The prevalence and effects of *Ergasilus* (gill lice) on fish species in two Northern Wisconsin lakes

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Mary Chivetta

Advisor: Todd Crowl, Ph.D.

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Abstract

Gill lice, *Ergasilus*, are common freshwater parasites that attach to the gill filaments of their host fish. They feed on blood and tissue of the gills causing extensive damage by reducing blood flow and surface area for oxygen exchange. The prevalence of gill lice in Tenderfoot Lake and Bay Lake in Northern Wisconsin, species, and sizes of fish were studied during a survey that spanned from early June to late July. The effect of gill lice on fish respiration was then tested by measuring the dissolved oxygen (DO) levels over the course of 30 minutes in closed containers containing fish with and without gill lice. Results of a Pearson chi-square test showed that there was a significant difference in species of fish infected with gill lice. *Ergasilus* were found in small mouth bass, rock bass, and bluegill. However, there was no significant difference found after running an ANOVA for different sizes of fish and presence of gill lice in bluegill. Contrary to previous studies, an ANCOVA showed that fish respiration in bluegill was not significantly affected by the presence of gill lice. This may be due to the small number of gill lice present in each fish, which may not cause extensive damage at low infection levels. Understanding the intensity of gill lice infection that causes a significant effect could prove to be important in treating freshwater systems and preserving the health of fish populations.

Introduction

Freshwater aquatic ecosystems are extremely susceptible to diseases, oxygen deprivation, and agricultural runoff (Scheffer 2001). Given their importance in providing food, freshwater, and a harbor for biodiversity, preservation of watersheds is particularly important (Rosi-Marshall and Wallace 2002). Parasites can act as sensitive, early indicators of the declining health of ponds, lakes, streams, or rivers (Bhuthimethee *et al.* 2005). In particular, ectoparasites in fish reflect changes in the environment, as poor water quality either increases food supply for parasites or decreases the immune system of the fish and their ability to fight parasites (Bhuthimethee *et al.* 2005).

Gill lice, a parasite of the genus *Ergasilus*, is a host-specific ectoparasite that infects many species of freshwater fish including yellow perch, walleye, brook trout, salmon, and large and small mouth bass (Roberts 1970). Eggs of the gill lice are in external egg sacs attached to the parasitic female. Once developed they hatch and the nauplii, (the first larval stage of many copepods) become free-living. Gill lice of the genus *Ergasilus* go through several copepodid stages (second larval stage) following various nauplii stages. After reaching maturity, adults mate and, while males remain free-living, females become parasitic (Hudson and Lesko 2003).

As their name suggests, gill lice attach themselves to the gills of fish and feed on their host's blood and tissue (Ojha and Hughes 2001). This attachment causes extensive tissue damage and inflammation, and may render fish susceptible to secondary infection by bacteria, fungi, and viruses (Dezfuli *et al.* 2003). Additionally, the damage to gill tissue can reduce the ability of the fish to maintain normal oxygen uptake by hindering water flow (Ojha and Hughes 2001).

The gills of fish are tissues specialized for gas exchange, circulation, ion and acid-base balance, hormone production, and nitrogenous waste secretion (Pelster and Bagatto 2010). Oxygen uptake is driven by diffusion of dissolved oxygen in surrounding water through small plates, called lamellae, with dense capillary networks, and into the blood (Pelster and Bagatto 2010). The counter-current exchange system in fish increases oxygen uptake up to five times than in co-current systems (Layton 1987). However, ectoparasites of the gill, like *Ergasilus*, may decrease oxygen exchange. For instance, earlier studies have found that fish with high numbers of gill parasites have reduced stamina, spend more time at the water surface, and may increase branching in the gills in order to increase surface area for gas exchange (Ojha and Hughes 2001).

In order to compensate for their poor oxygen exchange rates, fish infected with parasites may be pushed into areas with higher dissolved oxygen concentrations (Szalai and Terry 1991).

