Environmental Factors in Trematode Parasite Dynamics: Water Temperature, Snail Density and Black Spot Disease Parasitism in California Steelhead (*Oncorhynchus mykiss*)

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ABSTRACT

Climate change will raise water temperatures in rivers and streams that provide critical habitat to sensitive salmonid species like steelhead trout (*Oncorhynchus mykiss*). Corresponding changes in environmental conditions may lead to increases in rates of trematode parasitism in the form of black spot disease, an under-studied parasitic condition in steelhead. To better understand the parasite dynamics of black spot disease in steelhead, I examined the relationship between water temperature, snail density and black spot disease infection rates in steelhead and two comparable fish species, stickleback and roach, over the course of one summer season. By continuously recording water temperature at seven sample sites and capturing fish in three sampling efforts spread over the course of a summer, I determined that a positive relationship between water temperature and black spot disease infection rates existed in steelhead, but not in stickleback or roach. Water temperature, more than spatial and temporal differences in watershed characteristics, was the most significant factor associated with black spot parasitism rates in steelhead. I also determined that snail density was positively related to both water temperature and black spot infection rates in steelhead. Both snail density and infection rates in steelhead increased rapidly at a threshold temperature of 23° C, suggesting that high water temperatures lead to the ideal environmental and biotic conditions necessary for high rates of black spot parasitism in steelhead.

KEYWORDS

black spot disease, steelhead, trematode, parasite, *Neascus*, salmonid, climate change, Eel River

INTRODUCTION

As climate change is predicted to intensify drought in the western U.S. in the coming years (Cook et al. 2004), decreased water levels, decreased flows and increased water temperatures in rivers have the potential to pose a threat to many freshwater fish (Lake 2011). High water temperatures may be especially detrimental for salmonid fish (Richter and Kolmes 2005) and may result in increased rates of parasitism. High water temperatures have been associated with increased parasitism in many freshwater aquatic species, including salmonids (Karvonen et al. 2010, Marcogliese 2008, Poulin and Mouritsen 2006).

Trematode flukes are one type of freshwater parasites that proliferate in warm water temperatures (Paull and Johnson 2011 and Poulin 2006). Black spot disease is a condition caused in fish by parasitism of various species of trematode flukes of the genus *Neascus* (Berra and Au 1987, Markle et al. 2014, Meyers et al. 2008). The trematodes emerge from aquatic snails, their primary host, and penetrate the skin of fish, causing a melanin pigment reaction that produces a black spot around the parasitic cyst within 22 days (Berra and Au 1987, Northcote 1957, Teixeira-de Mello and Eguren 2008) that can persist for up to 4.5 years in some fish species (Hoffman and Putz 1965). The life cycle of the parasite continues as infected fish are eaten by birds, the final host (Lane and Morris 2000). The birds eventually defecate the parasite's eggs into river water where they emerge and swim into aquatic ram's horn snails (Genus *Planorbella*) (Turgeon et al. 1998), continuing the infection cycle.

Despite well-studied life cycle characteristics, the fitness effects of black spot disease in fish are still debated. Various species of infected freshwater fish have shown increased predation by birds and juvenile death rates (Harrison and Hadley 1982 and Markle et al. 2014) and significant weight loss at high levels of parasite load (Hunter and Hunter 1938 and Lemly and Esch 1984). Reports in infected coho salmon (*Oncorhynchus kisutch*) show possibly lower overwinter survival rates for heavily infected fish (Ferguson et al. 2010) but minimal direct effects on growth and metabolism (Ferguson et al. 2011). Because negative fitness effects seem to be more pronounced at high levels of parasitism, it is necessary to determine significant fish populations that may be exposed to environmental conditions and water temperatures that cause increased parasite loads.

Previous studies on salmonid fishes have demonstrated a positive correlation between water temperature and black spot disease, also known as black grub or *Neascus spp.* (Cairns et al. 2005, Ferguson et al. 2011, Ferguson et al. 2010). Steelhead trout (*Oncorhynchus mykiss*) are a federally protected salmonid species native to the west coast of North America (Good et al. 2005) that are susceptible to the trematodes that cause black spot disease. However, there is only minimal evidence of parasite incidence or prevalence in the species aside from a few informal reports from California (Morse 2003). Despite many investigations of black spot disease and environmental correlates in coho salmon, a close relative of steelhead (Cairns et al. 2005, Ferguson et al. 2011, Ferguson et al. 2010, Rodnick et al. 2008), no formal studies concerning the condition in steelhead, specifically, have been carried out and the prevalence and patterns of black spot disease parasitism in the species are unknown. Additionally, few studies have examined the prevalence of black spot disease in other freshwater fish that share streams with salmonids.

