The effects of the cestode worm *Diphyllobothrium dendriticum* on *Cyclops* copepods, and sockeye smolt (*Oncorynchus nerka*) predation rates on infected copepods

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ABSTRACT

Salmon are a species of ecologic and economic importance all over the world. Common prey items during their juvenile residence in lakes are zooplankton like *Cyclops* copepods and other zooplankton species. These zooplankton are common first intermediate hosts for helminth parasites, specifically cestode worms. One particularly widespread cestode that impacts many species of plankton and salmonids is *Diphyllobothrium dendriticum*. There have been studies on the altered behavior and physiology of the salmonids, but little is known about how the presence of *Diphyllobothrium* impacts the copepods. This study will determine whether infection by *Diphyllobothrium* will increase size, decrease speed, or increase rate of predation by juvenile sockeye salmon (*Oncorhynchus nerka*). Expected results will support these three hypotheses based on studies of similar subjects. Due to their reliance on eyesight for prey selection, juvenile sockeye salmon will prey selectively on larger, more nutritionally valuable copepods, so it would be beneficial to Diphyllobothrium to increase the size of its host. Decreased speed would also make infected copepods more easily caught by salmon. This study has applications in salmon fisheries, other natural populations, and in human health.

Keywords: [*Diphyllobothrium dendriticum*, parasite-host interactions, sockeye salmon]

INTRODUCTION

The ecologic and economic importance of salmon is undeniable, and anything that threatens their populations should be a major cause of concern. Economically, salmon are one of the most lucrative and valued commercial fishery in the world. Regions that heavily rely on salmon fisheries include Alaska, Norway, Japan, and countless others. Many salmon runs completely dominate local economies, provide thousands of jobs, and provide a huge protein source across the world. (Kendall and Quinn 2011; Hilborn et al. 2003). Salmon have a large impact in their environment because each life stage is an important prey source for other animals. Eggs and larvae in freshwater ecosystems provide food for other fishes, while adult salmon in the ocean support marine mammals and birds. It can be argued that salmon are a keystone species of their ecosystems (Hilborn et al. 2003).

Salmon also serve as predators later in their life cycle. Juvenile and adult salmon are highly predatory fish, and a study by Taylor and Gerking (1980) demonstrated that a large aspect of their success relies on keen eyesight. Because salmon are highly visual predators, factors like size, color, and behavior are important factors in their prey selection (Taylor and Gerking 1980; Hansen et al. 2013). Common prey item as juveniles in lakes are zooplankton (particularly copepods) and adult insects (Richardson et al. 2016).

The aquatic habitats occupied by salmonids can function as an ideal place for parasites to thrive (Barber et al. 2000; Henricksen et al. 2014). The relatively stable and buffered nature of water (especially in lakes and streams) provide a perfect environment for parasitic eggs and larvae to develop, and easy movement allows for these parasites to disburse across wide ranges (Barber et al. 2000). These planktonic prey items are commonly infected with parasites, acting as first intermediate host for many species of helminth worms.

Diphyllobothrium dendriticum is a cestode worm (Phylum Helminthes) distributed throughout the Northern hemisphere (Figure 1) with a complex, multi-host life cycle (Sharp 1990; Kuchta et al. 2013). This cestode begins its lifecycle as an egg in the feces of their definitive host—typically large piscivores including bears, wolves, and especially birds. The eggs are released through the feces of this host, and then hatch into fresh water (typically lakes) as free swimming coracidia. These coracidia then infect many species of freshwater copepods, their intermediate host. The procercoid infected copepods are then consumed by the second intermediate host, a wide range of fish species with a freshwater juvenile life stage (typically salmonid). In the fish, this life stage of the worm is called a plerocercoid, and once the fish is consumed by the predatory definitive host, the cestode's life cycle is complete (Sharp 1990).

Figure 1. The geographic range of *D. dendriticum* is shown in dark grey, with instances of human infection marked with black dots (Kuchta et al. 2013).

While the specific effects on the health of salmon are still unknown, there is evidence of a cestode parasite impacting fish behavior in a study done by Godin and Sproul (1988) on sticklebacks. Sticklebacks infected with a similar cestode worm (*S. solidus*) had a greater need for food and died more quickly of starvation, moving quickly back to areas where predators were recently, resume swimming more quickly after being startled by predators, and were in general more susceptible to predation (Godin and Sproul 1988). It is reasonable to assume there could be similar affects in salmonids, and potentially the first intermediate copepod hosts as well.