Similar studies have looked at gill parasites of fish populations in the Great Lakes, marine fish populations, and warm-water fish in Texas lakes (Roberts 1970). The University of Notre Dame Environmental Research Center provides a location with lakes that are both public and private and lakes that are connected to other sources of water, like the Great Lakes, through rivers. The presence of gill lice in different lake environments may have implications about the spread of this ectoparasite. Two questions about gill lice are being asked in this study. First, what is the prevalence of gill lice in fish species on different lakes? Second, is the rate of respiration and metabolism of fish infected with gill lice different than that of fish without gill lice? I hypothesized that lakes with more public access would have higher prevalence of gill lice than lakes with less public access. Within the lakes, I predicted that larger fish would have higher rates of gill lice infection than smaller fish, assuming that larger fish are older and would have circulated more water through the gills, and therefore had a greater chance of encountering *Ergasilus.* As *Ergasilus* damages gill tissue, the efficiency at which fish exchange oxygen is reduced (Ojha and Hughes 2001). When measuring DO levels in closed containers containing fish, I predicted that fish with gill lice would have impaired respiration and metabolic functioning, decreasing the rate at which they are able to use dissolved oxygen (DO) and causing the amount of DO to decrease more slowly.

Materials and Methods:

Study Site:

Surveying was done on two lakes at UNDERC in the Upper Peninsula of Michigan spanning from June to late July. Tenderfoot Lake and Bay Lake were sampled for a variety of fish species. Tenderfoot Lake (179 ha, 19.14 m maximum depth) is a large oligotrophic lake that is publically accessible and connects with Lake Superior via Tenderfoot Creek and the Ontonagon River. Bay Lake (68.9 ha, 13.7 m maximum depth) is an isolated, oligotrophic lake on the UNDERC property. It is mostly private, except for one branch, which has limited public access.

Prevalence:

A survey of the fish in the two lakes was taken over the course of 7 weeks from early June to late July, using line sampling, seine nets, minnow traps, and fyke nets. Information collected for each fish included: lake, species, length (cm), and presence or absence of gill lice, visible with the naked eye. Lake and fish length comparisons for species and number of fish with and without gill lice were made as well as the percentage of each fish species infected with gill lice. The initial findings led to choosing a species of fish for studying the impact of gill lice on respiration based on the ability to capture fish with and without gill lice.

Impact on Respiration:

To test whether or not gill lice affected a fish's ability to exchange oxygen, bluegill with and without gill lice were collected during sampling of the lakes. Fish were temporarily kept in cattle tanks containing water from Tenderfoot Lake. For each test, a fish (or empty control) was placed in a 1.89 liter sealed container filled with lake water and monitored using a YSI 55 Dissolved Oxygen (DO) meter to record the temperature and DO content every 5 minutes for thirty minutes, or until the system was approaching lethal limits of DO (1.5 mg/L). The length (cm) and mass (g) of the fish was recorded for each trial. Containers were placed on magnetic stir plates to ensure even mixing of oxygen throughout the water to obtain the most accurate reading of dissolved oxygen and temperature. Fish behavior was also noted to make sure fish were released before reaching mortality. Five replicates of each treatment (bluegill with gill lice from Tenderfoot Lake, bluegill without gill lice from Tenderfoot Lake, bluegill without gill lice from Bay lake, and empty container of lake water) were performed, with each fish only being used for one trial.

Statistical Analysis:

Data were analyzed using SYSTAT 13 software (Systat Software, Chicago, IL 2009). To look at the prevalence of gill lice in different fish species, a Pearson chi-square test was performed using numbers of fish captured and the number of fish infected with gill lice for each species. To see if there was a relationship between size of the fish (cm) and presence of gill lice, a one-way analysis of variance (ANOVA) was performed.

In order to account for any microbial respiration differences and provide an overall change in DO due to fish respiration, the DO difference for control containers for the first 20 minutes of the trial was subtracted from that of each experimental fish for the same time period. As body size was not held constant for fish and varied in each trial, the metabolic and respiration rate had to be scaled accordingly. A regression was run using a log-log transformation of fish mass, M, (g) and the difference in dissolved oxygen due to fish respiration at 20 minutes (SEDO). Body size (M) was then standardized by raising mass to the coefficient of the slope of the linear line (^{n}). To standardize the difference in dissolved oxygen (SDO20), the change of DO due to fish respiration was divided by the scaled body size (M^n).

A Shapiro-Wilk test was used to test for normality of SDO20 and average temperature over 20 minutes. An analysis of covariance (ANCOVA) was then run to look at the relationship between type of fish, average temperature, and SDO20. Fish type included fish with gill lice from Tenderfoot, fish without gill lice from Tenderfoot, and fish without gill lice from Bay. The average temperature was taken over the course of 20 minutes.

