variables except number of reproductive rediae were transformed using the *powerTransform* function in the R package car (Fox & Weisberg, 2011). Due to the low number of snails that acquired *Maritrema* infections, each response variable had to be analysed separately. Low numbers of snails also precluded *post hoc* analyses among exposure levels.

2.4.2 | Elimination of competitor

Cercarial production rates of each parasite colony were analysed using a linear mixed effect model with the *lmer* function in the R package *lme4* (Bates et al., 2015); note that cercarial shedding rates were taken from snails harbouring *Philophthalmus* only, *Maritrema* only or snails harbouring both *Maritrema* and *Philophthalmus*, in which case two shedding rates were obtained, one for each parasite species. Fixed effects were infection class (*Philophthalmus* only, *Maritrema* only, or both *Philophthalmus* and *Maritrema* together) and time, with snail number (from their individual tag) nested within time as a random effect to account for repeated measures of the same snail/parasite colony. The response variable, number of cercariae shed each month, was transformed to meet the assumptions of normality with the *powerTransform* function. *Post hoc* analysis was conducted using the *lsmeans* function in the R package *lsmeans* (Lenth, 2016).

A second analysis was performed on *Philophthalmus* colonies (i.e., excluding snails infected with *Maritrema* only) to assess differences in the numbers and caste ratio of rediae per colony after 12 months. The *cbind* function was used again in conjunction with a linear mixed effect model to simultaneously analyse multiple response variables (number of reproductive rediae, soldier rediae, total rediae and caste ratio) against a single predictor variable, that is, infection class (*Philophthalmus* only or both *Philophthalmus* and *Maritrema* together, that is, *Philophthalmus* exposed to competition or not). All response variables were transformed using the *powerTransform* function to meet the assumptions of normality.

A regression analysis was used to assess the effect of *Philophthalmus* soldier rediae numbers on the number *Maritrema* individuals in snails harbouring both species. This analysis excluded data from snails harbouring only one of the two parasite species. The fixed effect was the number of soldiers at the end of the experiment. The response variable was the number of *Maritrema* individuals, transformed using the *powerTransform* function.

Finally, regression analyses were used to test the effect of *Philophthalmus* caste ratio on the production of *Philophthalmus* cercariae, separately for snails harbouring *Philophthalmus* only and snails harbouring both species. The response variable was the number of *Philophthalmus* cercariae shed at 12 months and was again transformed using the *powerTransform* function. Caste ratio was the fixed effect, with a quadratic term also included to account for possible nonlinear relationships. A general additive model (GAM) with cubic regression splines in the package *mgcv* was also used to confirm the nature of the relationship between caste ratio and cercarial output in double and single infected snails (Wood, Pya, & Saefken, 2016). All analyses were completed using R v.3.3.1 (R Development Core Team, 2016) with $\alpha < 0.05$ indicating significance.

3 | RESULTS

3.1 | Prevention of competitor establishment

Upon dissection of snails in the zero exposure treatment (control), no snail harbouring *Philophthalmus* also harboured *Maritrema*, but one *Philophthalmus*-free snail was found to be infected by *Maritrema* (out of 100 surviving snails). It is likely that this snail was already infected at the beginning of the experiment but that the prepatent *Maritrema* infection was not producing cercariae at the time and could thus not be detected. No other parasite species was found during dissection, and preexperiment identification success of snail infection was thus close to 100%.

During the 7-month exposure to bird faeces, *Philophthalmus*-infected snails were invaded by *Maritrema* at greater rates than *Philophthalmus*-free snails. We found a significant effect of *Philophthalmus* colony presence/absence ($F_{1,783} = 8.15$, $p = 0.004$) and exposure level ($F_{3,783} = 11.18$, $p < 0.001$) on the success/failure of *Maritrema* invasion (i.e., prevalence; Figure 1). *Post hoc* analysis showed significant differences among all pair-wise combinations of exposure levels, except between low and medium levels (Table 1). However, there were significantly fewer individuals in *Maritrema* colonies that established in *Philophthalmus*-infected snails ($M \pm SE$ = 66.8 ± 6.6) than in *Philophthalmus*-free snails (201.6 ± 27.1; ANOVA, $F_{1,123} = 6.95$, $p < 0.0001$).

