

Black spot infection in juvenile steelhead trout increases with stream temperature in northern California

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Received: 23 September 2016 / Accepted: 27 March 2017 / Published online: 13 April 2017
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Abstract Climate change will increase water temperature in rivers and streams that provide critical habitat for imperiled species. Warmer water temperatures will influence the intensity and nature of biotic interactions, including parasitism. To better understand the factors influencing a neascus-type parasitic infection known as black spot disease, we examined the relationship between infection rate in juvenile steelhead trout (*Oncorhynchus mykiss*), abundance of another intermediate host (ramshorn snail, *Planorbella trivolvis*), and water temperature. We quantified infection patterns of trout at seven sites within the South Fork Eel River in northern California, visiting each site on three different occasions across the summer, and recording water temperature at each site. We also quantified infection patterns in trout captured from two tributaries to the South Fork Eel River. Overall, trout infection rates were highest in sites with the warmest temperatures. The abundance of ramshorn snails was positively related to both water temperature and black spot infection rates in juvenile trout. Both snail abundance and infection rates increased rapidly above a 23 °C daily maximum, suggesting a threshold effect at this temperature. We suggest that warmer temperatures are associated with environmental and biotic conditions that increase black spot disease prevalence in threatened steelhead trout. A

comparison of our results with similar data collected from a more northern latitude suggests that salmonids in California may be warm-adapted in terms of their parasite susceptibility.

Keywords Trematode · Digenea · Parasitism · Neascus · Salmonid · *Oncorhynchus* · *Planorbella trivolvis* · Cold-water refuge

Introduction

Climate change is expected to increase aridity, temperature, and drought frequency in many parts of the world over the coming decades (Dai 2011, 2013; Trenberth et al. 2014), including western North America (Cook et al. 2004; Seager et al. 2007; Cook et al. 2015). In fact, the recent drought in California may have been the most severe drought experienced in the region in the past 1200 years (Griffin and Anchukaitis 2014), and several studies predict that drought severity and frequency will continue to increase in this region (Diffenbaugh et al. 2015; Shukla et al. 2015). Decreased stream flow coupled with increased air temperature during periods of drought has the potential to dramatically increase water temperatures in rivers (Mantua et al. 2010; van Vliet et al. 2011), with many potential consequences for riverine taxa and ecosystems.

Beyond the direct effects of temperature on individual performance, elevated stream temperatures can alter and, in some cases, intensify ecological interactions. Increased parasitism and disease with warmer

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temperatures has been observed across a range of freshwater taxa from invertebrates (e.g., *Biomphalaria glabrata*, Marcogliese 2008) to amphibians (e.g., *Rana boylei*, Kupferberg et al. 2009) to fishes (e.g., *Micropterus salmoides*, Marcogliese 2001). For example, proliferative kidney disease, which is caused by a myxosporean (Hedrick et al. 1993), can increase mortality of brown trout (*Salmo trutta*) in Swiss rivers by 70% when water temperatures exceed 15 °C for over two weeks (Borsuk et al. 2006).

Cold-water taxa including salmonid fishes may be particularly vulnerable to the effects of increased water temperature. Warm temperatures can have direct physiological effects on salmonids with negative fitness consequences. For example, warm water temperatures reduce the ability of Chinook salmon (*Oncorhynchus tshawytscha*) to successfully undergo smoltification (Marine and Cech 2004) and increase chronic hormonal stress in juvenile coho salmon (*O. kisutch*, Thomas et al. 1986). Moreover, salmonids that are physiologically stressed by warm water temperatures may be unable to handle further stressors like pollutants or pathogens (Richter and Kolmes 2005). For example, the dual effects of warmer temperatures – including reduced physiological performance and increased parasite outbreaks – may result in increased mortality (Udey et al. 1975; Ray et al. 2012). In 2002, for example, a bacterial pathogen that proliferated in low flows and elevated water temperature in the Klamath River induced a significant die-off of Chinook salmon (Lynch and Risley 2003).

