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Documenting historical anchorworm parasitism of introduced warmwater fishes in the Willamette River Basin, Oregon

Running footer: Anchorworm Parasites On Freshwater Fishes

4 figures

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Abstract

Anchorworms (*Lernaea spp*.) are freshwater parasitic copepods that use a wide range of hosts. Yet, little is known about their prevalence, distribution, and which species are their primary fish hosts in the state of Oregon. Institutional fish collections serve as banks which allow investigators to look at historical fish specimens and ascertain their health status at the time of their collection. We examined 1,039 specimens collected between 1941 and 2016 from the Oregon State Ichthyology Collection to detect the presence of anchorworms on non-native warmwater fishes from the Willamette River Basin, Oregon. Adult female anchorworms were found on eleven of the seventeen fish species that we examined. The most infected species included Common Carp (*Cyprinus carpio*), Bluegill (*Lepomis macrochirus*), and Smallmouth Bass (*Micropterus dolomieu*). We suggest these introduced warmwater fishes can act not only as hosts, but also as potential reservoirs for these understudied parasites posing a potential risk for Endangered Species Act (ESA)-listed native fishes. Our findings reveal unique insights that will serve as a baseline to detect future changes in parasite loads in the Willamette River Basin.

Keywords: museum collections, climate change, invasive species, parasite ecology

Introduction

Research collections have long been an important resource due to their ability to preserve representative specimens over time. While initial uses of collections have focused on documenting new species, they have become valuable assets for a variety of research purposes (Meineke et al. 2019). For example, research collections have been used to generate new data about both fish and wildlife diseases (Harmon et al. 2019, Murphy et al. 2020, Welicky et al. 2021). Specifically, non-invasive methods such as the examination of specimens from collections have documented external parasite loads of salmonid fishes (Murphy et al. 2020). Re-examining collections can thus aid in gaining access to new data, including baseline datasets, that may provide historical and contemporary insights on population-scale metrics. This information is especially important to understand host-parasite dynamics under stressors such as climate change and invasive species.

Lernaea spp., commonly known as anchorworms, are parasitic freshwater copepods. Anchorworms are native to Asia, but they have been introduced globally (García-Berthou et al. 2007). These copepods have both free-living and parasitic life stages, making them particularly efficient in moving throughout freshwater environments where they can use multiple hosts. Fertilized mature anchorworm females attached to fish or amphibian skin and muscle are the visible indicators of infection (Demaree 1967).

The anchorworm life cycle is divided into nine stages delimited by molting (Grabda 1963, Al-Marjan and Abdullah 2008). Adult females release eggs into the water column that hatch as free-living nauplii, and they subsequently go through two additional free-living naupliar stages (nauplii I, II, and III, the latter stages sometime referred to as metanauplii). After that, they pass through five parasitic copepodid stages during which they are loosely associated with the gills or body surfaces of hosts and have the ability to swim and move between individual hosts and species (Grabda 1963). Finally, they enter the cyclopoid stage, which is the sexually mature adult stage. Initially males and females in this stage can swim and mate (Grabda 1963, Al-Marjan and Abdullah 2008). Shortly after mating, males die off, but females continue their parasitic lifestyle (Kearn 2004). The fertilized females find a final host, burrow their anterior cephalothoraces into host tissue and permanently implant using their anterior anchors. At this time, they change morphologically, growing asymmetrically into the worm-like body form typically observable. These implanted females produce pairs of egg sacs (Grabda 1963, Al-Marjan and Abdullah 2008) and typically develop several sets of egg sacs while attached to their host (Shields 1978). Anchorworm development is affected by temperature, with better survival and faster development at warmer waters with optimal conditions around 23-30 °C. Adult female anchorworms attached to their hosts are the stages that can survive over winter (Shields and Tidd 1968, Bednarska et al. 2009).

The status and distribution of introduced anchorworms have not been previously monitored in the state of Oregon, United States. While they are not native to North America or the Willamette Basin, *Lernaea*'s wide tolerance to temperature fluctuations, pH range, and oxygen levels make the parasite adept at establishing themselves in most types of freshwaters (Hossain et al. 2018). It is unknown when or by what methods these copepods were first introduced in the region, but their first documentation in the United States (i.e., states of Iowa and Ohio) occurred on farmed Goldfish *Carassius auratus* in 1915 (Wilson 1915). Anchorworms were documented on introduced and native fishes in the Pacific Northwest of North America in 1957 (Uzmann and Rayner 1958). These copepods can negatively impact native fishes as these new potential hosts have not yet had an opportunity to develop an acquired immune response to these novel parasites. A generalized inflammatory immune response to anchorworm infection is expected for both native and nonnative fish species (Khalifa and Post 1976). Ability to reject these infections and immune response has been shown to potentially be an acquired immunity and could last season to season between groups of exposed hots (Shields and Goode 1978, Reshmi et al. 2022). Infection by an introduced parasite can cause significant ecological and trophic impacts as well as effects on fish health, body condition, and reproduction (Torchin et al. 2003, Britton et al. 2011). Anchorworm infections can damage scales, skin, muscles, lead to the formation of ulcers and abscesses, and may penetrate the body deep enough to impact internal organs in smaller fishes (Bednarska et al. 2009). In some cases, infected hosts with high parasite loads can not only develop secondary bacterial and fungal infections, but also can succumb directly from anchorworm infections (Bednarska et al. 2009).