To better understand the parasite dynamics of black spot disease in steelhead, I examined the relationship between water temperature, secondary host (snail) density and black spot disease infection rates in steelhead and two comparable fish species, threespine stickleback (*Gasterosteus aculeatus*) and California roach (*Hesperoleucus symmetricus*), over the course of one summer season in a coastal watershed in Northern California. Specifically, I determined the relationship between water temperature and black spot disease prevalence in the different species with respect to the spatial and temporal differences in water temperature at differing locations throughout the watershed. I also determined the relationship between snail density and water temperature, including their combined influence on overall black spot disease prevalence. I hypothesized a positive association between water temperature and black spot prevalence in all species and at all sites along the main stem of the river, with downstream sites displaying the highest infection rates and upstream sites showing increasing rates of infection as the summer progressed and water temperature increased. I assumed that little to no black spot would be seen in steelhead occupying cooler tributaries, based on similar studies and results in coho salmon in Oregon (Cairns et al. 2005, Ferguson et al. 2011, Ferguson et al. 2010). Additionally, I predicted that sites with higher snail abundance would display highest frequency of black spot disease. These hypotheses were tested by sampling steelhead, stickleback and roach from pools with varying water temperature and snail densities in the main stem and various tributaries of the South Fork Eel River in Mendocino County, CA.

METHODS

Site description and background

From June 5 to August 21 2014, I sampled fish from an approximately 9 km section of the main stem of the South Fork Eel River (hereafter 'SFER') and two tributaries located on the UC Angelo Coast Range Reserve (39.733°N, 123.65°W), a UC Berkeley field station in Northern Mendocino County, CA (Figure 1). The reserve drains 4,320 square kilometers (acres) of mountain wilderness ranging from 378 to 1,290 meters in elevation (UC Natural Reserve System 2014). The site is typical of other spring-fed headwaters of coastal rivers in Northern California, with mixed hardwood forest (old-growth Douglas-fir as well as tan oak and madrone) atop impermeable shale and bedrock. On average, the site receives 215 centimeters of precipitation (Mast and Clow 2000), mostly during the winter months, but experiences hot and arid summers (with air temperatures averaging 29° C) that result in low flow and increased water temperature in the downstream reaches of the main stem of the river. The aquatic ecology of the SFER is similar to other Northern California coastal rivers, with coho and chinook salmon (*Oncorhynchus tshawytscha*), threespine stickleback, California roach, California sucker (*Catostomus occidentalis*), Pacific lamprey (*Lampetra tridentate*), non-native pikeminnow (*Ptychocheilus grandis*), and various species of frogs, salamanders and snails.

