DO SCAVENGERS INFLUENCE DERMO DISEASE *(PERKINSUS MARINU*S)
TRANSMISSION? EXPERIMENTS IN OYSTER PARASITE TROPHIC
INTERACTIONS

by

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ABSTRACT OF THE THESIS

Do Scavengers Influence Dermo Disease (*Perkinsus marinus*) Transmission?

Experiments in Oyster Parasite Trophic Interactions

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Perkinsus marinus is the protozoan endoparasite of the Eastern oyster

(*Crassostrea virginica*) responsible for Dermo disease. While not harmful to humans,

Dermo disease causes extensive oyster mortality, increasing annual natural mortality

from 10 to 35% or more in Delaware Bay annually. The disease spreads through the

water as parasites are shed from infected and moribund hosts. One prior study has

indicated that scavengers may spread the parasite to new hosts, but little information

exists as to how such trophic interactions affect host-parasite dynamics.

From July 2010 to September 2011, uninfected, or specific-pathogen free (SPF)

oyster hosts were exposed in the laboratory to four different species of scavengers

feeding on infected or uninfected oyster tissue. In each experiment, the accumulation of

P. marinus in oyster hosts was compared after 1-2 months as a measure of parasite

transmission. Results indicated that scavengers, regardless of species, increase the rate of

parasite transmission to new hosts when compared to passive shedding of parasites from infected tissue alone. These laboratory studies demonstrate that non-host organisms for the parasite have their own sets of interactions that can influence disease dynamics, and such interactions should be taken into consideration in future studies where transmission dynamics come into play.

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Introduction

Parasites hold important roles in ecosystems: they can alter host survival, reproductive output, and interactions among different species. But in traditional ecological food webs, the interactions of parasites tend to be overlooked. This exclusion from the traditional model is not without reason, as parasites are often cryptic and may have multiple hosts during their life cycle, making them difficult to identify and track through an ecosystem. Recent studies have demonstrated that community structure has the potential to significantly alter disease dynamics. Lafferty et al. (2006) demonstrated that when parasites were included in the food webs of the Carpinteria Salt Marsh, the degree of connectance, or interactions between species, was tripled. Since parasites seem to make up most of the links in any given food web (Lafferty et al. 2006, 2008), it would be to the benefit of all ecological studies to take the parasites into account.

To learn more about the role of parasites and their interactions with other organisms, I designed a set of laboratory experiments using the oyster parasite *Perkinsus marinus* as a model system. Uncovering the interactions between the parasite, its hosts, and non-hosts provided a more complete picture of its transmission, and in the case of *P. marinus*, elucidate some of the more subtle transmission dynamics behind the apparently direct waterborne transmission that has been traditionally so well-studied.

Background

Perkinsus marinus is a single-celled protozoan parasite of the Eastern oyster *Crassostrea virginica*. It has no specific vector and is directly transmitted between hosts through water currents. It has three distinct life stages, all of which are infective when introduced to oysters (Andrews 1965, Chu 1996, Bushek et al. 2002, Villalba et al. 2004). The proliferative stage within the host's cells, the trophozoite (also known as the meront, with a characteristic "signet ring" appearance) occurs as a 2-4 μm spherical cell within the host's hemocytes. Through progressive cell division, the immature trophozoite matures into a sporangium (schizont) of 8-32 cells contained within the mother cell membrane (Chu 1996, Villalba et al. 2004). When the membrane ruptures, a new generation of immature trophozoites is released, which go on to infect more host cells. When host tissues are incubated in Ray's fluid thioglycollate medium (RFTM), the trophozoites enlarge, develop a large vacuole and thick cell wall, and become prezoosporangia (formerly called hypnospores), a stage that is sometimes observed in dead or moribund oyster tissue in nature (Chu 1996). These prezoosporangia are resistant to low and high pH, as well as low temperature (Villalba et al. 2004), allowing them to survive until such time that conditions improve enough for zoosporulation and infection of new hosts. Dermo-infected oysters die primarily of tissue lysis and blockage of major blood vessels from the parasite's degradation of connective tissue (Andrews 1988, Burreson and Ragone Calvo 1996). As infective stages are released by dead and dying hosts, successive years incur increases in prevalence and mortality as more susceptible hosts become infected; researchers estimate that a full epizootic of naïve oysters in the

Chesapeake can be established within 1-3 years (Ewart and Ford 1993).

Perkinsus marinus can be detected in oysters through a microbiological or molecular assay. The first tests for *P. marinus* detection were developed by Ray (1952) when the parasite was classified as the fungus *Dermocystidium marinum* (while the classification has changed, the original moniker "Dermo disease" has remained). Because the parasite did not grow in the typical growth medium for fungi, it was assumed to be an obligate parasite that needed oyster tissue to proliferate. Pieces of antibiotic-treated oyster tissue were then incubated in fluid thioglycollate meduim (FTM) to establish a better growth medium, but examination of the tissues revealed enlarged round cells that could be easily stained with Lugol's iodine. These large cells were soon confirmed to be those of the parasite (Ray 1952). The addition of nystatin to the FTM, which was found to improve enlargement of the parasite and reduce fungal growth, became the formula for Ray's Fluid Thioglygollate medium (RFTM) that is still the standard diagnostic technique (Ray 1966, Villalba et al. 2004). This original "tissue squash" method, wherein pieces of oyster gill, mantle, and rectal tissue are incubated for a week in RFTM, stained with Lugol's iodine, and rated on the Mackin scale of infection intensity from 1-5 (Ray and Mackin 1954) is still the method of choice to determine parasite prevalence among oyster populations. However, this method depends on an individual's ability to estimate the percentage of the tissue sample is occupied by parasite cells. A more accurate method of detection, especially at low levels of infection intensity, was developed by dissolving the tissues in NaOH to count only *P. marinus* cells in solution (Choi et al. 1989, Bushek et al. 1994, Fisher and Oliver 1996). This technique,

known as a body burden, can be applied to the whole animal or a tissue biopsy and determines the number of parasite cells per gram of oyster tissue. It has been modified to include hemolymph as a non-fatal method of sampling and parasite detection (Gauthier and Fisher 1990) but body burdens with whole oysters are considered the most sensitive and accurate of all the RFTM assay methods (Bushek et al. 1994).

Transmission Dynamics

In general, warm temperature (>20 °C) and high salinity (>15) are the major contributing factors in epizootics (Andrews 1988, Burreson and Ragone Calvo 1996), and also account for the cyclical nature of Dermo outbreaks following the warm months. In mid-Atlantic estuaries, water temperature is the dominant factor in the intensity of Dermo outbreaks, whereas salinity is the dominant factor in the Gulf of Mexico (Andrews 1996). Mild winters allow the parasites to persist longer in infected oysters and extend the epizootic period throughout the year (Andrews 1996, Burreson and Ragone Calvo 1996, Villalba et al. 2004). Although cold winter temperatures decrease intensity and prevalence of infection in surviving oysters, *P. marinus* is readily capable of overwintering and releasing new infective stages once temperatures rise in the spring (Andrews 1996; Chu 1996; Burreson and Ragone Calvo 1996).

While the physical conditions and waterborne nature of the parasite are well documented, certain aspects of the life cycle of *P. marinus* and natural dynamics of transmission are still not fully understood (Villalba 2004). This includes how predators and scavengers may alter the dissemination of the parasite between discrete oyster reefs

of previously uninfected hosts (Figure 1). Only two prior studies have examined this subject. White et al. (1987) reported *P. marinus* transmission between live hosts is possible via the gastropod ectoparasite *Boonea impressa* (mosquito snail) which feeds on oyster hemolymph, though this gastropod is by no means required for transmission to occur. Hoese (1962) conducted a series of simple experiments with scavenging fish (including blennies, toadfish, and gobies) and crustaceans (blue crabs, Atlantic mud crabs, and others) and found that whole Dermo cells could be found in the digestive system and occasionally in the feces of the scavenging fish. He also demonstrated that infections could be transmitted to uninfected oysters from scavengers that had fed on infected oyster tissue, but the intensity of the acquired *P. marinus* infections was never quantified.

Hypotheses

Since there is little available data on how *P. marinus* might be transmitted to new hosts through the actions of scavengers, I posed the following questions:

1) Do secondary consumers have a significant effect on the transmission of Dermo compared to passive shedding of the parasite alone?

2) Do certain scavenger species have a greater impact on transmission than others?

3) What are the implications when studying the disease dynamics of oyster reefs?

To investigate these questions, I developed the following hypotheses:

H_0: Scavengers have no effect on Dermo transmission compared to passive

shedding alone.

H_A: Scavengers alter Dermo transmission compared to passive shedding alone, either by increasing or decreasing the number of parasites available for filtration by new hosts. Different scavengers may have different effects on the transmission of parasites.

If scavengers have no effect on the transmission of the parasite, I would expect to see no difference in the accumulated infections between oysters exposed to scavengers eating infected tissue, vs. infections in oysters exposed to infected tissue alone. Whereas if scavengers alter the transmission, I would expect to see a significant difference in the scavenger-exposed oysters (increase or decrease in parasite burden) when compared to the control. To address the hypotheses, I conducted four experiments: the first two compared the effects of different scavenger species against passive parasite transmission, and the last two addressed the same effect, but with the added impact of a dose-related response.

Methods

The first experiment exposed specific-pathogen free (SPF) oysters to whole shucked infected oysters in the presence or absence of scavengers (Table 1). Scavengers were allowed to feed on the shucked tissue twice per week, and at the end of two months, the SPF oysters were sacrificed and assayed for their parasite burden. The second experiment was intended to be a replicate of the first, but problems with the tank equipment necessitated fewer water changes per week. This, combined with an additional scavenger feeding per week, introduced enough variability to confound any comparisons, and so was considered a separate experiment of its own. The third experiment introduced the variable of parasite dosage, in addition to scavenger activity, having an effect on the final accumulated parasite burden in the SPFs. Four treatments, ranging from 0-3 feedings of infected shucked tissue where scavengers were either present or absent, controlled for different levels of introduced parasites (Table 2). This experiment ran for one month. The fourth and last experiment was identical in design to the third, but was allowed to run for two months rather than one, demonstrating how length of incubation time is also an important factor when measuring the final accumulated burden of the parasites.

Body Burden Assay

The methods for determining parasite burden in oyster tissue follow those described in Bushek et al. (1994) and Fisher (1996). To estimate the number of parasites fed to each treatment via oyster tissue, an RFTM body burden analysis was performed on

0.02 - 0.30 g subsamples of the tissue. Subsamples were taken from each shucked oyster and placed in 5 mL of sterile RFTM fortified with 0.5 mL of PenStrep (0.159g Penicillin, 0.33g Streptomycin in 0.5L sterile dH_2O) to prevent bacterial decay, and placed in the dark to incubate at room temperature. After one week, 5-7 mL of 2M sodium hydroxide (NaOH) were added directly to the subsample tubes, which were then placed in a 62°C drying oven for 2-4 hours. Once all the tissue had been digested, tubes were centrifuged for 15 minutes at 2000 rpm, the supernatant was discarded, and tubes re-filled to achieve a final volume of 10mL deionized water. No additional washes were necessary for this process. The same process was used for whole oysters when moribund individuals were removed, except tissues were incubated in 10mL RFTM with 1 mL PenStrep and then digested with 20 mL NaOH.

Once in water, subsamples and body burdens were stored in a refrigerator until such time that they could be analyzed. For analysis, tubes were inverted repeatedly , then mixed with a pipette, and a 1 mL sample was transferred to its own membrane filter grid (GN-6 Metricel, 0.45 μm, 47mm) on a vacuum aspirator. Parasite cells which remained on the top of the filter once all the liquid was drawn down were then stained with Lugol's iodine. The stained grids were immediately examined under the microscope for *P. marinus* density. Very dense samples with cells too numerous to be counted accurately were diluted serially to accurately count the cells present. Target counting density was 30-300 cells per filter; for the densest samples, as many as 4-5 dilutions were needed, or 1:10,000 to 1:100,000. Cell counts were used to calculate parasite burden normalized by tissue mass as follows:

$$\frac{\textbf{cell count x dilution factor x total sample volume (mL)}}{\textbf{tissue mass (g)}}$$

The tissue mass refers to two possible measurements: the mass of a whole oyster, as would be recorded upon experiment termination or whole-oyster weight of fed tissue, or the mass of a tissue subsample (biopsy) taken from the shucked oyster prior to every feeding.

Hemolymph assay

For experiments 1 and 2, a hemolymph sample was withdrawn from each SPF oyster before the start of the trials to verify that little or no parasite cells were initially present. The methods for non-lethal hemolymph sampling followed those outlined in Bushek et al. (1994). Oysters were notched in the shell near the adductor muscle using a benchtop grinder, and a 0.2 – 0.5 mL sample of hemolymph was withdrawn with a syringe from sinuses in the adductor muscle. Each sample was deposited into 2 mL tubes containing 1 mL of sterile Ray's Fluid Thioglycollate Medium (RFTM) and 0.5 mL PenStrep. After one week of incubation in the dark at room temperature, tubes were centrifuged for 15 minutes at 670 rcf. The supernatant was discarded and replaced with 2 mL NaOH, and all tubes were placed in a 62°C drying oven for ~2 hours. They were centrifuged a second time (15 min, 670 rcf), the supernatant was removed again and replaced with 2 mL deionized water. No additional washes were used in this process. These samples were then aspirated onto gridded membranes, stained and counted as

explained in the section above. Counts were normalized by hemolymph sample volume.