The pace that fish and their parasites are being introduced in freshwaters is increasing and could continue to cause impacts on native ecosystems (Britton 2013, Williams et al. 2013, Sheath et al. 2015). Many non-native warmwater fishes have been introduced in the Pacific Northwest of North America to create novel recreational fishing opportunities and have become popular with local anglers. Many of these nonnative fishes including bass, catfishes, and carp were spread to the Pacific Northwest in the late 1800's by the U.S. Fish Commission and other private parties (Lampman 1946). The U.S. Fish Commission distributed these species for legal aquaculture and sport fishing uses to create more unique fishing opportunities. Common Carp *Cyprinus carpio*, Bullhead Catfish *Ameiurus melas*, Channel Catfish *Ictalurus punctatus*, and Largemouth Bass *Micropterus salmoides* were first stocked into the Willamette River between the years of 1880-1893 by the fish commission (Lampman 1946). The aquarium trade also represents a possible invasion pathway for nonnative freshwater species in the Willamette Basin due to its dense human population, if people release pet fish (Strecker et al. 2011). Collectively, these introduced species represent suitable hosts for anchorworms and are likely the pathway that these parasites could have used to spread into North America including the Pacific Northwest (Uzmann and Rayner 1958, Calhoun et al. 2018).

The objective of our study is to examine specimens from the Oregon State Ichthyology Collection, Corvallis, Oregon, U.S., to help establish a baseline level of infection for freshwater introduced species in Oregon. We focused on non-native warmwater fishes as they provide insights about which potential hosts could have brought anchorworms to the region. In addition, these introduced warmwater fishes could act as vectors that may transfer anchorwoms into new areas and increase risks of infection in native species.

Methods

We examined 1,039 fish specimens contained in 226 jars all taken from the Willamette River Basin, Oregon (Figure 1). Specimens were collected between 1941 and 2016 and archived in the Oregon State Ichthyology collection https://ichthyology.oregonstate.edu/. Fish specimens were preserved initially in formalin and transitioned to isopropanol in the early 2000s. We examined specimens from 17 introduced fishes including Yellow Bullhead catfish *Ameiurus natalis,* Brown Bullhead catfish *Ameiurus nebulosus,* Goldfish *Carassius auratus,* Common Carp *Cyprinus carpio,* Banded Killifish *Fundulus diaphanus,* Western Mosquitofish *Gambusia affinis,* Green Sunfish *Lepomis cyanellus,* Pumpkinseed *Lepomis gibbosus,* Warmouth *Lepomis gulosus,* Bluegill *Lepomis macrochirus,* Smallmouth Bass *Micropterus dolomieu,* Largemouth Bass *Micropterus salmoides,* Spotted Bass *Micropterus punctulatus,* Oriental Weatherfish *Misgurnus* *anguillicaudatus,* Yellow Perch *Perca flavescens,* White Crappie *Pomoxis annularis,* and Black Crappie *Pomoxis nigromaculatus.*

Visual external inspections of each specimen were performed by removing the specimen from their lot and aligning them on a tray. Each specimen was assigned its own unique ID and then was carefully visually inspected. Special care was taken to inspect each specimen including inside of the mouth, the gills, and underneath the fins. Visual identification of *Lernaea* spp. was conducted following the key in *Parasites of North American Freshwater Fishes* (Hoffman 1999). We followed the descriptions of the anatomy of the ectoparasite and how the externally projecting portions of the female *Lernaea* spp. body would present and differentiate from other ectoparasite species. The choice to visually identify *Lernaea* to genus and not further speciate was made due to genetic findings suggesting that different species of *Lernaea* just correspond to morphological variations *L. cyprinacea* (Hua et al. 2019). Identification of *Lernaea spp*. was further confirmed by inspecting the infection site of the specimen underneath a microscope. Infected areas were recorded and then fork length (mm) was taken for each fish specimen. Fish specimens preserved with the methods listed above do experience shrinkage during the preservation process, but the body size change is proportional for length and depth of fish and shrinkage stops within the first year of preservation. Even though the specimens have shrunk from their true size at sampling date, we used fork length as an appropriate measure of body size (Gaston et al. 2013). The observed parasites were not extracted or dissected from the body of the sampled individuals as to not damage the collection specimens. Only areas where adult female *Lernaea* were attached were counted as infections as scars or previous injured areas could be due to other reasons.

For documentation purposes, digital images were taken of specimens that were infected and then the specimens were placed back into their respective jars. Digital images were obtained using a cell phone camera (e.g., Samsung Galaxy Amp 2 or iPhone 11). Additional images of the fish with the corresponding sample jar metadata sheet were also obtained. Data from the jars were also collected which included species of specimens, date of collection, and the geographic location of the collected lot. Illustrative images of anchorworms attached to hosts were obtained using a digital camera and macro lens (e.g., Canon 5D Mark IV, Canon L lens 100mm Macro). Images were cleaned up with a photo (e.g., Adobe Photoshop, version 23.4.2) and image plate (e.g., Adobe InDesign, version 17.3) editor.

