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Staying alive: The post-consumption fate of parasite spores and its implications for disease dynamics

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Abstract

Studies on the effects of selective predation by fish on disease dynamics in *Daphnia* generally assume that consumption by fish means the death of the parasite. I use a combination of feeding trials and infection assays to test this assumption using the host *Daphnia dentifera* and its common, virulent yeast parasite, *Metschnikowia bicuspidata*. Approximately 50% of *Metschnikowia* spores consumed by bluegill sunfish were released in fecal pellets. These spores remained infective to *D. dentifera*. Therefore, consumption of infected hosts by fish is not necessarily a dead end for the parasite, and in some cases, fish predation may actually help fuel epidemics in natural populations.

Parasites are increasingly recognized as integral components of food webs (Kuris et al. 2008; Lafferty et al. 2008), including in the planktonic food webs of lakes. While the parasites of the plankton were traditionally overlooked, a number of recent studies have demonstrated that they are common and can have important effects on the ecology and evolution of their host populations (Ebert 2005; Kagami et al. 2007).

Traditionally, studies on planktonic parasites have simply documented the presence and prevalence of parasites; more recently, studies have moved beyond this and have begun to explore the factors driving parasite prevalence (Hall et al. 2006; Johnson et al. 2009). One factor that has been proposed to be important is predation, particularly by predators that feed selectively on infected hosts (Kagami et al. 2004; Duffy et al. 2005; Johnson et al. 2006*b*). Such selective predation has been documented from several systems, and correlative evidence indicates that selective predators can influence the timing or location of disease epidemics in plankton (Duffy et al. 2005; Johnson et al. 2006*b*). This matches theoretical predictions that selective predation of infected hosts should make it more difficult for parasites to invade and persist in a host population (Packer et al. 2003; Ostfeld and Holt 2004; Hall et al. 2005).

However, these studies on the effects of selective predators on parasitism make an important assumption: the consumption of infected hosts by selective predators results in the death of the parasite. While this is likely to be true of some parasites, other parasites (such as those with thick-walled spores) may be able to survive passage through the gut of a predator. In these cases, predators may no longer prevent parasite epidemics but instead may actually facilitate them.

Here I present the results of a study in which I test whether spores survive gut passage and remain infective. These experiments use *Daphnia dentifera* as host. This daphniid is a common and important member of the plankton in stratified lakes in North America (Tessier and Woodruff 2002). The parasite used in these experiments is the yeast *Metschnikowia bicuspidata*. This yeast is highly virulent (Ebert et al. 2000; Hall et al. 2006; Duffy and Sivars-Becker 2007), has thick-walled asci (Lachance et al. 1976), and is a common parasite of *D. dentifera* (Cáceres et al. 2006) as well as other crustaceans (Ebert 2005). This parasite is horizontally transmitted; transmission occurs when spores are released from dead *Daphnia* and are then consumed by uninfected *Daphnia* (Ebert 2005). Finally, the predator used in this experiment is the bluegill sunfish, *Lepomis macrochirus*. Bluegill are the dominant predators on *D. dentifera* (Tessier and Woodruff 2002) and feed extremely selectively on infected *Daphnia* (Duffy et al. 2005; Johnson et al. 2006*b*), including those infected with *Metschnikowia* (Duffy and Hall 2008).

Passage of asci through fish guts—To determine the effect of fish gut passage on the quantity of *Metschnikowia* asci, I fed *Metschnikowia*-infected *D. dentifera* to bluegill and compared the number of spores released with the number of spores contained in infected *D. dentifera* that had not been fed to fish. To do this, I collected *Metschnikowia*-infected adult *D. dentifera* and distributed them into ten 50-mL centrifuge tubes filled with filtered lake water. There were five 'control' tubes and five 'fish' tubes. These individuals were collected and distributed 1 d prior to feeding the fish tubes to fish (*see* below). *D. dentifera* were not fed while in tubes. The control and fish tubes each received 15 and 30 infected *D. dentifera*, respectively. Twice as many *D. dentifera* were placed in the fish tubes to increase the likelihood of recovering asci after fish gut passage.