Results

Analysis of Prevalence of Gill Lice in Different Lakes and Fish Species

Several different species of fish were surveyed in Bay and Tenderfoot lakes, including large mouth bass, small mouth bass, rock bass, bluegill, black crappie, white suckers, northern pike, muskellunge, walleye, and yellow perch. In Bay Lake, no gill lice were present in any of the fish species. A comparison of fish species with gill lice in Tenderfoot Lake versus Bay Lake showed that gill lice were present in Tenderfoot, but absent in the same species in Bay (Fig 1). A Pearson chi-squared test was performed with number of fish captured for each species and how many of those fish were infected with gill lice. The p-value of 9.99200722E-016 was significant (Pearson chi-square value= 77.61773488, df=4).

Prevalence of Gill Lice in Different Sizes of Bluegill

Bluegill were chosen to look at the relationship between gill lice infection and size of fish due large sample size and variety of fish size. Fish were separated into different size categories (small= 5-10 cm, medium=10.5-16 cm, large= 16.5-22 cm). The ANOVA analysis showed that there was no significance between size classes and gill lice infection with a p-value of 0.23814805 (df=2, 96, F-Ratio=1.45652422,) (Fig 2).

Effect of Gill Lice on Respiration

Over time, the amount of DO in the closed containers decreased as the fish respired (Fig 3). The amount of respiration occurring was standardized for the size of the fish by finding the coefficient of the slope of the line of a log-log regression of fish size and dissolved oxygen difference at that time. The coefficient of the slope at time 20 was 0.63817237 (SE=0.09225187,

R^2= 0.76994425, p=0.00001057). Standardized dissolved oxygen was then found by taking the difference in DO at time 0 and time 20 and dividing it by size (g) raised to the 0.64 power (SDO20).

The Shapiro-Wilk normality test verified that the average temperature at 20 minutes and the SDO20 were normal (p_{SDO20}=0.14654043, $p_{avgtemp}$=0.46882256). An ANCOVA looking at the type of fish (with and without gill lice), average temperature at 20 minutes, and SDO20 resulted in an insignificant p-value for the interaction term between temperature and type of fish (p=0.14928647, R^2=0.38926817). Therefore an ANCOVA was run without the interaction term (R^2=0.06803078). This resulted in insignificant p-values for both average temperature at 20 minutes (p=0.62010047, df=1, 11, F-ratio=0.26014227) and type of fish (p=0.90828079, df=2, 11, F-ratio=0.09704797) (Fig 4).

Discussion

Gill lice (*Ergasilus*) were found to differ in prevalence in both lakes and in species of fish. This supports the notion that gill lice are both lake specific and species specific (Roberts 1970, Muzzall and Whelan 2011). Gill lice were found on rock bass, bluegill, and small mouth bass in Tenderfoot Lake. Given the species of fish infected, it is possible that the specific species of gill lice is *Ergasilus centrarchidarum (*Cloutman and Becker 1977*). E. centrarchidarum* is found in fish species other than bluegill, rock bass, and small mouth bass, and it is possible that in a larger sample, other centrarchids, such as large mouth bass, would also have gill lice.

The presence of gill lice in Tenderfoot Lake and not Bay Lake may be caused by the fact that Tenderfoot is publically accessible and has waterways connecting it to Lake Superior. Conversely, Bay is predominantly accessible only from the private UNDERC property and is an isolated lake with no incoming streams or rivers. Many of the Great Lakes, including Lake

Superior, already have confirmed accounts of *Ergasilus* spp. found on different species of fish (Muzzall and Whelan 2011). Since rivers connect the two lakes (Tenderfoot and Lake Superior) and people are allowed to move their boats between lakes, it is possible that *Ergasilus* either migrated through the waterways or were introduced to Tenderfoot from people's boats coming from the Great Lakes (Colorado DNR) . Future studies might include looking at different lakes with varying public access and connecting water systems, and looking for different species of *Ergaslius* in UNDERC lakes.

The results of the ANOVA analysis looking at size class and presence of gill lice were statistically insignificant. Bluegill in small, medium, and large classes all had some presence of gill lice, but there was no clear difference size class and gill lice prevalence. Although I predicted that larger and older fish were more likely to have gill lice, it may be there is a tradeoff between older fish immunity, though it has been circulating water through its gills longer, and younger fish being more susceptible to infection (Dezfuli 2011). This means that all size classes of fish may have an equal chance of encountering the free-swimming adult *Ergasilus* before they parasitize the gills. However, it would be interesting to see if this relationship holds true for all the species of fish infected with gill lice.

