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Parasite prevalence and composition in Tui Chub in Eastern California freshwater ecosystems

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Parasite prevalence and composition in Tui Chub in Eastern California freshwater ecosystems

A Thesis submitted in partial satisfaction of the requirements
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in

Biology

by

Daniella Ariel Fairbank

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Professor Jonathan Shurin, Chair
Professor Carolyn Kurle, Co-Chair
Professor Ryan Hechinger

2022

The Thesis of Daniella Ariel Fairbank is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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ABSTRACT OF THE THESIS

Parasite prevalence and composition in Tui Chub in Eastern California freshwater ecosystems

by

Daniella Ariel Fairbank

Master of Science in Biology

University of California San Diego, 2022

Professor Jonathan Shurin, Chair

Professor Carolyn Kurle, Co-Chair

Parasites and pathogens exert strong selection on their hosts and alter the structure, diversity, and productivity of communities of ecosystems. This paper presents results of a survey of parasite composition and prevalence observed on and within the freshwater hybrids Owens (*Siphateles bicolor snyderi*) and Lahontan (*Siphateles bicolor obesa*) Tui Chubs, a native minnow species, in the Eastern Sierra Nevada mountains of California. The Owen and Lahontan Tui Chub is present in many lakes and rivers in Northern California and its parasite community has yet to be characterized. My thesis asks what kinds of parasites are found in the freshwater Tui Chub, which lakes or streams held the highest parasitic loads, and which features of individual fish and the habitat influence parasite density and/or types of parasites. Fish samples were collected in Summer 2019 by PhD student Henry Baker at 10 different sampling sites including freshwater lakes and streams that vary in size, temperature, water chemistry and species present across Owens Valley, California. I dissected 134 individual fish to characterize the ecto- and endo-parasite communities. My results show that two of the locations had significantly higher parasite infection rates than the others, where few macroscopic parasites were observed. These two locations were both geothermal with warmer waters and distinct water chemistry with high salinity and alkalinity. This pattern suggests that some aspects of geothermal habitat favor the parasite life cycle and makes fish in these sites more easily accessible as a host, though the mechanism behind the pattern is unknown. Four main types of visually distinct parasites were found: one adult life-stage tapeworm, one adult life- stage nematode and two metacercaria trematodes, though none were identified taxonomically. The greater parasite infection rates in geothermal habitats may be related to the greater abundance of snails in these sites, which may serve as intermediate hosts to fish parasites. No differences in parasite infection rates or composition were observed between lake and stream habitats. My thesis suggests that the atypical thermal and chemical environment of geothermal springs promotes parasitism in Tui Chub, but that lakes and streams are similar in containing low rates of infection by any parasites among fish.

INTRODUCTION

Parasites are ubiquitous components of all ecosystems, sometimes capturing similar biomass and diversity as free-living species (Kuris & Hechinger et al. 2008). A parasite can be broadly described as an organism that lives on or within another species, aka its "host", and obtains its nourishment from that species and also uses it as a means to complete its life-cycle (Barber et al. 2000). Parasites play roles comparable to predators that eat their prey slowly and may have weak or strong effects on host fitness. The diversity and biomass of parasites in ecosystems is comparable to that of free-living species, indicating that parasites are ubiquitous, interact strongly with hosts and contribute greatly to community structure and function (Kuris et al. 2008). Parasites may utilize multiple hosts over their life cycle (Auld and Tinsley et al. 2015). Host populations represent a parasite metacommunity (Bolnick et al. 2020) that persists because parasites are transmitted from infected to uninfected hosts. Parasites with simple life cycles use one unique host, whereas those with a complex life cycle use two or more hosts (Sasal et al. 1998).

Parasites can manipulate the behavior of their hosts in ways that promote transmission among alternate hosts. For example, the California killifish (*Fundulus parvipinnis, Cyprinodontidae*), is an intermediate host of a brain-encysting trematode (*Euhaplorchis californiensis*) that has a strong effect on fish behavior making them more susceptible to predation (Lafferty & Morris et al. 1996). Parasites' ability to alter host behaviors and other aspects of phenotypes may help them to complete their life-cycle (Shaw et. al 2012). Examples of parasite-infected fish behavior modifications include swimming more actively, spending more time in exposed areas, or predator-avoidance behavior, promoting trophic infection of the parasite's next host (Crowden & Broom, 1980; Shaw et. al 2012). Hosts can evolve immunity against parasites over time. For example, Weber et al. (2017) showed that freshwater stickleback populations that coexist with the cestode *Schistocephalus solidus* evolve greater ability to suppress parasite growth than populations without the parasite. These examples portray the broad diversity of parasitism among host taxa and the significant role parasites play in their hosts' ecology and evolution.

Host exposure to parasitic infection is strongly affected by different aspects of the individual host, such as body size, as well as the environment including seasonal variation, chemistry, productivity, habitat size and/or temperature. Host body size can have an impact on parasite infection rates and the parasitic load (Morand et at., 1996; Sorci et al., 1997), leading to a tendency toward greater parasitism rates of larger hosts. Sasal and Monrand (1998) found greater loads of a flatworm, *Monogenea,* in larger fish, potentially because larger fish are older and more likely to have become infected (Winemiller and Rose et al.,1992). Parasite body size where consumer-resource body sizes correlate strongly with one another (i.e, when a predator or parasite is small, its prey or host will be as well). Exceptions to this rule have been found with no correlation or opposite findings (smaller fish having higher parasite infection rates than larger) between predator-prey size (Poulin and Monrand et al. 2000). The traits of hosts that affect infection rate are unknown in many cases.

Studies have found that an aquatic surface area, diversity of species and a pH were all positively correlated with parasite prevalence in fish (Calhoun et al. 2018). Large habitat area has been shown to correlate with higher species diversity, thus providing more potential encounters with more definitive host species like birds and mammals (Kennedy et al. 2009). A larger area means more definitive hosts that spread parasites, favoring higher infection rates of fish. Johnson et al. (2007) found that more productive ponds contained higher densities of snail hosts of trematode parasites, resulting in greater infection rates for amphibian hosts. Features of the environment that favor greater contact rates between hosts and infectious stages of pathogens should result in higher infection rates.

The environmental factors such as temperature, elevation, or salinity can affect parasite loads and transmission rates (Richgels et al. 2013; Moss et al. 2020). Smith (1973) examined sensitivity of cestodes to climate over 15 years and found a trend of warming temperatures and an increase in host, the sockeye salmon, infection rates. Warmer temperatures may generally result in greater infection (Paull et al., 2011). Temperature not only affects parasite communities, but also plays a strong role in freshwater fish behavior, survival rates, feeding rate, migration and population dynamics (Farkas et al., 2001). For example, a study of growth rates and mortality of cyprinids across seasons found that temperature affects food availability and is the driving force behind migration between lakes and streams (Brönmark et al., 2008). Cyprinids leave lakes and migrate into streams in the winter to find food and avoid predation (Jobling et al., 1994). Such migrations can be a strategy for avoiding parasitic infection in many species. With warming temperatures, migration behaviors among animals that use seasonal cues can potentially reduce the transmission of infectious diseases by separating hosts and pathogens (Altizer et al, 2013). For instance, migrating juvenile salmon do not normally encounter wild adults or their parasites, but the presence of salmon farms in coastal inlets bring them into contact, resulting in greater loads of sea lice and higher mortality for juveniles (Krkosek et al. 2006). Climate change and warming temperatures is a threat to migratory species as it can cause them to stay within a more temperate environment where pathogens and parasites reside, enhancing parasite survival and transmission rates (Alizer et al., 2013).