Higher levels of exposure to the *Maritrema* competitor caused an increase in the number of soldier rediae in *Philophthalmus* colonies at the end of the 7-month exposure ($F_{3,407} = 3.45$, $p = 0.017$), although *post hoc* analysis only showed a significant difference between the zero and low exposure levels (Figure 2a). There was a tendency for lower number of *Philophthalmus* reproductive rediae with increased exposure level, but the effect was not significant ($F_{3,407} = 2.54$, $p = 0.056$). Overall, the cumulative effect of these alterations to redial counts resulted in a significant increase in caste ratio with differences in exposure level ($F_{3,407} = 7.33$, $p < 0.001$; Figure 2b). *Post hoc* analysis revealed significant differences between the zero and all other exposure levels (zero–low, $|Z| = 3.39$, $p = 0.004$; zero–medium, $|Z| = 3.66$, $p = 0.004$; and zero–high, $|Z| = 4.20$, $p < 0.001$; Figure 2b). No significant effect of exposure level was found on the total number of rediae ($F_{3,407} = 0.74$, $p = 0.527$) or cercariae produced ($F_{3,398} = 1.26$, $p = 0.286$).

The composition of *Philophthalmus* colonies differed between snails that acquired *Maritrema* and those that did not (Figure 3). Competition with *Maritrema* resulted in significantly more *Philophthalmus* soldiers ($F_{1,398} = 37.00$, $p < 0.001$; Figure 3a), more rediae (total number; $F_{1,398} = 7.68$, $p = 0.006$), higher caste ratio ($F_{1,404} = 73.05$, $p < 0.001$; Figure 3c) and a lower cercarial output ($F_{1,398} = 64.90$, $p < 0.001$; Figure 3d); there was no significant difference for the number of reproductives ($F_{1,404} = 3.02$, $p = 0.083$; Figure 3b). Exposure level alone had no significant effect on any response variable.

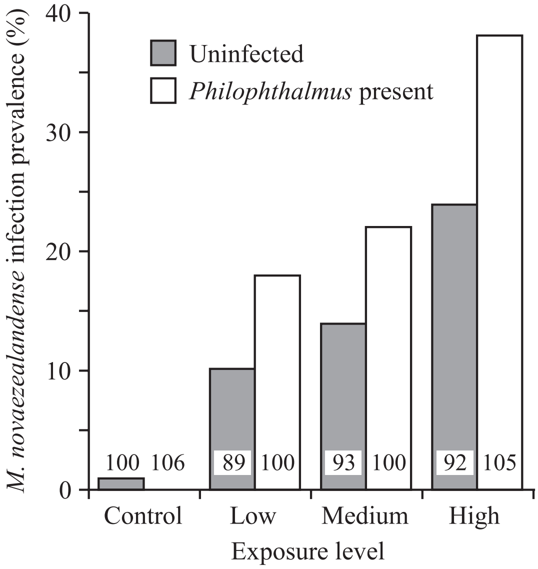

FIGURE 1 Proportion of snails that acquired *Maritrema novaezealandense* (infection prevalence in %) during the 7-month experiment under four levels of exposure to eggs of *Maritrema* [control (zero), low, medium and high], shown separately for uninfected snails and those harbouring *Philophthalmus* colonies. Numbers inside bars are sample sizes (i.e., number of snails)

TABLE 1 *Post hoc*, pair-wise comparisons among levels of exposure to *Maritrema novaezealandense*

Treatment pairs	Estimate	*SE*	*Z*-value	*p*
High–Control	4.5609	1.0145	4.496	**<0.001**
Low–Control	3.5345	1.024	3.452	**0.00267**
Medium–Control	3.8289	1.02	3.754	**<0.001**
Low–High	−1.0264	0.2603	−3.943	**<0.001**
Medium–High	−0.732	0.2437	−3.004	**0.01153**
Medium–Low	0.2944	0.2811	1.047	0.696

Note. *p* values in bold indicate significant differences between exposure level treatments.