Contrary to my expectations, the presence of gill lice did not significantly affect fish respiration. Other studies looking at the gills of fish infected with gill lice have found significant changes in gill tissue and respiration rates (Ojha and Hughes 2001, Dezfuli *et al* 2003, Szalai and Terry 1991). In this study, respiration remained unaffected. Fish respiration was the main cause for decrease in DO as temperature was not a significant factor. Bluegill were chosen as the experimental species of fish due to the abundance of fish with and without gill lice. However, the number of gill lice present in bluegill was qualitatively lower than other infected species (Fig 5).

Ideally, rock bass would have been used as the experimental species when studying respiration because of the large number of gill lice infecting the gill tissue, but only one rock bass specimen was found without gill lice over the course of the study. It may be the case that small numbers of gill lice are not noticeably harmful to fishes' ability to respire, but large numbers of *Ergasilus* are detrimental to their health (Piasecki 2004).

Copepods are extremely important in aquatic ecosystems; they can be a food source for small fish, intermediate hosts of fish parasites or fish parasites themselves, and serve as vectors of disease (Piasecki 2004). Ergasilus are found in many freshwater sources and adversely affect aquaculture, a growing business that helps reduce the overexploitation of wild fish populations. (Piasecki 2004). Gill lice infections in high number can cause reduce the fitness of the fish and even cause it to die of asphyxia (Dezfuli 2011). Current treatments for *Ergasilus* include "organophosphate compounds, pesticides, or mixtures of copper sulfate and ferric sulfate" however; these treatment methods are not very effective (Schaperclaus 1992). This study has shown that small numbers of gill lice do not cause an adverse affect in fish respiration. By understanding the limits and numbers of *Ergasilus* in a fish that cause harm to the host, we may find that reducing *Ergasilus* and their ability to attach to the gills may be more efficient in preserving the health of fish populations.

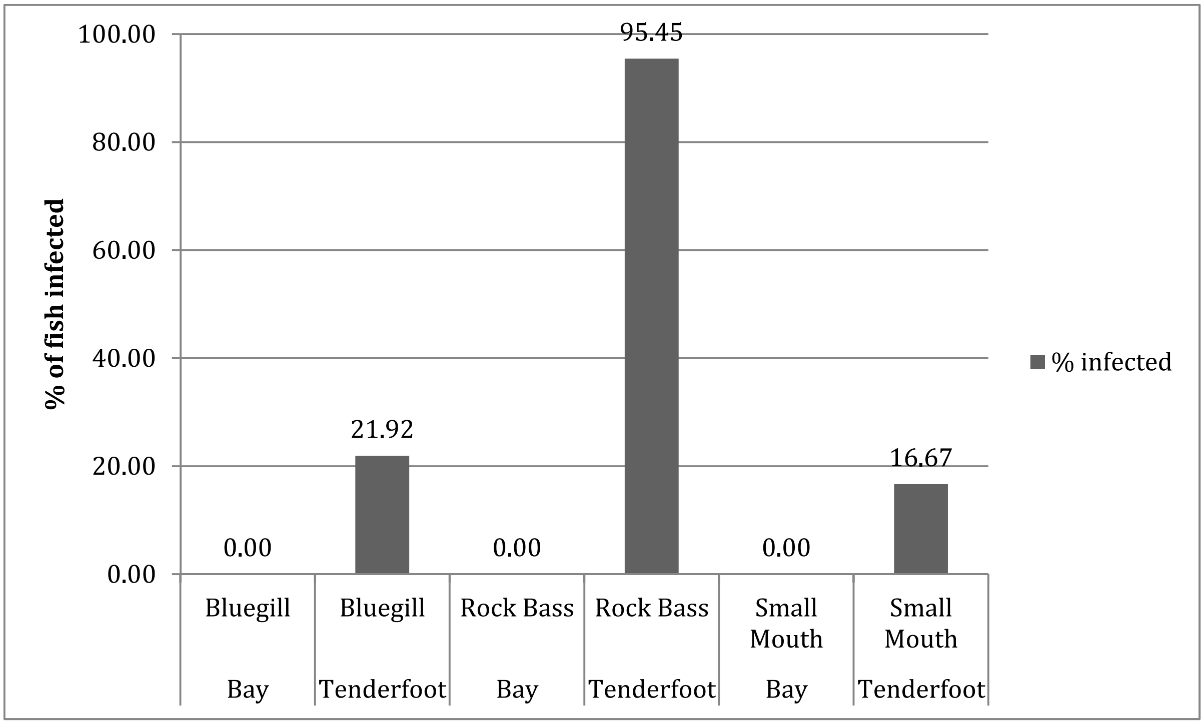

Figure 1: Percentage of Fish with Gill Lice (Ergasilus) in Bay and Tenderfoot Lakes. Species of fish with gill lice found in Tenderfoot Lake were not found in the same species in Bay Lake.