MOTIVATION FOR RESEARCH

Few studies have been conducted on whether there are any specific physical changes in copepods infected with *Diphyllobothrium dendriticum*, and there have also been no behavioral studies on copepods infected by this worm. Little is known also about the mechanisms of transmission from *Diphyllobothrium* in copepods to salmon, and so it could be helpful to determine whether the parasite changes the behavior or physiology of its host. There's also not much known about how this parasite impacts the health of salmon once successfully transmitted. A study by Rahkonen et al. (1996) found Diphyllobothrium to result in high mortality in sea and brown trout by causing their hearts to rupture and become infected. This mortality was found to be made more frequent and likely by increased temperatures, so as climate change progress, the rate of mortality in infected salmon could increase. Economically vital salmon fisheries could be negatively impacted by increased predation on infected prey items, potentially leading to a higher rate of infection. Interestingly, this worm has also been found infecting humans who consumed raw or undercooked salmon (Kuchta et al. 2013). These cases have occurred in the typical northern range of the parasite but have also spread to areas like Chile and Brazil by transport of or contact with fish from infected areas (Cabello 2007). The more we know about how this worm is transmitted and the rate of infection in salmon and other frequently consumed fishes, the better we can understand how to contain and recognize infection.

RESEARCH QUESTIONS

This study will attempt to answer the following questions:

1. Does infection by *D. dendriticum* lead to any observable differences in size between infected and uninfected copepods?
2. Are infected copepods slower than uninfected copepods?
3. Are infected copepods more susceptible to predation by sockeye smolts?

RESEARCH HYPOTHESES

1. Infected copepods will be larger in size than uninfected copepods.
2. Infected copepods will be slower than uninfected copepods.
3. Salmon will select infected copepods over uninfected copepods.

METHODS/EXPERIMENTAL DESIGN

Sample collection

To collect the *D. dendriticum* eggs, herring gull (*L. argentatus*) fecal samples will be collected from three lakes in Snohomish County, Washington; Goodwin, Shoecraft, and Silver lakes (Becker and Brunson 1967) where *Diphyllobothrium* is prevalent. Additionally at these lakes, a beach seine will be used to capture juvenile sockeye salmon (*Oncorhynchus nerka*). To test the feces for the presence of eggs, a fecal float will be conducted, using the Sheather's sugar flotation methods described in Dryden et al. 2005. This involves mixing the gull feces with a floatation solution (saturated salt solution) and letting it stand until any eggs float to the surface. To separate the eggs from any fecal matter, the samples will be pushed through a series of progressively smaller sieves (10-200 meshes/inch) until only the eggs remain (Meyer 1967).

Live copepods (*Cyclops strenuus*) will be purchased from a fish food supplier (AlgaeBarn), and to ensure the copepods don't have any previously existing infections (by *Diphyllobothrium* or another parasite), a subsample will be taken from the purchased copepods to undergo genetic testing. This genetic testing will involve a QPCR to get the DNA sequence of the copepod and quantify the presence or absence of parasites in the copepods (Melo et al. 2015).

The eggs will then be left in a tank at 18-20ºC for 14 days to mature and hatch into free swimming coracidia (Wright and Curtis 2000). The coracidia, which infect the copepods through contact and absorption, will be put in the same Petri dish as the purchased *Cyclops* copepods (n=500) for 2 hours. After this time, exposure will be ended by putting the Petri dish contents through a sieve (212 µm mesh), and the presumably infected *Cyclops* will be placed into tanks for 14 days and fed twice daily with bacteria (Sharp 1990). The remaining uninfected *Cyclops* will represent the control group of uninfected copepods.

Measuring length of infected copepods

To find length measurements for the copepods, ImagePro software will be used. This software uses a camera that is connected to a dissecting scope, and groups of copepods will be photographed and measured using the software's precise measuring tool. Size will be measured using the length of the longest part of the copepod body, not including their antennae. Averages will be taken of the copepods infected with *Diphyllobothrium* and the control (uninfected) copepods.