Results

Out of the 1,039 specimens examined, 48 individuals from 11 different species were infected with adult female *Lernaea spp*. Some of these specimens had infections located in multiple places including the dorsal, pectoral, and anal fin areas (Figure 2). The five species with the highest infection prevalence (Figure 3) were Goldfish *Carassius auratus* (20%; n = 5), Common Carp *Cyprinus carpio* (18.8%; n = 16), Bluegill *Lepomis macrochirus* (15.4%; n = 117), Brown Bullhead *Ameiurus nebulosus* (9.3%; n = 54), and Yellow Bullhead *Ameiurus natalis* (6.4%; n = 63). The highest number of infected specimens occurred in September (Figure 4) and the oldest infections were detected in 1950 for Brown Bullhead catfish *Ameiurus nebulosus*, Pumpkinseed *Lepomis gibbosus*, Bluegill *Lepomis macrochirus*, and Largemouth Bass *Micropterus salmoides*. Infection prevalence by size (Figure S1) or over time (Figure S2) did not show an apparent pattern among species and years.

Discussion

We document anchorworm (*Lernaea spp.*) infections in 11 of the 17 introduced warmwater fishes we examine in this study. Although some of these species has limited samples, the highest infection prevalence found in Common Carp and Goldfish suggest that these fishes could represent important vectors for the introduction and spread of anchorworms in Oregon as Common Carp and Goldfish are in sympatry with anchorworms in Asia (Kabata 1963, Raicu et al. 1981, Balon 1995). These fishes are also likely responsible for the spread of *Lernaea* into Oregon considering their historical introduction efforts. Common Carp have been introduced in the Willamette River in the late 1800's (Lampman 1946), and the related Goldfish species is responsible for the first documentation of *Lernaea* in the United States (Wilson 1915). It is highly plausible that anchorworms spread into Oregon using these species as main original hosts. Both fishes are widespread and remain popular in the aquarium and aquaculture industries (Lampman 1946, Strecker et al. 2011). Anchorworms appear to be similarly widely distributed within the Willamette River basin, except for areas of higher elevation such as the Cascade Mountain Range. This apparent absence from high elevation reaches could be due to a variety of reasons including both the limited ability of many non-native warmwater fishes to move upriver to higher gradient habitats, and the reduced availability of specimens at upriver areas. We show that most infections have historically occurred during spring, summer, and early fall; infections are apparently absent in late fall and winter. This pattern of infection would be consistent with the preferred warmer conditions that anchorworms need to complete their life cycles (Shields and Tidd 1968, Bednarska et al. 2009).

The impacts that these introduced parasites might have on native fishes could be significant considering their lack of prior experience and the likely limited immune response of the native fauna (Britton et al. 2011). The Willamette River Basin has many important native fishes including the Endangered Species Act-listed Chinook Salmon (*Oncorhynchus tshawytscha*) and Steelhead Trout (*O. mykiss*) (U.S. Endangered Species Act of 1973: 16 U.S. Code Chapter 35; ODFW and NMFS Northwest Region 2011). These salmonids have great economic and cultural importance in the region with extensive recovery plans in place to rebuild their habitats and populations. Currently, Chinook Salmon and Steelhead Trout populations from the Willamette River Basin face many challenges due to habitat degradation and past fishing pressures that have put them at a severe disadvantage (Lundin et al. 2019). Important life history stages of salmonids occur in the spring, summer, and fall, for example, adult Chinook Salmon migrating back into freshwater (Groot and Margolis 1991). The juvenile stages and maturation of salmonids also occur within their freshwater rearing habitats (Mattson 1962). Potential seasonal overlaps between native salmonids and anchorworms could not only result in juvenile salmonid mortalities, but also negatively affect the growth and fitness of the native fauna (Britton et al. 2011).

Lernaea presence on salmonids in the Willamette Basin has been confirmed on Cutthroat (*Oncorhynchus clarkii*) and Rainbow trout (*Oncorhynchus mykiss*) specimens in the OSU research collection, with very low prevalence rates compared to what we found on the introduced warmwater species (see supplementary table in Murphy et al. 2020). Previous monitoring of *Lernaea* spp. infections on salmonids in the state of Utah, U.S., showed that high infections occur in October in a reservoir setting (Berry et al. 1991). Increased chance of coinfections by anchorworms and bacterial diseases such as *Aeromonas hydrophilia* are also possible (Shields and Tidd 1968). Other ectoparasites have also already begun to seriously affect salmonids within the Willamette Basin. *Salmincola californiensis* has been shown to be negatively affecting fitness and increasing mortality rates of juvenile salmonids in reservoirs (Herron et al. 2018, Neal et al. 2021). *Lernaea* has also been shown to already be seriously affecting native fish species in Oregon. In the Upper Klamath Lake, Klamath County, Oregon, two native Sucker species have been in decline due to serious infestations of *Lernaea* and other parasites (Janik et al. 2018). To understand the full picture of potential negative impacts of these ectoparasites, the monitoring of anchorworm levels across populations at risk in the Willamette River Basin and other Oregon basins is warranted.