The bluegill used for this experiment had been maintained in 900-liter cattle tanks for several months prior to the experiment. These bluegill, which were 35–46-mm standard length, were moved to large indoor aquaria 2 d prior to the experiment and were fed uninfected *Daphnia* to acclimate them to laboratory conditions. One bluegill was then placed in each of ten 4-liter aquaria filled with filtered lake water. Bluegill were starved for 24 h prior to feeding them infected *Daphnia*. Half of these bluegill were assigned to the fish treatment, and half were assigned to the control treatment; these latter bluegill were not fed infected *D.*

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dentifera but were instead used to create water that contained fish kairomones to use in the infection assay (*see* below). The fish-treatment bluegill were each fed one tube of *D. dentifera* (i.e., 30 *Metschnikowia*-infected *D. dentifera*). After the bluegill consumed all of the *Daphnia* they were transferred to beakers filled with 300 mL of filtered lake water. Each bluegill ate all the *D. dentifera* and was moved to a beaker within 15 min. The control-treatment bluegill were treated identically except that they were not fed infected *D. dentifera*. Instead, the tube of *Metschnikowia*-infected *D. dentifera* was left in the laboratory overnight. All bluegill were left in beakers overnight.

The next morning, bluegill were removed from the beakers with a net while water agitation was limited to the extent possible. Fecal pellets were collected from the bottom of the fish beakers, resuspended in 10 mL of filtered lake water, ground to release asci, and counted to determine spore abundance. The water in the beaker was then mixed well, and samples were collected to determine the abundance of asci released directly into the water. The number of asci in the control *D. dentifera* (that is, the *D. dentifera* that had not been fed to fish) was obtained by grinding the *D. dentifera* in each tube to release asci. Asci were counted using a hemocytometer at 200× magnification. To correct for the different numbers of *D. dentifera* in the fish and control treatments, values were divided by the number of *D. dentifera* (that is, 30 and 15, respectively). Because the *D. dentifera* used in this experiment had well-developed infections, this experiment produced a conservative estimate of the reduction in asci after fish gut passage. In natural lake populations, more asci might be digested if fish consume infected *D. dentifera* before the *Metschnikowia* asci have fully developed.

Forty-eight percent of the *Metschnikowia* asci were retrieved after bluegill gut passage (Fig. 1). On average, the control *D. dentifera* contained 69,667 asci *Daphnia*$^{-1}$ (range: 61,667–72,500 asci *Daphnia*$^{-1}$). An average of 33,458 asci *Daphnia*$^{-1}$ (range: 15,208–52,916 asci *Daphnia*$^{-1}$) were retrieved from the fish treatments. Of those asci, most (on average, 25,958 asci *Daphnia*$^{-1}$; range: 2708–40,417) were contained within fecal pellets; comparatively few (on average, 7500 asci *Daphnia*$^{-1}$; range: 0–12,500) were released directly into the water column.

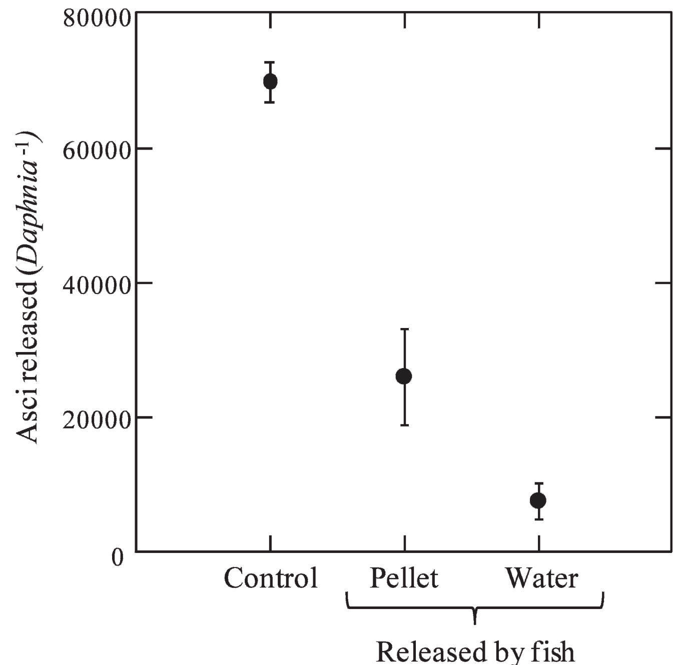

Fig. 1. Number of *Metschnikowia* asci (±1 SE) in *Daphnia* that were not fed to bluegill (control) or that were released after passage through a fish gut, either in fish fecal pellets (pellet) or directly into the water (water). Note that the total number of asci released by fish includes both the pellet and water asci.