In addition to temperature, the water chemistry may influence not only parasite abundance but also the community of organisms that may be intermediate hosts for fish parasites. In freshwater aquatic environments, parasite transmission is often between snails, birds and fish via the food-web (Barber et al., 2000; Bolnick et al., 2020). Possible host such as snails are highly sensitive to salinity levels, as calcifying organisms they are dependent upon calcium and carbonate to form their shells out of the mineral calcium carbonate. Previous studies have shown an an increasing alkinity positively influences the occurrence of a freshwater snail species, *Bulinus Globosus* (Olkeba et al. 2020). Under low salinity levels, concentrations of minerals may limit gastropod and crustacean populations. We therefore could expect the transmission between parasites, snails and fish to be higher within more saline ecosystems. Another example is shown in the California killifish is infected by the trematode *E. californiensi*s, (Shaw et al. 2009) where the California horn snail, *Cerithidea californica*, is locally abundant.. Water chemistry conditions can be a key variable driving parasitism rates.

Lake and stream fish serve as intermediate hosts to diverse parasites that exhibit a wide variety of life-cycle strategies (Loehle et al., 1995; Wilkins et al., 2002). Habitat differences between lakes and streams can also impact fish-parasite relationships. Fish consume different prey and may be exposed to different trophically transmitted parasites in lakes vs. streams (Northcote et al., 1978). In lakes, many fish consume a mixture of zooplankton and benthic invertebrates, while stream fish diets tend to consist primarily of benthic organisms. Some of the species that potentially interact with fish may be intermediate hosts for parasites. Other community members influence freshwater fish in other ways as well, for example Xi He & James F. Kitchell (1990) showed that in response to piscivore introduction, freshwater fish migrate out of a lake into streams to avoid predation, potentially influencing exposure to different parasites.

Differences in ecological interactions and physical conditions may drive variation in parasite-host interactions and infection rates between lake and river populations of fish species that occupy both environments.

The two hybrid species, Owens (S*iphateles bicolor snyderi*) and Lahontan (*Siphateles bicolor obesa*) Tui Chub are a small minnow fish in the family *Cyprinidae,* found in aquatic habitats in Eastern California, Nevada and Oregon, (Moyle 2002). The Owens Tui Chub is a Federal and State listed endangered species due to introgressive hybridization with the Lahontan species that was introduced as fishing bait. Little is known about its ecology or genetics. The Tui Chub is an omnivore that typically forages on detritus, small aquatic plants, periphyton and biofilm, arthropods, insect larvae and smaller fish (Williams et. al., 1985). Predators of the chub include birds, larger fish and some mammals within the lake and stream ecosystems (Williams et al., 1985; Leunda et. al., 2013). Tui Chub are common within freshwater food webs and serve as a trophic link between the lower trophic level species to the top level-predators (Loehle et al., 1995). Little information is available in the literature regarding parasites infecting Tui Chub. One study documented the Asian fish tapeworm (*Bothriocephalus acheilognathi*) infecting the Tui Chub in California and Arizona (Archdeacon et at., 2010). Researchers have commented on the lack of studies done on parasite occurrences in freshwater fish communities from California (Calholn et al., 2018), which should push for more research within this field. Not only is this kind of research important for fish health and learning more about freshwater ecosystems, it's also important to examine to determine the role of parasites and the risks of infection in different habitats for the threatened Owens Tui Chub.

In this study, I quantified the parasite load on Tui Chubs from 10 populations in the Owens Valley. Five populations were found in lakes, five in streams, and one of each (Hot Creek and Warm Lake) was a geothermal site with high temperature and salinity, and anomalous water chemistry including high alkalinity. These samples were collected by PhD student Hank Baker and frozen until processing and dissection in the lab. These sites varied in temperature, salinity, depth and elevation, and water flow velocity. These freshwater sites contain a great diversity of other species that interact with Tui Chub, which could be potential intermediate hosts for parasites. I sampled 134 fish, at least 10 from each site. Samples were dissected to examine the numbers and identities of parasites found in different tissues of their bodies. Each major organ was removed, separated and examined under a dissecting microscope. The identity of the parasites was based on morphology with no taxonomic identification. My goal was to ask if parasite prevalence and composition varies between lakes and streams, or between geothermal and non-geothermal sites. The project aimed to illuminate the environmental features that determined the kinds and numbers of parasites infecting Tui Chub in the Owens Valley.

METHODS

Sampling Sites

Hybrid Owens (S*iphateles bicolor snyderi*) and Lahontan (*Siphateles bicolor obesa*) Tui Chubs were collected by PhD student Henry Baker from July 2019 to August 2019 by backpack electrofishers from lake sites and beach seines from stream sites. The location types were five freshwater lakes and five streams, and a total of 10 different sites were used. These sites varied in temperature, elevation and salinity (Fig. 5,6). Fish collected were hybrids between Lahontan and Owens species. Fish were kept in the lab freezer until processing. All fish were collected in accordance with UCSD IACUC protocol S14140 and under a California Fish and Wildlife Scientific Collection Permit.

Dissections

The fish samples were dissected beginning in Fall 2019 until the Spring of 2021. The main organs of the fish were removed for investigation. They were frozen and then thawed when ready for dissection and processing. I dissected the body cavity and main organs (liver, brain, swim bladder, gonads, eyes) and opened the gut contents and digestive tract. I separated each organ, stomach and gut contents. Ectoparasites were examined on top of the fish scales, within the sample bag contents and partially embedded within the fins. I removed the gill arches to examine in greater detail.

To investigate parasites, the organs were separated, intestines sliced open, squashed under dissection slides and examined under the scope one at a time. The larger parasites that were visible to the naked eye were separated from the organs, placed on a petri dish or weighing boat and weighed (wet weight) using an analytical balance. The numbers of smaller parasites seen under the microscope were counted then estimated by visual inspection. Along with investigating parasites, parts of the fish samples were also collected and preserved for future research and records including otoliths, gut contents, gill arches/rankers, fin clips, gut length and stomach contents.

Each fish was assigned a unique ID number by Hank Barker. At least ten fish from each site were dissected and the order in which they were selected was by random. After those dissections were complete, the fish IDs were randomized and given blind ID's that hid the location they were collected from to avoid biasing the dissections. A total of 134 samples were dissected.

The parasites were removed from the fish and placed in a petri dish. Parasite wet mass was recorded by morphotype in grams using an analytical balance to the nearest + 0.001 g. In cases where parasites were too small and numerous to remove, only ½ of the fish on one side was examined, then the biomass load was estimated and multiplied by 2. Length of larger parasites were recorded in millimeters and counts of the smallest parasites (e.g tapeworms in lengths, trematodes in counts). Parasite types that were too numerous to count were weighed. Most nematodes were found within the fish body but some that were occasionally found in the sample bag with the Tui Chub sample in it were assumed to have come from the fish and included in the data.