There are some limitations for the use of fish collection specimens to answer questions about parasite-host dynamics. Among these limitations are the inconsistent sampling methods and efforts over time in sampling areas, species, and number of available specimens. Many of the specimens we examine are originated from past projects not designed to collect infection data. In addition, biases against fish that appear to look 'unhealthy,' due to external parasites or other apparent physical damage could underestimate infection prevalence as researchers might choose not to keep a specimen that has adult anchorworms attached. Although we cannot characterize trends of infection prevalence over time, our findings are valuable as baseline of patterns of infection among non-native warmwater species in Oregon.

This baseline dataset could be expanded in a variety of ways in the future. New sampling efforts at the same stream reaches may allow for a better understanding of current anchorworm infection levels within the basin. Monitoring parasites along with other covariates such as fish community composition and environmental information could also be used to understand relationships between fish health and habitats (Marcogliese 2004, Nachev and Sures 2016, Sures et al. 2017). Ultimately, one of the values of museum collections, such as those used in this study, is to create baseline datasets that can be used in the future to better understand how species and ecosystems change over time.

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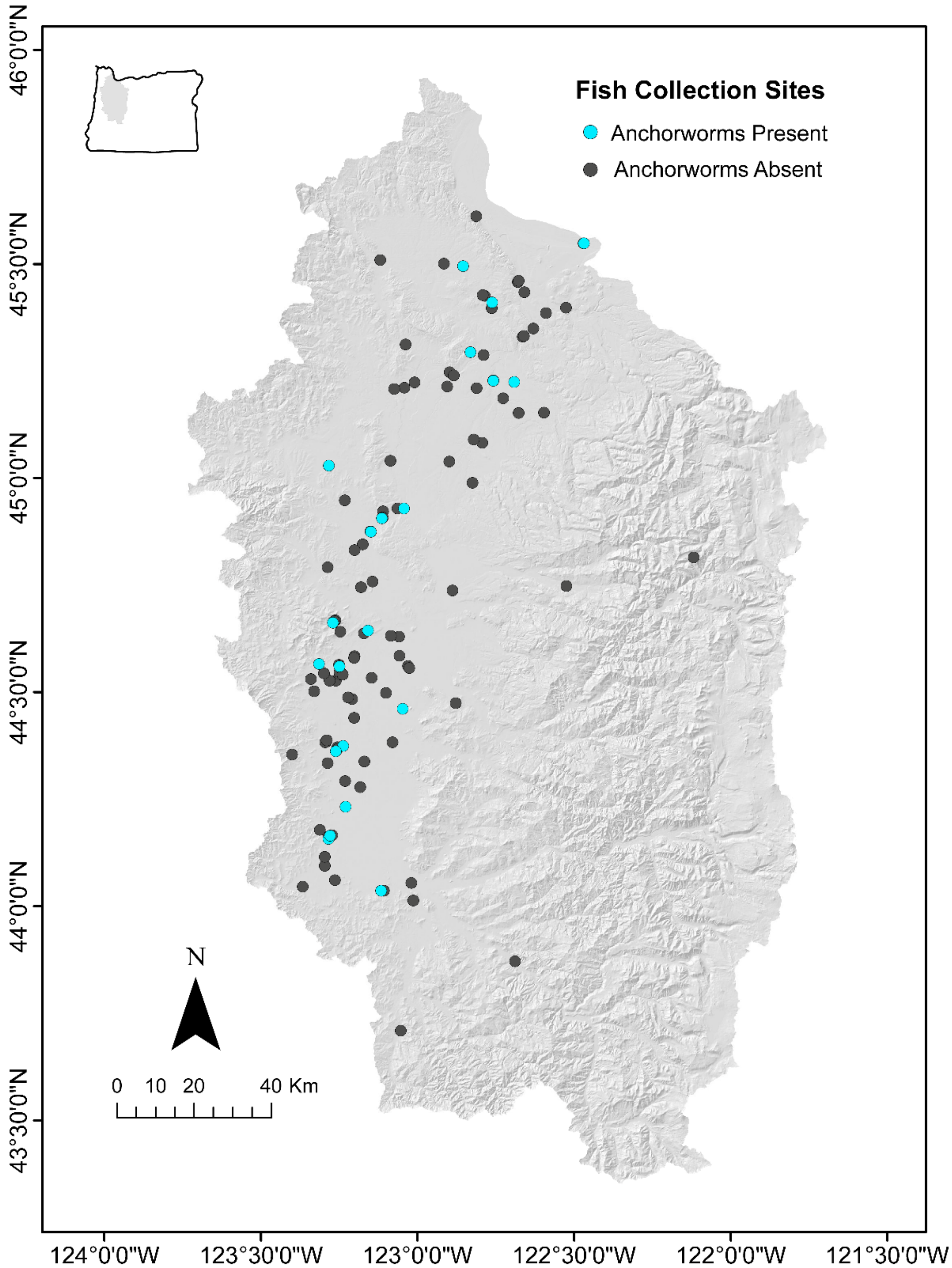
Figure Captions


Fish Collection Sites
Anchorworms Present
Anchorworms Absent
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44°30'0"N
44°0'0"N
43°30'0"N
124°0'0"W
123°30'0"W
123°0'0"W
122°30'0"W
122°0'0"W
121°30'0"W

395

Figure 1. Map of locations where freshwater fishes (n = 1,039) were collected between 1941 and 2016 including presence/absence of infected specimens with anchorworms (*Lernaea spp.*) in the Willamette River Basin, Oregon, U.S.

