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Bold, Sedentary Fathead Minnows Have More Parasites

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Abstract

Parasites that rely on trophic transmission can manipulate the behavior of an intermediate host to compromise the host's antipredator competence and increase the probability of reaching the next host. Selection for parasite manipulation is diminished when there is significant risk of host death to causes other than consumption by a suitable definitive host for the parasite. Consequently, behavioral manipulation by parasites can be expected to be subtle. *Ornithodiplostomum ptychocheilus* (*Op*) is a trematode parasite that has a bird–snail–fish host life cycle. Fathead minnows are a common intermediate host of *Op*, where metacercariae encyst in the minnow brain. In this study, we report a link between metacercarial intensity and behavior in fathead minnows. In the field, we found that roaming distance by free-living minnows over 24 h was negatively correlated with parasite intensity. In the laboratory, we found that boldness in an open field test was positively correlated with parasite intensity. These parasite-induced behavioral changes may render infected minnows more susceptible to predators, which would serve to facilitate trophic transmission of parasites to the bird host.

Introduction

PREDATOR–PREY INTERACTIONS are more complex than is commonly understood because the role of parasites is usually ignored or at least not quantified.[1–3] This oversight is especially important when prey and predator are intermediate and final hosts of trophically transmitted parasites because parasites can compromise antipredator behavior of the prey (intermediate host) to facilitate their own transmission to the predator (final host).[4–10] Trophic transmission of parasites presents an interesting dynamic that confounds conventional predator–prey theory. Prey hosts are generally assumed to be under selection to behave in ways that reduce the probability of predation[11]; however, trophically transmitted parasites are under selection to manipulate their intermediate host to increase the probability of being eaten.[4]

One of the early descriptions of this phenomenon is the manipulation by the acanthocephalans, *Polymorphus marilus* and *Polymorphus Paradoxus*, of gammarid amphipod hosts to seek—rather than avoid—light and to cling to surface vegetation. These behavioral changes greatly increase the probability of predation of the gammarid (and acanthocephalan cystacanths therein) by mallard ducks, the definitive host.[12]

Another celebrated example is host manipulation of berry ants (canopy ants: *Cephalotes atratus*) by nematodes.[13] In this system, nematode parasites (*Myrmeconema neotropicum*) induce depigmentation of the exoskeleton of the abdomen, which in combination with nematode eggs inside the gaster (abdomen) causes the normally black gaster to swell and approximate the size and bright red color of the berries of *Hyeronima alchorneoides* favored by the avian definitive hosts.[14] Moreover, the attachment of the gaster to the rest of the ant's body is weakened to allow it to detach easily. In addition, this remarkable mimicry is accompanied by behavioral changes in infected ants that cause the ant to climb a berry bush and raise its reddened abdomen, become sluggish, and suppress defensive biting behavior and production and secretion of noxious alarm pheromone.[13]

Although most parasite-induced manipulations of host behavior are more subtle than these examples, even subtle manipulations can effectively increase the probability of predation of an intermediate host by the final host. Subtle changes in host behavior will be selected when parasite-induced burden on host metabolism or immunocompetence renders the host (and resident parasites) likely to die from causes other than consumption by a suitable final host.

One manifestation of subtle manipulations of host behavior is a shift toward boldness or risk-taking behavior. Shy–bold is a major axis of variation in animal behavior, in which individuals consistently respond as risk-prone or risk-

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averse to novel stimuli. The shy–bold continuum was first noted by Huntingford,[15] formalized as a factor in evolutionary ecology by Wilson *et al.*[16] and Magurran,[17] and is currently recognized as one axis of correlated behavioral traits among several such axes collectively referred to as behavioral syndromes, behavioral profiles, or personality.[18–21] A subtle shift in host behavioral phenotype increases host susceptibility to predation without compromising host immunocompetence or reproductive success.[6,9,22]

Evidence is accumulating that behavioral syndromes are indeed a common vehicle for parasite modification of host behavior to favor trophic transmission.[9] Boldness is a natural behavioral trait that is adaptive in some contexts (i.e., foraging, courtship), but a liability in the context of predation.[20] This liability may be exploited by parasites to subtly shift the odds of host predation in their favor.