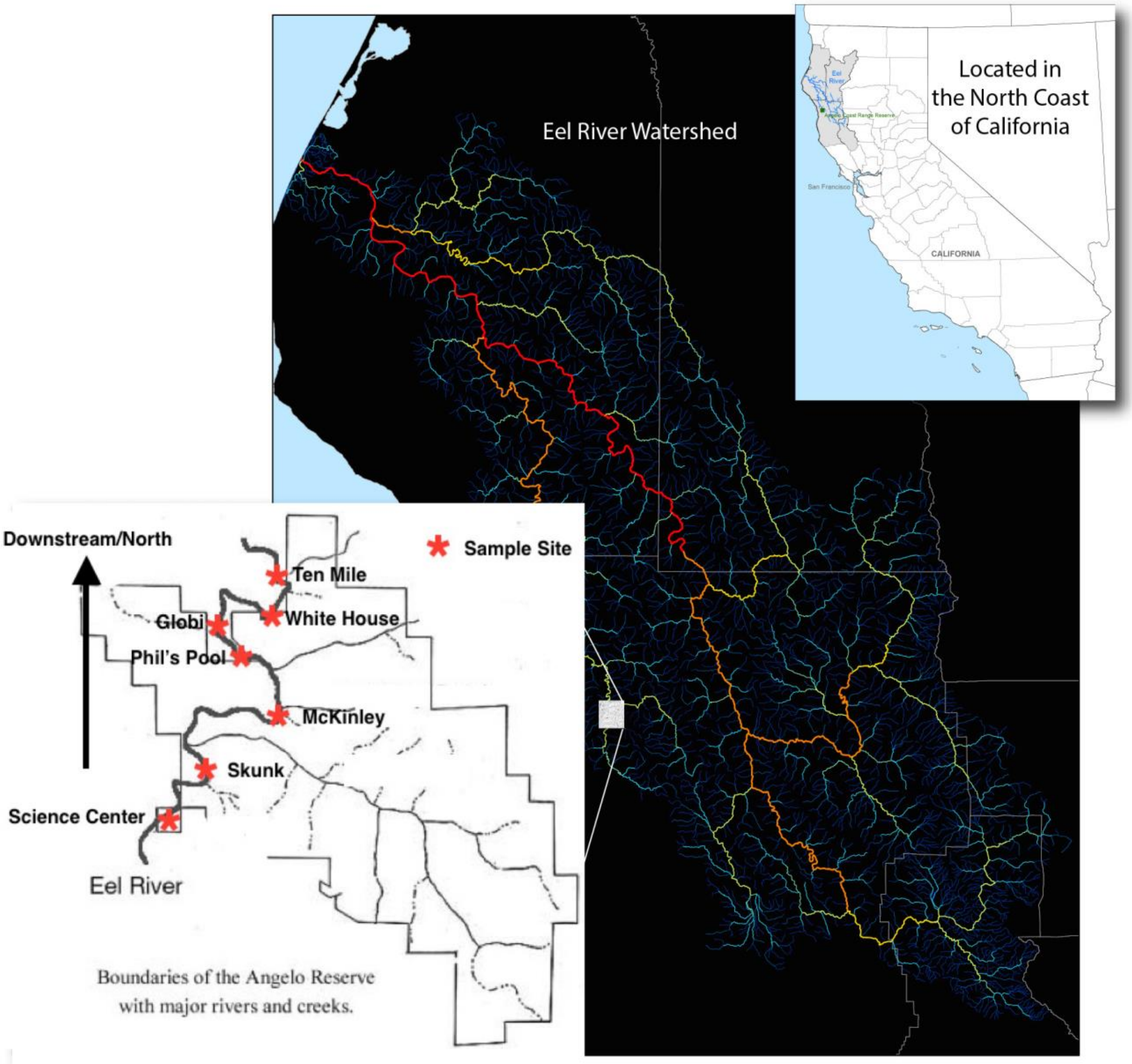

Figure 1. Study site location in the upper headwaters of the SFER in Northern California. Red stars denote sample sites along the main stem SFER and an arrow pointing downstream/north represents the flow of water through the study site.

I sampled from seven sites on the main stem, spaced from 1 to 2.8 km apart, chosen for best accessibility, fishing success, and to reflect a gradient of increasing water temperature as I moved from the upstream to the downstream sites (Figure 1). I, along with other researchers, also sampled fish throughout the length of two tributaries linked to the main stem by coordinating with a simultaneous research project that required a comprehensive sampling of these waters. At each site, and in each tributary, I placed temperature data loggers (HOBO Pendant Temperature/Light Data Logger 64K - UA-002-64) set to record water temperature every 10 minutes about 10 cm from the substrate during the first sampling effort.

Fish and snail sampling

At all main stem sample sites during each sampling effort, we caught fish with a team using large dip nets, seine nets, and, at times, a backpack electrofisher unit (Smith-Root LR-24). Captured fish were placed in in-stream buckets with mesh siding before being anesthetized with a tab of effervescent Alka Seltzer Gold (1000 mg Anhydrous Citric Acid, 344 mg Potassium Bicarbonate, and 1050 mg Sodium Bicarbonate) in a separate bucket. For each anesthetized fish, I weighed (in grams), measured fork length (FL) (in mm, from nose of fish to middle tail fork), and visually assessed for black spot disease infection. If the fish had any level of infection, I categorized it as low, moderate, or high and took a photo of the fish before placing it in a separate freshwater bucket to recover before being released. A similar sampling process was carried out in the tributaries, where another research project demanded a more comprehensive sampling of the reaches. This meant that there were no concrete sample sites, but all fish caught at any location within a tributary were quickly assessed for black spot infection before being analyzed for the separate project.

During data analysis, visual parasite load estimations from the field were confirmed as being accurate by using photos to count number of infections per fish at each categorical infection level. By comparing the photos of six fish from each infection level (low, moderate and high), I determined that fish categorized as low had a sample range of 0 to 6 spots (Avg: 3, SD: 2), moderate had 6 to 23 spots (Avg: 12, SD: 7) and high had 16 to 151 and above spots (Avg: 79, SD: 58). Although there was some overlap between categories in terms of number of spots per fish, the majority of fish were clearly defined as being within the correct infection category.

We captured 886 fish (steelhead, stickleback and roach) from the main stem over three sampling efforts (June 5 –June 30, June 30 –July 13, and August 21) representing early, mid and late summer. In addition, over two thousand steelhead were captured and assessed in the ongoing tributary samplings. Infection rate is reported as percentage/proportion of fish infected per site and parasite load (hereafter: intensity) is the relative number of parasites per fish based on a categorical scale. Because samples during the first two efforts consisted of mostly juvenile steelhead, we used an electrofisher during the third effort to obtain larger and older steelhead that were too difficult to catch using netting techniques.

To determine the density of ram's horn snails at each site, I carried out a snail survey mid-study (July 14) at all main stem sites and at one site in one of the sampled tributaries. Because snails were most commonly found grazing algae near the banks, I surveyed a quadrat rectangle that measured 3m along the bank and extended 2m out into the water. I selected feasible sites along the bank at each sample location and set up the quadrat before taking exactly 15 minutes to collect all the snails I could see and feel on the substrate. Collected snails were put into a jar and counted to determine the number of snails observed in each site's quadrat.

Data analysis

To determine a water temperature metric to correspond to each sampling effort, I summarized water temperature data for each site and each sampling effort by averaging the maximum daily water temperature that was recorded during a seven-day timeframe centered on the sampling date. This resulted in three average daily maximum (ADM) temperature metrics for each sample site, one for each sampling effort, representing early, mid and late summer (similar to the 7-day ADM of Cairns et al. (2005)). A separate 30-day ADM was calculated by averaging the maximum temperature at each site for a 30-day period centered on the date of a one-time snail density survey. This was done to include more days of temperature data in the metric since there was only one sampling (as opposed to the three samplings in fish).

For the first early summer sampling effort, I placed temperature data loggers at most sites on the date of the initial sampling. Thus, for the early summer ADM for each site, the seven-day average includes the initial sampling date and the six following days. Because temperature loggers were removed during the last sampling, the late summer ADM I calculated in a similar fashion, except I averaged the six days leading up to and including the final sampling.