Experimental Design

Four laboratory experiments were conducted from July 2010 to September 2011.
All experiments focused on the actions of common oyster-associated species of
Delaware Bay known to scavenge dead or moribund oysters—specifically, mud crabs
(*Panopeus herbstii*), mud snails (*Ilyanassa obsoleta*), mummichogs (*Fundulus
heteroclitus*), and blue crabs (*Callinectes sapidus*). These species were not only chosen
for their association with oyster reefs, but also for differences in rates and modes of
feeding. The snails and mud crabs, for example, fed more slowly and less aggressively
than blue crabs or mummichogs, which tended to destroy tissue quickly while feeding.
According to the alternate hypothesis, different feeding modes may contribute to
differences in final parasite burdens, hence the variety of common species. All animals
were collected from Delaware Bay and the Maurice River in Port Norris, NJ as they were
needed: blue crabs and mud crabs were obtained from the Maurice River in traps off the
Haskin Shellfish Research Laboratory dock, or from oyster bushels as bycatch from the
laboratory's Delaware Bay oyster seedbed monitoring project. Mummichogs were
collected from a tidal, brackish creek that flows into the Maurice River, and mud snails
were taken from the Rutgers Cape Shore Facility mud flats in Cape May, NJ.

All infected oysters used in these experiments were collected in trawls from the
Delaware Bay seedbeds on the New Jersey side of the bay. Because their precise
infection status was unknown upon collection, infected oysters were held separately in a

warm, high-saline tank at 25°C and S = 25 to provide an ideal environment for *Perkinsus*

marinus, and to maintain or build sufficient infections until the oysters were needed.

Oysters were selected to be approximately the same size and weight across experiments

(average wet weight = 8.29 g, SEM = 0.15, n = 661).

All SPF oysters were ordered from from the Pemaquid Oyster Co. on the

Damariscotta River, or from the Bagaduce River, ME. These were maintained in a

separate tank under quarantine in a recirculating system at 11-15°C and S = 10-15, and

were acclimated for one week before the start of experiments. Once acclimated, oysters

were sorted by size. Small two-inch oysters (average wet weight = 4.07 g, SEM = 1.11 n

= 915) were placed in trays to be exposed to the conditions of each tank, while large

"cocktail" oysters (average wet weight = 7.66 g, SEM = 0.13, n = 667) were used as

uninfected food for the negative controls. All oysters in treatments and holding tanks

were fed a maintenance diet of 10mL concentrated algae (Phyto Feast®) daily.

All seawater used in the current study was collected from Sea Isle, NJ and stored

in an outside storage tank until needed, then passed through three cartridge filters of 10,

5, and 1 μm, plus a UV light filter before use to ensure as little *P. marinus* contamination

as possible. Filtered seawater was mixed with fresh water to achieve a salinity of 25 in a

clean reservoir.

Experiment 1— July 30 - September 28, 2010

Eight ~227L recirculating aquaculture tanks were set up at 25°C and S = 25 to

hold treatments and controls (Table 1): positive (infected) scavenger treatments, a

positive control (no scavengers present), and four corresponding negative controls. Scavengers in the positive and negative treatments were separated by species to determine if different scavengers had different effects on parasite transmission (Table 1). Each tank was stocked with 30 adult specific pathogen free (SPF) oysters from Damariscotta, ME for a total of 240 oysters. These oysters were separated from the scavengers by plastic mesh barriers, to prevent them from being eaten during the experiment (negative controls containing no scavengers also had a plastic barrier for the sake of consistency). To ensure that these oysters were indeed uninfected before the start of the experiment, a 0.5 mL sample of hemolymph was taken from each and incubated for one week in RFTM to detect any initial presence of the parasite. Water in the recirculating tanks was maintained at 25°C and S = 25 for two months, and 20% water changes for all tanks took place 3 times per week to maintain water quality. Twice weekly, tanks were given whole shucked oyster meats appropriate to the treatment (infected oysters in the positive treatments, uninfected SPF oysters in the negative control treatments). The whole shucked oysters were weighed and biopsies were taken prior to each feeding for RFTM body burden analysis as described above. Total possible dosage was calculated by multiplying biopsy parasite burden by the whole oyster mass for each oyster placed in a tank. A final dosage estimate (the approximate number of parasites actually released from the tissue) was obtained by multiplying the previous total dosage value by the percentage of tissue consumed or decayed. Any tissue that remained after one day was removed and weighed to obtain this percentage. Tanks were checked daily for any SPF mortalities, which were removed and incubated for one week in RFTM to

determine parasite burden. Mortalities that occurred within the first six days of the experiment were not factored into the final analysis, and were instead counted as non-disease, or stress mortalities. After 60 days, all remaining SPF oysters were sacrificed and incubated for parasite burden analysis (Bushek et al. 1994). A two-way ANOVA and Bonferroni multiple comparison was conducted to compare infection burdens across treatments.

Experiment 2— November 3, 2010 – January 6, 2011

The methods for the second experiment were identical to those outlined in experiment 1, except for the frequency of feedings and water changes. Feedings occurred three times weekly and 20% water changes took place twice weekly. In addition, a smaller hemolymph sample (0.2 mL) was taken from each oyster and incubated in RFTM to detect any parasites present before the start of the experiment. Any oyster mortalities within the first six days of the experiment were discarded and replaced with a new SPF oyster that had previously been sampled for its hemolymph; these stress mortalities were not taken for body burdens and not factored into the final analysis. A two-way ANOVA and Bonferroni multiple comparison was conducted to compare infection burdens across treatments.

Experiment 3— June 23 - July 23, 2011

The third experiment was devised to test for possible effects of initial parasite dosage on transmission to new hosts. As before, eight recirculating, ~227 L aquaculture

tanks were set up at 25°C and S = 25, and were designated into pairs. Each pair

represented a different dosage treatment (Table 2). Within each of these dosage

treatments, one tank was stocked with scavengers (positive) and the other tank was left

with none (negative). Instead of separating scavengers by species, a small community

consisting of 1 blue crab, 5 mummichogs, ~50 mud snails, and 1-2 mud crabs was

present. Each tank was also stocked with 30 adult specific pathogen free (SPF) oysters

from the Bagaduce River, ME for a total of 240 SPF oysters. In lieu of initial

hemolymph sampling from each oyster, a random sample of 20 individuals from the

shipment were sacrificed and incubated for one week in Ray's Fluid Thioglygollate

Medium (RFTM) to detect any significant initial presence of the parasite. Water in the

recirculating tanks was maintained at 25°C and S = 25 for one month, and 20% water

changes for all tanks took place three times per week to maintain water quality. Three

times weekly, tanks were given whole shucked oyster meats appropriate to the treatment

(Table 2). Tanks were checked daily for any SPF mortalities; those that occurred within

the first six days of the experiment were considered stress mortalities, discarded and

replaced with a new SPF oyster from the quarantine tank. These stress mortalities were

not taken for body burdens and not factored into the final analysis. After 30 days, all

remaining SPF oysters were sacrificed and incubated for parasite burden analysis

(Bushek et al. 1994). A two-way ANOVA and Bonferroni multiple comparison was

conducted to compare infection burdens across treatments.

Experiment 4— July 28 – September 26, 2011

The methods for the fourth experiment were identical to those outlined in experiment 3, but the experiment was allowed to run for two months instead of one. At the end of one month, ten oysters were selected randomly from each tank and taken for analysis to determine the progression of *P. marinus* infection. At the end of the second month, all remaining oysters were taken for burden analysis. Data were analyzed with a two-way ANOVA and Bonferroni multiple comparison to compare infection burdens across treatments. Because oysters were taken for body burdens at the midpoint and at the termination of the experiment, the dosage and body burden results for each month were analyzed separately to demonstrate any progression of the infections over time.

Results

Experiment 1

Scavengers, regardless of species, always increased the rate at which the fed

oyster tissue was lost, in both the infected and uninfected treatments. In all scavenger

groups, an average of 90-100% of all fed tissue was consumed over the two months of

the experiment, compared to the control's passive decay average of 15-22% (Figure 3).

As a result, the total estimated parasite dosage in the infected treatments with scavengers

averaged an order of magnitude higher than the estimated parasite dosage in the control

treatment ($2.7 - 4.2 \times 10^7$ versus 1.1×10^6 cells/g). Dosages among all infected

treatments did not differ significantly from each other ($p>0.05$, Table 3). The dosages of

the infected treatments were significantly higher than all uninfected treatments, which

had an estimated dosage of $0.32 – 3.2 \times 10^4$ cells/g. The uninfected groups' dosages did

not differ significantly from each other ($p>0.05$, Table 3).

All SPF oysters began the experiment with infection levels below the average

detection limits of the standard RFTM tissue assay (Bushek et al. 1994), based on the

average cell count from individual hemolymph samples (Figure 2). Between days 1-59, a

total of 65 oysters exposed to infected tissue were removed as mortalities (gapers), and a

total of 47 oysters exposed to uninfected tissue were removed as mortalities. The

cumulative mortality rate, particularly in the early days of the experiment, did not

correlate strongly with infection burden, though over time the infection burdens in the

gapers removed from infected scavenger treatments did exhibit an increasing trend

relative to the infected and uninfected controls (Figure 5). Mortalities within days 1-6

were considered stress deaths unrelated to parasite infection. Mortalities were not replaced with new oysters at any point in the experiment.

Upon termination, oysters exposed to infected tissue plus scavengers, regardless of scavenger species, did not exhibit significantly different body burdens from each other, averaging approximately 1.1 - 2.2 x 10^5 cells/g (p>0.05, Table 4). Oysters in all three infected scavenger treatments did exhibit significantly higher parasite burdens than the infected control, which averaged 89 cells/g (Figure 6). This infected control was distinct not only from the infected scavenger treatments (p<.0001 Table 4), but all uninfected treatments as well. All parasite burdens in the four uninfected treatments averaged 7-13 cells/g, and none were significantly different from each other (Figure 6).

Experiment 2

As in experiment 1, scavengers always increased the rate at which the fed oyster tissue was consumed in both the infected and uninfected treatments, but the percentage of tissue consumed was not uniform across species. Mud crabs and mud snails together consumed an average of 58% of infected tissue, and 86% of uninfected tissue. Mummichogs consumed 100% of both infected and uninfected tissue, and blue crabs consumed 65-70% of tissue in both groups. The control group had an average decay of 18% of infected tissue, and 28% of uninfected tissue (Figure 7). The total estimated parasite dosage in the infected control, infected blue crab treatment, and infected mud crab+mud snail treatment averaged 0.5 – 0.66 x 10^6 cells/g, while the infected mummichog treatment dose averaged 1.9 x 10^6 cells/g (Figure 8). Dosages among all the

infected treatments did not differ significantly from each other (p>0.05, Table 5). The infected treatment dosages were significantly higher than the uninfected controls, which had an estimated average dosage of ~4-18 cells/g. The uninfected groups' dosages did not differ significantly from each other (p>0.05, Table 5).

All SPF oysters began the experiment with infection levels below the average detection limits of the standard RFTM tissue assay (Bushek et al. 1994), based on the average cell count from individual hemolymph samples (Figure 2). Between days 1-63, a total of 13 oysters exposed to infected tissue were removed as gapers (mortalities), and a total of 11 oysters exposed to uninfected tissue were removed as mortalities. The cumulative mortality rate, particularly in the early days of the experiment, did not correlate strongly with infection burden, though over time the infection burdens in the few gapers removed from all infected treatments (infected control included) did exhibit an increasing trend relative to the uninfected controls only (Figure 9).

At the end of two months, oysters in the infected mummichog, infected blue crab, and infected control had an average parasite burden of $1.2 - 7.3 \times 10^6$ cells/g, and the infected mud crab+mud snail treatment had an average burden of 1.6×10^7 cells/g (Figure 10). A Bonferroni post-hoc analysis indicated that the infected control was not significantly different from the infected blue crab treatment, but both of these treatments were significantly different from the mummichog and mud crabs/mud snail treatments (Table 9). All infected treatments exhibited significantly higher burdens than the four uninfected treatments. Parasite burdens in the uninfected treatments averaged 1.8-4.3 cells/g, and none were significantly different from each other (Table 6).

Experiment 3

As in experiments 1 and 2, the presence of scavengers always increased the percentage of oyster tissue consumed. Across all dosage groups with scavengers, 100% of the fed tissue was consumed. By comparison, the passive decay of food in treatments without scavengers averaged between 19-27% across all groups (Figure 11). The estimated average parasite dosage in all groups increased with more frequent feedings of infected oyster tissue per week, ranging from 2.18-2.95 cells/g in the group receiving only uninfected oyster tissue, to 318 - 803 cells/g in the treatment receiving 3 infected tissue feedings per week. Although the estimated dose in the scavenger groups was consistently higher than the controls without scavengers, this difference between pairs in the same feeding regimen did not differ significantly (Figure 12, Table 7).

Between days 1-29, a total of 22 oysters exposed to scavengers were removed as mortalities (gapers), and a total of 24 oysters in the scavenger-absent controls were removed as mortalities. The cumulative mortality rate did not correlate strongly with infection burden, nor did feeding dosage seem to have any correlation with the burdens of these few mortalities (Figure 13). Mortalities within days 1-6 were considered stress deaths unrelated to parasite infection, and only stress mortalities were replaced with new SPF oysters during this period.

After one month, average infections in the control groups ranged from ~27-406 cells/g and did not correlate with increasing frequency of dosage (Figure 14). Average infections in the scavenger treatments ranged from ~0.008 - 8×10^3 cells/g, and tended to increase with increasing frequency of dosage. The results of a Bonferroni post-hoc

analysis indicated that among the controls, only the oysters receiving 1 infected dose per week had significantly higher burdens than all other control oysters. Among the scavenger treatments, oysters receiving 3 infected doses/week had significantly higher parasite burdens than those in the other three scavenger treatments, while those receiving 1 or 2 infected doses per week were not significantly different from each other. Significant differences between the scavenger/control pairs in each feeding regimen were only apparent in the 3 infected doses/week treatment: oysters with scavengers accumulated significantly higher infections than the corresponding control (Table 10, Figure 8), but this effect was not observed in the 0, 1, and 2 infected doses/week groups.