Trematode flukes are freshwater parasitic flatworms that proliferate in warm temperatures (Poulin 2006; Paull and Johnson 2011). Several digenetic trematode species (e.g., *Uvulifer ambloplitis*, *Crassiphilia bulboglossa*) have metacercarial stages that induce a condition known as black spot disease in fish (Hoffman and Putz 1965; Berra and Au 1978; Harrison and Hadley 1982). These black spot-causing trematodes are often referred to as neascus-type or neascus-like in recent work (Ferguson et al. 2010; Markle et al. 2014). Many families of fish are vulnerable to neascus-induced black spot disease including Cyprinidae (Evans and Mackiewicz 1958; Mahon et al. 1979; Wisenden et al. 2012), Gasterosteidae (Fitzgerald et al. 1994; Karvonen et al. 2013), and Salmonidae (Cairns et al. 2005; Rodnick et al. 2008; Ferguson et al. 2010, 2012), among others. Neascus-type trematodes have a complex life cycle with multiple

hosts and life stages. The first intermediate host is often a snail (e.g., *Planorbella* (aka *Helisoma*) *trivolvis*, in California streams, Turgeon et al. 1998). After emerging into the water column from the snail, infectious cercariae penetrate the skin of a fish, the second intermediate host, and encyst as metacercariae that produce a melanin-induced black spot around the raised, fibrous cyst (Berra and Au 1978; Teixeira-De Mello and Eguren 2008; Ferguson et al. 2010). The life cycle of the parasite is completed as infected fish are eaten by a belted kingfisher (*Megaceryle alcyon*), the definitive host, and the trematode carries out reproduction in the bird's intestine (Berra and Au 1978; Lane and Morris 2000). The trematode's eggs are shed with bird feces into the river where larvae hatch and infect a snail, continuing the cycle of infection.

Parasites and pathogens, in combination with increased temperatures, have been considered a potential factor in the decline of wild Pacific salmon populations (Miller et al. 2014). Moreover, previous studies have noted that parasites, including those that cause black spot disease, are more prevalent in coho salmon in warmer stream reaches of the Pacific Northwest (Cairns et al. 2005; Rodnick et al. 2008; Ferguson et al. 2010, 2012). However, the relationship between temperature and neascus-type infection prevalence has yet to be explored in salmonids at their more southern range in California, where salmonids are already on the brink of extinction (Katz et al. 2013).

To better understand the factors influencing black spot prevalence in salmonids near the southern end of their range, we examined the relationship between water temperature and black spot disease infection rates in juvenile steelhead trout (*O. mykiss*) in the South Fork Eel River in northern California. We also explored other variables that may influence infection rates, including intermediate host (i.e., snail) abundance and fish size. We predicted that black spot prevalence in *O. mykiss* would increase with water temperature, leading to spatial variation in infection rates between sites with different temperature histories. We also predicted that infection rates would increase as the summer progressed and water temperature and exposure history increased. We predicted that sites with high infection rates would have high abundance of snails (the first intermediate host). Finally, we predicted that infection rates would be higher in older/larger fish, which have had a longer period of exposure than younger/smaller fish.

Materials and methods

Study system

Our sampling sites were distributed along the mainstem of the South Fork Eel River (SFER) and two of its tributaries in Mendocino County, California, USA (Fig. 1), including sites in and around the UC-Berkeley Angelo Coast Range Reserve (39.733°N, 123.65°W). The SFER experiences Mediterranean seasonality characterized by wet winters and dry, hot summers (with daily summer air temperatures averaging 29 °C in the reserve, Mast and Clow 2000). The dry season extends from May through October and is associated with low river flows and warm water temperatures. The fish community in this region includes our focal organism, steelhead trout (*O. mykiss*), as well as threespine stickleback (*Gasterosteus aculeatus*), Sacramento sucker (*Catostomus occidentalis*), California roach (*Hesperoleucus symmetricus*), and non-native Sacramento pikeminnow (*Ptychocheilus*

grandis). Steelhead trout from this region are part of the Northern California Coast Distinct Population Segment (DPS), which is currently listed as threatened under the U.S. Endangered Species Act.