Parasites were identified by morphospecies and grouped into four broad taxonomic categories (Fig. 7-10, the photos) due to the difficulty of identifying lower taxonomic levels. The morphotypes identified included a nematode, two distinct trematodes and tapeworm. Morphological examinations were made under a dissecting microscope at 0.8X to 6.3X magnification or without magnification for larger parasites such as the tapeworms.

Statistical analysis

All statistical analyses and data visualizations were performed using excel and R Studio (Version 1.2.1335). Linear regression was used to test for association between fish body mass and parasite aggregate biomass. Differences in parasite mass per unit fish mass between lakes and streams, and between geothermal and non-geothermal habitats were tested by mixed effects models ("lme" in the nlme package in R) including sampling location as a random factor.

RESULTS

Of a total of 134 fish dissected, parasites were found in 35%. Two sites, Hot Creek and Warm Lake, had the highest prevalence (79 and 74%, respectively), with Layton Springs having only two parasitized fish and Crowley Lake only one (Fig. 1). No parasites were observed in any of the other six sites.

Parasite mass per unit fish mass was greatest in the Warm Lake and Hot Springs sites, and significantly greater in geothermal sites than non-geothermal ($P = 0.03$, Table 1, Figure. 2). There was no significant difference in parasite load between lake and stream habitats ($P = 0.96$, Table 1).

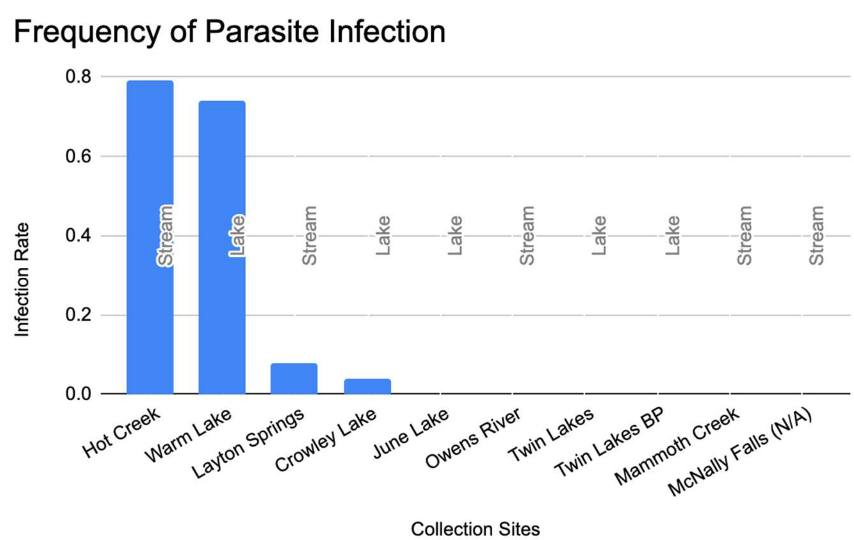

Figure 1: Infection rates of the 10 collection sites. Results indicate the total amount of fish with parasites found divided by the total amount of samples dissected for each site.

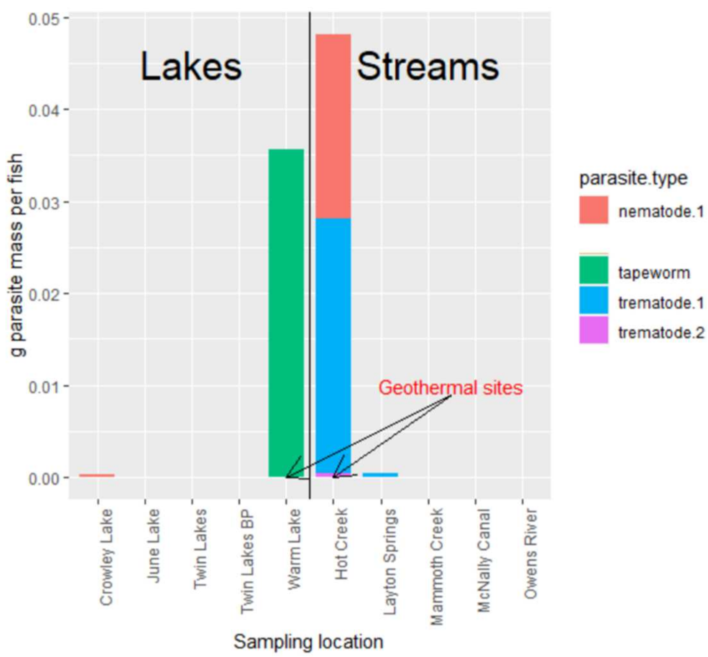

Figure 2: Stacked box plot showing parasite types and mean biomass load per location site. Hot Creek had the higher diversity of parasite types and highest mean mass per fish, while Warm Lake only had tapeworms. No other site had findings of parasites.

Table 1: Results of a linear mixed effects model testing the effects of habitat types (lakes vs. streams and geothermal vs. non-geothermal) on parasite mass per unit fish mass. Sampling location (the five lake and five stream sites) was included as a random effect in the model.

Source	DF	F	P
Intercept	1,7	4.62	0.07
Lake vs. stream	1,7	0.003	0.96
Geothermal vs. non-geothermal	1,7	7.65	**0.03**

Four morphologically distinct types of parasites were found (Fig. 7-10, the photos). Based on their morphology they were given four names to reference through dissections: tapeworm, nematode 1, trematode 1 and trematode 2. Hot Creek and Warm Lake fish were often infected with one or more parasite taxa (Fig. 2). Nematode 1, trematode 1 and trematode 2 were only found at Hot Creek, while the tapeworm was only found at Warm Lake. Crowley Lake had only one fish found with parasites (nematode 1) and Layton Springs had two observations (both trematode 1) while no parasites were observed at the other six sites. Trematode 1 had the highest average mass per fish of ±1.192 g, nematode 1 had a mass averaged of ±0.655 g per fish, and the tapeworm had an average mass of ±0.520 g per fish.