In this study, we measured differences in behavioral risk taking associated with parasitism in fathead minnows, *Pimephales promelas.* The parasite that is the main focus of this study is the trematode, *Ornithodiplostomum ptychocheilus* (*Op*). *Op* adults reside in the gut of piscivorous birds. Eggs pass with the feces into the water where ciliated larvae (miracidia) penetrate a *Physa gyrina* snail and undergo asexual reproduction.[23] Cercariae released from the snail penetrate the skin of any fathead minnow they encounter, migrate to the optic tectum and meninges of the brain,[24,25] and encyst there as metacercariae awaiting ingestion by the final host.

Op infection is associated with alteration of normal minnow behavior, manifest as reduction in shoal cohesion[26] and impaired ability to visually track moving objects at the height of metacercarial metamorphosis.[27,28] However, in a test with a mechanical model of a simulated heron attack, no correlation between *Op* intensity and response time was detected.[29]

We studied this fathead minnow-*Op* system in two small lakes, Budd Lake and Deming Lake, near the University of Minnesota Itasca Biological Field Station within the boundaries of Itasca State Park, Minnesota. Fathead minnows in these lakes have been the focus of study for chemical ecology of predator–prey interactions,[30–34] host–parasite interactions,[29] and reproductive behavior.[35] Large piscivorous fish are absent from these populations, although there is presumably some predation in Deming Lake by eastern mudminnows (*Umbra limi*) and black bullhead catfish (*Ameiurus melas*). The principal vertebrate predators in both systems are birds such as great blue herons (*Ardea herodias*), loons (*Gavia immer*), belted kingfishers (*Megaceryle alcyon*), and garter snakes (*Thamnophis sirtalis*).

Trophically transmitted trematodes in these fathead minnows that use birds as a final host are *Op* (metacercariae encyst in the brain), *Ornithodiplostomum* body cavity (*Obc*; metacercariae encyst in the viscera), black spot *Crassiphiala bulboglossa* (metacercariae encyst in the epidermis), and *Diplostomum* sp. in the lens of the eye. Because fathead minnows have been studied extensively for their antipredator responses to chemical alarm cues,[36] including populations within our study system, they are a good model system for studying the three-way interaction between prey, predator, and the parasites that use both prey and predator as intermediate and final hosts.

Previous work by Pellegrini *et al.*[34] provided the impetus for the current study. Fathead minnows generally do not enter traps that contain a piece of sponge soaked in chemical alarm cue derived from minnow skin extract because these cues reliably indicate the presence of an actively foraging minnow predator.[32,33,37]

Nevertheless, some fish do enter alarm cue-labeled traps and these fish are significantly bolder in the face of staged encounters with potential predators than minnows captured in water sponge traps.[34] Moreover, minnows from alarm cue-labeled traps had on average 43.2 ± 12.2 ($n = 19$) *Op* metacercariae per fish compared with 24.5 ± 7.3 ($n = 19$) metacercariae for fish caught in water traps (all fish captured had at least one metacercaria). Although the small sample size in the study by Pellegrini *et al.*[34] precluded statistical distinction between these two means, the data were suggestive of parasite-induced boldness in their intermediate host (or alternatively, a relatively higher risk of parasitism for bold individuals).

In this study, we performed a test of the effect of parasitism on roaming behavior and two independent tests for associations between parasite intensity and boldness. Roaming behavior is akin to exploratory behavior, which is generally correlated with boldness in behavioral syndromes.[39] Bold individuals should, all else being equal, incur higher risk of predation than shy individuals.[20,38] We predicted that minnows with relatively high parasite intensity would roam more and exhibit greater boldness than minnows with low parasite intensity.