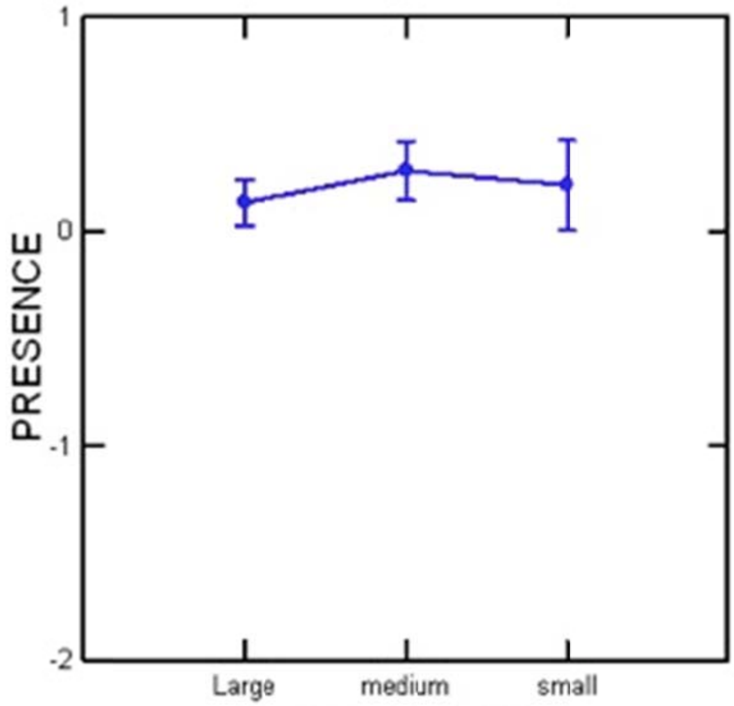

Figure 2: Presence of gill lice in different size classes of bluegill analyzed using ANOVA. Size was classified by small (5-10 cm), medium (10.5-16 cm), and large (16.5-22). There was no significance in size class of fish and infection with gill lice (p=0.23814805 df=2, 96, F-Ratio=1.45652422)

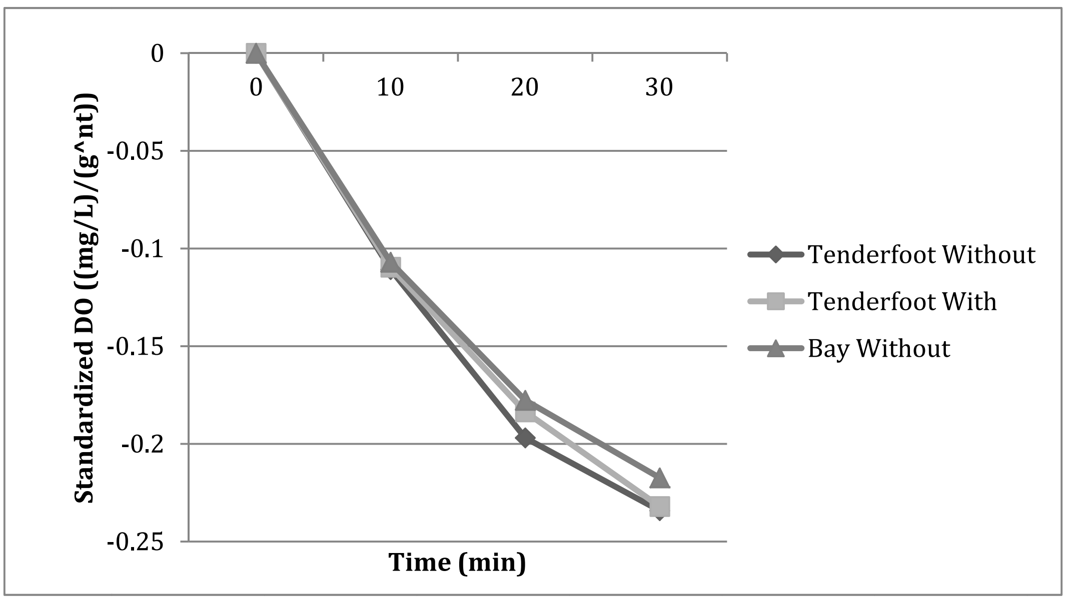

Figure 3: Decrease in Standardized DO over time. Standardized DO was calculated using DO difference caused by fish respiration in mg/L divided by the mass of the fish in grams raised to the coefficient to scale for size at that time (nt).