Fish and snail sampling

From June 5 to August 21 2014, we sampled juvenile trout from a 9 km section of the SFER mainstem. We sampled seven sites, spaced from 1 to 2.8 km apart, chosen to represent a gradient of increasing water temperature from upstream to downstream (Fig. 1).

To assess individual fish for black spot infection, we captured young of year (YOY) trout (≤ 80 mm fork length) using a combination of seine and hand nets. At capture, each fish was weighed (± 0.01 g), measured (fork length, mm), and assessed for black spot disease infection, always by the same author (CJS). Infection was confirmed via a tactile examination of the black spot to determine whether it was a natural marking or a metacercarial infection (infection spots are raised off the skin,

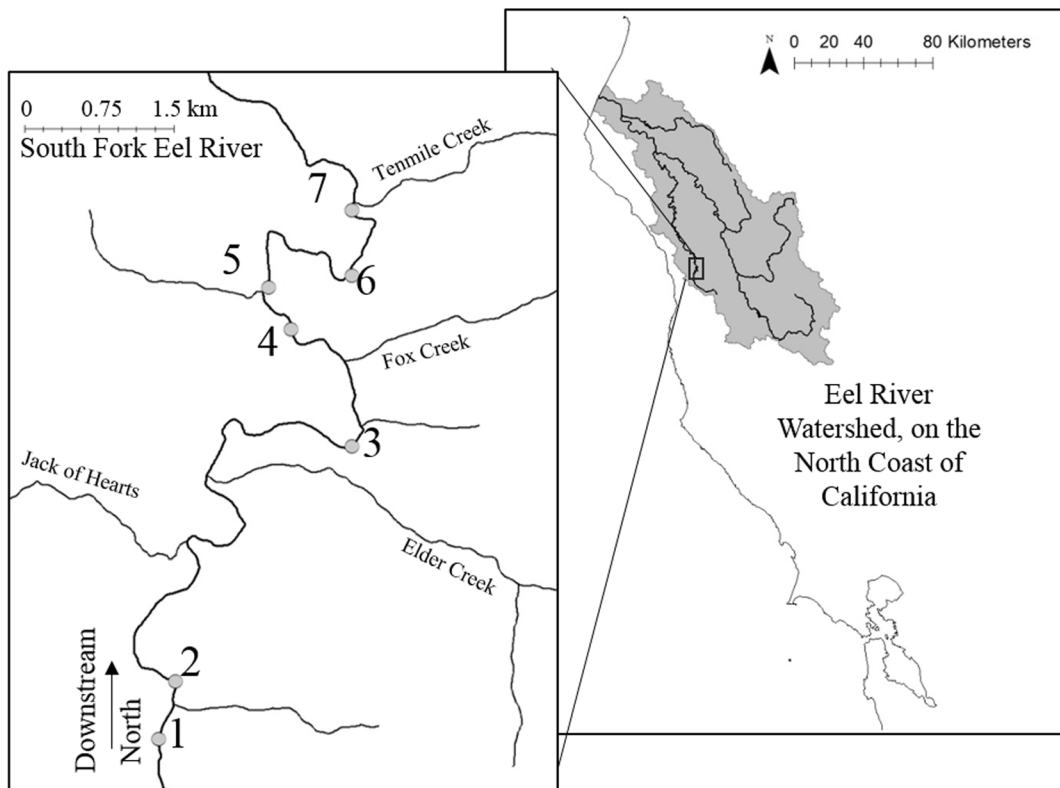


Fig. 1 Map of our study reach on the South Fork Eel River (SFER) in northern California. Numbered circles represent sample sites distributed along the mainstem SFER

Our tributary sample sites on Elder and Fox creeks are labelled . The arrow pointing downstream/north represents the direction of water flow

Vaughn 1962; Ferguson et al. 2010). While the vast majority of metacercarial cysts were black, raised nodules that were yellow or brown (Fig. 2) were also considered infectious, since these likely represented new infections that had not yet produced a melanin pigment reaction (Ferguson et al. 2010; Markle et al. 2014).