Educational Context

All work was conducted as part of summer programs at the University of Minnesota's Itasca Biological Station and Laboratories (IBSL). Program goals follow the recommendations of the American Association for the Advancement of Science (AAAS) *Vision and Change* report, which articulates several priorities for biology education, among them the incorporation of authentic research experiences into biology education.[40] One author (E.D.) conducted work during a summer REU program, Global Change Ecology at the Headwaters of the Mississippi. Another (T.P.) was conducting research as part of a self-initiated directed study. K.G. was enrolled in a summer course, Animal Behavior. Animal Behavior engages students in authentic science through course-based undergraduate research experiences (CUREs; see Auchincloss *et al.*[41] for further information on CUREs).

In all cases, research is conducted without external funding for equipment or supplies, and desired student outcomes include increased self-efficacy in science process skills, gains in skills themselves, and work that (such as the work described herein) contributes meaningfully to existing disciplinary knowledge.

Materials and Methods

Study sites

Budd Lake is located within Itasca State Park, Minnesota (N 47°9′59.1638″, W 95°10′28.418″), near the Itasca Biological Field Station of the University of Minnesota. Budd Lake is ~3 ha in area and contains only two fish species: fathead minnows (*P. promelas*) and northern redbelly dace (*Chrosomus eos*). In June 2011, we conducted two experiments using fathead minnows from Budd Lake. The first experiment was conducted in the field and measured distance moved by fish over 24 h.

The second experiment on Budd Lake fish was conducted in the laboratory and measured fish activity and boldness. The test for a link between boldness and parasite intensity was conducted a second time on fathead minnows from nearby Deming Lake (N 47°10′15.5489″, W 95°9′59.6819″), also in Itasca State Park. Deming Lake is ∼5 ha in area and contains fathead minnows, redbelly dace, and, during this study, this lake also contained brook stickleback (*Culaea inconstans*), mudminnows (*U. limi*), Iowa darters (*Etheostoma exile*), and black bullhead catfish (*A. melas*).

Roaming behavior in the field

We seined 800 fathead minnows from about 30 m of shoreline. We clipped the upper lobe of the caudal fin from each fish with a pair of dissecting scissors and released all the fish from one central location within the area that had been seined. To estimate roaming behavior, we resampled the following day. Specifically, we returned 24 h later and set three standard wire-mesh minnow traps, without bait, at each of 10 sample stations. Stations were 10 m apart and continued for 50 m in each direction along the shore from the release point the previous day. We retrieved the traps after 2 h and inspected the catch of each trap for clipped fish, which were taken to the laboratory for dissection.

We repeated the experiment 7 days later on the opposite side of the lake. In the second sample, we captured 600 fathead minnows and gave each fish a clip on the lower lobe of the caudal fin and released them from a central location within the new seining site. We returned after 24 h to set 30 minnow traps, 3 per station at 10-m intervals running 50 m in each direction from the release site. After 2 h, we inspected the catch for recaptured fish, which we retained for analysis. Fish bearing a clip on the upper lobe of the caudal fin from the previous week were also retained for analysis.

All clipped fish were brought to the laboratory where we recorded total length (TL), sex, and the number of metacercariae of *Op*. Each fish was killed by an overdose with the anesthetic, methane tricaine sulfonate (MS222). The brain was removed and squashed between two glass slides. Infection intensity was recorded, as parasites per individual, with the aid of a dissecting microscope.

Boldness in the laboratory: Budd Lake fish

Fathead minnows from Budd Lake were transported to the laboratory where they were assessed for boldness using a standard open field test.[42] Latency to exit from an enclosed space is reliably shorter for bold individuals than for shy individuals.[43] In our study, individual minnows were placed in an opaque box (10 × 10 cm base) in a test aquarium and given 15 min to acclimate. The box was fitted with a removable door that slid upward to create an opening 1.8 cm wide through which the minnow could exit. Latency to emerge was recorded up to a maximum of 15 min.

To establish that latency to exit was part of a behavioral syndrome of correlated behavioral traits, we also measured activity. Boldness and activity are commonly correlated traits.[18] To measure activity, individual fish were placed in a standard 37-L aquarium. The tank was filled to a depth of 15 cm. A grid with cells measuring 2.5 × 2.5 cm was drawn on the front pane of each aquarium. After a 15-min acclimation period, activity was recorded in real time, by a tank-side observer, as the number of grid lines crossed in 5 min. This protocol permitted the measurement of vertical and horizontal movement, but back-and-forth activity was not recorded; thus, our estimate of total fish activity was based on a sample measured in two dimensions. A curtain of black plastic kept the tanks visually isolated from the movements of the observer.