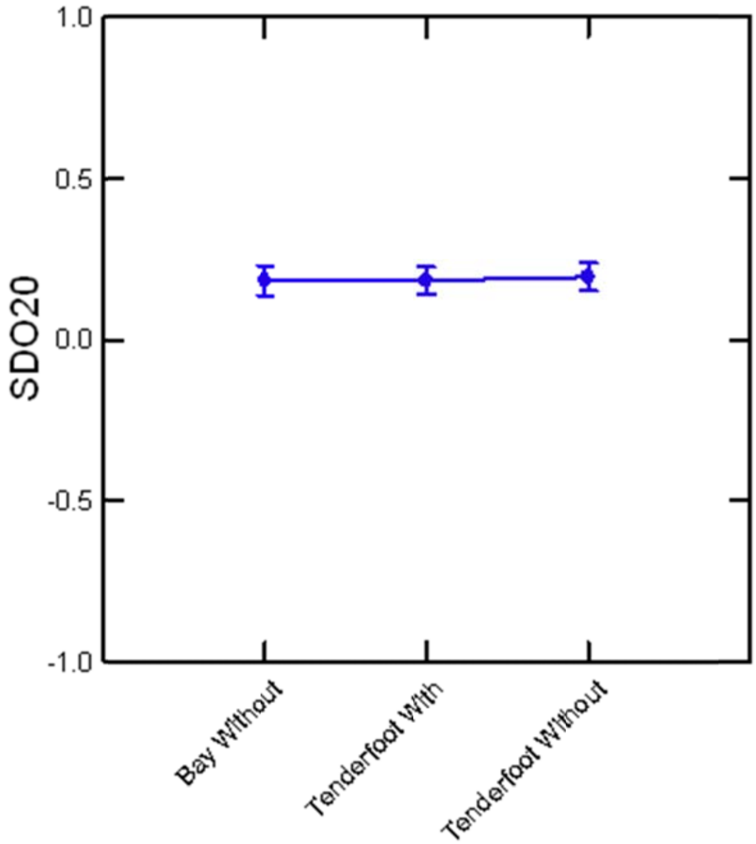

Figure 4: ANCOVA looking at Standardize Decrease of DO at 20 minutes for different fish types. There was no significance in decrease of SDO at time 20 for fish with and without gill lice (p=0.90828079, df=2, 11, F-ratio=0.09704797)