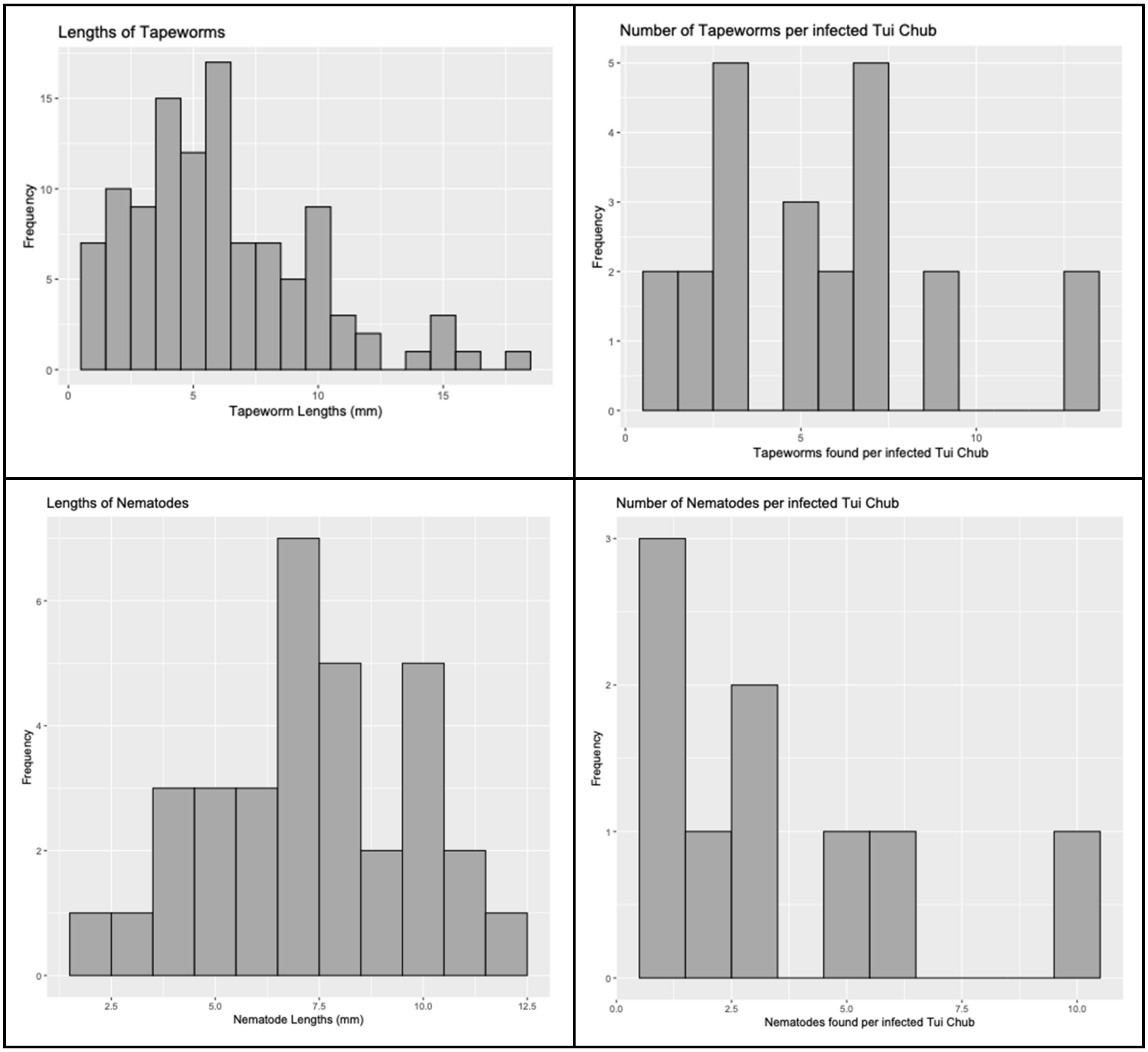

Figure 3: Histograms of lengths (mm) and number of parasites (nematodes or trematodes) found per individual Tui Chub.

A total of 109 tapeworms were found in the Warm Lake samples. The tapeworms' lengths ranged in sizes from 1.3 mm to 15.6 mm with an average of 6.22 mm (Fig. 3). When tapeworms were found, the average number was 6 tapeworms per fish. The nematodes ranged in sizes from 2.4 mm to 11.3mm with an average 6.39 mm (Fig. 3). Nematode-infected Tui Chub averaged 4 nematodes per fish.

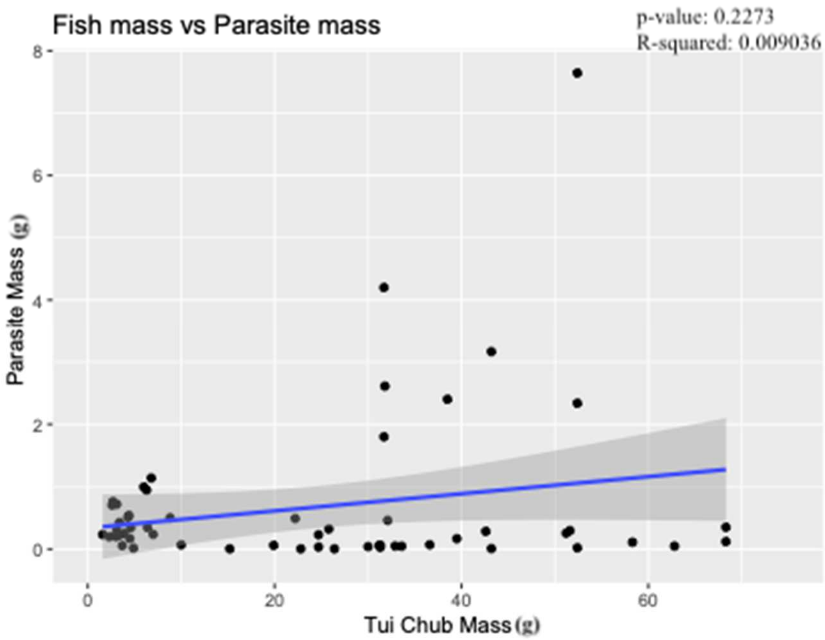

Figure 4: Scatterplot showing the mass of Tui Chub samples and the total parasite mass per infected fish. Each data point represents the whole parasite mass per individual infected fish. Line of best fit is the correlation between fish mass and parasite mass (p-value=0.2273) (R-squared value=0.009036).

Tui Chub weights prior to dissection ranged from 0.5g to 164.3grams. Parasite masses recorded ranged from 0.0205g to 7.644g among fish where parasites were observed. The mass of the fish was not significantly related to the mass of parasites (Fig. 4, p=0.2273)).

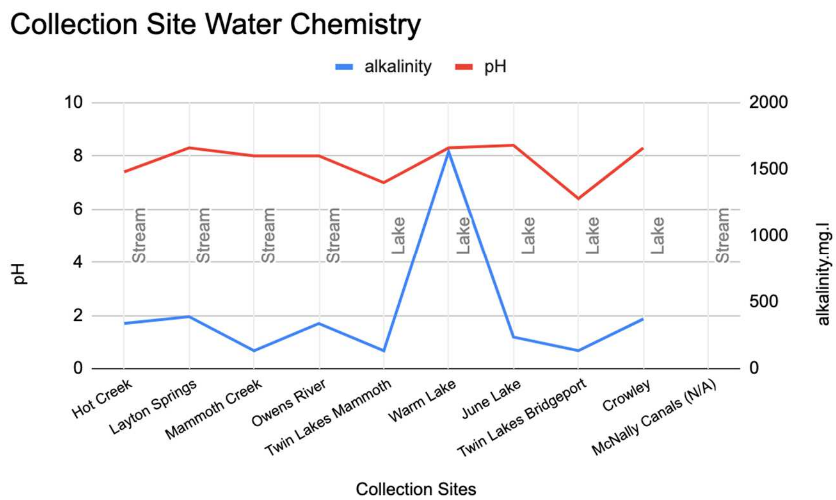

Figure 5: Alkalinity and pH at the ten collection sites where Tui Chub were collected from a single sampling occasion (McNally Falls information not collected). Sites vary by stream or lake.