To relate water temperature to rates of parasitism, I conducted a mixed effects logistic regression model in which water temperature (ADM) predicted the response of infection rates in the various species. Logistic regression was the chosen statistical model because samples of fish were not independent due to multiple samplings at the same site and the fact that infection rate was reported as a binomial variable (as a proportion). Site location for each sampling was included as a random effect and the ranking of sites as being upstream or downstream in relation to the central point of the sample reach was included as a fixed effect. I then performed a stepwise model selection to determine whether sampling date, site location within the watershed, or water temperature had the most significant influence in the model. Additionally, I conducted an analysis of variance (ANOVA) to determine whether there were differences in infection rates between fish (of all species) of different size and weight classes.

To determine the relationship between snail density and water temperature, I performed a simple linear regression on snail density (logarithmically transformed) and the 30-day ADM for each site. I also performed a simple linear regression on snail density and the average overall infection intensity in steelhead for each site to explore the relationship between snail abundance and steelhead infection rates throughout the watershed over the course of the study.

All data analyses were carried out using R and R commander (R Development Core Team 2014).

RESULTS

7-day ADM water temperature in the main stem ranged from a minimum of 18.96° C to a maximum 25.32° C over the course of the study. ADM water temperature for one site (locally known as “Phil’s Pool”) exceeded 27° C for the last sampling effort and was excluded from my data analysis because the data logger was exposed to unrealistically warm in-stream conditions due to sever water level drop. Water temperatures increased over the summer at most sample sites (Figure 2a), with the most significant increase occurring between the first and second sampling efforts (Figure 3). Regardless of the sampling event, water temperature was always highest at downstream sites (Figure 2b). Water temperature in the tributaries was significantly lower and remained relatively constant throughout the summer, ranging from approximately 13 to 18° C.

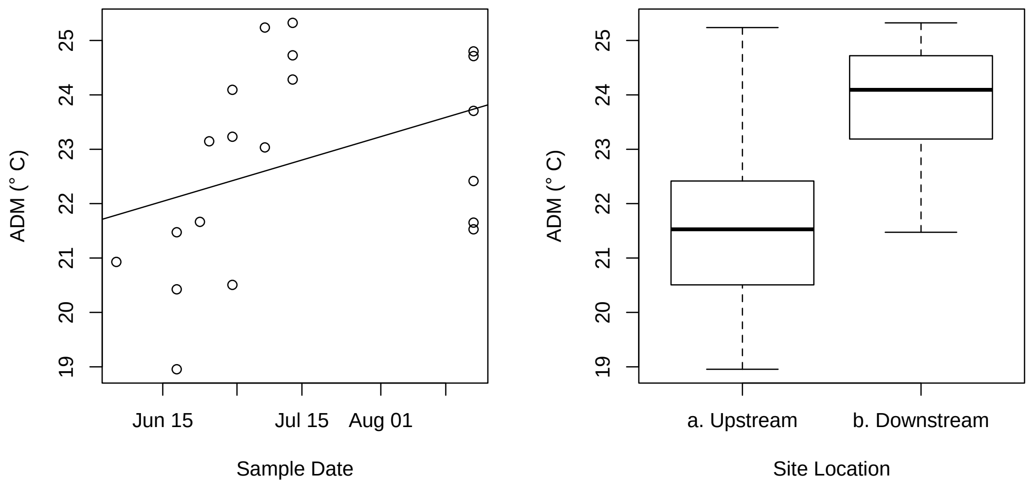

Figure 2 (a & b). Water temperature in relation to sample date and site location. Increases in water temperature were tied to trends in sample date and upstream/downstream site location. As the summer progressed, water temperature generally increased. Downstream sites (n=4) showed significantly higher ADM water temperatures. Upstream sites (n=3) were those located upstream of Phil's Pool (see Table 1 for site reference).

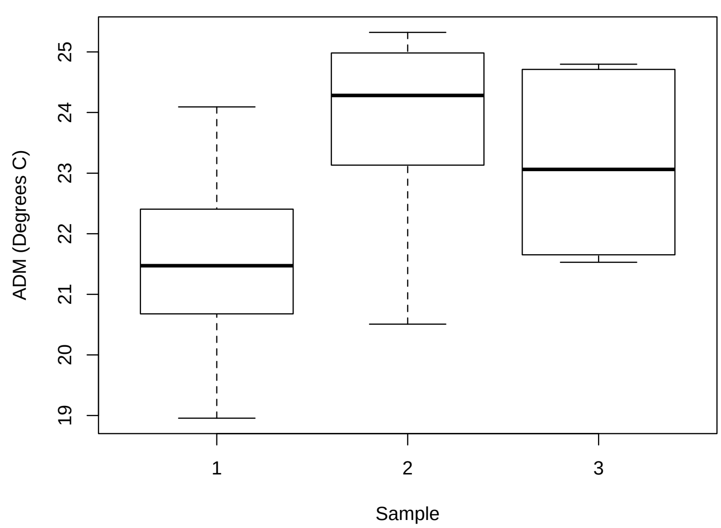

Figure 3. Temporal summer water temperature trends. The early, mid, and late summer sampling are represented here as 1, 2 and 3, respectively. The last two sampling efforts, carried out in mid and late summer, saw higher ADM water temperatures than the first. The greatest temperature increase was between the first and second sampling event. ($P < 0.0001$)