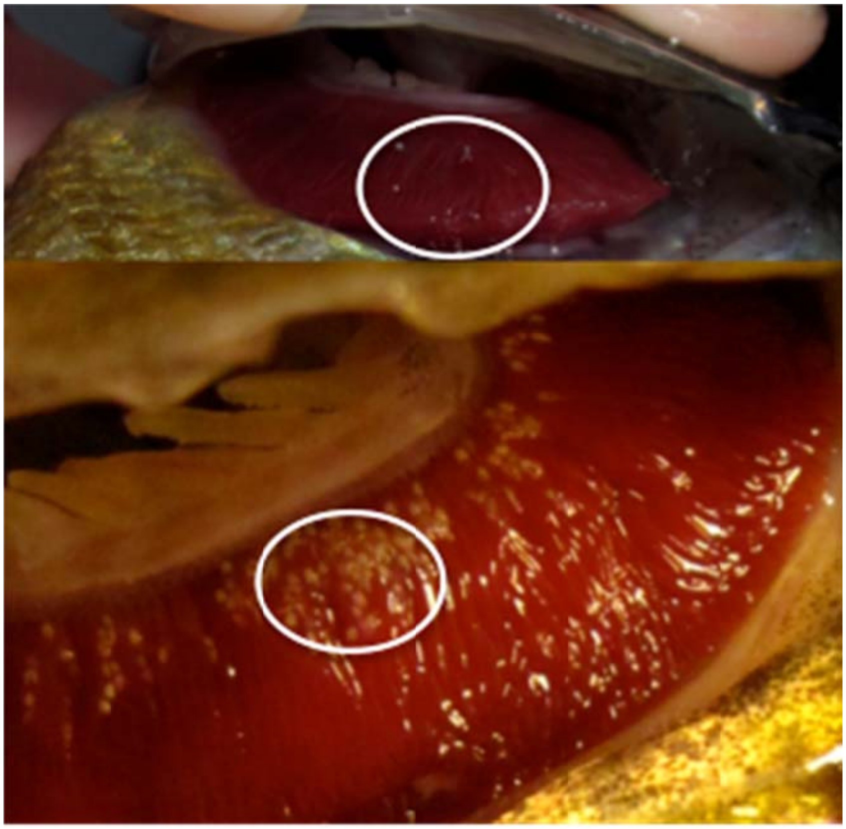

Figure 5: (Top) Gill lice in a bluegill. (Bottom) Gill lice in a rock bass. There are more gill lice present in the gills of rock bass than in the gills of bluegill

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References Cited

Bhuthimethee, Mary, Dronen, Norman O., Neill, William. 2005. Metazoan parasite communities of sentinel bluegill caged in two urbanizing streams, San Antinio, Texas. *Journal of Parasitoid* 91: 1358-1367.

Clouthman, Donald G., and Becker, David A. 1977. Some Ecological Aspects of Ergasilus Centrarchidarum Write (Crustacea: Copepoda) on Largemouth and Spotted Bass in Lake Fort Smith, Arkansas. *The Journal of Parasitology* 63: 372-376.

Colorado Department of Natural Resources. Gill lice in Colorado. 2012. http://wildlife.state.co.us/Fishing/Management

Dezfuli, Bahram S., Giari, Luisa, Lui, Alice, Lorenzoni, Massimo, Noga, Edward J. 2011. Mast cell responses to Ergasilus (Copepoda), a gill ectoparasite of sea bream. *Fish and Shellfish Immunology* 30: 1087-1094.

Dezfuli, Bahram Sayyaf, Luisa Giari, Robert Konecny, Paul Jaeger, and Maurizio Manera. 2003. Immunohistochemistry, ultrastructure and pathology of gills of Abramis brama from Lake Mondsee, Austria, infected with Ergasilus sieboldi (Copepoda). *Diseases of Aquatic Organisms* 53: 257–262.

Hudson, Patrick L., and Lynn T. Lesko. 2003. Free-living and Parasitic Copepods of the Laurentian Great Lakes: Keys and Details on Individual Species. Ann Arbor, MI: Great Lakes Science Center Home Page.

Layton, H.E. 1987. Energy Advantage of Counter-current oxygen transfer in fish gills. *J. Theor. Biol* 125: 307-316.

Muzzall, Patrick M., and Whelan, Gary. 2011. Parasites of Fish from the Great Lakes: A Synopsis and Review of the Literature, 1871-2010. *Great Lakes Fishery Commission*.

Ojha, Jagdish and G. M. Hughes. 2001. Effect of branchial parasites on the efficiency of the gills of a freshwater catfsh, Wallago attu.. *J. Zool*. 255:125-129.

Pelster, Bernd and Brian Bagatto. 2010. Respiration. *Fish Physiology* 29: 289-309.

Piasecki, Wojciech, Goodwin, Andrew E., Eras, Jorge C., Nowak, Barbara F. 2004. Importance of Copepoda in Freshwater Aquaculture. *Zoological Studies* 43: 193-205.

Roberts, Larry S. 1970. Ergasilus: Revision and Key to Species in North America. *Transactions of the American Microscopical Society* 89: 134-161.

Rosi-Marshall, Emma J. and Wallace, J. Bruce. 2002. Invertebrate food webs along a stream resource gradient. *Freshwater Biology* 47: 129-141.

Schaperclaus, W. 1992. Fish Diseases. Rotterdam: AA Balkema.

Scheffer, Marten, Carpenter, Steve, Foley, Jonathan A., Folke, Carl, Walker, Brian. 2001. Catastrophic shifts in ecosystems. Nature 413: 591-596.

Szalai, Alexander J. and Terry A. 1991. Evaluation of Gill Nets, Fyke Nets and Mark-Recapture Methods to Estimate the Number of Hirundinea and Crustacea on Fish. *Journal of Parasitology* 77: 914-922.