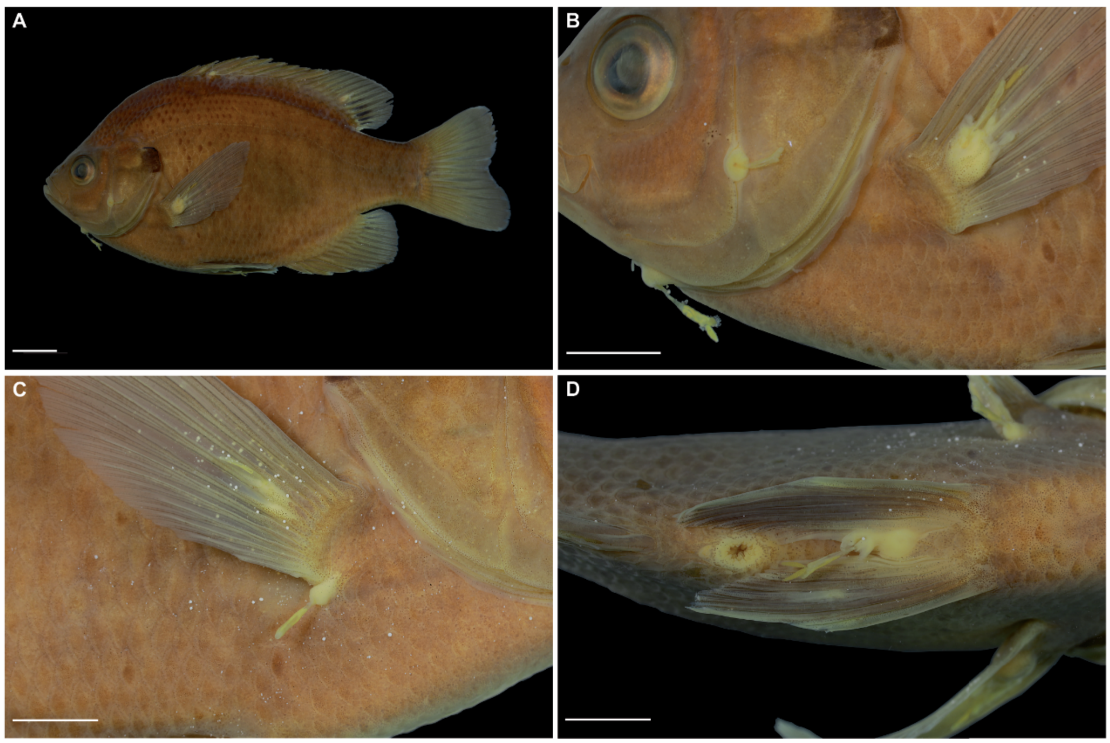

Figure 2. Common locations of multiple infections by *Lernaea spp*. on a Bluegill specimen (*Lepomis macrochirus*; 102mm standard length; OS 16633) collected from the Willamette Basin, Oregon (see Figure 1). **A** Overview of the left lateral side of the specimen. **B**. Infected right pectoral fin base and rays. **C**. close up of *Lernea sp.* attached to the isthmus of the gills, preopercle, and pectoral fin. **D**. *Lernea spp*. medial between the pelvic fins. Scale bars in **A** 10 mm, **B** – **D** 5 mm. Small white spots in images are crystallizations that develop due to older fish preservation methods. Image taken with Canon 5d Mark IV with a Canon L-series 100mm macro lens by P. Konstantinidis.