Table 1. Total summary of ADM water temperature and infection frequencies at each sample site. This table summarizes the 7-day ADM water temperature (° C) for each site and the infection rates (%) of each species (SH=Steelhead, R=Roach, SB=Stickleback) over the course of the three sampling efforts of the study. Sites are listed in order from furthest upstream to furthest downstream. For most sites, ADM water temperature was highest during the second sampling effort. ADM water temperature was consistently highest at downstream sites. Steelhead infection rates were consistently higher at downstream sites than rates roach and stickleback. An NA value means that the sample size was too small to be representative (< 3 fish).

	Sample 1 (June 5 –June 30)				**Sample 2 (June 30 –July 13)**				**Sample 3 (August 21)**			
Mainstem Site	**ADM**	**SH**	**R**	**SB**	**ADM**	**SH**	**R**	**SB**	**ADM**	**SH**	**R**	**SB**
Science Center	20.929	0	NA	NA	20.507	8.57	NA	NA	21.528	33.33	100	75
Skunk	18.956	0	NA	100	23.033	7.58	100	33.33	21.652	36.36	88.24	68.18
McKinley	20.425	0	NA	NA	25.237	52	100	75	22.417	100	66.67	100
Phil's Pool	21.473	0	66.7	NA	23.229	11.54	NA	NA	NA	92.86	33.33	35.71
Globi	21.665	6.25	NA	NA	24.727	100	56.25	7.69	24.712	100	50	91.67
White House	24.093	96.15	NA	NA	25.323	86.96	55.56	100	24.798	100	65.91	78.57
Ten Mile	23.146	76.47	NA	NA	24.281	100	12.5	NA	23.705	100	87.5	NA

Over half (52.8%) of the fish (n=886) we sampled from the main stem SFER showed black spot infection. Infection rates were highest in stickleback (66.7%, n=120), followed by roach (65.7%, n=210) and steelhead (44.8%, n=556). Infection rates in steelhead were consistently higher at downstream sites where water was warmest, but in stickleback and roach, infection trends were much less obvious (Table 1). There was minimal black spot infection (< 0.5%) seen in fish sampled from the two tributaries. Infection intensity (number of black spots per fish) was highest in longer (Figure 4a) and heavier (Figure 4b) fish of all species sampled.

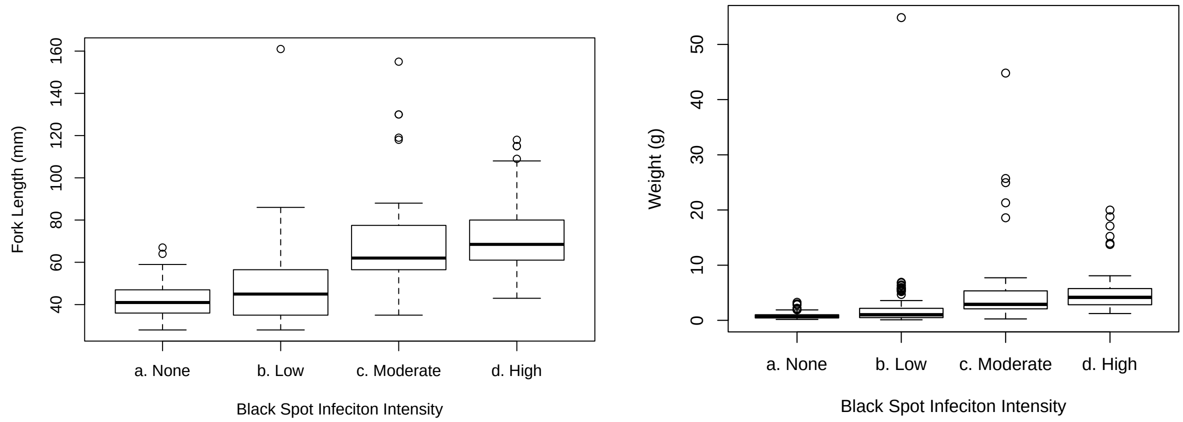

Figure 4 (a & b). Infection intensity is highest in fish of greater size and weight. Fish (of all species) with High and Moderate black spot infection intensity had longer fork lengths (FL) than fish with None and Low infection intensity. ($P < 0.0001$) Fish with High and Moderate black spot infection intensity were heavier than fish with Low or None. ($P < 0.0001$)

Black spot infection rates in steelhead showed a significant positive association with ADM water temperature at all sites throughout the study ($P < 0.05$), with the majority of high infections at sites with an ADM above 23° C (Figure 5). The stepwise logistic regression model incorporating the factors of water temperature, sampling location (upstream vs. downstream), and sampling date confirmed that water temperature and sampling date were the most significant variables influencing steelhead infection rates. Out of these two factors, water temperature had a significant effect ($P < 0.05$), confirming that the relationship between ADM and infection rates was most powerful, considering all other factors.