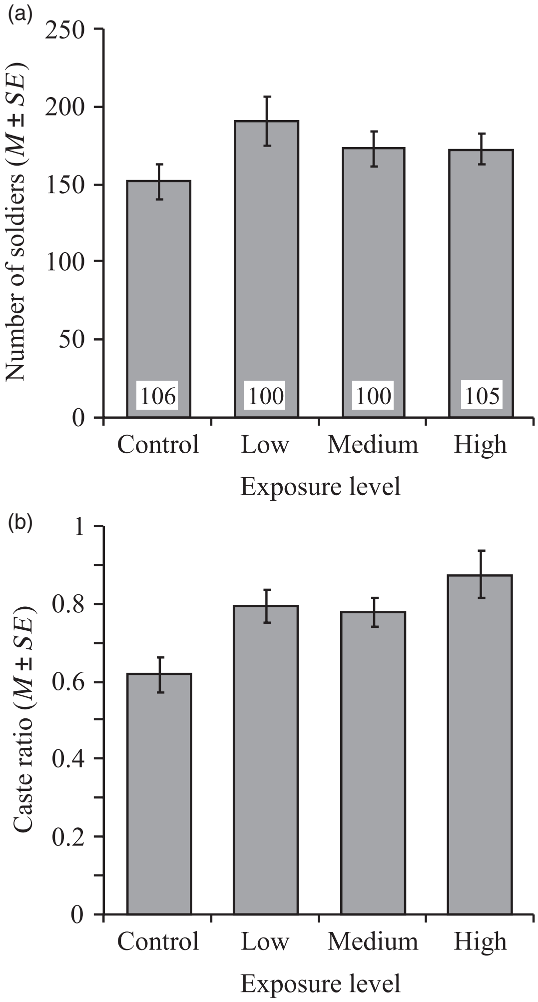

FIGURE 2 (a) Number ($M \pm SE$) of soldier rediae and (b) caste ratio ($M \pm SE$) in *Philophthalmus* colonies at the end of the 7-month experiment under four levels of exposure to eggs of the competitor *Maritrema novaezealandense* [control (zero), low, medium and high]. Numbers inside bars are sample sizes (i.e., number of *Philophthalmus* colonies); samples sizes are the same in both graphs

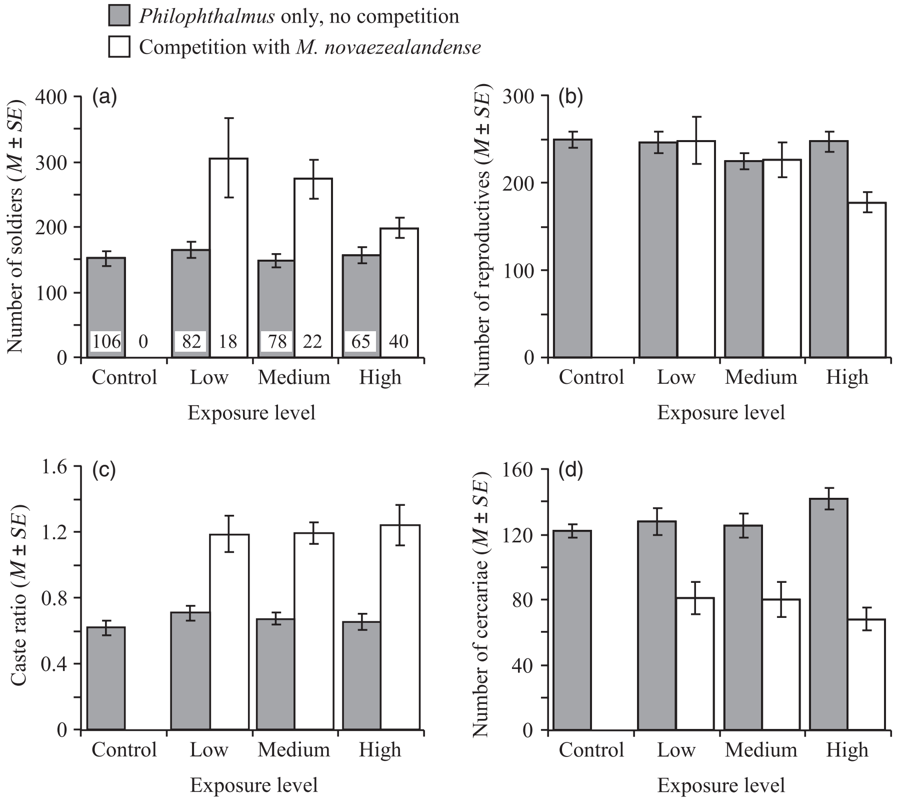

FIGURE 3 (a) Number ($M \pm SE$) of soldier rediae, (b) reproductive rediae, (c) caste ratio ($M \pm SE$) and (d) cercarial production (M number ± SE) in *Philophthalmus* colonies from snails that were or were not invaded by *Maritrema novaezealandense* during the 7-month experiment under four levels of exposure to eggs of *Maritrema* [control (zero), low, medium and high]. Numbers inside bars are sample sizes (i.e., number of *Philophthalmus* colonies); sample sizes are the same in all graphs