Boldness in the laboratory: Deming Lake fish

As part of a larger study on Deming Lake fish, fathead minnows were collected with a seine net from Deming Lake in June 2014. We sampled only enough fish per sample to run two complete trials and limit time in captivity of test subjects to 2 days. Captured fish were transported to holding tanks at the Itasca Biological Field Station.

Six experimental plastic tanks (50 × 35 cm) were each filled to a depth of 15 cm with 26 L of water from Lake Itasca. A curtain of black plastic kept the tanks visually isolated from movements of observers. Inside each tank there was a pair of nested inverted plastic tubs (25 cm tall, diameter of base was 15 cm). The outer tub was fully intact. The inner tub had a circular hole of diameter 3.2 cm cut into the side. When the tubs were nested, the outer tub covered the hole in the inner tub. A string attached to the top of the outer tub allowed the experimenter to surreptitiously raise the outer tub using a pulley system and uncover the hole.

Individual fish were placed inside the nested tubs for 15 min to acclimate. Trials began when the outer tub was raised to uncover the exit hole. The latency for fish to exit the tub was recorded up to a maximum of 20 min. Test fish were then moved to individual aerated containers for 20–28 h before being retested. Water in the test tanks was replaced for each trial. After completing the behavioral trial, each fish was killed by an overdose of MS222 and necropsied. We recorded metacercaria intensity from tissue squashes as described above for the brain (*Op*), internal organs (Obc), the skin (black spot *C. bulboglossa*), and trematodes that occur in the lens of the eyes (*Diplostomum* sp.).

Data analysis

We used generalized linear modeling (GLM) to determine whether parasite counts could be related to distance traveled for minnows and to model parasite counts as a function of latency or activity scores as proxies for boldness. We controlled for fish TL, sex, and date of collection as covariates where appropriate. Models were fit using both Poisson and negative binomial error structures, and Akaike's Information Criteria (AIC) model selection procedure was used to choose the better fitting model. In all cases, the negative binomial error structure fit best (ΔAIC >> 20). The negative binomial GLM was fit using the glm.nb function from the MASS package in R.

Global models were generated with all covariates and the joint significance of the model relative to a null was tested using a likelihood ratio test (LRT), which was performed using the lrtest function in lmtest package in R. For models that explained significantly more deviance than the null, marginal probabilities for individual terms were interpreted when they were significant by exponentiation of regression coefficients to generate incidence rate ratios (IRR) as a measure of effect size.

Results

Roaming movements of free-living minnows in Budd Lake

A total of 75 clipped fathead minnows were recaptured 24 h after release. The mean ±1 SE (standard error) TL was 59.70 ± 0.83 mm ($n = 50$) and 53.52 ± 1.02 mm ($n = 25$) for males and females, respectively (Student's *t*-test $t_{73} = 4.49$, $p < 0.001$). Overall, mean *Op* intensity was 44.92 ± 5.12 metacercariae per fish. A global model predicting *Op* counts as a function of distance traveled, size, sex, and date was significant (LRT: $\chi^2 = 12.697$, df = 3, $p < 0.05$).

In general, smaller fish and fish that roamed farther distances had fewer parasites after controlling for size, sex, and date. We computed the incidence rate ratio (IRR) by exponentiating the regression coefficients and found that for each meter increase in distance traveled, the *Op* count for a fish decreased on average 0.986 (95% CI 0.983–0.988) relative to the *Op* count at the previous distance while holding all other factors constant. As expected, larger fish had higher *Op* counts (IRR 1.044, 95% CI 1.038–1.051). Neither sex nor date of sample influenced *Op* count. We separately tested for any interactions between predictor terms in the model and found none ($p > 0.05$).