We sampled fish at each of the seven mainstem sites on three different dates across the summer, resulting in a total capture of 556 steelhead. To explore infection rate in fish of different size classes, we used a backpack electrofisher (Smith-Root LR-24) to capture 36 additional larger steelhead (i.e., > 80 mm fork length) from our seven mainstem sampling sites during the last sampling effort. Due to length between sample sites (> 1 km), it is highly unlikely that any fish moved between sites during the study period (Edmundson et al. 1968; Keely 2000).

To compare infection rates in the SFER with its reliably cooler tributaries, we report black spot infection rates of 1426 trout that were captured in Elder and Fox creeks (Fig. 1). These fish were captured as part of another ongoing study via a backpack electrofisher between July 20 and Aug 17, 2014 in Elder Creek and July 10–11, 2014 in Fox Creek. Observations from these sites were not included in any statistical models.

To determine the abundance of intermediate snail hosts (*Planorbella trivolvis*), we surveyed snails once (on July 14, 2014) at all seven mainstem sites. We

qualitatively observed that snail abundance was highest near the stream bank, where the snails graze algae. Consequently, we surveyed one random site of dimensions 3 m × 2 m at each site, with the long axis paralleling the bank. Within this area, we counted and collected all *P. trivolvis* encountered within a 15-min period, including those visible to the naked eye and those that were found via touch within algae-covered substrates.

Water temperature

At each study site, and in each tributary, we placed one thermistor (HOBO Pendant Temperature/Light Data Logger 64 K - UA-002-64) 10 cm from the substrate, which recorded water temperature at 10-min intervals between the first and last sampling events (6/5/2014–8/21/2014). We use a two-pronged approach to explore temperature variation through space and time.

First, we calculated the daily average temperature at each site to visualize continuous trends in temperature throughout the summer at each sampling site.

Next, we 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 including the sampling date (details below). We chose to use the average daily maximum (ADM) water temperature as a metric because it has been used previously in other studies

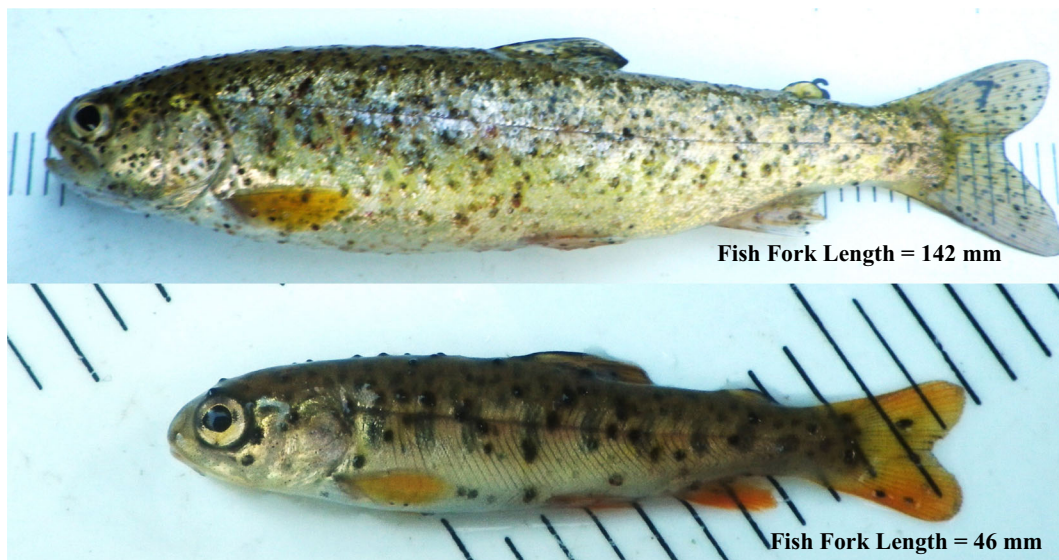


Fig. 2 Photos showing large (panel **a**) and small (panel **b**) trout with evidence of black spot infection. Cysts on infected trout were small, fibrous, and rounded black spots that were raised off the