3.2 | Elimination of competitor

Cercarial production increased over the course of the experiment in colonies of either parasite species on their own, and in *Philophthalmus* colonies competing with *Maritrema*, but it decreased over time for *Maritrema* competing with *Philophthalmus* (Figure 4). Repeated measures analyses showed a significant effect of whether the two species were alone or together in the same snail ($F_{3,171} = 598.85$, $p < 0.001$), time ($F_{1,171} = 75.92$, $p < 0.001$) and the interaction of these factors ($F_{3,171} = 134.01$, $p < 0.001$). Further, *post hoc* analysis showed significant differences among all pair-wise comparisons of infection classes (*Philophthalmus* only, *Maritrema* only or both species together; Table 2).

At the end of the 12-month experiment, the composition of *Philophthalmus* colonies differed significantly between those under competition from *Maritrema* and those without competition (Figure 5). The effect of competition resulted in significantly more soldiers ($|T \text{ value}| = 6.369$, $p < 0.001$; Figure 5a), more total rediae ($|T \text{ value}| = 3.438$, $p < 0.001$; Figure 5a) and higher caste ratios ($|T \text{ value}| = 8.121$, $p < 0.001$; Figure 5c), but no significant difference in the number of reproductive rediae ($|T \text{ value}| = 0.286$, $p = 0.776$; Figure 5a). In contrast, the number of *Maritrema* individuals was significantly lower in *Philophthalmus*-infected than *Philophthalmus*-free snails (ANOVA, $F_{1,81} = 127.6$, $p < 0.0001$; Figure 5b). Regression analyses showed a weak but significant reduction in the number of *Maritrema* individuals ($R^2 = 0.108$, $p = 0.021$) as a function of increasing number of *Philophthalmus* soldiers.

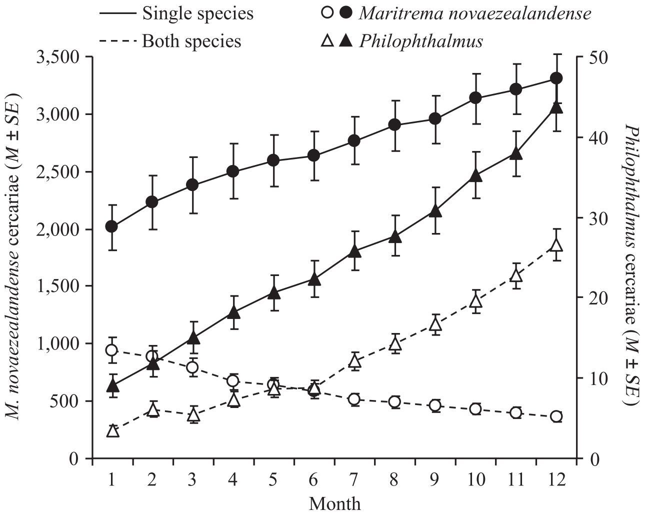

FIGURE 4 Monthly production of cercariae ($M \pm SE$) by *Maritrema novaezealandense* and *Philophthalmus* colonies in snails harbouring one species only ($N = 43$ and 34 for *Philophthalmus* and *Maritrema* colonies, respectively) and both species ($N = 49$ colonies for each parasite species)

In terms of colony fitness, at the end of the 12-month experiment, the number of cercariae produced was not associated with caste ratio in *Philophthalmus* colonies not under competition ($F_{1,42} = 1.325$, $p = 0.257$, $r = 0.047$; Figure 6a), but showed a curved relationship with caste ratio, with a significant quadratic term, in colonies under competition from *Maritrema* ($F_{1,48} = 15.88$, $p = 0.0002$, $r = 0.162$; Figure 6b). In other words, under

TABLE 2 *Post hoc*, pair-wise comparisons of cercarial output between infection classes (*Philophthalmus* only, *Maritrema* only or both species together)

	df	|*T* ratio|	*p*
DMn – DPh	171.67	18.231	**<0.0001**
DMn – Mn	171.67	9.787	**<0.0001**
DMn – Ph	171.67	13.996	**<0.0001**
DPh – Mn	171.67	26.289	**<0.0001**
DPh – Ph	171.67	3.631	**0.0021**
Mn – Ph	171.67	22.263	**<0.0001**

Note. DMn: *Maritrema* cercariae originating from snails harbouring both species together; DPh: *Philophthalmus* cercariae originating from snails harbouring both species together; Ph: *Philophthalmus* cercariae originating from snails harbouring only that species; Mn: *Maritrema* cercariae originating from snails harbouring only that species. *p* values in bold indicate significant differences between groups.