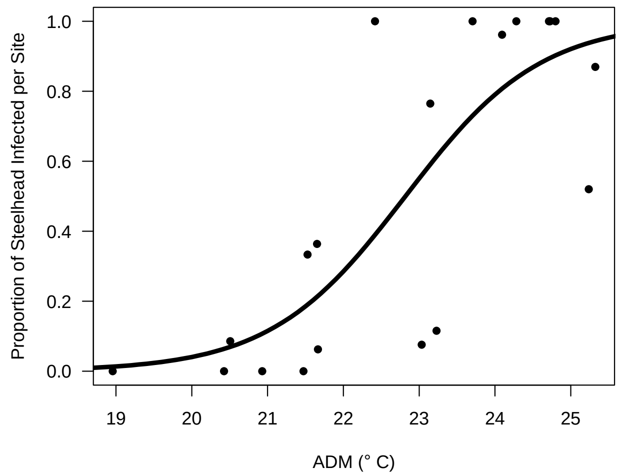

Figure 5. Relationship between ADM water temperature and steelhead infection rates. There was a positive logistic trend between black spot infection rates in steelhead and ADM water temperature at all sites throughout the study. At 23° C, a threshold exists where infection rates rapidly increase and remain high. (P < 0.05)

In stickleback, black spot infection rates were not significantly associated with ADM water temperature, sampling location or sampling date (P > 0.05). In roach, there was a significant negative association between water temperature and sampling location (P < 0.05) (Figure 6), but not sampling date (P > 0.05). Both of these species did show much higher infection rates in locations where steelhead infection rates were lower in the early summer despite less noticeable associations between environmental factors and parasitism rates (Table 1).

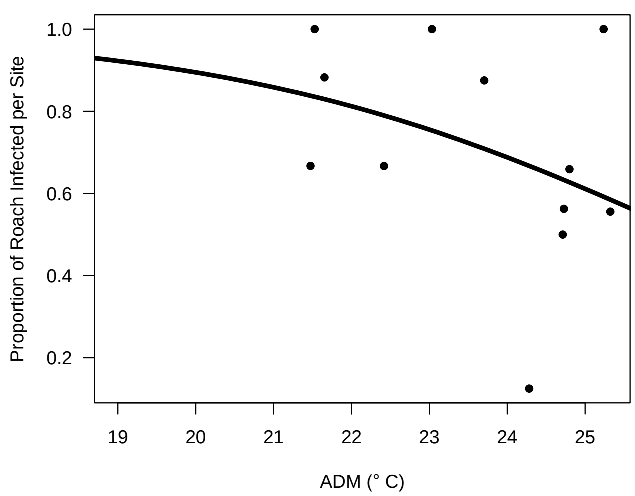

Figure 6. Relationship between ADM water temperature and roach infection rates. There was a negative logistic trend between black spot infection rates in roach and ADM water temperature at all sites throughout the study. (P < 0.05)

Ram's horn snail density showed a highly significant positive association (R-squared: 0.926, Adjusted R-squared: 0.9112, p: 0.0005) with 30-Day ADM water temperature. There was a rapid increase in snail density above 23° C (Figure 7). Snail density was lowest at upstream main stem sites (n=0), and increased steadily as we sampled further downstream at increasingly warm water sites. The most snails (n=41) were observed at the furthest downstream site. In addition, overall average black spot infection rates at each site showed a significant positive association with snail density at the corresponding site (R-squared: 0.8307, Adjusted R-squared: 0.7969, p: 0.00427) (Figure 8).

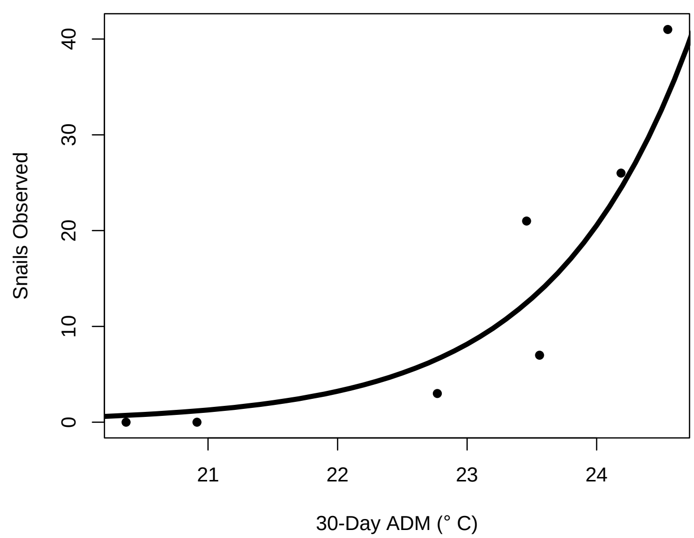

Figure 7. Relationship between ADM water temperature and snail density (Snails Observed). There was a significant positive correlation between water temperature (30-Day ADM) and snail density. (R-squared= 0.926) (P < 0.001) A rapid increase in snail density occurs at 23° C, similar to the threshold temperature in steelhead infection rates. Snails Observed is logarithmically transformed in this figure.