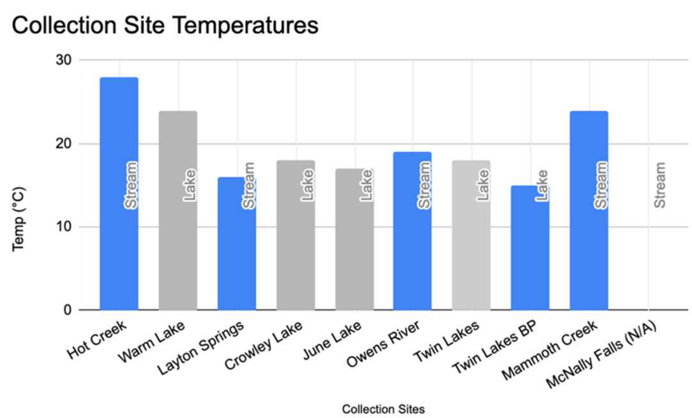

Figure 6: Temperatures of the ten collection sites where Tui Chub were caught are from a one-time sampling (McNally Falls information not collected).

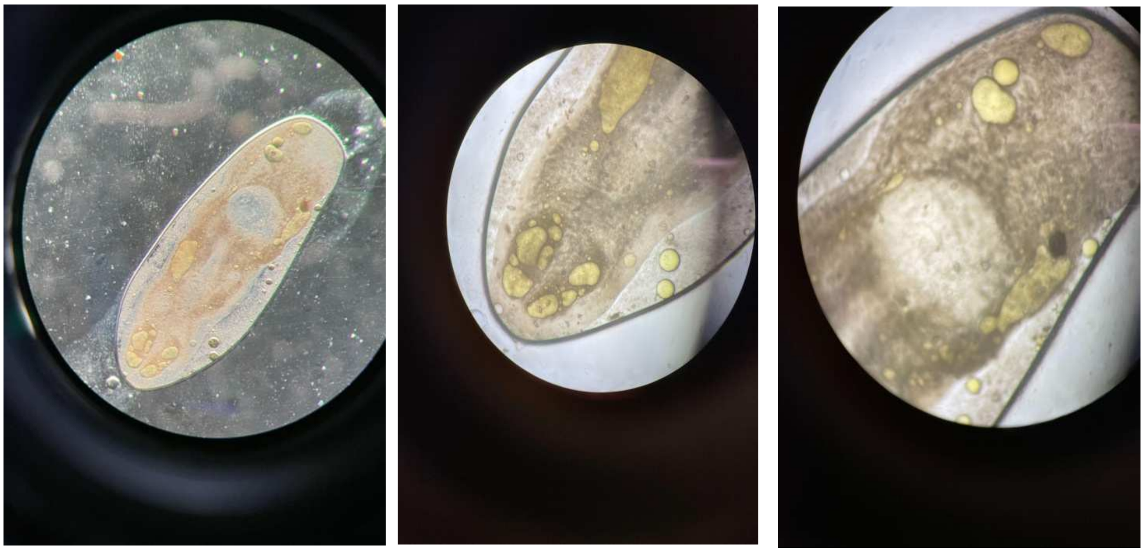

Figure 7: Microphotographs of Trematode 1, extracted from gill arches of Tui Chub sample # 77 (Hot Creek). Magnification at 4.0X. A- Excysted full body B- Excretory bladder C- Oral sucker

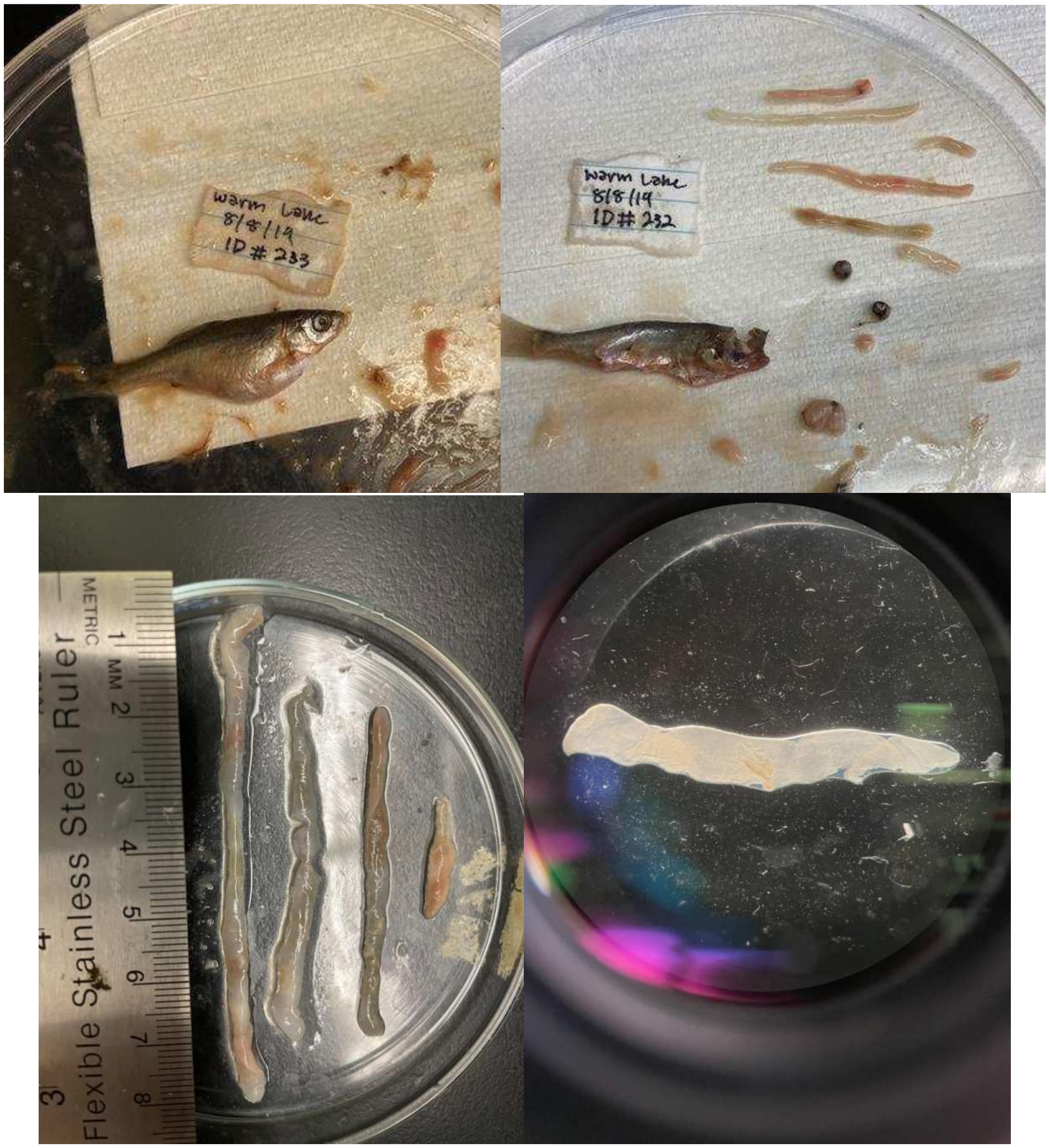

Figure 8: Image of infected Tui Chub samples (ID#283 and #282) from Warm Lake. Left- Prior to dissection, a bulge in the belly indicates tapeworms are present. Right- Post-dissection, tapeworms externally shown.
Images of tapeworms extracted from Tui Chub sample ID# 212 (Warm Lake). Left- Tapeworms on a petri dish with a ruler for size comparison. Fig._ shows average lengths of tapeworms. Right- Tapeworm at magnification of 0.8X.