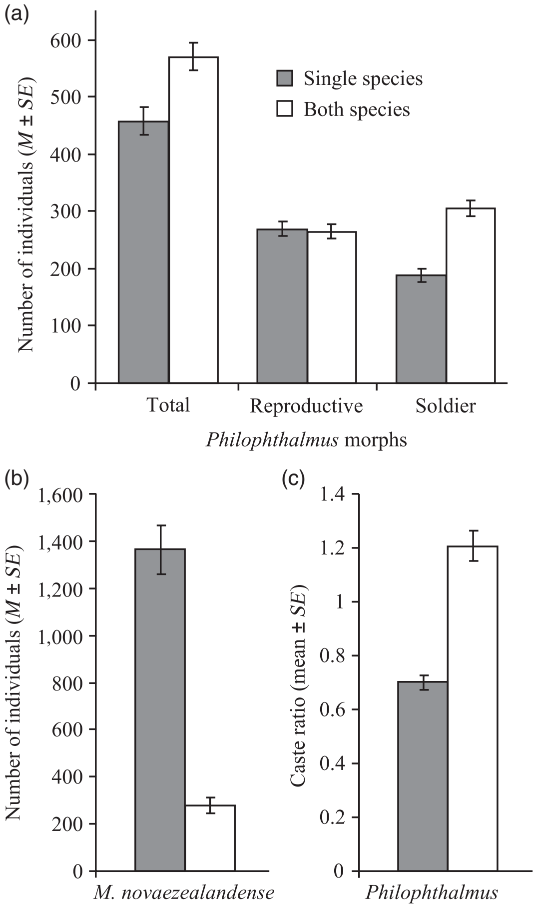

FIGURE 5 Number of parasite individuals (*M* ± *SE*) in (a) *Philophthalmus* colonies (total, reproductive and soldier morphs) and (b) *Maritrema novaezealandense* colonies in snails harbouring one species only (*N* = 43 and 34 for *Philophthalmus* and *Maritrema* colonies, respectively) and both species (*N* = 49 colonies for each parasite species). (c) Caste ratio (*M* ± *SE*) in *Philophthalmus* was also estimated and compared between snails harbouring *Philophthalmus* only and those harbouring *Philophthalmus* and the competitor *Maritrema*