skin. A few of the heavily infected fish had parasitic cysts that were yellow and brown due to developing infections (e.g., top photo)

exploring black spot infection rates (Cairns et al. 2005). This resulted in three ADM temperature metrics for each sample site, i.e., one for each of three sampling events that were used in analyses.

For our first sampling effort, we deployed thermistors 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. During our mid-summer sampling event, the seven-day ADM was centered on our sampling date. Because temperature loggers were removed on our last sampling event, the late summer ADM included the six days leading up to and including the final sampling date.

ADM temperature at one site (site three on figures and table) was excluded from analyses because the data logger was exposed to unrealistically warm in-stream conditions due to severe water level drop at that site.

Statistical analyses

To determine the effect of water temperature (measured as the seven-day ADM) on black spot infection rate in YOY trout from our seven mainstem sampling sites, we used a mixed modeling approach, with infection status (infected/not infected) as the response variable (logistic regression). We chose to use mixed models so that temperature and sample date could be included as fixed effects, and site could be included as a random effect on the intercept to avoid pseudo-sampling (Lindstrom and Bates 1990). We tested the fit of three candidate models to determine the effects of temperature and sample date on the proportion of infected fish: 1) temperature as a fixed effect, 2) sample date as a fixed effect, and 3) both as fixed effects. Sample site was included as a random effect for all models. We used Akaike Information Criteria (AIC) (Akaike 1974) to choose between the three candidate models (Sakamoto et al. 1986). All analyses were performed in R (R Core Team 2016). Mixed models were fitted with the *glmer()* function from the package *lme4* (Bates et al. 2015).

To determine the relationship between snail count and water temperature, we performed exponential regressions on snail abundance, i.e., abundance as the response variable, and e to the power of temperature as the explanatory variable, this time using the 30-day ADM for each site. We used a longer temperature history for this analysis since there was only a single sampling event for the snails. The 30-day ADM was calculated by averaging the maximum temperature at each site for a 30-day period centered on the date of the

snail abundance survey (July 14, 2014). To test the hypothesis that trout infection rates increase with snail abundance, we used a logistic regression with snail count as the independent variable and the proportion of infected steelhead at each site as the response variable, focusing on YOY trout and combining data from all seven mainstem sites from all three sampling events.

Finally, we tested whether infected trout were on average larger than non-infected trout using a Welch two-sample t-test accounting for heteroscedasticity. For this analysis, we only used fish captured in the final sampling event, to include the age-1 and older fish captured during this event.

Results

Water temperature varied among sites and across the summer (Table 1, Fig. 3). In general, downstream sites

Table 1 Summary of the 7-day average daily maximum temperature (ADM, °C) and black spot infection rates (%) in young of the year steelhead trout at each site and each sampling event (n = sample size). Sites are listed in order from upstream (Site 1) to downstream (Site 7)

Site	Date	ADM	% Infected	n
1	6/5/2014	20.9	0	18
	6/30/2014	20.5	8.6	35
	8/21/2014	21.5	33.3	18
2	6/18/2014	19.0	0	44
	7/7/2014	23.0	7.6	66
	8/21/2014	21.7	36.4	11
3	6/18/2014	20.4	0	28
	7/7/2014	25.2	52	25
	8/21/2014	22.4	100	19
4	6/18/2014	21.5	0	45
	6/30/2014	23.2	11.5	26
	8/21/2014	NA	92.9	28
5	6/23/2014	21.7	6.4	16
	7/13/2014	24.7	100	22
	8/21/2014	24.7	100	42
6	6/25/2014	24.1	76.5	17
	7/13/2014	25.3	100	13
	8/21/2014	24.8	100	11
7	6/30/2014	23.1	96.2	26
	7/13/2014	24.3	87.0	25
	8/21/2014	23.7	100	21