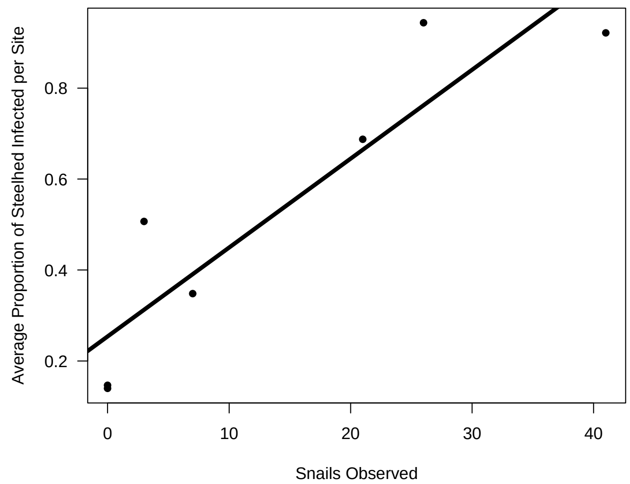

Figure 8. Relationship between snail density (Snails Observed) and steelhead infection rates. Snail density at each site showed a positive association with the corresponding average black spot infection rates in steelhead at those sites. (R-squared= 0.8307) ($P < 0.05$)

DISCUSSION

The complex relationship between black spot disease parasitism, water temperature, snail density, and spatial and temporal variations in the headwaters of the SFER offer a model system to examine parasite dynamics in aquatic systems. Through stepwise modeling, I determined that water temperature, more than varying spatial and temporal factors in a watershed, is the most significant influence on black spot disease infection rates in steelhead trout. In light of this, black spot infection rates were highest in steelhead occupying downstream, warm water sample sites and incidence of infection moved further upstream as water temperatures increased through the summer season. This pattern is similar in other salmonid species (Cairns et al. 2005, Ferguson et al. 2011, Ferguson et al. 2010), but it is the first time the relationship has been documented in steelhead. In roach and stickleback, infection rates were highest overall; however, the slight decrease in infection rates over the course of the summer is intriguing, suggesting that infection rates are likely influenced by a number of complex ecological factors and not simply water temperature, location or seasonal changes in site characteristics. Abundance and distribution of snails and piscivorious birds (two intermediate hosts in the parasitic life cycle) significantly influences black spot prevalence in a watershed (Steedman 1991, Poulin 2006) and is likely a contributing factor that we were unable to model in this study. Additionally, differences in infection trends among species may be explained by different host specificities of the trematode parasite among different fish species within the same system (Quist et al. 2007).

Spatial and temporal infection variability

Despite water temperature being the most significant factor influencing infection rates, location of fish within the river system (in terms of residing in upstream or downstream sample sites) was also statistically significant. In steelhead, it was clear that fish in the furthest upstream sites and tributaries displayed little to no infection (Table 1), even late in the summer. Conversely, in stickleback and roach, location of fish in the main stem SFER had a negligible influence on infection rates. Although studies have noted that fish in slow moving water display higher rates of parasitism (Berra and Au 1987, Evans and Mackiewicz 1958), I often found high rates of infection in fish collected from both fast riffles and slower pools in the SFER. Larger and heavier fish likely showed higher rates of parasitism because they have been in the river system longer than young of the year fish and have been exposed to black spot causing agents longer than younger fish (Berra and Au 1987).

Water temperature and its influence on the biotic processes necessary for parasite transmission

More than spatial and temporal variability, water temperature had the most significant influence on infection rates. It is likely that water temperature influences the biotic factors necessary to achieve parasite proliferation and thus high infection rates. Downstream sites with abundant algae and snail populations displayed highest infection rates (Table 1). A significant factor in this pattern is likely the increased snail density that comes with the temporal cycle of summer low flow algae blooms that are common in the main stem SFER (Power 1990). Additionally, high downstream productivity and nutrient input can lead to optimal conditions for parasitic hosts and transmission conditions, including high water temperature, algae and snail densities (Vinikour 1977). In the SFER, there was a threshold around 23° C at which both snail density (Figure 7) and steelhead infection rates (Figure 5) began to rapidly rise. It is possible that at this threshold temperature in similar watersheds, optimal conditions for parasite transmission are achieved and high parasitism rates occur.

Algae as a possible influence on infection rates and water temperature

Differing algal densities may explain some variation in infection rates between species and locations. I observed the most snails on substrates with high densities of algae at downstream sites; SFER roach and stickleback forage on and in algae beds while steelhead usually feed away from algae in open surface water (Power 1990). This difference in feeding ecology offers a possible explanation for the high infection rates in roach and stickleback at sites where steelhead infection rates were low at the beginning of the summer. Roach and stickleback feeding in dense algae are thus more likely to be infected since they are in closer proximity to high densities of grazing snails hosting trematode parasites. The decreased infection rates in roach and stickleback at the end of the summer seem to contradict this mechanism and may be due to differing host specificities (Quist et al. 2007). Regardless, extended drought and low flow periods brought about by projected climate change conditions will likely increase abundance of algae blooms, snail densities and black spot-causing parasites in the future.