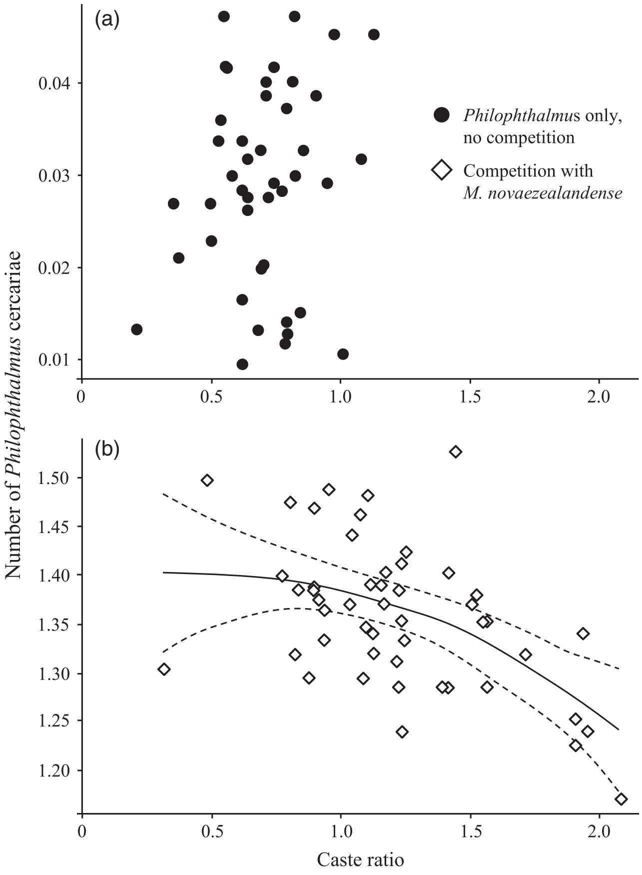

FIGURE 6 Transformed cercarial output (number of cercariae emitted per snail) as a function of caste ratio. Estimated smoothing curves (cubic regression splines) and point-wise 95% confidence bands are shown when significant. (a) *Philophthalmus* colonies alone in their snail host (*Philophthalmus* only, no competition; *N* = 43; data transformed using the exponent −0.987) and (b) colonies under competition from *Maritrema* (competition with *M. novaezealandense*; *N* = 49; data transformed using the exponent 0.0977). All data are from the end of the 12-month experiment

competition, colony fitness was improved by a more soldier-biased caste ratio, but only to a point beyond which further soldiers lowered reproductive output.

4 | DISCUSSION

In colonial organisms with division of labour, natural selection acting at the colony level is expected to favour flexible caste ratios, in response to variation in external conditions, in particular threats such as competition or resource shortage (Gordon, 1996; Hasegawa, 1997; Herbers, 1980; Oster & Wilson, 1978; Robinson, 1992). Empirical evidence from studies on social insects supports this expectation (Harvey et al., 2000; Passera et al., 1996). Here, we extend the phylogenetic generality of this prediction to social trematodes, in which colonies consist of a reproductive caste and a soldier caste (Garcia-Vedrenne et al., 2016, 2017; Hechinger et al., 2011; Leung & Poulin, 2011a; Miura, 2012). Our findings demonstrate that trematode colonies can adjust their caste ratios in favour of more soldiers when facing both the perceived risk of competition and actual competition.

Our first experiment consisted in exposing *Philophthalmus* colonies to different levels of risk of invasion by its main natural competitor. The experimental procedure indeed created a gradient of competition threat, with higher levels of exposure to eggs (in bird faeces) of the competitor *Maritrema* resulting in a greater proportion of snails acquiring this parasite. For any given level of exposure, *Maritrema* was more successful at establishing colonies in *Philophthalmus*-infected than in *Philophthalmus*-free snails. It is likely that infection by one species of trematode is facilitated through immunosuppression of the host caused by earlier infection with a different trematode species (Bayne, 1983; Southgate, Brown, Warlow, Knowles, & Jones, 1989; van der Knaap & Loker, 1990). However, the size of *Maritrema* colonies, measured as total number of individuals, was smaller in *Philophthalmus*-infected than in *Philophthalmus*-free snails, a likely consequence of interspecific competition for limited resources placing a cap on total colony size (Hendrickson & Curtis, 2002; Walker, 1979).

Importantly, although total *Philophthalmus* colony size was similar, caste ratios were significantly more biased towards soldiers in colonies exposed to invasion of the host by a competitor than in control colonies. This indicates an adaptive adjustment of colony structure in the face of a perceived threat, a response similar to that observed in social insects (Harvey et al., 2000; Passera et al., 1996). Overall, the level of invasion risk by a competitor, measured as the relative concentration of *Maritrema* eggs to which snails were exposed, had no influence on the response of *Philophthalmus* colonies. Thus, the reorganisation of *Philophthalmus* colonies was an all-or-nothing response that appears insensitive to the degree of threat facing the colony. Furthermore, *Philophthalmus* colonies that failed to prevent infection by *Maritrema* and ended up sharing their host with a competitor achieved smaller sizes, more soldier-biased caste ratios and lower reproductive output (i.e., lower cercarial production) than *Philophthalmus* colonies whose snail host was not invaded by the competitor. This confirms the fitness cost of competition in this pair of trematode species.

After the 12-month competition experiment, *Philophthalmus* colonies with a competitor showed a significant caste ratio shift towards more soldiers. More telling, the greater the number of soldiers per *Philophthalmus* colony, the smaller the size of the competing *Maritrema* colony. This result, combined with the gradual decline in cercarial output by *Maritrema* colonies in shared hosts, suggests not only that *Philophthalmus* is the dominant competitor (i.e., it is apparently capable of reducing the size of *Maritrema* colonies over time), but also that adjustments in caste ratios can accelerate this process. Of course, in this experiment, we used naturally infected snails and therefore did not control for the order in which the two species became established in their shared snail host. Priority effects can sometimes influence the outcome of interspecific competition in trematodes (Leung & Poulin, 2011b). However, past research on competitive hierarchies in trematodes within snails indicates clearly that species with rediae, like *Philophthalmus*, are dominant over species with individuals, called sporocysts, which lack mouthparts, like *Maritrema* (Kuris & Lafferty, 1994; Sousa, 1993). Nevertheless, total elimination of *Maritrema* by *Philophthalmus* seems unlikely or at least very slow: Joint infections by both species are fairly common in nature and have been anecdotally documented to persist up to 2 years in snails kept in the laboratory (Lloyd & Poulin, 2013). In the present study, we observed only one incidence of total elimination of *Maritrema* over 12 months.