Experiment 4, month 1

As in all previous experiments, the presence of scavengers always increased the percentage of oyster tissue consumed. In all groups where scavengers were present, 100% of the fed tissue was consumed. In contrast, the passive decay of food in treatments without scavengers averaged between 13-28% across all groups (Figure 15a). The estimated average parasite dosage in all groups increased with more frequent feedings of infected oyster tissue per week, ranging from 11.84 - 31.37 cells/g in the group receiving only uninfected oyster tissue, to $0.408 – 5.96 \times 10^6$ cells/g in the treatment receiving 3 infected tissue feedings per week. Although the estimated dose in the scavenger groups was consistently higher than the controls without scavengers, this difference was only statistically significant between the two "heavy" dosage treatments receiving 3 infected meals per week (Figure 16a, Table 9).

Between days 1-30, a total of 14 oysters exposed to scavengers were removed as mortalities (gapers), and a total of 9 oysters in the scavenger-absent controls were removed as mortalities. The cumulative mortality rate did not correlate with infection burden, but parasite burdens of the existing mortalities did tend to increase over time in all treatments where infected tissue was present in any dosage (Figure 17). Mortalities within days 1-6 were considered stress deaths unrelated to parasite infection, and only stress mortalities were replaced with new SPF oysters during this period.

At the end of one month, average infections in the scavenger-free controls ranged from 7-71 cells/g and did not correlate with increasing frequency of dosage. Average infections in the scavenger treatments ranged from 0.009 - 4.8×10^3 cells/g (Figure 18a). The results of a Bonferroni post-hoc analysis indicated that among the controls, oysters receiving 1, 2, and 3 infected doses per week were not significantly different from each other, but all did have significantly higher burdens than the control oysters exposed to uninfected tissue only (Table 10). Among the scavenger treatments, parasite burdens in oysters receiving 1 and 3 infected doses/week were not significantly different from each other, but did have significantly higher parasite burdens than those receiving 2 infected doses/week. Scavenger groups with doses 1, 2, and 3 were all significantly higher than the scavenger treatment receiving 0 infected doses/week (Table 10). Significant differences between the scavenger/control pairs in each feeding regimen were only apparent in the 1 and 3 infected doses/week treatments: oysters with scavengers in these groups accumulated significantly higher infections than the corresponding control (Table 10), but this effect was not observed in the 0 and 2 infected doses/week groups.

Experiment 4, month 2

As in all previous experiments, the presence of scavengers always increased the percentage of oyster tissue consumed. In all groups where scavengers were present, 100% of the fed tissue was consumed. In contrast, the passive decay of food in treatments without scavengers averaged between 16-25% across all groups (Figure 15b). The estimated average parasite dosage in all groups increased with more frequent feedings of infected oyster tissue per week, ranging from 1.96-3.73 cells/g in the group receiving only uninfected oyster tissue, to $0.524 – 3.07$ x 10^6 cells/g in the treatment receiving 3 infected tissue feedings per week. Although the estimated dose in the scavenger groups was consistently higher than the controls without scavengers, this difference was only statistically significant between the two "heavy" dosage treatments receiving 3 infected meals per week (Figure 16b, Table 11).

Between days 31-59, a total of 5 oysters exposed to scavengers were removed as mortalities (gapers), and a total of 11 oysters in the scavenger-absent controls were removed as mortalities. The cumulative mortality rate did not correlate with infection burden, although in treatments receiving any dose of infected tissue, body burdens of these few mortalities did exhibit a slight increase since the first month (Figure 17).

At the end of the second month, average infections in the control groups ranged from ~$0.0010 – 1.7$ x 10^4 cells/g and displayed a positive correlation with the increasing frequency of dosage. Average infections in the scavenger treatments ranged from ~$0.00021 – 3.09$ x 10^5 cells/g, but showed no correlation with the increasing frequency of dosage (Figure 18b). The results of a Bonferroni post-hoc analysis indicated that among

the controls, oysters receiving 1 and 2 infected doses per week were not significantly different from each other, but did have significantly higher burdens than the control oysters exposed to 0 infected doses per week. The control oysters receiving 3 infected doses per week had significantly higher burdens than all other controls (Table 12). Among the scavenger treatments, parasite burdens in oysters receiving 1, 2, and 3 infected doses/week were not significantly different from each other, but did have significantly higher parasite burdens than those receiving 0 infected doses/week (Table 12). Significant differences between the scavenger/control pairs in each feeding regimen were apparent in the 1, 2, and 3 infected doses/week treatments: oysters with scavengers in these groups accumulated significantly higher infections than the corresponding control (Table 12), but this effect was not observed in the 0 infected doses/week group.

Discussion

Apart from the present study, only Hoese (1962) and White et al. (1987) investigated how *P. marinus* infections could be transmitted from secondary consumers that fed upon infected oyster tissue. Hoese's experiment with parasite transmission from scavenger feeding, involved three species of fish; *Gobiosoma bosci (*goby), *Chasmodes bosquianus* (striped blenny) and *Hypsoblennius hentzi* (feather blenny). These fish were allowed to feed on infected oyster meat and were then placed in a tank containing uninfected oysters. Infections in oysters that had been transmitted from the scavenging fish were allowed to progress for 4-6 weeks, and final parasite burdens were not counted, but instead recorded as "light." The disease transmission aspect of this study was limited in the variety of scavenging fish used, the small sample size of oyster and scavenger groups, and the amount of time that infections were allowed to progress. Viable *P. marinus* cells had also been previously observed on the setae of blue crabs and on the bodies of mud crabs that had fed on infected oysters (Hoese 1964), but no species of crab was used in any of the transmission experiments. In the study by White et al., uninfected oysters were exposed to the ectoparasitic snail *Boonea impressa* that were previously allowed to feed on oysters infected with *P. marinus*. At the end of one month, oysters were examined for transmitted infections. *Perkinsus marinus* burdens in oysters that were parasitized by infected snails were significantly greater than infections in control oysters, which had been parasitized by snails that had only fed on low-infection oysters. Only oysters that had been attacked by snails exhibited *P. marinus* infections; individuals that remained free of snails did not develop infections. Infections seemed confined to the

mantle tissue where the snail pierced the tissue and fed upon the host's fluids.

While it was abundantly clear from White's study that *Boonea impressa* could serve as a vector for *P. marinus*, and therefore secondary consumers could have an effect on parasite transmission, the effect of the snail was not compared to more typical infections acquired by passive shedding of *P. marinus* from infected hosts, as would typically occur on a reef where the snail might also be present. Neither Hoese's nor White's study made use of a passive shed control, nor did they allow scavengers to feed on infected tissue in the same tanks as the uninfected oyster hosts— any feeding on infected tissue was conducted in a separate tank, and scavengers were then moved to the tanks containing the oysters. In spite of these limitations, both prior studies did demonstrate that *P. marinus* could be transmitted through the actions of secondary consumers.

Hoese also documented the feeding behavior of a variety of species in detail. He noted whether they tended to tear pieces of oyster tissue (as the crabs did) or eat pieces that had already been torn by previous scavengers (as the gobies were observed to do). By plotting the rate of passive decay of newly-killed oysters on reefs vs. the rate of consumption by scavengers, he found that scavengers greatly increased the percentage of tissue lost per unit time, and concluded that the scavenging of moribund oysters may facilitate the release of parasites into the water.

Current study

In all four of my experiments, specific pathogen free (SPF) oysters in negative

control treatments never developed intense infections, indicating that SPF oysters began experiments uninfected or with very low starting infections and that cross-contamination from adjacent tanks with infected oysters did not occur (Figure 2). Although the results of the hemolymph assays in experiments 1 and 2 versus the initial whole body burdens in experiments 3 and 4 would seem to indicate that oysters began with higher infections in the latter two experiments (Figure 2), this may be the result of false negatives that commonly occur at low parasite intensities (Bushek et al. 1994, Fisher and Oliver 1996). Hemolymph sampling, while generally non-lethal, does place stress on the experimental animals and is time-consuming. In studies like this one, where the goal is to simply begin with uninfected animals and measure their infection accumulation, a whole body burden assay of 20 randomly selected individuals from the entire group of oysters provided an equally useful estimate of starting infection, was more efficient, and was more sensitive in detecting parasites (Figure 2). In either sampling method, all average starting infections of the SPF oysters fell below detectable levels of the standard RFTM tissue assay (Bushek et al. 1994). Below 10^3 cells/g, Bushek et al. (1996) found a high number of false positives using the standard RFTM tissue assay (Ray 1952, 1966). Above that level, positives were typically ranked as very light, light, or light to moderate infections. Therefore, since all individuals examined before the start of parasite exposure fell below this value, even with the more accurate body burden method in experiments 3 and 4, the SPF oysters in this study were considered uninfected individuals at the start of all four experiments.

Experiment 1 indicated that scavengers had an effect on the transmission of *P.*

marinus in a lab setting: the body burdens of oysters kept with scavengers plus infected tissue meals were significantly higher than the passive shedding of parasites from infected tissue alone (Figure 6). The body burdens among scavenger treatments were not significantly different from each other, indicating that the type of scavenger species made no difference in the transmission of the parasite. According to the graph of tissue consumed or decayed in the various treatments (Figure 3), more tissue was always consumed in the treatments containing the feeding scavengers. As in Hoese's study, observations of the different scavengers during feeding revealed an accelerated breakdown of the tissue: crabs and fish had a tendency to tear oyster tissue into smaller pieces before eating, and snails grazed on the remaining tissue pieces that settled to the bottom. Oyster meat placed in the passive shed control treatments decayed much more slowly, and pieces removed after 24 hours remained largely intact. The body burdens in the SPF oysters suggested that, in the presence of oyster-feeding scavengers, more parasites were being released into the surrounding water through the accelerated breakdown of the infected tissue meals, resulting in more intense infections for new hosts.

However, experiment 2, while similar in its design, yielded a different pattern, wherein the body burdens from the passive shed control were equally intense as the infected scavenger treatments (Figure 10). This suggested that while the scavengers were still breaking down the infected tissue at an accelerated rate (Figure 7), the number of parasites present in the passive shedding control was sufficient to infect the SPFs with equally intense infections. Indeed, experiment 2 had a higher average parasite dosage

than the first experiment (Figure 8). In addition, the less-frequent water changes (twice a week instead of three times) may have allowed for a longer recirculation time of parasites, allowing the oysters to accumulate more parasites and resulting in a saturated infection, regardless of scavenging activity. Collectively these results suggested that there may be a parasite threshold of infection intensity, above which the accumulated infections of new hosts begin to saturate and scavenging behavior has no additional effect.

To explore the possibility of a dosage-dependent effect, experiments 3 and 4 were devised. Since the separated scavenger species had no discernible effect on any differences in Dermo transmission, all four species were placed in the scavenger tanks as a community. The total number of scavengers was reduced from the first two experiments to prevent excessive fouling and overcrowding, but having all species present increased the percentage of consumed oyster tissue to 100% (Figures 11, 15). Due to some early, non-disease mortality of the SPF oysters in the "very light" and "light" dose treatment of experiment 3 (Figure 13), plus several oyster deaths as the result of predation from a single errant blue crab in the light treatment (not shown), there were only sufficient oysters to run the trial for one month rather than two. To add further complications, the parasite doses in the fed tissue were not nearly as intense as they were in the previous two experiments (Figure 12), owing to the fact that it was still early in the season, and overwintering infections in Delaware Bay oysters had not yet begun their typical seasonal increase.   Nevertheless, the body burden data from experiment 3 revealed an emerging pattern of increasing infection intensity with increasing frequency

of infected feedings when scavengers were present (Figure 14).

In contrast to Experiment 3, the passive shed controls of Experiment 4 showed a gradual increase in infection intensity with increasing dosages. This held true in both months of the experiment (Figure 18), and overall parasite burdens increased in all groups from the first to the second month. Parasite burdens in the tanks containing scavengers were higher when compared to the controls, but a pattern of infection intensity did not clearly emerge until the second month: burdens from oysters exposed to scavengers and infected tissue, regardless of the dosage, were equally high by the end of two months.

All results from these four experiments collectively indicate that the scavenging actions of secondary consumers increase the breakdown of infected tissues and subsequent release of more parasites into the water, where they can be filtered by new hosts. In addition, the effect of scavenging activity appears to be independent of initial parasite dosage, since it liberates sufficient parasites even in low dosage treatments to cause infections equal to those where the dose was two or three times greater (Figure 18). However, as experiment 2 demonstrated, it is possible to saturate the system with enough parasites that the effects of scavengers are not observed (Figure 10), that is, when the dosage exceeds a threshold, the effect of scavengers is overwhelmed and therefore undetectable. In the case of experiment 3, not enough time was provided for infections to take a strong hold in the new hosts (Figure 14). Thus, the scavenger effect seems dependent on larger factors in the system such as frequency of water flushing and amount of parasites initially released.

Conclusion

The highly contagious and virulent nature of *P. marinus* makes it a simple, yet formidable parasite of the Eastern oyster. Since it does not require any vector other than water currents to spread to new oyster hosts, it is highly communicable. Although a limited genetic resistance has been demonstrated in oyster populations exposed to high abundances of the parasite, (Bushek and Allen 1996), *P. marinus* continues to infect nearly all oyster populations of the mid-Atlantic and Gulf coasts.

Of all the molluscan diseases, it is likely that Dermo has resulted in the most severe commercial losses in terms of oyster yield (Villalba et al. 2004). Several types of Dermo-like diseases caused by various species of *Perkinsus* affect a variety of molluscs worldwide, including several commercial species of oysters, abalones, clams, scallops, and mussels (Villalba et al. 2004). Dermo is such a wide-ranging and virulent disease, it has already significantly reduced oyster catches since the 1950s (Andrews 1996), thereby decreasing the profit for the fishing industry and ultimately harming the ecology of the estuarine communities along much of the East Coast and throughout the Gulf of Mexico. Although a great deal of the parasite's life history, transmission and virulence is already known, studies like this one have uncovered an additional, more subtle role of other species in the transmission of *P. marinus*. The role of scavengers, at least in the scope of this study, would appear to be small by comparison to larger factors such as the frequency of water flushing or intensity of parasite dosage, but under the right conditions, the actions of scavengers feeding on infected oysters do increase the number of Dermo parasites available for transmission to new oyster hosts when compared to passive

shedding from decaying tissue. For example, it may be possible when Dermo intensity is low, such as in early spring after overwintering, scavengers feeding on infected oysters facilitate *P. marinus* transmission, spreading it more rapidly than would occur from passive shedding. If this holds true, one would expect to see a saturation effect as these infections take hold and grow more intense later in the year, with scavenger activity having no further discernible effect once infections are high.