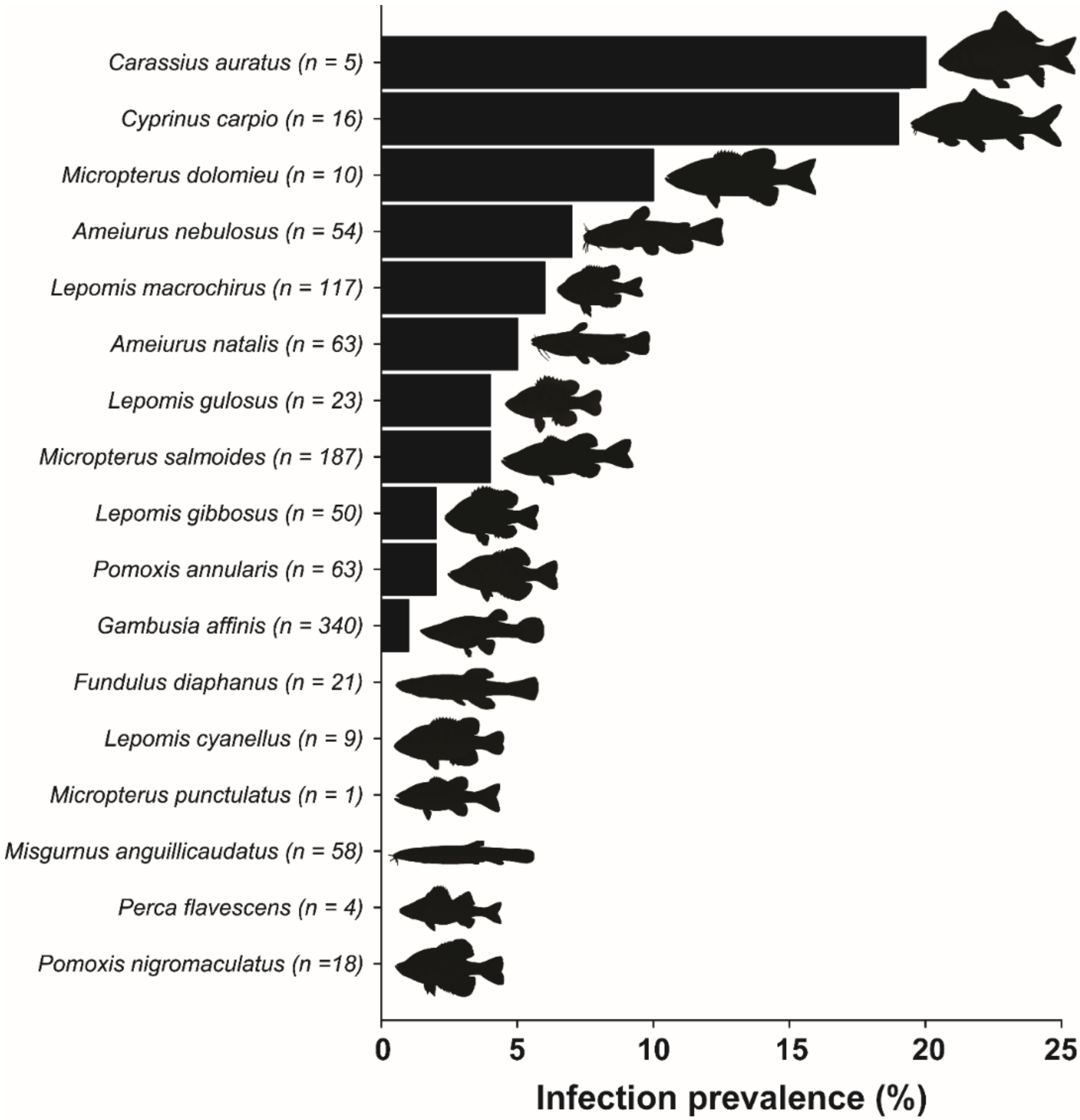

Figure 3. Anchorworm (*Lernaea spp*.) infection prevalence (%) of specimens by fish species collected from the Willamette River Basin, Oregon, United States (see Figure 1) and stored in

the Oregon State Ichthyologic Collection, Corvallis, Oregon (sample sizes shown in parentheses) between 1941 and 2016 (Detailed information can be found in Table S1).