Earlier studies suggested that whether or not competitors are present, the presence of at least some soldiers can benefit the colony, possibly by making certain host resources available to reproductive rediae (Lloyd & Poulin, 2012). However, in short-term experiments and in the absence of competition, more soldiers do not benefit the colony and can even lead to reduced cercarial output (Kamiya & Poulin, 2013). Here, we found that after months of competition with coinfecting *Maritrema*, *Philophthalmus* colonies with relatively more soldiers achieved roughly the same cercarial output as those with fewer soldiers, but only up to a point, as the curvilinear pattern we observed suggests that an excess of soldiers is costly. No such pattern was seen in the absence of competition, though without competition colonies generally do not have soldier-biased caste ratios. Thus, although there may be fitness costs associated with maintaining many soldiers in the absence of competition (Kamiya & Poulin, 2013), under competitive situations greater numbers of soldiers are only costly beyond some threshold. Soldiers are over one order of magnitude smaller than reproductives; therefore, an increase in their numbers should cost much less than producing a single new reproductive. Subsequently, nutrients from competitors killed by soldiers become available to reproductive rediae, thus compensating for the costs of producing soldiers. However, beyond a certain number of soldiers, maintaining this army requires resources that are traded-off against colony reproduction. *In vitro* studies tracking the fate of fluorescently labelled compounds in *Maritrema* when cultured with *Philophthalmus* colonies might shed light on this possibility. It must be noted that cercarial output over a short time period is only a rough proxy of actual fitness; the benefits gained through the elimination of competitors by soldiers may in fact lead to much larger fitness gains over the years that a *Philophthalmus* colony can survive.

In summary, competition shifts caste ratios in *Philophthalmus* colonies towards more soldiers. Although this may not prevent establishment of the competitor, because the host is immunologically compromised, it may prime the colony for defense against an imminent threat. Subsequently, under actual competition, *Philophthalmus* colonies investing into more soldiers can accelerate the reduction in colony size of their competitor. In addition, investments in defense are not necessarily costly, at least under immediate competition, as shown by the trade-off between numbers of soldiers and cercarial output only becoming manifest when soldiers become too numerous. It will be interesting to see whether these adaptive responses also occur in other social trematodes, including those where division of labour takes place among age classes of rediae (see Galaktionov et al., 2015) rather than among distinct castes. More generally, our results provide additional evidence of phenotypic responses to competition in parasites and of state-dependent strategies in the face of changing and unpredictable conditions (Thomas, Brown, Sukhdeo,

& Renaud, 2002). For example, the malaria parasite *Plasmodium chabaudi*, which multiplies asexually in its mammalian host to produce clones, can respond to the presence of competing genotypes within the same host by increasing its replication rate, and therefore its virulence (Taylor, MacKinnon, & Read, 1998), as well as adjusting its sex allocation strategy (Reece, Drew, & Gardner, 2008). In addition, compared to social insects, our results suggest parallel adaptations of colonial organisms against competitive threats in phylogenetically disparate organisms. The frequent and independent evolutionary origins of division of labour across distantly related taxa (Simpson, 2012) may reflect the efficiency and flexibility of this strategy to perform various functions to different degree over time and meet the changing demands of colonial life.

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AUTHORS' CONTRIBUTIONS

R.P., C.L. and L.K. conceived the ideas and the study; C.L. and R.P. designed the methodology; C.L. collected the data; C.L. and C.D.M. analysed the data; C.L., C.D.M. and R.P. shared the writing of the manuscript. All authors contributed critically to the drafts and approved submission.

DATA ACCESSIBILITY

Data used in this manuscript is available in Dryad Digital Repository: https://doi.org/10.5061/dryad.gb2bm7h (Lagrue, MacLeod, Keller, & Poulin, 2018).

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