These findings, while valuable to ecology studies, should not be applied to restoration efforts of oyster reefs—I do not wish to imply that scavengers would have a deleterious effect on oysters where disease is already present. Rather, these studies are meant to uncover how parasites can move through their environments, and to understand how species diversity might alter disease transmission in aquatic ecosystems. While this study was limited to laboratory research only, future researchers may benefit from designing similar experiments in the field. Such field studies could be valuable in determining if species diversity has any role in aquaculture, where, if the spread of parasites is undesirable among the stock, it may be necessary to keep oysters separate from other organisms that may increase the spread of the disease. Field studies in areas where Dermo is already prevalent, such as the Delaware and Chesapeake Bay, would aid in creating a kind of "parasite map" across different areas: if a given scavenger is more or less abundant in certain places that overlap with variable abundances of the parasite, it may point to even deeper relationships between secondary consumers and the spread of disease. Dermo will likely always be a problem for Eastern oyster communities, but future studies into all aspects of the parasite's biology and interactions will allow for

better understanding of epizootics and how they may be controlled.

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Table 1. Design for experiments 1 and 2. Positive treatment tanks were provided with infected oyster tissues while negative control tanks were provided with tissues from SPF oysters to control for cross-contamination and validate pathogen status of SPF oyster in experimental systems.

Experiment Tanks	Control Tanks
2 blue crabs	2 blue crabs
10-15 mud crabs, 100 mud snails	10-15 mud crabs, 100 mud snails
20 mummichogs	20 mummichogs
No scavengers (positive control)	No scavengers (negative control)

Table 2. Design for experiments 3 and 4. The relative dosage intensity (very light, light,

etc.) refers to the number and types of oyster meats given to each treatment per week,

and the relative dosage of parasites introduced to the treatments. Positive treatments

contained a community of the four scavenger species, while controls had no scavengers

present (see text for details on scavenger community).

	Very light	Light	Moderate	Heavy
Scavengers present	3 uninfected feeds 0 infected feeds	2 uninfected feeds 1 infected feed	1 uninfected feed 2 infected feeds	0 uninfected feeds 3 infected feeds
Scavengers absent (control)	3 uninfected feeds 0 infected feeds	2 uninfected feeds 1 infected feed	1 uninfected feed 2 infected feeds	0 uninfected feeds 3 infected feeds

Table 3. ANOVA values for dosage data of experiment 1.

Source of variation	SS	df	MS	F	P value
2-way ANOVA					
Food (infected vs. uninfected)[1]	867.05	1	867.05	355.20	0.0000
Scavengers (present or absent)[1]	74.74	1	74.74	30.62	0.0000
Food x Scavengers[1]	2.80	1	2.80	1.15	0.2849
Model	1300.28	3	433.43	177.56	0.0000
Residual	654.20	268	2.44		
Total	1954.48	271	7.21		
1-way ANOVA					
Food (within infected)[2]	0.58	2	0.29	0.32	0.7282
Model	0.58	2	0.29	0.32	0.7282
Residual	90.89	99	0.92		
Total	91.47	101	0.91		
Food (within uninfected)[2]	15.87	2	7.94	2.18	0.1182
Model	15.87	2	7.94	2.18	0.1182
Residual	360.02	99	3.64		
Total	375.89	101	3.72		

[1] Model: $\log_{10}(\text{dosage}+1) = \text{food} + \text{scavenger} + \text{food*scavenger}$

[2] Model: $\log_{10}(\text{dosage}+1) = \text{tank}$

Table 4. ANOVA values for body burden data of experiment 1.

Source of variation	SS	df	MS	F	P value
Two-way ANOVA					
Food (infected vs. uninfected)[1]	139.25	1	139.25	449.54	0.0000
Scavengers (present or absent)[1]	60.09	1	60.09	194.00	0.0000
Food x Scavengers[1]	47.76	1	47.76	154.18	0.0000
Model	373.15	3	124.38	401.54	0.0000
Residual	31.60	102	0.31		
Total	404.75	105	3.85		
One-way ANOVA					
Food (within infected)[2]	108.23	3	36.08	113.74	0
Model	108.23	3	36.08	113.74	0.0000
Residual	14.59	46	0.32		
Total	122.82	49	2.51		
Food (within uninfected)[2]	0.47	3	0.16	0.51	0.6794
Model	0.47	3	0.16	0.51	0.6794
Residual	16.16	52	0.31		
Total	16.63	55	0.30		

[1] Model: $\log_{10}$(cells per gram+1) = food + scavenger + food*scavenger

[2] Model: $\log_{10}$(cells per gram+1) = tank

Table 5. ANOVA values for dosage data of experiment 2.

Source of variation	SS	df	MS	F	P value
Two-way ANOVA					
Food (infected vs. uninfected)[1]	1826.66	1	1826.66	486.58	0.0000
Scavengers (present or absent)[1]	7.45	1	7.45	1.98	0.1597
Food x Scavengers[1]	0.11	1	0.11	0.03	0.8648
Model	2440.85	3	813.62	216.73	0.0000
Residual	1606.73	428	3.75		
Total	4047.58	431	9.39		
One-way ANOVA					
Food (within infected)[2]	14.53	3	4.84	0.86	0.4657
Model	14.53	3	4.84	0.86	0.4657
Residual	900.17	159	5.66		
Total	914.7	162	5.65		
Food (within uninfected)[2]	5.29	2	2.64	1.01	0.3674
Model	5.29	2	2.64	1.01	0.3674
Residual	417.06	159	2.62		
Total	422.35	161	2.62		

[1] Model: $\log_{10}(\text{dosage}+1) = \text{food} + \text{scavenger} + \text{food*scavenger}$

[2] Model: $\log_{10}(\text{dosage}+1) = \text{tank}$

Table 6. ANOVA values for body burden data of experiment 2.

Source of variation	SS	df	MS	F	P value
Two-way ANOVA					
Food (infected vs. uninfected)[1]	1541.10	1	1541.10	2131.43	0.0000
Scavengers (present or absent)[1]	5.96	1	5.96	8.24	0.0045
Food x Scavengers[1]	0.07	1	0.07	0.1	0.7500
Model	1991.77	3	663.92	918.24	0.0000
Residual	0.72	210	0.72		
Total	2143.6	213	10.06		
One-way ANOVA					
Food (within infected)[2]	19.77	3	6.59	16.84	0.0000
Model	19.77	3	6.59	16.84	0.0000
Residual	39.92	102	0.39		
Total	59.69	105	0.57		
Food (within uninfected)[2]	2.42	3	0.81	0.88	0.4556
Model	2.42	3	0.81	0.88	0.4556
Residual	95.74	104	0.92		
Total	98.16	107	0.92		

[1] Model: $\log_{10}(\text{cells per gram}+1) = \text{food} + \text{scavenger} + \text{food*scavenger}$

[2] Model: $\log_{10}(\text{cells per gram}+1) = \text{tank}$

Table 7. ANOVA values for dosage data of experiment 3.

Source of variation	SS	df	MS	F	P value
Two-way ANOVA					
Food (dosage)[1]	184.13	3	61.38	12.69	0.0000
Scavengers (present or absent)[1]	11.33	1	11.33	2.34	0.1273
Food x Scavengers[1]	7.54	3	2.51	0.52	0.6693
Model	203.00	7	29.00	6.00	0.0000
Residual	1044.62	216	4.84		
Total	1247.62	223	5.59		
One-way ANOVA					
Tank (0 infected doses/week)[2]	0.03	1	0.03	0.03	0.8634
Model	0.03	1	0.03	0.03	0.8634
Residual	60.59	54	1.12		
Total	60.62	55	1.10		
Tank (1 infected dose/week)[2]	1.20	1	1.20	0.19	0.6635
Model	1.20	1	1.20	0.19	0.6635
Residual	338.02	54	6.26		
Total	339.22	55	6.17		
Tank (2 infected doses/week)[2]	15.23	1	15.23	2.76	0.1026
Model	15.23	1	15.23	2.76	0.1026
Residual	298.24	54	5.52		
Total	313.46	55	5.70		
Tank (3 infected doses/week)[2]	2.41	1	2.41	0.37	0.5432
Model	2.41	1	2.41	0.37	0.5432
Residual	347.78	54	6.44		
Total	350.19	55	6.37		

[1] Model: $\log_{10}(\text{dosage}+1)$ = food + scavenger + food*scavenger

[2] Model: $\log_{10}(\text{dosage}+1)$ = tank

Table 8. ANOVA values for body burden data of experiment 3.

Source of variation	SS	df	MS	F	P value
Two-way ANOVA					
Food (dosage)[1]	91.11	3	30.37	36.9	0.0000
Scavengers (present or absent)[1]	15.54	1	15.54	18.88	0.0000
Food x Scavengers[1]	30.6	3	10.2	12.39	0.0000
Model	136.01	7	19.43	23.61	0.0000
Residual	133.33	162	0.82		
Total	269.34	169	0.59		
One-way ANOVA					
Tank (0 infected doses/week)[2]	2.96	1	2.96	3.25	0.0782
Model	2.96	1	2.96	3.25	0.0782
Residual	41.89	46	0.91		
Total	44.84	47	0.95		
Tank (1 infected dose/week)[2]	1.4	1	1.4	2.51	0.1246
Model	1.4	1	1.4	2.51	0.1246
Residual	15.61	28	0.56		
Total	17.01	29	0.59		
Tank (2 infected doses/week)[2]	7.15	1	7.15	6.63	0.0133
Model	7.15	1	7.15	6.63	0.0133
Residual	49.62	46	1.08		
Total	56.76	47	1.21		
Tank (3 infected doses/week)[2]	35.62	1	35.62	57.08	0.0000
Model	35.62	1	35.62	57.08	0.0000
Residual	26.21	42	0.62		
Total	61.83	43	1.44		

[1] Model: $\log_{10}(\text{cells per gram}+1) = \text{food} + \text{scavenger} + \text{food*scavenger}$
[2] Model: $\log_{10}(\text{cells per gram}+1) = \text{tank}$

Table 9. ANOVA values for dosage data of experiment 4, month 1.

Source of variation	SS	df	MS	F	P value
Two-way ANOVA					
Food (dosage)[1]	629.25	3	209.75	39.23	0.0000
Scavengers (present or absent)[1]	24.11	1	24.11	4.51	0.0349
Food x Scavengers[1]	6.19	3	2.06	0.39	0.7634
Model	59.55	7	94.22	17.62	0.0000
Residual	1069.21	200	5.35		
Total	1728.76	207	8.35		
One-way ANOVA					
Tank (0 infected doses/week)[2]	2.08	1	2.08	0.85	0.3597
Model	2.08	1	2.08	0.85	0.3597
Residual	121.49	50	2.43		
Total	123.56	51	2.42		
Tank (1 infected dose/week)[2]	3.34	1	3.34	0.38	0.5378
Model	3.34	1	3.34	0.38	0.5378
Residual	434.02	50	8.68		
Total	437.36	51	8.58		
Tank (2 infected doses/week)[2]	3.88	1	3.88	0.49	0.4886
Model	3.88	1	3.88	0.49	0.4886
Residual	398.24	50	7.96		
Total	402.12	51	7.88		
Tank (3 infected doses/week)[2]	21.01	1	21.01	9.10	0.0040
Model	21.01	1	21.01	9.10	0.0040
Residual	115.46	50	2.31		
Total	136.46	51	2.68		

[1] Model: $\log_{10}(\text{dosage}+1) = \text{food} + \text{scavenger} + \text{food*scavenger}$
[2] Model: $\log_{10}(\text{dosage}+1) = \text{tank}$

Table 10. ANOVA values for body burden data of experiment 4, month 1.

Source of variation	SS	df	MS	F	P value
Two-way ANOVA					
Food (dosage)[1]	40.2	3	13.4	26.18	0.0000
Scavengers (present or absent)[1]	18.24	1	18.24	35.63	0.0000
Food x Scavengers[1]	12.65	3	4.22	8.24	0.0001
Model	71.1	7	10.16	19.84	0.0000
Residual	36.86	72	0.51		
Total	107.95	79	1.37		
One-way ANOVA					
Tank (0 infected doses/week)[2]	0.09	1	0.09	0.12	0.7294
Model	0.09	1	0.09	0.12	0.7294
Residual	12.51	18	0.69		
Total	12.59	19	0.66		
Tank (1 infected dose/week)[2]	13.58	1	13.58	52.56	0.0000
Model	13.58	1	13.58	52.56	0.0000
Residual	4.65	18	0.26		
Total	18.23	19	0.96		
Tank (2 infected doses/week)[2]	0.19	1	0.19	0.24	0.6331
Model	0.19	1	0.19	0.24	0.6331
Residual	14.52	18	0.81		
Total	14.71	19	0.77		
Tank (3 infected doses/week)[2]	17.04	1	17.04	59.16	0.0000
Model	17.04	1	17.04	59.16	0.0000
Residual	5.18	18	0.29		
Total	22.22	19	1.17		

[1] Model: $\log_{10}$(cells per gram+1) = food + scavenger + food*scavenger
[2] Model: $\log_{10}$(cells per gram+1) = tank

Table 11. ANOVA values for dosage data of experiment 4, month 2.