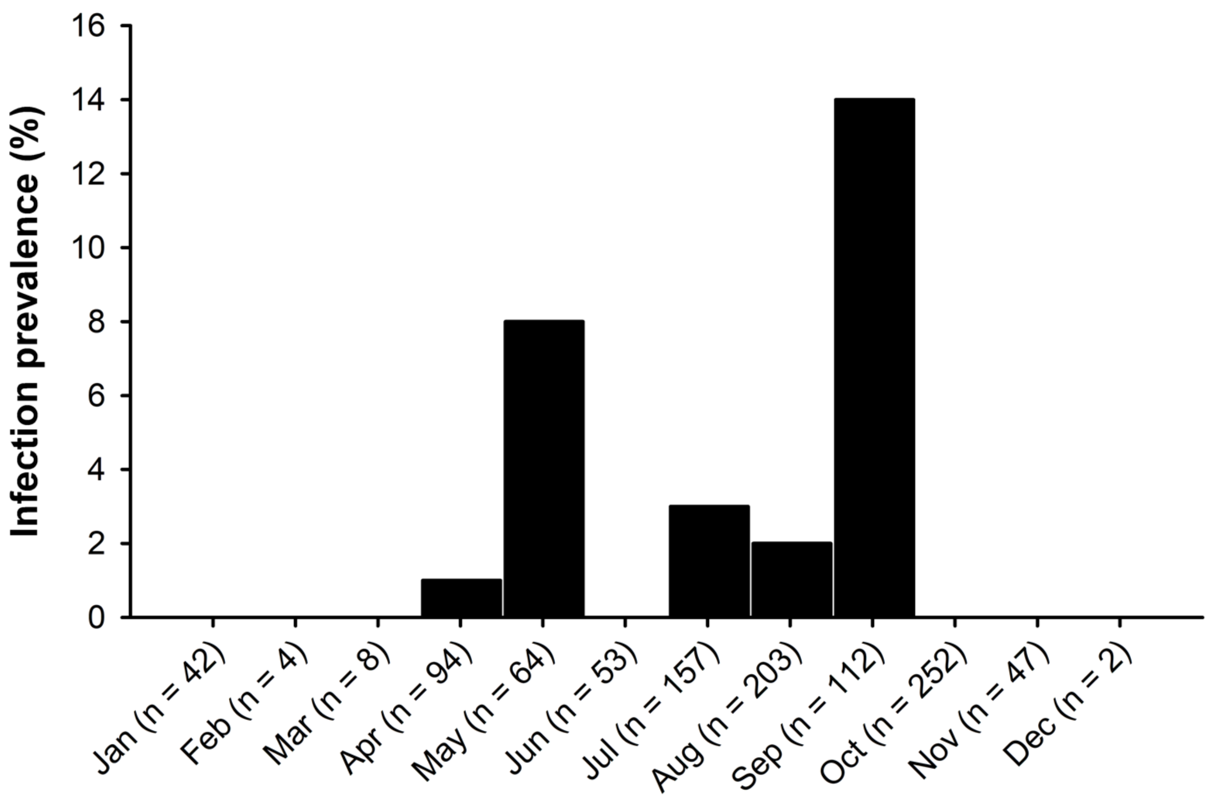

Figure 4. Anchorworm (*Lernaea* spp.) infection prevalence (%) by month for all examined specimens collected from the Willamette River Basin, Oregon between 1941 and 2016 and stored in the Oregon State Ichthyologic Collection, Corvallis, Oregon, U.S.

Supplemental Material for

Documenting historical anchorworm parasitism of introduced warmwater fishes in the Willamette River Basin, Oregon

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Supplemental Figures

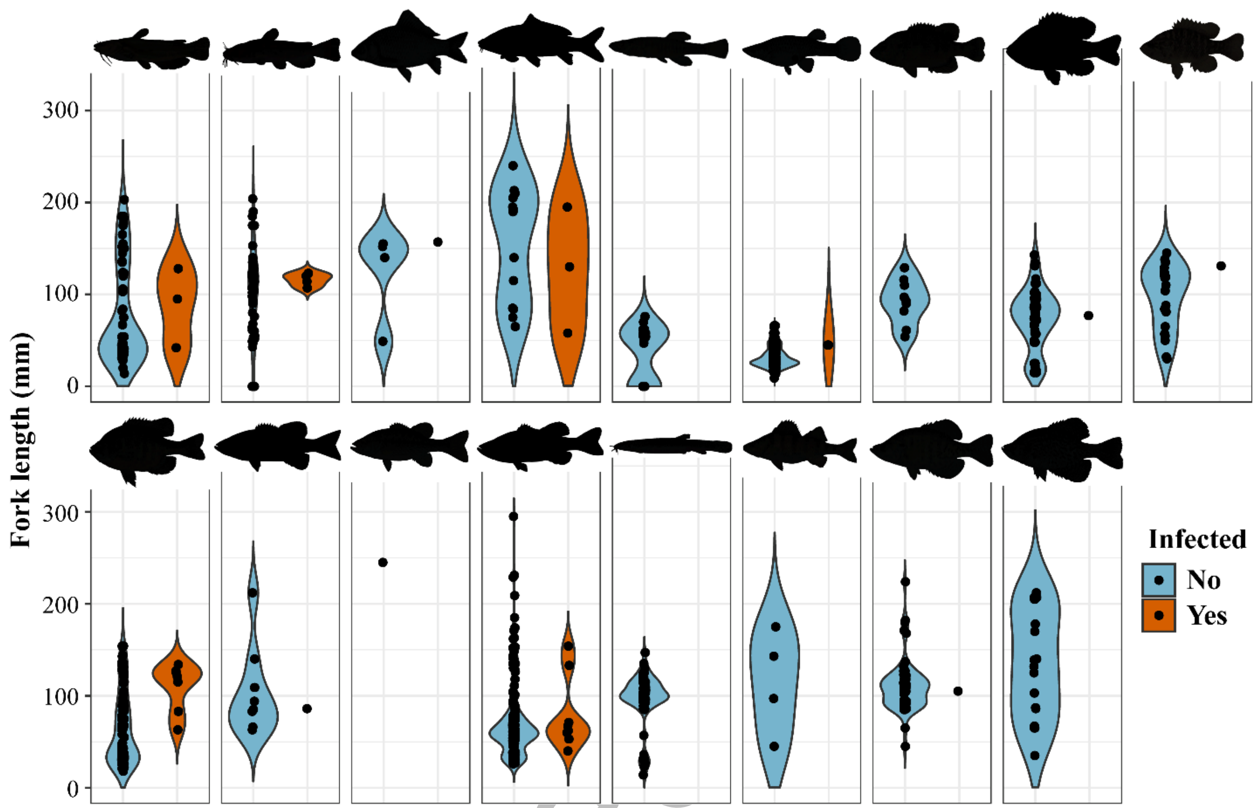

Figure S1. Exploration of the relationship between body size (fork length (mm)) and *Lernaea* infection for each species collected from the Willamette River Basin, Oregon, U.S. (see Figure 1). Species listed Top (Left to Right) Yellow Bullhead catfish *Ameiurus natalis,* Brown Bullhead catfish *Ameiurus nebulosus,* Goldfish *Carassius auratus,* Common Carp *Cyprinus carpio,* Banded Killifish *Fundulus diaphanus,* Mosquitofish *Gambusia affinis,* Green Sunfish *Lepomis cyanellus,* Pumpkinseed *Lepomis gibbosus,* and Warmouth *Lepomis gulosus.* Bottom (Left to Right) Bluegill *Lepomis macrochirus,* Smallmouth bass *Micropterus dolomieu,* Spotted Bass *Micropterus punctulatus,* Largemouth bass *Micropterus salmoides,* Oriental weatherfish *Misgurnus anguillicaudatus,* Yellow Perch *Perca flavescens,* White Crappie *Pomoxis annularis, and* Black Crappie *Pomoxis nigromaculatus.*