Figure 9: Photos of Tui Chub Sample DVS (ID#93 from Hot Creek) infected externally and internally by Trematode 1. A- Tui Chub infected with Trematode 1 prior to dissection B-Embedded in the mouth region and under the chin of Tui Chub C- Embedded within gill arches.

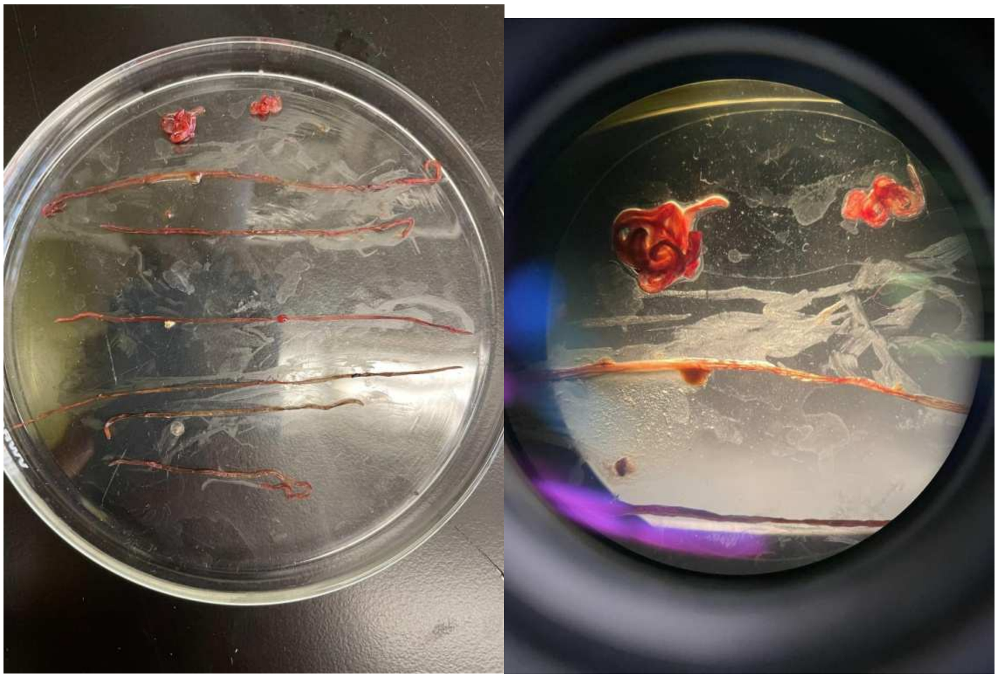

Figure 10: Nematodes removed from Tui Chub sample 82 (Hot Creek site). Left- Right- Magnification at 1.25X.

Trematode 1 was found both internally and externally. Externally trematode 1 was embedded in the fish muscle throughout the body, embedded within the fins, the buccal cavity, and abundant in the gill arches. Internally, when dissected, this parasite was found in the body cavity. Tapeworms and nematode 1 were found only within the body cavity.

DISCUSSION

My thesis research found four parasites present within hybrid Tui Chub species, Owens (*Siphateles bicolor snyderi*) and Lahontan (*Siphateles bicolor obesa*) Tui Chub at ten freshwater collection sites in Eastern California. The infection rate and parasite composition (Fig.2) both varied among sampling sites, ranging from no parasites found at six sites to >70% of fish containing parasites at the two geothermal sites. Although they were not the warmest of the ten sites at the single time of observation, the two sites where parasites were found, Hot Creek and Warm Lake, were both geothermal with high salinity and alkalinity. Warm Lake was an outlier with anomalously higher alkalinity compared to the others. Of these two sites, one was a stream (Hot Creek) and the other a lake (Warm Lake). The results showed no indication of a difference in parasite load between lakes or streams. Fish size and parasite load or infection rate also showed no correlation, as smaller fish had similar parasite loads as larger ones. These results indicate that temperature and/or water chemistry most likely plays a strong role in the abundance and composition of parasites infecting the hybrid Tui Chub species in lakes and streams. I found no indication of consistent differences between lakes and streams in parasite loads. My findings suggest that the thermal and chemical environment of geothermal springs has the greatest impact on parasitism in Tui Chub.

Among the 10 collection sites, parasites were most common in two, Hot Creek and Warm Lake, which, as their names indicate, are influenced by geothermal groundwater. Only two other sites, Crowley Lake and Layton Springs, had just two and one instances where parasites were found and the other sites had none at all. One key variable that differentiated the sites, Hot Creek and Warm Lake, where parasites were found most common, was that they were warmer than the other sites (Fig. 6). Although Warm Lake was not warm at the single time of sampling, it is frequently considerably warmer than ambient temperatures. The temperature varies over time as a function of the fluctuating input of geothermal groundwater and runoff of surface water from the watershed. The warmer environments and higher parasitic load are consistent with some previous studies such as Paull et al. (2011), which also showed a positive relationship between parasitism and water temperature. The warmer temperature may be a more suitable habitat for these certain parasites to thrive. My results agree with Alizer et al. (2013) who predicted that climate change and warming temperatures should increase parasite transmission. While warming may generally favor parasitic infection, the scope of my study is limited to only investigating fish during the summertime. The environmental temperature was only recorded once by PhD student Henry Baker, so the averages and variability are unknown. In addition to being warmer, a key feature of Warm Lake that differentiated it from the other sites was the high alkalinity level. Warm Lake's alkalinity level was significantly higher than any of the other sites (Fig.5). Alkalinity is a measure of acid buffering capacity that is determined by concentrations of ions like bicarbonate and other minerals of geologic origin that also produce high salinity. High abundances of snails were observed in the geothermal sites. Snails may increase infection of fish by providing intermediate hosts for different life stages of parasites. As stated by Shaw (2009) where there's higher parasitic loads in fish in areas where the California horn snail, *Cerithidea californica*, is abundant**.** Whether water chemistry or temperature is the primary driver of parasitism rates is impossible to determine from my data.