Source of variation	SS	df	MS	F	P value
Two-way ANOVA					
Food (dosage)[1]	755.76	3	251.92	55.68	0.0000
Scavengers (present or absent)[1]	8.99	1	8.99	1.99	0.1603
Food x Scavengers[1]	4.36	3	1.45	0.32	0.8098
Model	769.12	7	109.87	24.28	0.0000
Residual	832.49	184	4.52		
Total	1601.61	191	8.39		
One-way ANOVA					
Tank (0 infected doses/week)[2]	0.14	1	0.14	0.15	0.6983
Model	0.14	1	0.14	0.15	0.6983
Residual	41.29	46	0.90		
Total	41.43	47	0.88		
Tank (1 infected dose/week)[2]	0.30	1	0.30	0.03	0.8526
Model	0.30	1	0.30	0.03	0.8526
Residual	400.47	46	8.71		
Total	400.78	47	8.53		
Tank (2 infected doses/week)[2]	5.83	1	5.83	0.82	0.3706
Model	5.83	1	5.83	0.82	0.3706
Residual	328.06	46	7.13		
Total	333.89	47	7.10		
Tank (3 infected doses/week)[2]	7.08	1	7.08	5.20	0.0273
Model	7.08	1	7.08	5.20	0.0273
Residual	62.66	46	1.36		
Total	69.75	47	1.48		

[1] Model: $\log_{10}(\text{dosage}+1)$ = food + scavenger + food*scavenger
[2] Model: $\log_{10}(\text{dosage}+1)$ = tank

Table 12. ANOVA values for body burden data of experiment 4, month 2.

Source of variation	SS	df	MS	F	P value
Two-way ANOVA					
Food (dosage)[1]	217.46	3	72.49	129.65	0.0000
Scavengers (present or absent)[1]	52.74	1	52.74	94.33	0.0000
Food x Scavengers[1]	19.8	3	6.6	11.8	0.0000
Model	277.53	7	39.65	70.92	0.0000
Residual	57.03	102	0.56		
Total	334.56	109	3.07		
One-way ANOVA					
Tank (0 infected doses/week)[2]	0.65	1	0.65	1.6	0.2165
Model	0.65	1	0.65	1.6	0.2165
Residual	10.97	27	0.41		
Total	11.62	28	0.42		
Tank (1 infected dose/week)[2]	43.12	1	43.12	53.66	0.0000
Model	43.12	1	43.12	53.66	0.0000
Residual	18.48	23	0.8		
Total	61.6	24	2.57		
Tank (2 infected doses/week)[2]	16.94	1	16.94	32.55	0.0000
Model	16.94	1	16.94	32.55	0.0000
Residual	11.97	23	0.52		
Total	28.9	24	1.2		
Tank (3 infected doses/week)[2]	7.55	1	7.55	14.03	0.0008
Model	7.55	1	7.55	14.03	0.0008
Residual	15.61	29	0.54		
Total	23.16	30	0.77		