Measuring speed of infected copepods

To compare the speed of infected and uninfected copepods, the copepods will be placed under a dissecting scope in a petri dish with gridlines (mm^2). Individual copepods will be followed for 10 seconds, and the squares they move through in 10 seconds will be counted. This provides a mm/sec measurement, which can be used to determine the speed of infected and uninfected copepods.

Salmon copepod prey selection

To determine whether copepods infected with *Diphyllobothrium* are eaten more frequently than uninfected copepods, a juvenile sockeye salmon smolt will be placed in a large circular tank with a closed system with equal proportions of infected (n=500) and uninfected copepods (n=500). After 5 hours, remove the smolt and count the remaining number of infected and uninfected copepods to determine any preference between uninfected and infected copepods.

ANTICIPATED RESULTS

The expected results of the size experiment are a higher average size (mm) in *Cyclops* infected with *Diphyllobothrium* than uninfected *Cyclops* (Figure 2). This increased size is expected because a study by Taylor and Gerking (1980) found a salmonid (*Salmo gairdneri*) to feed preferentially on *Daphnia pulex* that were larger in size. Because salmonids are such highly visual predators that are size-biased feeders, it makes sense that *Diphyllobothrium* procercoids would increase the size of their *Cyclops* host to increase their chances of being consumed by their second intermediate hosts.

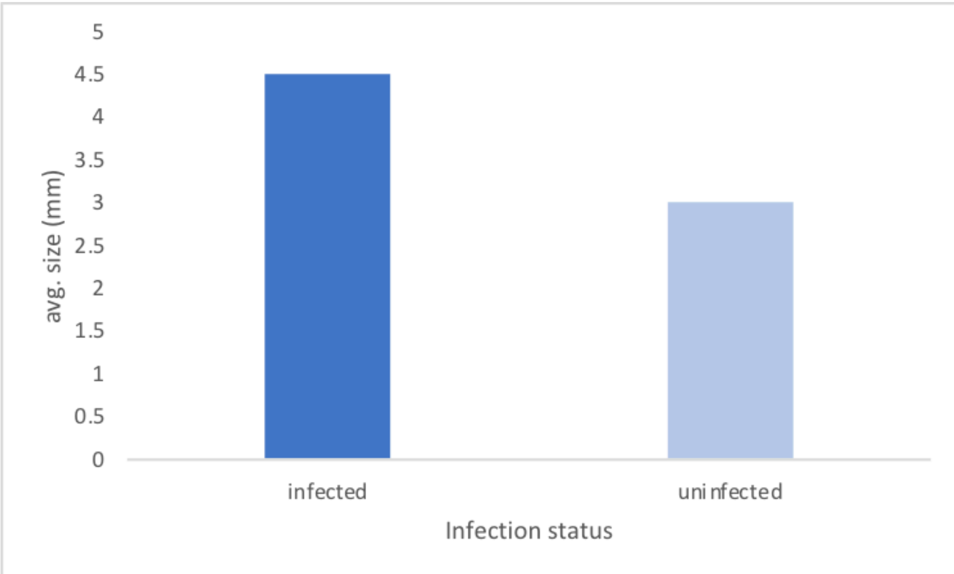

Figure 2. The anticipated difference in average size between *Cyclops* infected with *Diphyllobothrium* and uninfected *Cyclops* (sizes of copepods were estimated from study by Coker 1933).

It is expected that the average speed of infected *Cyclops* would be slower on average than uninfected *Cyclops* (Table 1). This result is expected because a study by Bethel (1972) found several species of cestode larvae to significantly alter the behavior of their amphipod hosts to move with decreased speed away from their predators or other disturbances.

Table 1. The anticipated results for the experiment comparing the speeds of infected and uninfected *Cyclops* (Data approximated from study by Kerfoot 1978).