There were 13 clipped fish (8 females 53.54 ± 1.47 mm and 5 males 56.40 ± 2.94 mm) from the first sample that were recaptured a week later in the second sample. The minimum distance moved between the two sites is about 100 m if these fish crossed the lake directly in a straight line. If instead they followed the shoreline between the sites, then minimum distance is about 300 m. Mean intensity of *Op* in minnows that dispersed between sites in 1 week was 21.8 ± 6.9 *Op* per fish. We plotted these data in Figure 1, but we did not include them in our full model analyses because travel distance and time interval were unknown.

Boldness in the laboratory: Budd Lake fish

We tested 37 females (TL = 53.2 ± 0.5 mm) and 18 males (56.6 ± 1.5 mm). Mean ±1 SE intensity of infection was 31.91 ± 3.27 metacercariae per minnow. We used separate regression models to predict *Op* counts relative to latency and activity given that both measures were of interest, but significantly correlated with one another ($r = -0.433$, $p = 0.001$, $n = 55$; Fig. 2). For both model formulations, we used sex and TL as covariates. The global model predicting *Op* counts as a function of latency, sex, and TL was not significant using the LRT ($\chi^2 = 6.683$, df = 3, $p = 0.083$). The global model predicting *Op* counts as a function of activity, sex, and TL was significant ($\chi^2 = 9.198$, df = 3, $p < 0.05$). None of the terms from the joint model showed marginal significance; however, activity ($p = 0.067$) and sex ($p = 0.087$) were trending toward significance.

If we eliminate the TL term from the model, we only find minimal improvement (AIC = 486.6) relative to the model that included the TL term (AIC = 486.8) and no difference between the two models using the LRT ($\chi^2 = 2.247$, df = 1, $p = 0.134$); however, the more parsimonious model does provide for a probability that is significant for sex ($p < 0.05$) and nearly so for activity ($p = 0.053$). These general trends indicated that *Op* counts on fish were higher on more active fish and were higher on males relative to females. We tested for an interaction between sex and activity, but found no relationship ($p = 0.894$; Fig. 3).

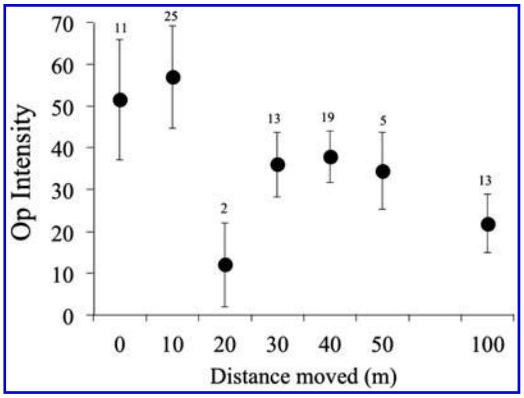

FIG. 1. Mean (±SE) (*n* shown above each point) *Op* infection intensity per fish as a function of dispersal distance. Distances ≤50 m are for 24 h. The data for >100 m are for dispersal over an interval of 7 days. *Op*, *Ornithodiplostomum ptychocheilus*; SE, standard error.

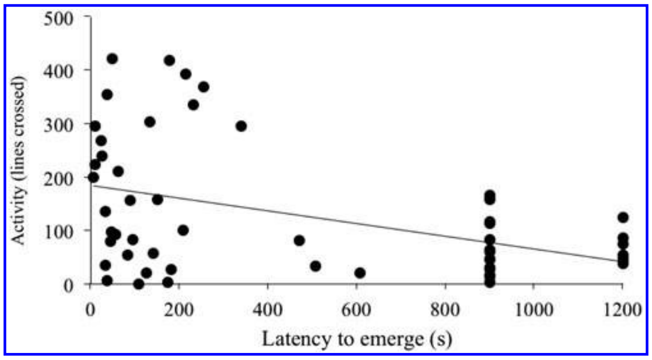

FIG. 2. Correlated behaviors indicating a behavioral syndrome in Budd Lake fish. Line of best fit: Activity = 184.33; Latency = 0.119, $R^2 = 0.1877$.