[1] Model: $\log_{10}$(cells per gram+1) = food + scavenger + food*scavenger
[2] Model: $\log_{10}$(cells per gram+1) = tank
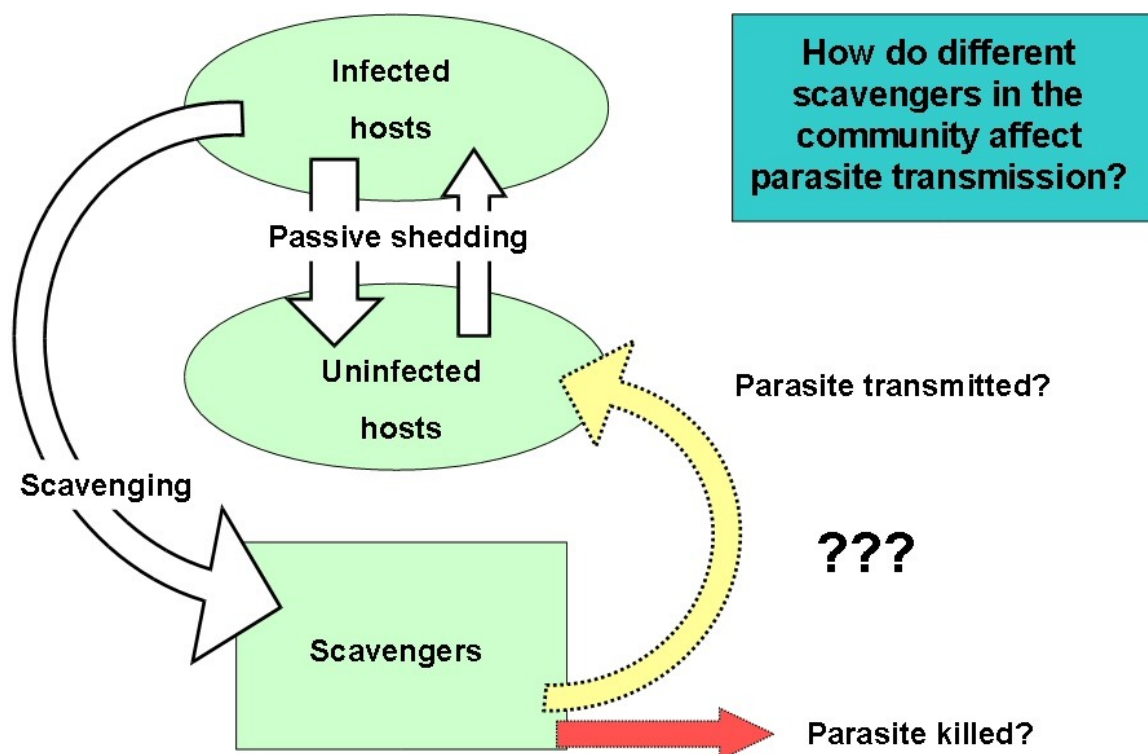
Figure 1. Simplified box model demonstrating possible fates of *Perkinsus marinus* when consumed by a scavenger, and how it might affect overall transmission.
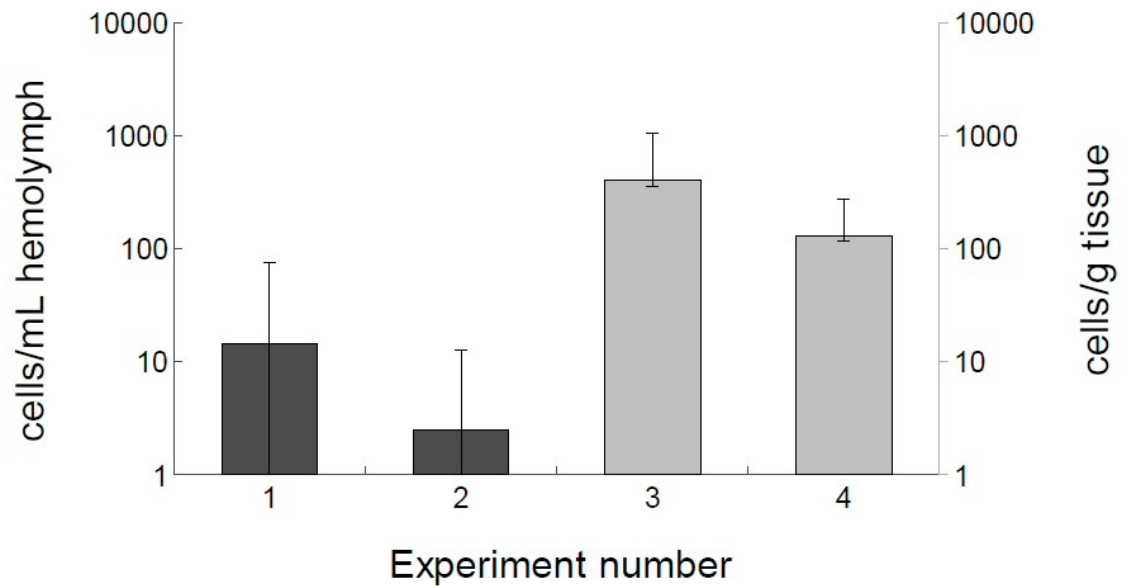
Figure 2: Initial average parasite burdens of the SPF oysters before any experimental exposure to *Perkinsus marinus*. Dark bars representing experiments 1 and 2 are the average parasite burden of 240 individuals, as determined from hemolymph sampling. Light bars representing experiments 3 and 4 are the average parasite burden of 20 sampled individuals taken for body burdens before the start of the experiments. Note that all values are below the minimum detectable infection level of 10^3 cells/g for the standard RFTM tissue squash assay not used in these experiments (Bushek et al. 1994). Error bars are ± 1 standard deviation (SD).
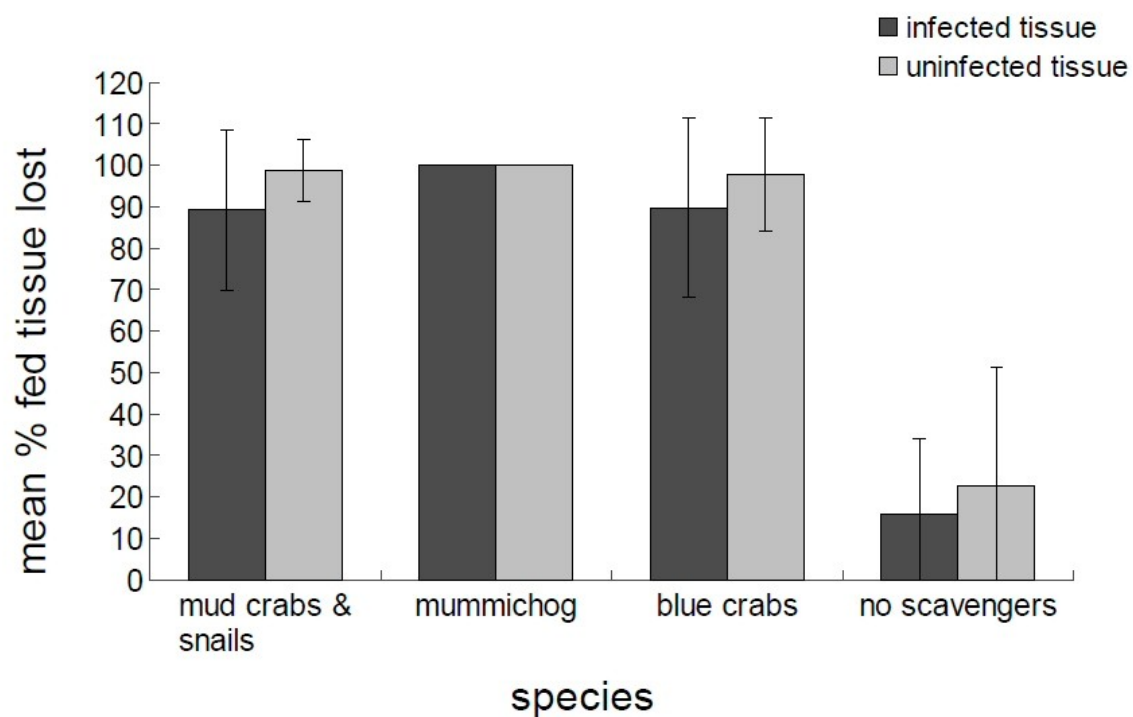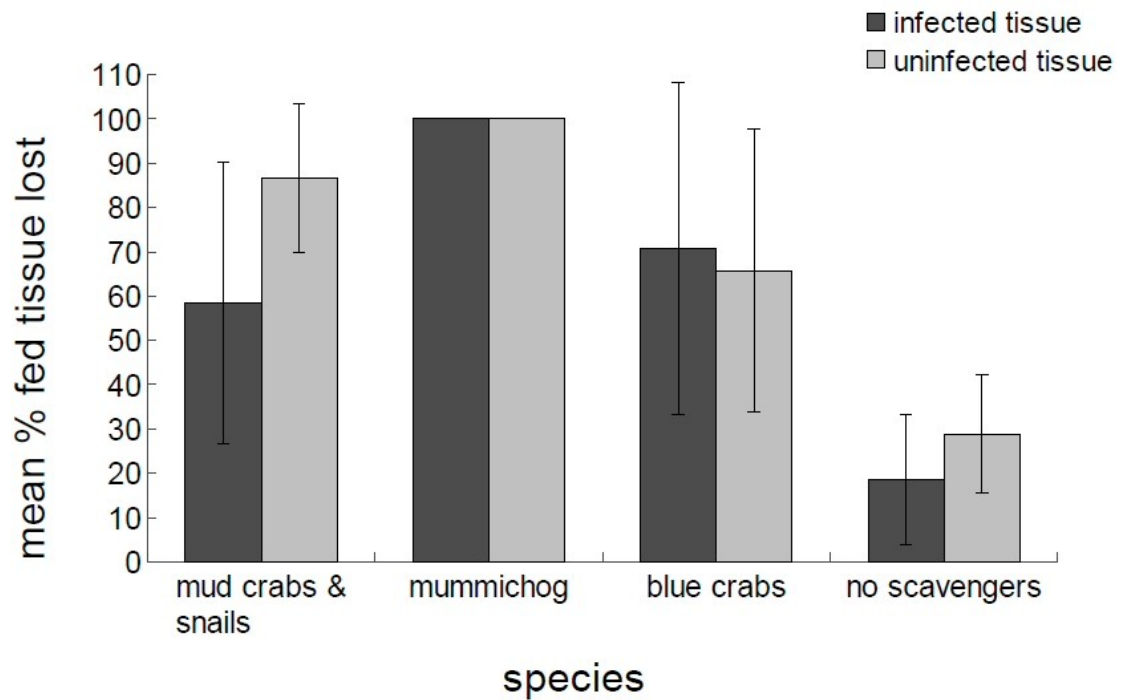
Figure 3. Mean percentage of fed tissue lost (consumed by scavengers or passively decayed) in experiment 1. Values are calculated from the initial wet weight of the oyster tissue at the start of each feeding, minus the final wet weight of the same tissue recovered from tanks after approximately 24 hours. Error bars are ± 1 standard deviation (SD), n=34.
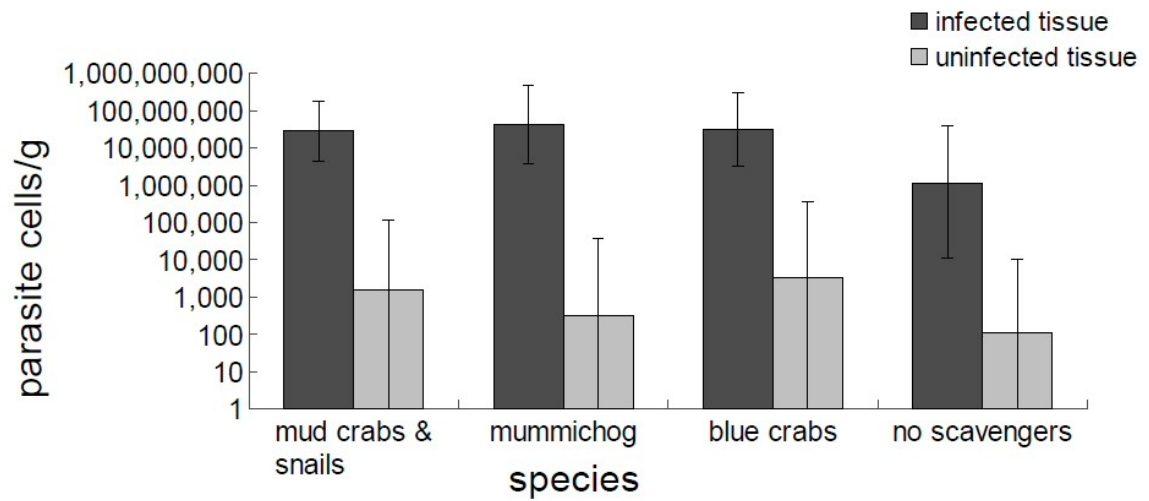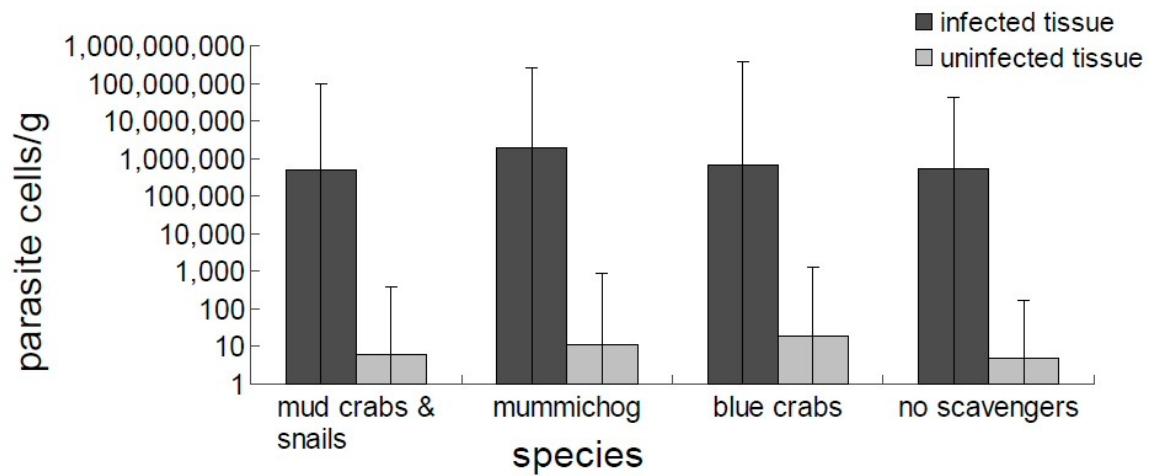
Figure 4. Parasite dosage for experiment 1 over the course of two months. The estimated dosage is a geometric mean calculated from subsample body burdens multiplied by the percentage of tissue remaining in tanks after approximately 24 hours. Error bars are ± 1 standard deviation (SD), n=34.
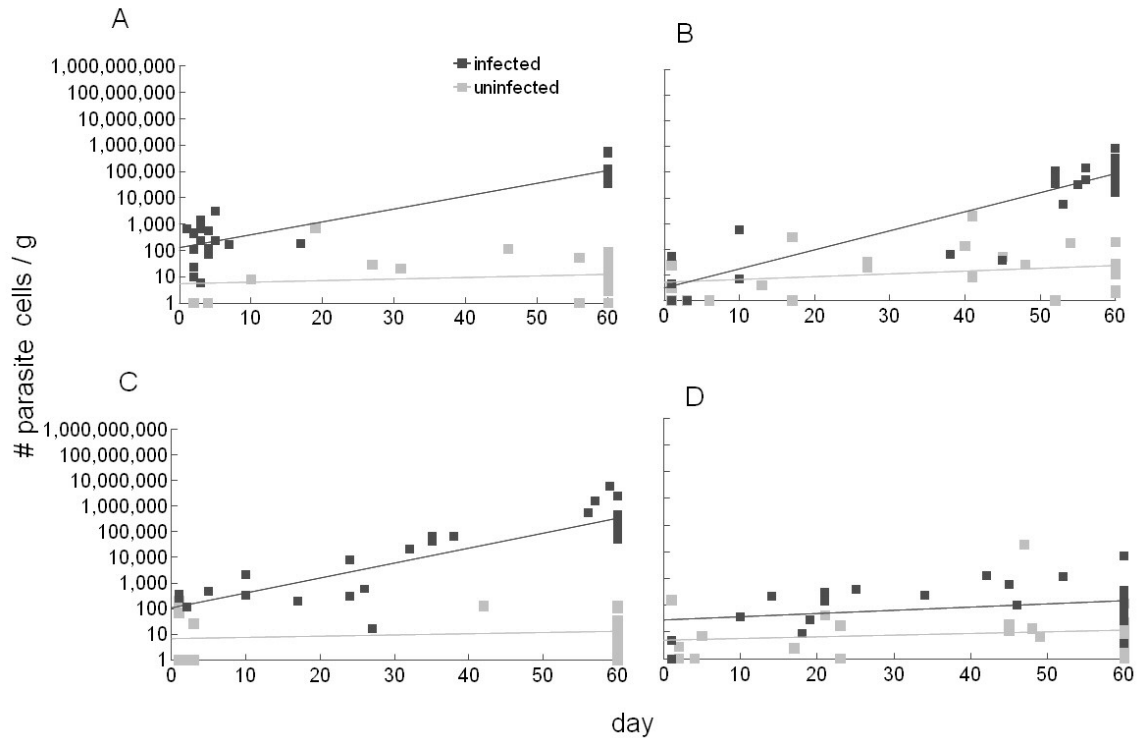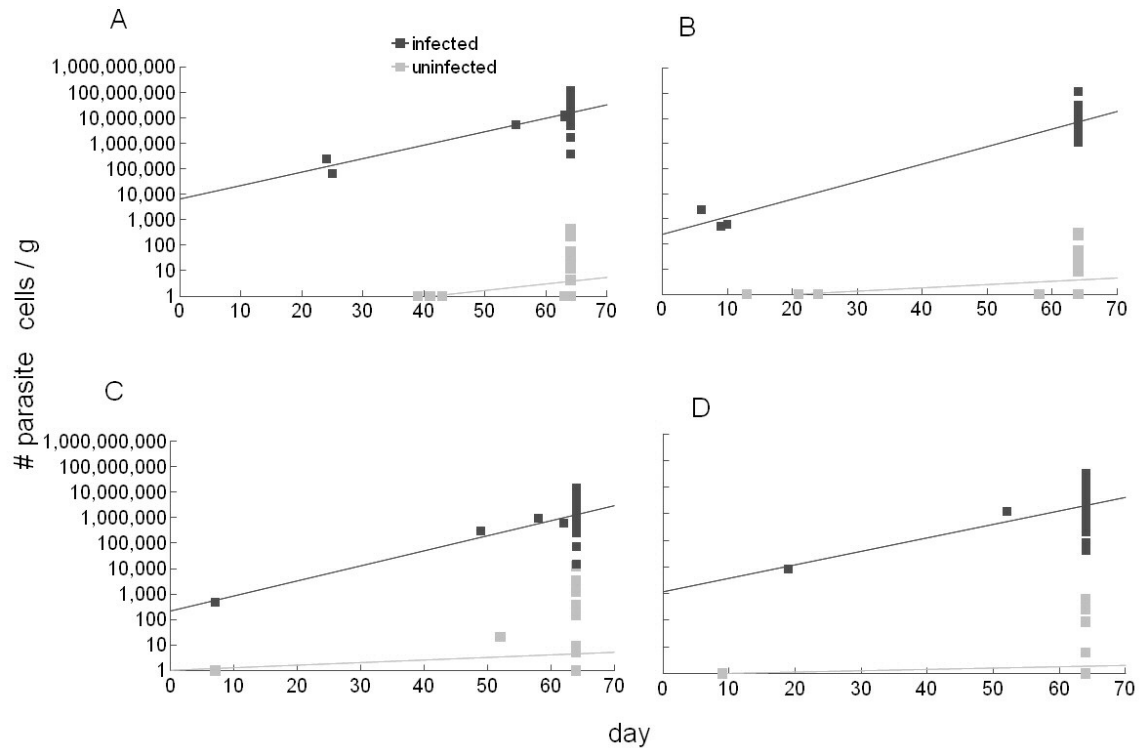
Figure 5. Body burdens of all oyster mortalities from each treatment over the course of experiment 1. A) mud snails and mud crabs present; B) mummichogs present; C) blue crabs present; D) no scavengers present. Each point represents a gaping oyster that was found during the daily mortality check and taken for body burden analysis of parasites, except points at day 60, which represent all remaining oysters sacrificed at the experiment termination. Mortalities taken during days 1-6 of the experiment are shown, but are considered stress deaths and not the result of disease mortality.
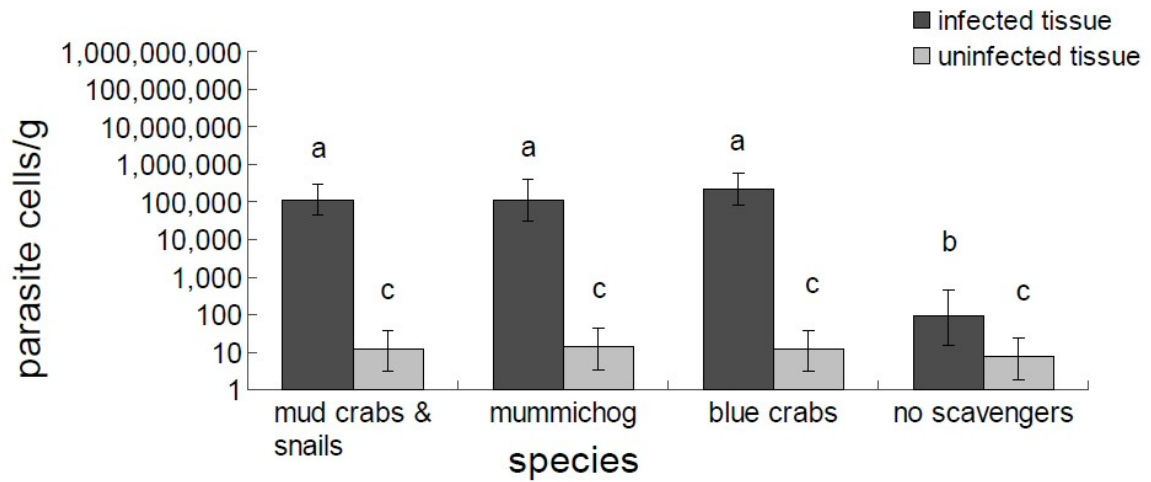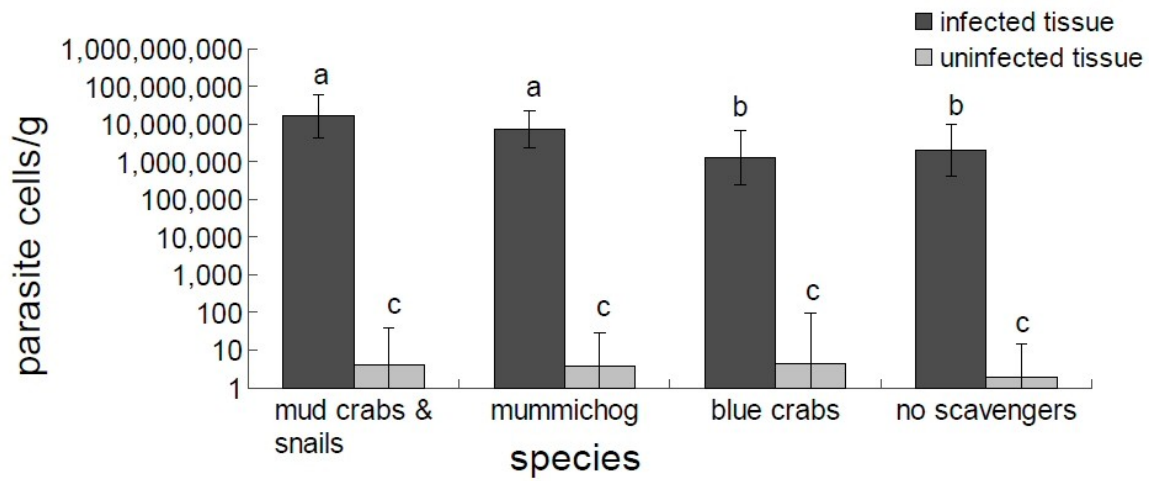
Figure 6. Termination body burdens of experiment 1. Values are geometric means calculated from body burdens of all remaining oysters at experiment termination (two months). Letters indicate results of Bonferroni post-hoc analysis. N values: mud snails and mud crabs, $n_{infected}$=10, $n_{uninfected}$=17. Mummichogs, $n_{infected}$=13, $n_{uninfected}$=11. Blue crabs, $n_{infected}$=12, $n_{uninfected}$=17. D) no scavengers present, $n_{infected}$=15, $n_{uninfected}$=14. Error bars are ± 1 standard deviation (SD).
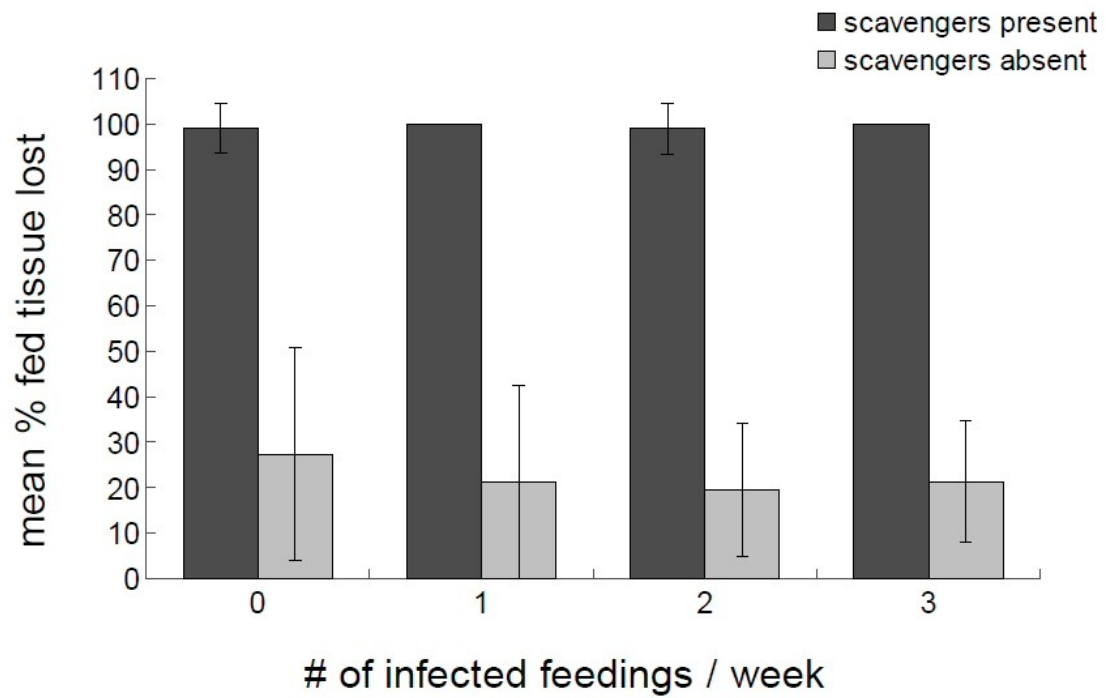
Figure 7. Mean percentage of fed tissue lost (consumed by scavengers or passively decayed) in experiment 2. Values are calculated from the initial wet weight of the oyster tissue at the start of each feeding, minus the final wet weight of the same tissue recovered from tanks after approximately 24 hours. Error bars are ± 1 standard deviation (SD), n=54.
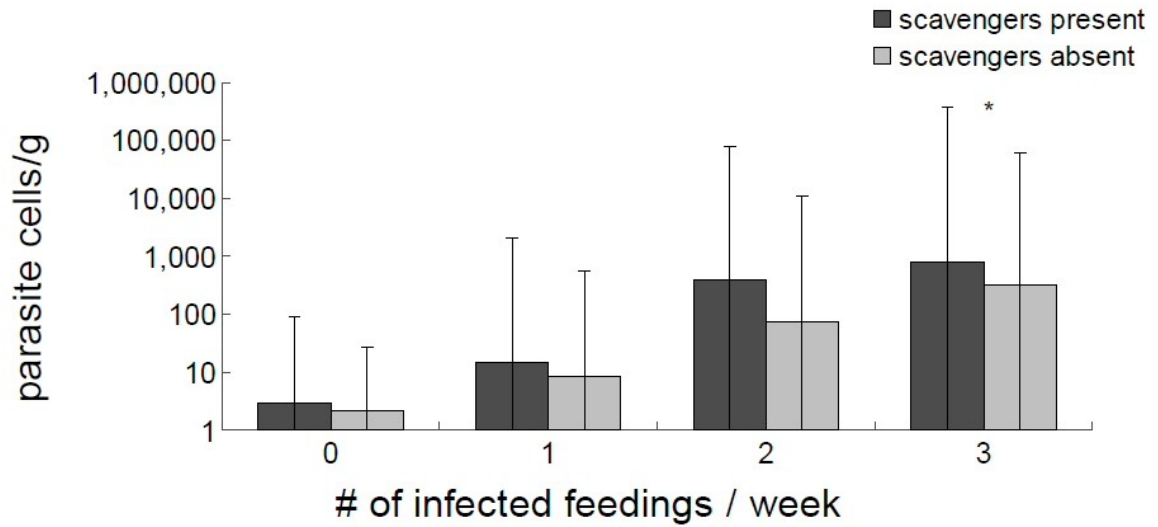
Figure 8. Parasite dosage for experiment 2 over the course of two months. The estimated dosage is a geometric mean calculated from subsample body burdens multiplied by the percentage of tissue remaining in tanks after approximately 24 hours. Error bars are ± 1 standard deviation (SD), n=54.