Infectivity of asci after passage through fish guts—To determine whether the asci retrieved after passage through a fish gut remain infective, I conducted an infection assay in which I exposed *D. dentifera* of a single highly susceptible clone to asci that were collected either from the fish fecal pellets or from the control *Daphnia*. There were once again two treatments, 'fish' and 'control.' There were five replicates of the control treatment, which contained asci from the *D. dentifera* in the five corresponding control tubes above. There were four replicates of the fish treatment, each of which contained asci from the fecal pellet of a fish-treatment bluegill; there was not a fifth replicate because the fecal pellet of the fifth fish did not contain enough asci. Asci were resuspended in water from the control fish treatments so that all beakers, control and fish, received the same volume of water containing fish kairomones. This was done to control for any possible effects of kairomones on *Daphnia* behavior that might affect infectivity (Decaestecker et al. 2002).

This experiment followed a standard infection assay protocol (Duffy and Sivars-Becker 2007; Duffy et al. 2008). Briefly, *D. dentifera* were exposed to 500 asci mL^{-1} in 110-mL beakers overnight and were then moved to clean water. Each beaker contained five 6–9-d-old *D. dentifera*, and there were six beakers per replicate (that is, 54 beakers total). Animals were scored for infection after 11 d, and the proportion of *D. dentifera* infected per beaker was calculated. I analyzed the results of the infection assay with a mixed-model ANOVA using Proc Mixed in SAS 9.1 and restricted maximum likelihood estimation (Littell et al. 2006). Proportion infected per beaker was the dependent variable; data were arcsin square root–transformed prior to analysis. Treatment (fish vs. control) was a fixed effect and replicate (fish identity or tube of *D. dentifera*, nested within treatment) was a random effect. I tested for significance of the random effect by using differences in the -2 restricted log likelihood, which are χ^2 distributed with 1 degree of freedom, between models with and without the random effect included.

Fish gut passage did not significantly affect the infectivity of *Metschnikowia* asci (Fig. 2; $F_{1,7} = 1.98$, $p = 0.20$). There was significant variation among replicates within treatments ($\chi^2 = 4.1$, $p = 0.04$). This was driven by significant variation within the fish treatment ($\chi^2 = 4.6$, $p = 0.03$).

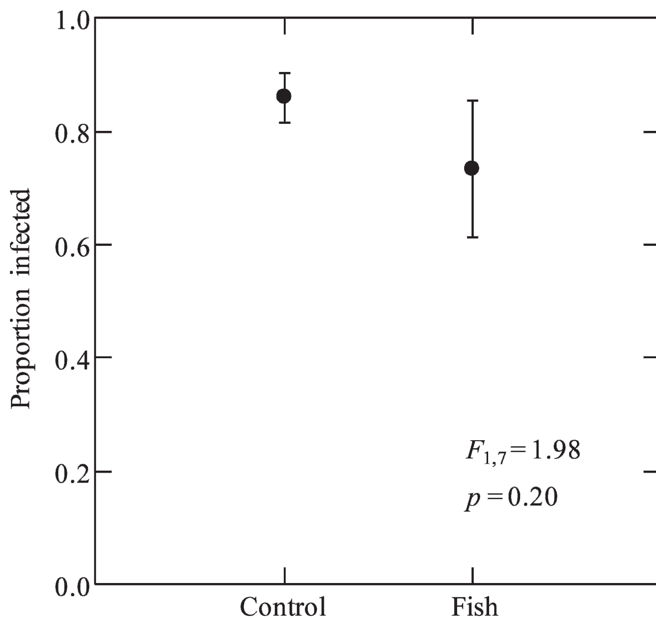

Fig. 2. Infectivity of *Metschnikowia* asci that have (fish) or have not (control) passed through a bluegill. Differences in infectivity are not significant ($F_{1,7} = 1.98$, $p = 0.20$).