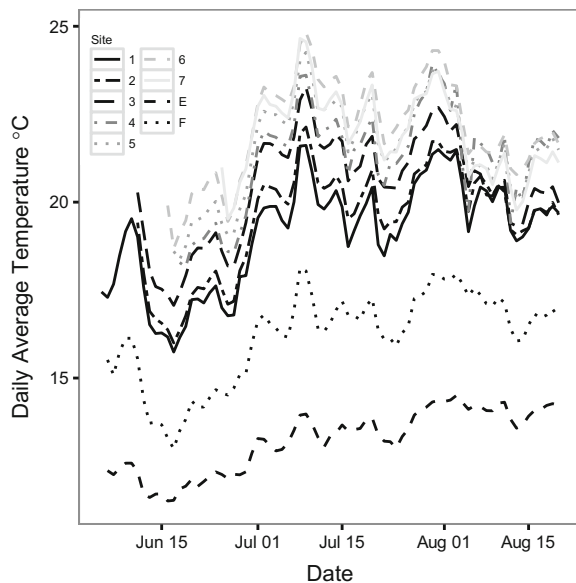


Fig. 3 Average daily water temperature at each site over the course of the summer. E and F refer to Elder Creek and Fox Creek, respectively. Refer to Fig. 1 for the location of sites 1–7 along the mainstem South Fork Eel River

were consistently warmer than upstream sites (Table 1, Fig. 3), and temperatures increased rapidly from June to July, after which temperature remain elevated and decreased slowly through our final sampling event in mid-August (Table 1, Fig. 3). Water temperatures in the two tributaries were considerably lower than all mainstem sites, ranging from 13 to 18 °C over the course of the summer. The seven-day ADM water temperature in the mainstem ranged from a minimum of 19.0 °C to a maximum 25.3 °C from June 5 to August 21, 2014 (Table 1).

Nearly half (249 of 556, 44.8%) of the juvenile trout that were sampled from the seven mainstem SFER sites showed evidence of black spot infection. In contrast, we observed minimal black spot infection (2 of 1426, < 0.1%) among trout sampled from the cool tributary sites in Elder and Fox creeks.

The mixed models of the seven mainstem sampling sites revealed that both temperature and sample date are important covariates in determining the proportion of infected steelhead at each site. Temperature was significant in the first (i.e., temperature [fixed] and site [random]) model ($z = 6.86$, $p < 0.01$, $AIC = 178.4$). Sample date was significant in the second (i.e., sample date [fixed] and site [random]) model ($z = 9.88$, $p < 0.01$, $AIC = 124.6$). In the final model (temperature [fixed], sample date [fixed], and site), both fixed effects were

statistically significant, and this model was associated the lowest AIC value (temperature: $z = 4.35$, $p < 0.01$, sample date: $z = 7.22$, $p < 0.01$, $AIC = 98.0$). From these comparisons, we base our interpretations on the final model, including both sample date and temperature as fixed effects, and site as a random effect.

Overall, the top-supported model suggests that temperature and sample date both influence observed infection rates, with infections accumulating over the course of the summer, and infection rates increasing with temperature. The model suggests that at 23.3 °C, 50% of fish will be infected with black spot disease. In general, trout infection rates tended to be higher at downstream sites (Figs. 4 and 5), reaching infection rates of 95–100%, where water temperatures were the highest (Fig. 3), in comparison to cooler sites, where infection rates never exceeded 40% (Table 1, Fig. 5). In addition, at every site, infection rates increased over the course of the study, peaking at the last sample event on August 21, 2014 (Table 1, Fig. 5).