Fitness and health effects on steelhead

Whether black spot disease presents a serious risk to steelhead populations is unknown and difficult to assess based on the few studies of the condition in salmonid species. There is documented evidence of lower overwinter survival in heavily infected salmon from watersheds similar to the SFER (Ferguson et al. 2010). However, similar studies have noted no apparent impact in salmon metabolism and growth (Ferguson et al. 2011). Heavily parasitized steelhead in my study were rough to the touch, suggesting a possible loss in streamlining and increased swimming burden based on studies of ectoparasites in coral reef fish (Binning et al. 2013).

Infection was most commonly seen on the central body, matching other reports of infection in a wide variety of species excluding salmonids (Evans and Mackiewicz1958). It is unclear whether infection is more damaging in certain areas of the body, but metacercarial cysts can harm the operculum (cheeks) of fish, causing holes and depressions in the cartilage of this tissue (Quist et al. 2007). Although many fish were infected with cysts on this area of the body, I am unsure of whether operculum damage had occurred in fish captured for my study. Effects in stickleback and roach are likely similar to those in steelhead and the literature displays a wide variety of conclusions in terms of fitness impacts of black spot disease on these and other species. In one report, high rates of parasitism in stickleback resulted in reduced egg counts and slightly lower fitness than non-parasitized fish (Fitzgeral et al. 1994). Literature is very sparse concerning black spot disease in roach.

Limitations

The relationship between snail density, water temperature, and infection rate must be cautiously interpreted due to a lack of continuous data on snail densities. My one-time snail sampling was by no means exhaustive, and it is possible that I missed smaller juvenile snails in some sites with thick algae on rocks, but the repeated timed effort for each site was a way to normalize this and account for some of this oversight. In terms of discrepancies in my large dataset on fish and infection rates, the last sampling was carried out using only an electrofisher, allowing us to catch more large fish that have been in the river system longer, possibly skewing our infection rates for the final sampling and making the increase in infection rates over the summer seem more dramatic than it actually was. However, observationally, there was certainly a progressive increase in infection at the upstream sites as the summer went on. Also, very few stickleback and roach were sampled at the beginning of the study, resulting in many null values for the first sampling. This did not give me a good estimate of the true infection pattern in these species in the early summer and may again present an underestimate of the true infection rate.

Water temperature data may only be an estimate of the conditions experienced by fish and snails at each site. Water temperature loggers were exposed to complex variations in habitat at each site (including substrate, depth, tree canopy, and algae variations). Thick algae blooms have been known to significantly increase temperature in certain pools in the SFER (Power 1990), and many of the temperature loggers placed in downstream sites were covered with algae by the end of the study, possibly leading to inaccurate recordings. Additionally, it was impossible to standardize placement of loggers, resulting in varying depths and locations of loggers at each site. In the case of one site (Phil's Pool), a temperature logger became exposed to unrealistically high water temperature because it was placed in an area that got disconnected from the main stem flow by the end of the study.

Future directions

Future studies on the fitness effects and implications of the parasitic condition in steelhead are warranted given the potential for black spot to be lethal in some species (Harrison and Hadley 1982, Markle et al. 2014), including other salmonids (Ferguson et al. 2010). Clean and healthy river systems provide the optimal conditions necessary for algae, snail, bird, and parasite abundance, suggesting that healthy watersheds, where salmonids flourish, are likely to be candidates for high rates of infection in the future (Flores-Lopez and Thomaz 2011). However, characterization of the complete set of ecological factors involved - snail and algae abundance, temperature ranges, water quality, host specificity, host (snail, bird and fish) distribution, etc. - are necessary before assumptions about the possible spread of the condition to other watersheds are made. Although we did not identify the trematode parasite the species level, based off of similar studies in an Oregon watershed, it is likely that it is either *Apophallus sp*. or *Neascus* that is moving through host snails and into fish (Rodnick et al. 2008). For a more robust and critical assessment of black spot disease in steelhead in California, the specific trematode should be isolated, identified and confirmed in a variety of different fish species and watersheds.

Broader Implications and conclusion

The prevalence of black spot disease in endangered steelhead and other fish species is relatively high in a headwater basin of a large northern California river, the SFER. Most significantly, steelhead seem to experience a rapid increase in infection rates around 23° C, suggesting that other watersheds that achieve this threshold temperature in the future due to drought or climate change may result in steelhead populations with extremely high prevalence of black spot disease. Steelhead comprise over one million dollars of the conservation and licensing funds dedicated to the California Department of Fish and Wildlife (California Department of Fish and Wildlife 2013) and are an extremely important biological component in maintaining healthy and diverse river food webs in Northern California (Power et al. 2008). In turn, their health and future viability are a significant management priority since the species faces extinction risks associated with future drought and increased water temperatures (Lindley et al. 2007 and Moyle et al. 2008). State fisheries management agencies should be aware of high prevalence of black spot disease in steelhead given the lack of current information on its health impacts. Mixed and sparse reports on salmonid fitness impacts as a result of black spot parasitism deem it necessary to move towards a better understanding of black spot disease and the possible spread of the condition in warming West Coast rivers and streams that provide habitat for sensitive and endangered steelhead populations.

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