Boldness in the laboratory: Deming Lake fish

We tested 29 females (TL = 54.5 ± 0.5 mm) and 20 males (TL = 55.9 ± 1.3 mm). There were four species of trematode metacercariae found in fathead minnows in Deming Lake: *Op* located in the brain, *Obc* located in the viscera, and

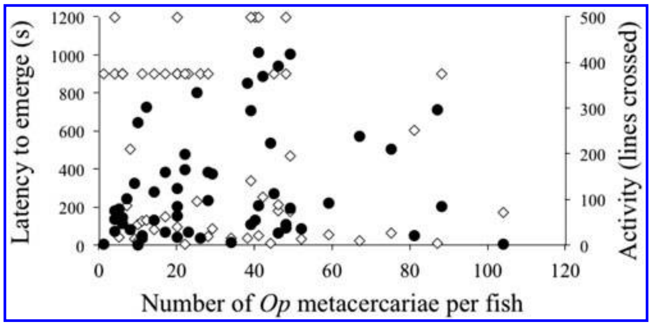

FIG. 3. Time to emerge in an open field test (*open diamonds*) and activity (*closed circles*) as a function of the number of *Op* metacercariae per fish from Budd Lake, June 2011.

Diplostomum sp. located in the lens of the eyes. Of the 10,827 parasites tallied in this part of the study, only 45 (0.4%) were *Diplostomum* sp., therefore we did not use this species in our analyses. In addition, only one individual in our sample presented black spot metacercariae, therefore this species was deleted from further analysis. Overall, mean ±1 SE intensity of infection was 221.06 ± 19.3 metacercariae per fish (Table 1). Of these, *Op* was the numerically dominant species. Latency to emerge from the open field test was repeatable, indicated by a significant correlation between the first and second times each fish was tested ($r = 0.644$, $p < 0.001$, $n = 49$). We used the average latency time from the two trials as our measure of boldness for all subsequent analyses. A GLM to predict total parasite load as a function of latency, sex, and TL was significant (LRT: $\chi^2 = 8.197$, df = 3, $p < 0.05$), but the marginal significance showed that there was no effect of sex ($p = 0.289$) or TL ($p = 0.543$).

Total parasite intensity was significantly and negatively correlated with latency to emerge (IRR = 0.9996, $p < 0.05$), meaning that highly infected minnows were bolder than lightly infected minnows and emerged more quickly from the refuge in the open field test. Because of their numerical dominance, the effect of *Op* infection followed this general pattern (Fig. 4). The joint model to predict *Op* as a function of latency, sex, and TL was significant (LRT: $\chi^2 = 8.217$, df = 3, $p < 0.05$), but the marginal significance indicated no effect of sex ($p = 0.282$) or TL ($p = 0.559$). Infection by *Obc* did not generate any significant relationships (LRT: $\chi^2 = 0.610$, df = 3, $p = 0.894$).

Discussion

These data indicate that infection by *Op* is associated with a reduced tendency to roam (contrary to our initial prediction) and, at high levels of infection, *Op* intensity is associated with bold risk-taking behavior. There are multiple ways to interpret these data. One interpretation is that these shifts in behavior are the result of parasite manipulation of host behavior to facilitate trophic transmission to the final host. A second interpretation is that individual differences in minnow behavior predisposed them to acquire or avoid trematode infection. A third possibility is that boldness reduces the antipredator benefits associated with shoaling and leads to increased vulnerability to predation in heavily infected fish. It is also possible that unmeasured causal variables are independently correlated with intensity of infection and minnow behavior.

TABLE 1. DESCRIPTIVE STATISTICS FOR METACERCARIAL ABUNDANCE AND INTENSITY FOR THREE SPECIES OF TREMATODES IN FATHEAD MINNOWS IN DEMING LAKE, MINNESOTA, JUNE 2014

	Abundance					*Intensity*		
Parasite	n	*Min*	*Max*	*Mean*	*SE*	*Mean*	*SE*	n
Obc	49	0	24	4.08	0.6	4.88	0.7	41
Op	49	7	704	216.06	18.8	216.06	18.8	49
Eye	49	0	14	0.92	0.3	3.46	1.0	13
Black spot	49	0	3	0.06	0	3	0	1
Total	49			221.06	19.3			

Obc, *Ornithodiplostomum* body cavity; *Op*, *Ornithodiplostomum ptychocheilus*; SE, standard error.