Figure 9. Body burdens of all oyster mortalities from each treatment over the course of experiment 2. A) mud snails and mud crabs present; B) mummichogs present; C) blue crabs present; D) no scavengers present. Each point represents a gaping oyster that was found during the daily mortality check and taken for body burden analysis of parasites, except points at day 64, which represent all remaining oysters sacrificed at the experiment termination. Mortalities that occurred during days 1-6 of the experiment are not shown, as they were considered stress deaths and were not taken for body burden analysis.

Figure 10. Termination body burdens of experiment 2. Values are geometric means calculated from body burdens of all remaining oysters at experiment termination (two months). N values: mud snails and mud crabs, $n_{infected}=25$, $n_{uninfected}=26$. Mummichogs, $n_{infected}=27$, $n_{uninfected}=26$. Blue crabs, $n_{infected}=26$, $n_{uninfected}=27$. No scavengers present, $n_{infected}=28$, $n_{uninfected}=29$. Letters indicate results of Bonferroni post-hoc analysis. Error bars are $\pm$ 1 standard deviation (SD).

Figure 11. Mean percentage of fed tissue lost (consumed by scavengers or passively decayed) in experiment 3. Values are calculated from the initial wet weight of the oyster tissue at the start of each feeding, minus the final wet weight of the same tissue recovered from tanks after approximately 24 hours. Error bars are ± 1 standard deviation (SD), n=28.