The mass of the Tui Chub samples ranged from 0.5g to 164.3g. From previous literature I expected that the larger fish would have more parasites. But unlike the findings from Sasal and Monrand et al. (1998) that larger fish host a greater parasitic abundance, I found no correlation between size of fish and parasitic load (Fig.4; p-value 0.2273), as smaller fish at times had a higher parasite load in mass than fish that were larger. This indicates that smaller fish have just as likely a chance as larger fish to become infected. The parasites found at both Warm Lake and Hot Creek did not vary in masses in relation to how large the fish was. This shows that the parasites do not differentially infect fish based on size.

Four morpho-types of parasites were found, of which three, tapeworm, nematode 1 and trematode 1, were the most common. Trematode 2 was only found in three instances. They were differentiated by their morphology. Based on morphological characteristics and location (freshwater environments in California) possible identities of the parasites found for the tapeworms are the cestode tapeworm, *Ligula intestinalis*, or the Asian fish tapeworm, *B. acheilognathi*. For the nematode found, its possibly in the same genus as *Eustrongylides*. The cestode tapeworm (*B.acheilognathi*) matched with previous studies, and has been found to infect Tui Chub (Archdeacon et al. 2018; Klinger et al. 2009). *L. intestinalis* has also been found to infect fish such as the cyprinid species, but there is nothing in the literature about infected Tui Chub (). The tapeworms were found only in the higher alkalinity site, Warm Lake, so it's possible that this type of environment is most suitable for them. Although I have not found studies of alkalinity levels and either of the tapeworms correlating together, my research may be an indicator that in these higher alkaline environments, tapeworms are most prevalent and able to infect host. The nematode was only found at Hot Creek. Morphologically it appears to be similar to the genus *Eustrongylides*. This genus of parasite has also been found infecting a common prey item of Tui Chub, which are soft-bodied worms (Klinger and Floyd et al. 2009). These studies have led me to believe that the parasites found in my research are the same, if not similar. I did not find similar studies that would indicate the likely identities of trematode 1 or trematode 2. These parasites could be identified by DNA sequencing in future research but are difficult to resolve morphologically without specialized training. There are frozen samples achieved that can be used for future DNA sequences.

The locations of the parasites on or within the Tui Chub varied among parasites and sampling locations. The tapeworms present within the Warm Lake Tui Chub were mostly within the body cavity and were found when the underbelly was dissected. They occupied the whole area of the body cavity and organs were slightly squashed. The tapeworms within the body cavity were visible when examining the exterior of the fish prior to dissection, as it left a bulge under the abdomen (Fig. 8). Although I found nothing in the literature about L. i*ntestinalis* infecting Tui Chub directly, there have been studies done showing that this tapeworm has an intense cellular response for fish host they infect (Hoole et al. 2010). Although I was unable to monitor fish behavior or health for my research, the impact of these parasites on host behavior, health and ecology is a fruitful avenue for future research. Previous literature suggests that fish parasites may greatly affect their hosts' fitness (Lafferty & Morris et al. 1996). The parasites I examined could be identified by genetic analysis to determine whether other studies have shown their effects on fish. Examining parasite infected vs. uninfected fish in a controlled environment would be ideal to identify whether parasites affect host behavior. The potential impact of parasitism may have major implications for recovery of threatened Owens Tui Chub and identifying suitable sites for population re-introduction.

My research found four parasites that infect the hybrid Tui Chub in freshwater lakes and streams of the Owens Valley in the Eastern Sierra Nevada. The major environmental features correlated with parasite infection loads were temperature and alkalinity. There could be multiple explanations for the absence of parasites at the other sites. Archdeacons et al. (2008) suggested that parasites may not be found within fish if the parasite's life-cycle was complete. On the other hand, these findings could potentially support Weber et al. (2017) if the Tui Chub species at the eight other locations have evolved immunity against the parasites, suppressing their growth enough to explain their absence during dissections. My study was based on Tui Chub collected during summer, so further research could determine whether parasitism varies seasonally. In addition, the temperature, pH and alkalinity were measured only once, and no information was taken for one of the sites (McNally Falls). Long term monitoring would provide a more accurate representation of environmental variability at the sites. Consistent patterns are often difficult to detect in parasite communities, and this study was of a short duration so it’s difficult to truly determine the cause of why parasites are found in some sites while not in others. Despite the limitations, my research showed that parasites use the Tui Chub as a host at these freshwater sites. The thermal and chemical environments of geothermal springs were the most important drivers of parasitism for the Tui Chub. Further research is needed to understand causes of variation and effects of parasitism in Tui Chub.

REFERENCES

Altizer, Sonia & Ostfeld, Richard & Johnson, Pieter & Kutz, Susan & Harvell, Catherine. (2013). Climate Change and Infectious Diseases: From Evidence to a Predictive Framework. Science (New York, N.Y.). 341. 514-9. 10.1126/science.1239401.

Archdeacon, T. P., Iles, A., Kline, S. J., & Bonar, S. A. (2010). Asian fish tapeworm Bothriocephalus acheilognathii in the desert southwestern United States. Journal of Aquatic Animal Health, 22(4), 274–279. https://doi.org/10.1577/H09-009.1

Auld, S. K., & Tinsley, M. C. (2015). The evolutionary ecology of complex lifecycle parasites: linking phenomena with mechanisms. *Heredity*, *114*(2), 125–132. https://doi.org/10.1038/hdy.2014.84

Barber, I., Hoare, D. & Krause, J. 2000 Effects of parasites on fish behaviour: a review and evolutionary perspective. Rev. Fish Biol. Fish. 10, 131–165. (doi:10.1023/A:1016 658224470)

Bolnick, D. I., Resetarits, E. J., Ballare, K., Stuart, Y. E., and Stutz, W. E.. 2020. Scale□ dependent effects of host patch traits on species composition in a stickleback parasite metacommunity. Ecology 101(12):e03181. 10.1002/ecy.3181

Brönmark C, Skov C, Brodersen J, Nilsson PA, Hansson LA (2008) Seasonal Migration Determined by a Trade-Off between Predator Avoidance and Growth. PLOS ONE 3(4): e1957. https://doi.org/10.1371/journal.pone.0001957

Calhoun, D. M., McDevitt-Galles, T., & Johnson, P. T. J. (2018). Parasites of invasive freshwater fishes and the factors affecting their richness. Freshwater Science, 37(1), 134–146. https://doi.org/10.1086/696566

Crowden, A. E., & Broom, D. M. (1980). Effects of the eye fluke, Diplostomum spathaceum, on the behaviour of dace (Leuciscus leuciscus). Animal Behaviour, 28(1), 287–294. https://doi.org/10.1016/S0003-3472(80)80031-5

Farkas, T., Fodor, E., Kitajka, K., & Halver, J. E. (2001). Response of fish membranes to environmental temperature. Aquaculture Research, 32(8), 645-655.

Hoole, D., Carter, V., & Dufour, S. (2010). Ligula intestinalis (Cestoda: Pseudophyllidea): An ideal fish-metazoan parasite model? *Parasitology, 137*(3), 425-438. doi:10.1017/S0031182010000107

Jobling M (1994) Fish bioenergetics. London: Chapman and Hall.

Lafferty, Kevin & Morris, Kimo. (1996). Altered Behavior of Parasitized Killifish Increases Susceptibility to Predation by Bird Final Hosts. Ecology. 77. 1390-1397. 10.2307/2265536.