Implications for epidemics in lake populations—The experimental results show that *Metschnikowia* spores survive predation and remain infective. While there may be a slight (though nonsignificant) reduction in infectivity (Fig. 2), it is clear that the spores are still largely infective. This ability of *Metschnikowia* spores to survive predation and remain infective may have important implications for disease dynamics, given the physical structure of lakes and the behavior of bluegill sunfish. *D. dentifera*, the focal host in this study, lives primarily in deep, stratified lakes (Tessier and Woodruff 2002). In these lakes, infected *D. dentifera* that die from direct virulent effects of the parasite would be expected to sink down to the hypolimnion. There, the spores would be released after decomposition, but would be unlikely to get resuspended until the lake turns over (in spring or fall; Lampert and Sommer 1997).

Spores that are contained within *Daphnia* consumed by fish, however, may meet a different fate as a result of the foraging behavior of bluegill. Bluegill feeding on plankton peaks around sunrise; bluegill then return to the littoral zone of lakes during the day (Mittelbach 1981). Thus, while approximately half of the *Metschnikowia* spores will be killed by fish predation (Fig. 1), the remaining spores are likely to be released in the littoral zone. There they can more easily be resuspended into the water column, where they can be consumed by foraging *Daphnia* (Hall et al. 2007).

Thus, while fish predation will have at least some inhibitory effects on epidemics (as a result of reductions in host density, the digestion of some parasite spores, ingestion of infected individuals before spores have fully developed, and selection for smaller-bodied hosts, which are less susceptible; Hall et al. 2007), they may also facilitate epidemics of some parasite species. Whether, on balance, selective predation by fish promotes or inhibits epidemics is likely to depend on both the structure of lakes and the digestion resistance of parasites. In polymictic lakes, the balance would likely shift toward inhibition of epidemics, since there the primary effect of predation would be to reduce host and parasite density, without any benefits due to increased resuspension of spores. In addition, many different parasites can infect lake *Daphnia* (Ebert 2005; Johnson et al. 2006*a*), and the morphologies of these parasites differ greatly (Ebert 2005). Thus, the effect of fish gut passage on spore survival and infectivity is likely to vary among parasite species. For parasites with easily digested transmission stages, fish predation is more likely to inhibit epidemics; this likely includes the bacterial parasite *Spirobacillus cienkowskii*, which appears to be digested by fish (M. A. Duffy unpubl.; unfortunately, we have been unable to successfully carry out infection assays with this parasite, and so cannot do a direct comparative study). However, for parasites that are at least partially digestion-resistant and in lakes that are strongly stratified, selective predation may actually fuel parasite spread.

Another factor that has the potential to influence the effect of fish predation on parasitism is the size of fish predators. Body size is positively correlated with gut length, which, in turn, is positively correlated with digestion ability (Benavides et al. 1994; Yang and Joern 1994). Thus, the size of fish predators may influence the proportion of spores that are digested and, therefore, the effect of fish predation on parasitism.

Together, the seasonality of stratification and seasonal variation in fish size (due to ontogeny; Rettig and Mittelbach 2002) should combine to render the effects of fish predation on parasitism seasonally variable. In late summer, when small bluegill are abundant and the lake is stratified, fish predation is more likely to increase parasite prevalence. Conversely, in autumn, when lakes are mixing and fish are larger (and perhaps more likely to digest spores), fish predation is more likely to negatively affect parasitism.

In this study I show that parasites can survive fish gut passage and remain infective to *Daphnia*. Because spores released by fish are more likely to be resuspended in the water column (where they can infect additional hosts), this indicates that in some cases selective predation may actually increase parasite prevalence. The sum of the facilitative and inhibitory effects of predation will likely depend on a number of factors—including the selectivity of predation, the fraction of spores that get digested, and the likelihood of spore resuspension. Because these will differ between lakes, predators, parasites, and seasons, the overall effect of predation on parasitism will be system specific and season specific.

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