Figure 12. Parasite dosage for experiment 3 over the course of one month. The estimated dosage is a geometric mean calculated from subsample body burdens multiplied by the percentage of tissue remaining in tanks after approximately 24 hours. Error bars are ± 1 standard deviation (SD), n=28.
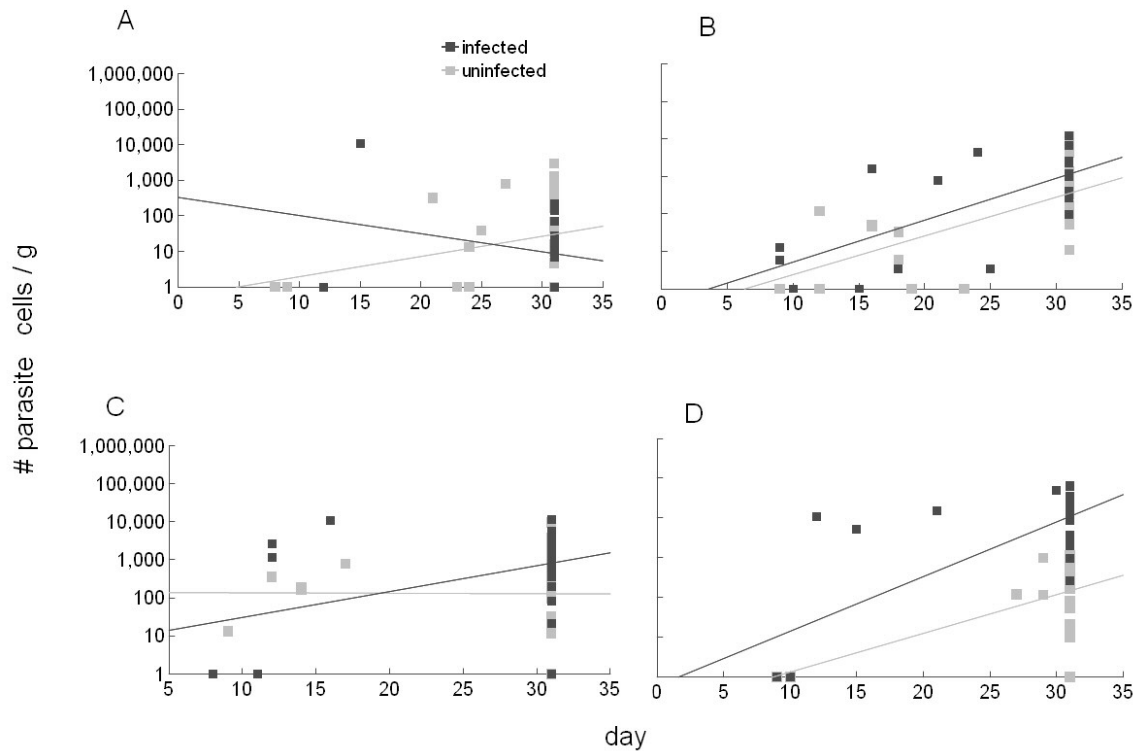
Figure 13. Body burdens of all oyster mortalities from each treatment over the course of experiment 3. A, 0 infected feedings per week; B, 1 infected feeding per week; C, 2 infected feedings per week; D, 3 infected feedings per week. Each point represents a gaping oyster that was found during the daily mortality check and taken for body burden analysis of parasites, except points at day 31, which represent all remaining oysters taken at the experiment termination. Any mortalities that occurred during days 1-6 of the experiment are not shown, as they were considered stress deaths and were simply replaced with a new SPF oyster.
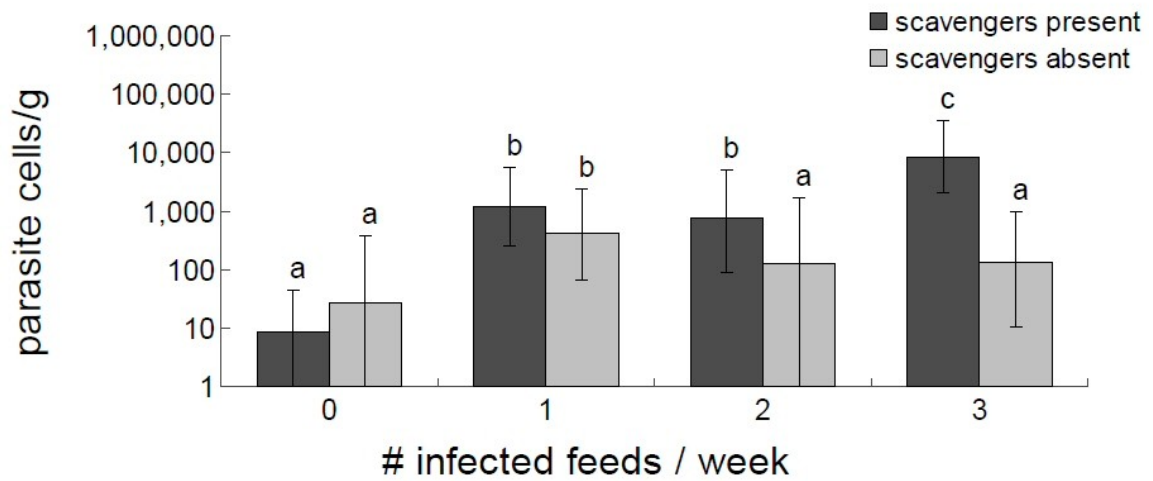
Figure 14. Termination body burdens of experiment 3. Values are geometric means calculated from body burdens of all remaining oysters at experiment termination (one month). Letters indicate results of Bonferroni post-hoc analysis. N values: 0 infected feedings per week, $n_{scavengers\ present}=27$, $n_{scavengers\ absent}=21$. 1 infected feeding per week, $n_{scavengers\ present}=9$, $n_{scavengers\ absent}=21$. 2 infected feedings per week, $n_{scavengers\ present}=23$, $n_{scavengers\ absent}=25$. 3 infected feedings per week, $n_{scavengers\ present}=20$, $n_{scavengers\ absent}=24$. Error bars are ± 1 standard deviation (SD).
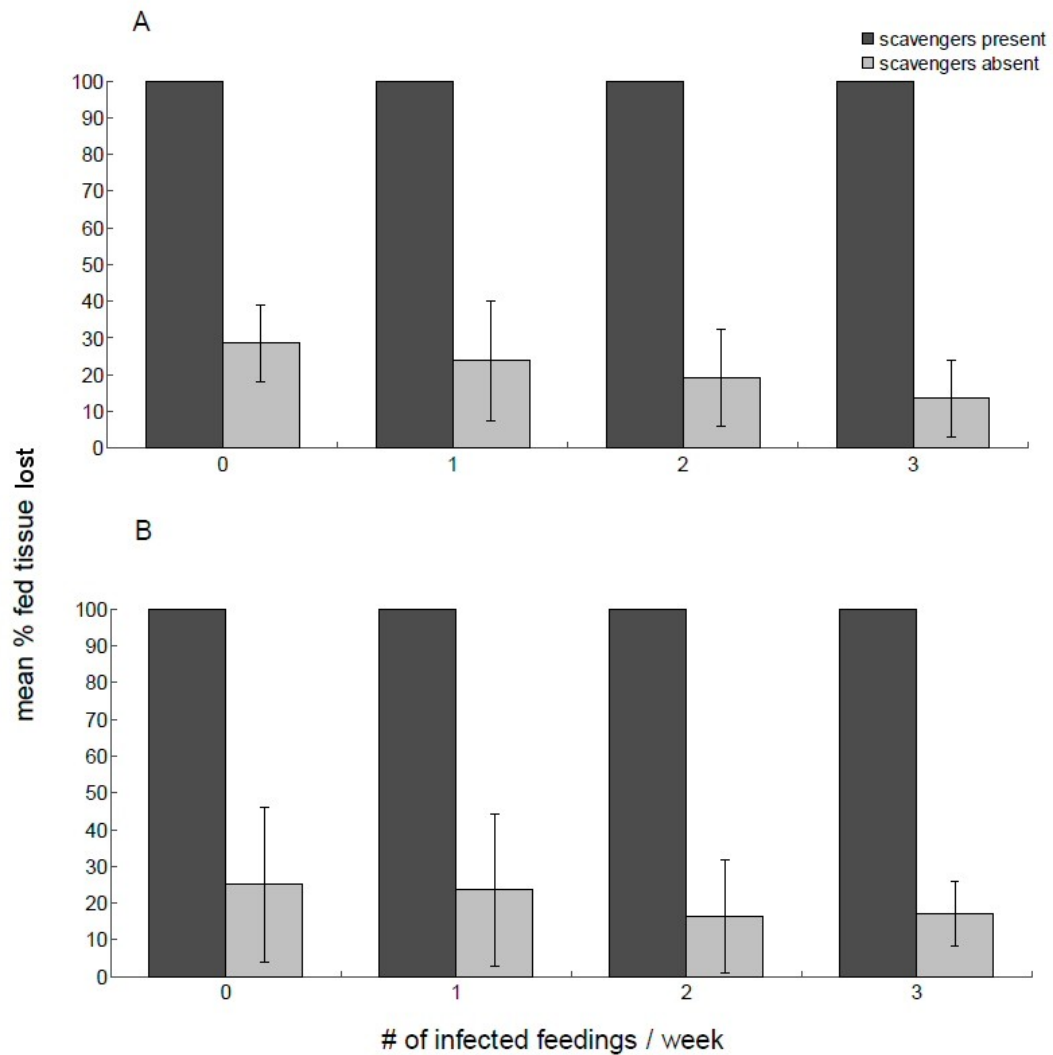
Figure 15. A) Mean percentage of fed tissue lost (consumed by scavengers or passively decayed) in experiment 4, 1st month. B) Mean percentage of fed tissue consumed by scavengers or passively decayed in experiment 4, 2nd month. All values are calculated from the initial wet weight of the oyster tissue at the start of each feeding, minus the final wet weight of the same tissue recovered from tanks after approximately 24 hours. Error bars are ± 1 standard deviation (SD), n_A=26, n_B=24
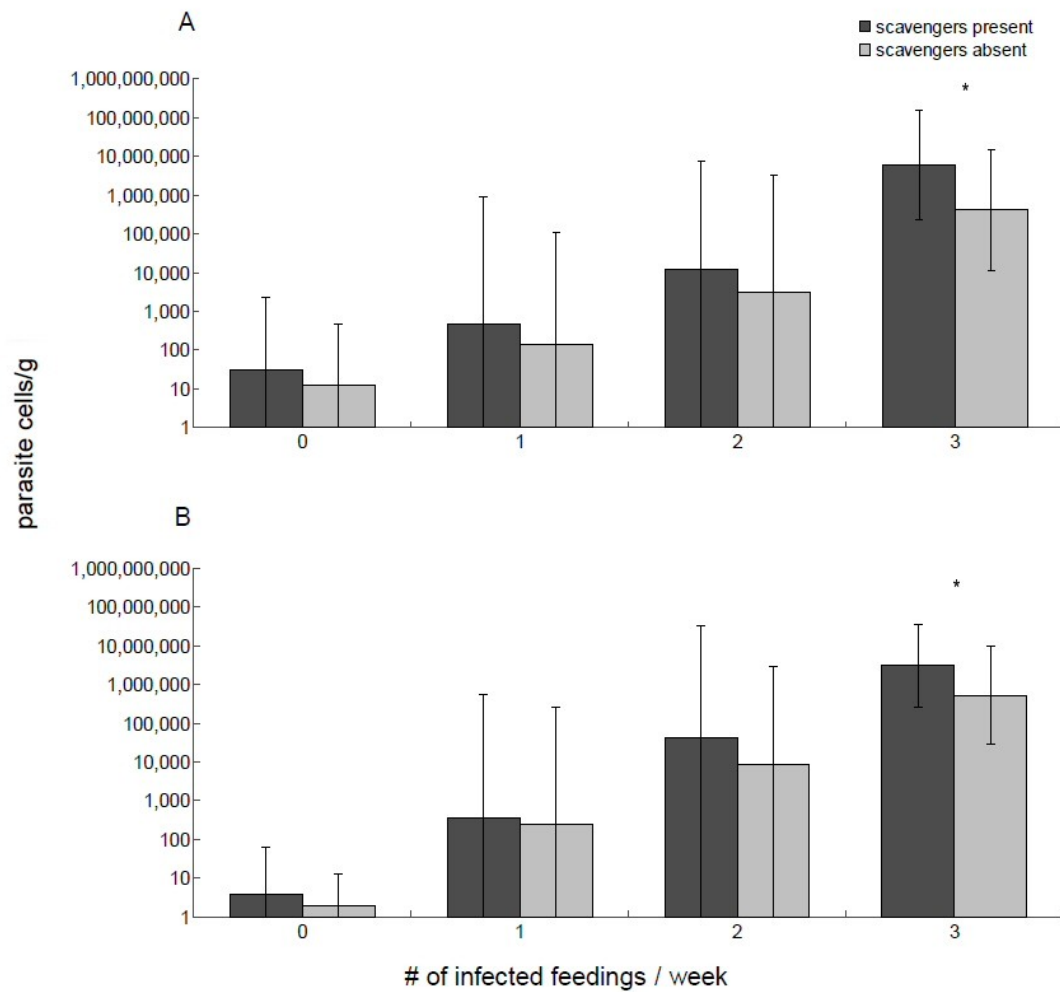
Figure 16. A) Parasite dosage for experiment 4 over the course of the first month. B) Parasite dosage for experiment 4 over the course of the second month. The estimated dosage is a geometric mean calculated from subsample body burdens multiplied by the percentage of tissue remaining in tanks after approximately 24 hours. Asterisk indicates where dosage given to tanks with scavengers vs. without scavengers was statistically different. Error bars are $\pm$ 1 standard deviation (SD), n_A=26, n_B=24
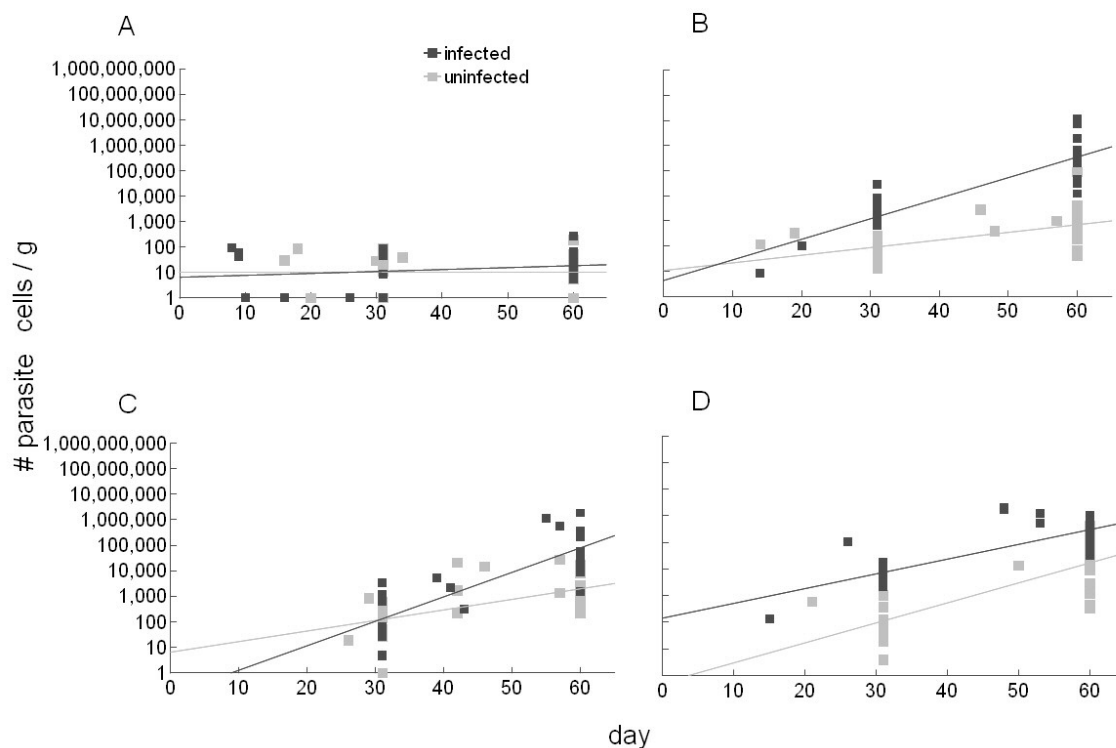
Figure 17. Body burdens of all oyster mortalities from each treatment over the course of experiment 4. A, 0 infected feedings per week; B, 1 infected feeding per week; C, 2 infected feedings per week; D, 3 infected feedings per week. Each point represents a gaping oyster that was found during the daily mortality check and taken for body burden analysis of parasites, except points at days 31 and 60. Points at day 31 represent oysters taken at the midpoint of the experiment to determine progression of the infections; points shown at day 60 represent all remaining oysters taken at the experiment termination. Any mortalities that occurred during days 1-6 of the experiment are not shown, as they were considered stress deaths and were replaced with a new SPF oyster.
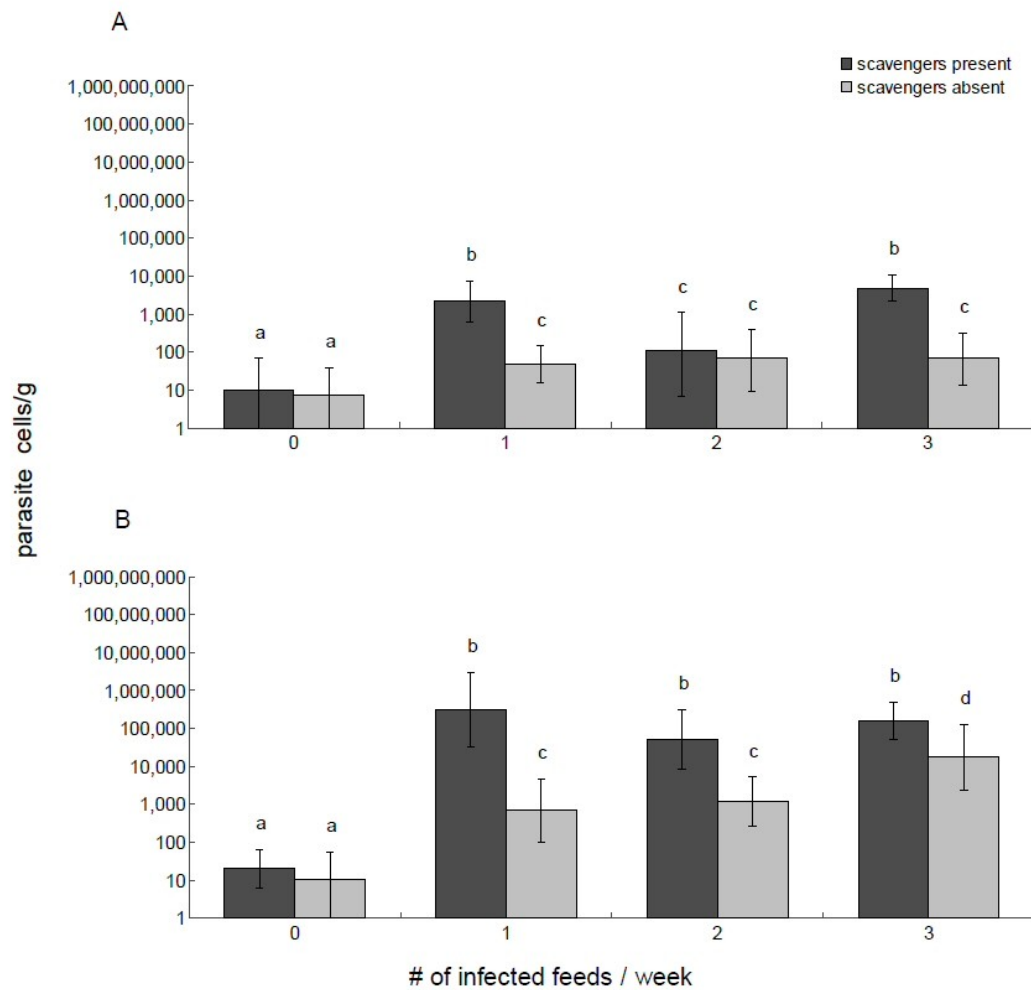
Figure 18. A) Midpoint body burdens for experiment 4, first month. N=10 for all groups. B) Termination body burdens for experiment 4, second month. N values: 0 infected feedings per week, $n_{scavengers\ present}$=14, $n_{scavengers\ absent}$=15. 1 infected feeding per week, $n_{scavengers\ present}$=11, $n_{scavengers\ absent}$=14. 2 infected feedings per week, $n_{scavengers\ present}$=13, $n_{scavengers\ absent}$=12. 3 infected feedings per week, $n_{scavengers\ present}$=14, $n_{scavengers\ absent}$=18. Values are geometric means calculated from body burdens of sacrificed oysters at the midpoint of the experiment (one month) and termination (two months). Letters indicate results of Bonferroni post-hoc analysis. Error bars are ± 1 standard deviation (SD).