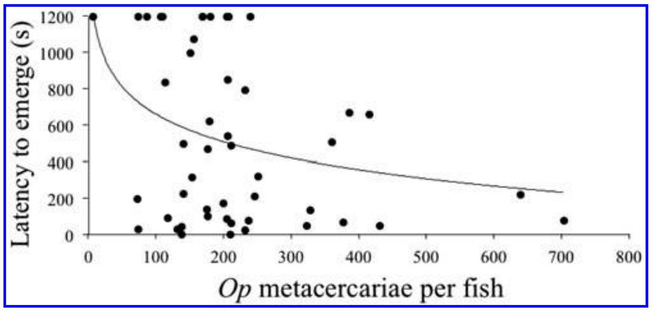

FIG. 4. Time to emerge from the shelter in the first trial of the open field test as a function of intensity of *Op* infection in Deming Lake fish, June 2011. Line of best fit: Latency = 1676.6–220.1 Ln(*Op*), $R^2 = 0.1047$.

The second possibility that individual differences in minnows predispose them to acquire or avoid cercarial infection is not the most likely explanation in this instance. Cercariae are weak swimmers and rely on chance contact with passing minnows for infection, therefore roaming behavior is likely to increase, not decrease, the encounter rate between minnows and cercariae.[38] Moreover, minnows evade cercarial infection by reduction in movement[44] and increase in shoal cohesion.[45] The mechanism by which bold individuals might have relatively high exposure to cercarial infection is not clear. Bold minnows may be more inclined than shy individuals to enter areas where risk of infection and predation are both high, but there are no data in support of this hypothesis.

One mechanism for changing the behavioral phenotype of an intermediate host is through parasite-induced pathology. For example, Shirakashi and Goater[27] reported that fathead minnows infected with *Op* showed significantly reduced activity at 2 weeks postinfection when cercarial metamorphosis into metacercariae is at its peak. This effect occurred only for experimental infections of 300 worms per host, but not for fish that were exposed to 120 worms.

Given that *Op* intensity in Budd Lake reached a maximum of 104 in our sample, direct pathology is not likely to be the explanation for the reduced distances traveled by infected fish in our study. Alternatively, suppression of roaming behavior may increase the probability of trophic transmission because site fidelity makes prey location more predictable for sit-and-wait predators such as herons and kingfishers. Vulnerability to predation would be enhanced further by bold behavior causing fish with a high intensity of *Op* to move from cover into the open. This is the most likely interpretation of the observed associations.

Previous measures of association between infection intensity and shy–bold behavior have been mixed. Pumpkinseed sunfish (*Lepomis gibbosus*) that entered unbaited traps were classified as bold by Wilson *et al.*,[16] relative to sunfish captured passively with a seine net. In their study, bold individuals had double the number of black spot metacercariae, but white grub metacercariae were twice as abundant in seined fish. A third trematode species, yellow grub (*Clinostomum* sp.), was equally abundant in each group of sunfish. In another study, common bullies (*Gobiomorphus cotidianus*) infected with metacercariae of four trematode species found no link between intensity and individual variation in boldness or activity.[46] Thus, parasites do not uniformly manipulate behavioral syndromes in all cases or even across all levels of host ontogeny or intensity of infection.

In the current study, we found no association between intensity of *Op* infection and behavior in minnows collected from Budd Lake. These data indicate that the effect of *Op* on minnow behavior may be dependent upon the size or age of the host, starting initially with a decrease in roaming behavior and eventually manifesting as a shift in the host behavioral profile along the shy–bold continuum.