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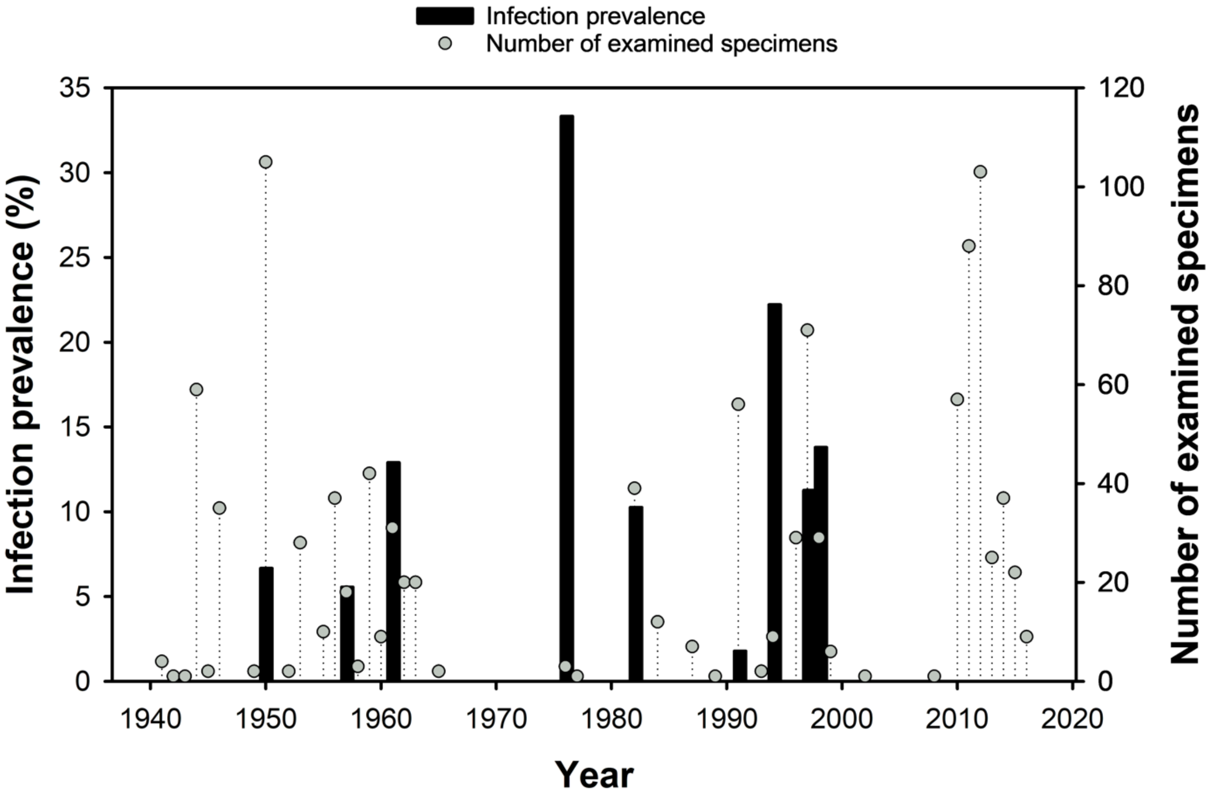

Figure S2 Annual infection prevalence (%) of warmwater fishes from the Willamette Basin, Oregon, U.S. by *Lernaea* collected between 1941 and 2016 including the number of examined specimens. Sampled fish were accessed from the Oregon State Ichthyologic Collection, Corvallis, Oregon, U.S.

Note: This article has been peer reviewed and accepted for publication in *Northwest Science*. Copy-editing may lead to differences between this version and the final published version.

Supplemental Tables

Table S1 Infection prevalence by *Lernaea* for warmwater fishes from the Willamette Basin, Oregon, U.S. collected between 1941 and 2016 including the number of examined specimens. Sampled fish were accessed from the Oregon State Ichthyologic Collection, Corvallis, Oregon, U.S.

Species	Number of examined specimens	Number of infected specimens	Infection prevalence (%)
Ameiurus natalis	63	3	4.76
Ameiurus nebulosus	54	4	7.41
Carassius auratus	5	1	20
Cyprinus carpio	16	3	18.75
Fundulus diaphanus	21	0	0
Gambusia affinis	340	2	0.59
Lepomis cyanellus	9	0	0
Lepomis gibbosus	50	1	2
Lepomis gulosus	23	1	4.35
Lepomis macrochirus	117	7	5.98
Micropterus dolomieu	10	1	10
Micropterus punctulatus	1	0	0
Micropterus salmoides	187	8	4.28
Misgurnus anguillicaudatus	58	0	0
Perca flavescens	4	0	0
Pomoxis annularis	63	1	1.59
Pomoxis nigromaculatus	18	0	0