Infection status	**Average speed (mm/sec^{-1})**
uninfected	1.8
infected	1.3

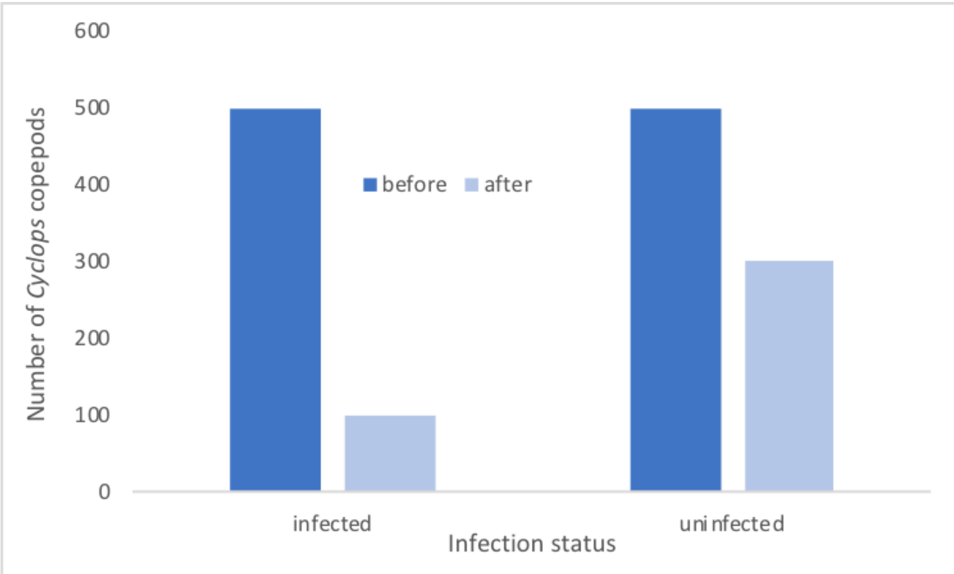

Figure 3. The anticipated difference in predation on *Cyclops* infected with *Diphyllobothrium* and uninfected *Cyclops* by juvenile sockeye salmon.

Infected *Cyclops* were expected to be eaten more frequently than uninfected *Cyclops*, because the expected physiological and behavioral changed in infected copepods should lead to increased predation (Figure 3). There are few studies exploring selective predation on copepods infected with cestode procercoids, but if the other results of the study hold true, it would make sense for juvenile sockeye (*Oncorynchus nerka*) would feed preferentially on slow and large copepods.

DISCUSSION

The anticipated and potentially negative differences in speed size, and rate of predation all benefit *Diphyllobothrium*. By altering the behavior of the copepod, this worm increases its rate of transmission to its second intermediate host, juvenile sockeye salmon. Other studies have found *Diphyllobothrium* to impact the behavior of its host copepod by lowering respiration rate and impairing escape responses (Pasternak et al. 1995). The findings of Pasternak et al. (1995)

reinforce the assumed results of this study and demonstrate the ability of *Diphyllobothrium* to modify at least some behavioral aspects of copepods.

One interesting thing to consider is whether the net cost to salmon of increased parasite transmission is negative. While infection by *Dipyllobothrium* can be negative for salmon smolts, making infected copepods easier to catch indirectly increases the amount of food available to them. *Diphyllobothrium* and similar parasitic worms have also been found directly influencing the behavior of the salmonid second intermediate host (Ferguson et al. 2012). *Eubothrium salvelini*, another cestode, was found to impair swimming abilities, growth, rate of survival, ability to adapt to saltwater, and impairing orientation of smolts headed to sea (Ferguson et al. 2012; Boyce 1979; Garnick and Margolis 1990). Whether these negative modifications outweigh the positive potential benefit of increased food supply could be an interesting topic for further study.

An important application of this study is *Diphyllobothrium* as an emerging human health concern. There are 14 documented species of *Diphyllobothrium* found to cause diphyllobothriosis, with *D. latum* and *D. dendriticum* being the main infectious agents. Infection can occur by consuming improperly cooked or raw salmon, and while the infection isn't life-threatening, symptoms can be uncomfortable if allowed to persist (Kuchta et al. 2013). While infections are more common in temperate and artic regions in the Northern hemisphere, there are multiple cases in SE Asia and South America (Kuchta et al. 2013; Wiwanitkit 2016). The spread of infected salmonids from its geographical range is easy due to transport of frozen salmon across the world, and human travel (Kuchta et al. 2013).

This study is novel, interesting, and important because of its relevance to human health and the fishing industry. Learning more about how *Diphyllobothrium* is transmitted between its hosts can help contain and prevent further spread of the disease to new regions. Understanding the mechanisms of transport and more about the physiological changes caused by cestodes can improve our understanding of potential damages to salmon fisheries, fish farms, and many other salmonid populations.

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