There are several mechanisms for parasite manipulation of host behavior that will require further study to resolve. In this system, there is selection against severe reduction in the fitness of the fish host, which could occur through selection on parasites to be less virulent or through selection on hosts to be more tolerant.[5] *Op* intensity in Deming Lake can exceed 1000 metacercariae in the cranium of a single minnow and involves 100% prevalence in sampled populations.[29] Fathead minnows steadily accumulate *Op* metacercariae throughout their entire lives with no evidence of immune resistance to infection through repeated exposure.[29] Therefore, *Op* infection begins at host sizes too small to be a preferred prey item by piscivorous birds, which generally consume prey >50 mm.[47]

Because there are no piscivorous fish species in Budd and Deming Lakes, minnow population densities are very high in both lakes, and consequently, overall per capita risk of predation to individual minnows is low. Parasite-induced pathology is selected against because it would increase the probability of mortality. Mortality could result from starvation, low dissolved oxygen conditions during the 5 months of the year when these lakes are covered by ice,[48] or bacterial infection of penetration wounds following cercarial attack.[49,50]

Similarly, minnows with antipredator competence badly compromised by parasite infection, especially small minnows, may fall prey to nonhost odonate predators abundant in these systems. Selection for parasite modification of host behavior is therefore constrained and manifest as a decrease in roaming behavior and an increase in boldness, but only at high levels of infection. Selection in favor of behavioral manipulation of the minnow host may increase near the end of the host's life.[51,52] In the final season of a minnow's life, the risk of minnow death by natural causes without trophic transmission is a relatively high cost to parasite fitness; simultaneously, parasite manipulation leading to predation presents only a modest cost to minnow reproductive fitness, thus reducing selection for minnow resistance to manipulation.

The proximate physiological mechanisms for inducing the observed behavioral changes are beyond the scope of this study, but we assume that the intensity of the effect on minnow behavior is proportional to the *Op* intensity, which accumulates over time,[29] but at different rates in Budd Lake and Deming Lake.

Op intensity in Deming Lake is approximately seven times higher than it is in Budd Lake, possibly due to factors such as snail density, risk of bird predation, and various environmental parameters not measured in this study. It may be the case that parasite-induced boldness in Budd Lake fish was not detectable in our test apparatus given the inherent variability in behavioral data. The difference in *Op* intensity between our two study lakes generates some new questions for future study on the three-way interaction between a well-studied predator–prey system[36] and a parasite that uses prey and predator as intermediate and definitive hosts.[53]

For example, what is the relationship between *Op* intensity and bird predation? What is the effect on behavioral modification of nonhost predators that target different life stages? Does coinfection by other parasite species reduce longevity and therefore increase selection for behavioral modification? Do different parasite species within a single minnow host that have different definitive hosts compete for control of minnow behavior to guide the minnow to be eaten by the correct host? Until these questions can be tested, these data provide insight into dynamics that may govern the three-way predator–prey–parasite interactions in this study system and potentially inform workers studying other systems.

Ethics Note

The experiments described herein comply with the current laws of the United States and were reviewed and approved by the University of Minnesota Institutional Animal Care and Use Committee in protocol number 0604A84261.

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Authors' Contributions

T.P. is a junior undergraduate student in the Department of Ecology, Evolution, and Behavior (College of Biological Sciences) at the University of Minnesota. As part of a directed research summer project, T.P. designed and conducted the boldness tests on minnows from Deming Lake. K.G. is a recent graduate of the University of Minnesota. As part of her undergraduate work in the Animal Behavior course at the field station, Kelsey designed and conducted the roaming tests in Budd Lake. E.D. is a recent graduate of Humboldt State University. In 2011, E.D. was a participant in the NSF-supported ''Global Change Ecology'' REU program, led by J.C. and S.C.; funds were awarded to J.C. and S.C. Professor McEwan conducted the GLM analysis for the latency and roaming data. Professor Cotner codirected the NSF-REU program at the field station and collaborated with T.P., E.D., and B.D.W. during summer field and laboratory work. Professor Cotner codirected the NSF-REU program at the field station and selected student participants. Professor Wisenden has several years' experience studying the fathead/*Op* system at IBSL.

Disclosure Statement

No competing financial interests exist.

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