Lafferty, K. D., & Kuris, A. M. (2002, November 1). Trophic strategies, animal diversity and body size. Trends in Ecology and Evolution. https://doi.org/10.1016/S0169-5347(02)02615-0

Leunda PM, Galicia D, Miranda R, Madoz J, Parmenter S. 2013. Bone-to-Body biometric relationships for Owens and Lahontan tui chubs and their hybrids in California. Journal of Fish and Wildlife Management 4(2):326–331; e1944- 687X. doi:10.3996/022013-JFWM-018

Loehle, C. (1995), Social Barriers to Pathogen Transmission in Wild Animal Populations. Ecology, 76: 326-335. https://doi.org/10.2307/1941192

Kennedy, C. (2009). The ecology of parasites of freshwater fishes: The search for patterns. Parasitology, 136(12), 1653-1662. doi:10.1017/S0031182009005794

Klinger, R. & Floyd, R.. (2009). Introduction to Freshwater Fish Parasites. University of Florida, Fisheries and Aquatic Sciences Department, Florida Cooperative Extension Service, IFAS Extension Document CIR716.. 716.

Krkosek, M., M. Lewis, A. Morton, L.N. Frazer & J. Volpe. 2006. Epizootics of wild fish induced by farm fish. Proceedings of the National Academy of Sciences of the USA. 103, 15506-15510.

Kuris, A. M., Hechinger, R. F., Shaw, J. C., Whitney, K. L., Aguirre-Macedo, L., Boch, C. A., Dobson, A. P., Dunham, E. J., Fredensborg, B. L., Huspeni, T. C., Lorda, J., Mababa, L., Mancini, F. T., Mora, A. B., Pickering, M., Talhouk, N. L., Torchin, M. E., & Lafferty, K. D. (2008). Ecosystem energetic implications of parasite and free-living biomass in three estuaries. Nature, 454(7203), 515–518. https://doi.org/10.1038/nature06970

Moss, W. E., T. McDevitt-Galles, D. M. Calhoun, and P. T. J. Johnson. 2020. Tracking the assembly of nested parasite com- munities: using b-diversity to understand variation in parasite richness and composition over time and scale. Journal of Animal Ecology 89:1532–1542.

Moyle PB. 2002. Inland fishes of California. Berkeley and Los Angeles: University of California Press.

Northcote TG (1978) Migratory strategies and production in freshwater fishes. In: Gerking SD, editor. Ecology of freshwater fish production. Oxford: Blackwell. pp. 326–359.

Olkeba, B. K., Boets, P., Mereta, S. T., Yeshigeta, M., Akessa, G. M., Ambelu, A., & Goethals, P. (2020). Environmental and biotic factors affecting freshwater snail intermediate hosts in the Ethiopian Rift Valley region. *Parasites & vectors*, *13*(1), 292. https://doi.org/10.1186/s13071-020-04163-6

Paull, S. H., & Johnson, P. T. J. (2011). High temperature enhances host pathology in a snail-trematode system: Possible consequences of climate change for the emergence of disease. Freshwater Biology, 56(4), 767–778. https://doi.org/10.1111/j. 1365-2427.2010.02547.x

Johnson, P. T. Johnson, Jonathan M. Chase, Katherine L. D_Dosch, Richard B. Hartson, Jackson A. Gross, Don J. Larson, Daniel R. Sutherland, Stephen R. Carpenter. "Aquatic Eutrophication Promotes Pathogenic Infection in Amphibians." Proceedings of the National Academy of Sciences, vol. 104, no. 40, 2007, pp. 15781–15786., doi:10.1073/pnas.0707763104.

Richgels, K. L. D., J. T. Hoverman, and P. T. J. Johnson. 2013. Evaluating the role of regional and local processes in struc- turing a larval trematode metacommunity of Helisoma trivol- vis. Ecography 36:854–863.

Sasal, P., Desdevises, Y., & Morand, S. (1998). Host-Specialization and Species Diversity in Fish Parasites: Phylogenetic Conservatism? Ecography, 21(6), 639-643. Retrieved June 5, 2021, from http://www.jstor.org/stable/3682856

Smith, H. D. (1973). Observations on the cestode Eubothrium salvelini in juvenile sockeye salmon (Oncorhynchus nerka) at Babine Lake, British Columbia. Journal of the Fisheries Research Board of Canada 30, 947–964.

Shaw, Jenny & Korzan, Wayne & Carpenter, R.E. & Kuris, Armand & Lafferty, Kevin & Summers, Cliff & Øverli, Øyvind. (2009). Parasite manipulation of brain monoamines in California killifish (Fundulus parvipinnis) by the trematode Euhaplorchis californiensis. Proceedings. Biological sciences / The Royal Society. 276. 1137-46. 10.1098/rspb.2008.1597.

Shaw, J C, and Ø Øverli. "Brain-encysting trematodes and altered monoamine activity in naturally infected killifish Fundulus parvipinnis." Journal of fish biology vol. 81,7 (2012): 2213-22. doi:10.1111/j.1095-8649.2012.03439.x

Weber JN, Steinel NC, Shim KC, Bolnick DI. Recent evolution of extreme cestode growth suppression by a vertebrate host. Proc Natl Acad Sci U S A. 2017 Jun 20;114(25):6575-6580. doi: 10.1073/pnas.1620095114. Epub 2017 Jun 6. PMID: 28588142; PMCID: PMC5488926.

Welicky, R. L., Demopoulos, A. W. J., & Sikkel, P. C. (2017). Host-dependent differences in resource use associated with Anilocra spp. parasitism in two coral reef fishes, as revealed by stable carbon and nitrogen isotope analyses. Marine Ecology, 38(2). https://doi.org/10.1111/maec.12413

Wilkins, J.L., T.J. DeBates, and D.W. Willis. 2002. Food habits of yellow perch, Perca flavescens, in West Long Lake, Nebraska. Transactions of the Nebraska Academy of Sciences 28: 49-56.

Williams, J.E., J.E Johnson, D.A. Hendrickson, S. Contreras-Balderas, J.D. Williams, M. Navarro-Mendoza, D.E. McAllister, and J.E. Deacon. 1989. Fishes of North America Endangered, Threatened, or of Special Concern. 14(6):2-20.

Winemiller, K. 0. and Rose, K. A. 1992. Patterns of life Humphery-Smith, I. 1989. The evolution of phylogenetic story- diversification in North American fishes: implicacificity among parasitic organisms. - Parasitol. Todatyion5s: for population regulation. - Can. J. Fish. Aq. Sci. 49: 385-387. 2196-2218.

Xi He & James F. Kitchell (1990) Direct and Indirect Effects of Predation on a Fish Community: A Whole-Lake Experiment, Transactions of the American Fisheries Society, 119:5, 825-835, DOI: 10.1577/1548-8659(1990)119<0825:DAIEOP>2.3.CO;2

Zedler, Joy. (2000). Progress in Wetland Restoration Ecology. Trends in ecology & evolution. 15. 402-407. 10.1016/S0169-5347(00)01959-5.