The relationship between snail abundance at each site and water temperature (as measured by the 30-day ADM) was best described by an exponential relationship ($F_{1,5} = 51.6$, $p < 0.001$, $r\text{-squared} = 0.91$). We found <10 snails per plot until water temperatures exceeded 23 °C (Fig. 6a). Snail counts were lowest at upstream (cool) mainstem sites ($n = 0$) and increased at the

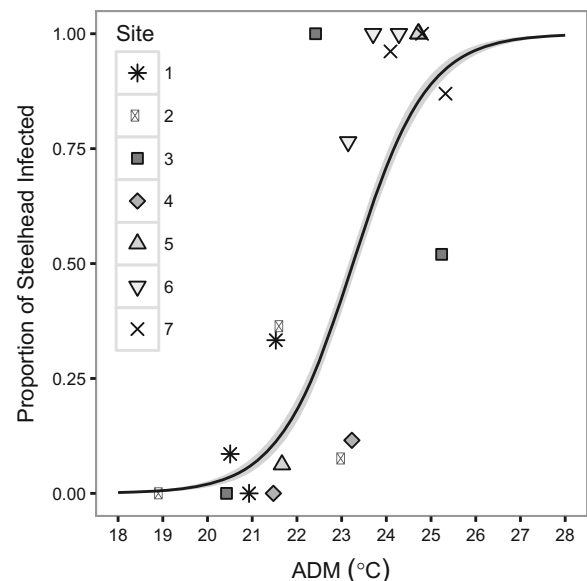


Fig. 4 Relationship between trout infection rates and average daily maximum (ADM) water temperature calculated over 7-day windows

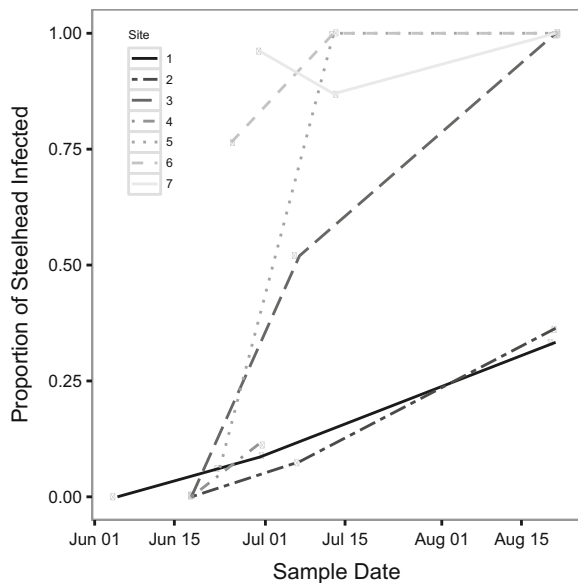


Fig. 5 Temporal trend in trout infection rates at each site over the study period, highlighting differences among early, middle, and late sampling events at each site. Refer to Fig. 1 for the location of sites 1–7 along the mainstem South Fork Eel River

warmer downstream sites (Fig. 6a). The highest number of snails encountered in any given survey ($n = 41$) occurred at the furthest downstream site (i.e., site 7). The

relationship between snail abundance and proportion of infected fish was best described by a logistical regression relationship ($z = 12.15$, $p < 0.001$). When there were 11 snails per plot, at least 50% of the steelhead at that site were infected with black spot disease.

Finally, we found that the mean size of infected fish was significantly larger than the mean size of not-infected fish (infected: 72.8 ± 23.3 mm, not-infected: 64.6 ± 13.4 mm, $t_{43} = 2.24$, $p = 0.03$).

Discussion

Black spot infection rates in juvenile steelhead trout increased with maximum water temperature in the South Fork Eel River watershed of northern California (Fig. 4). Moreover, spatiotemporal variation in water temperature was associated with spatiotemporal variation in blackspot infection rates. Black spot infection rates were highest in juvenile steelhead trout occupying warmer downstream sites than cooler upstream sites, and infection rates increased over the course of the summer season at every site, possibly due to increased exposure or increased cercarial releases.

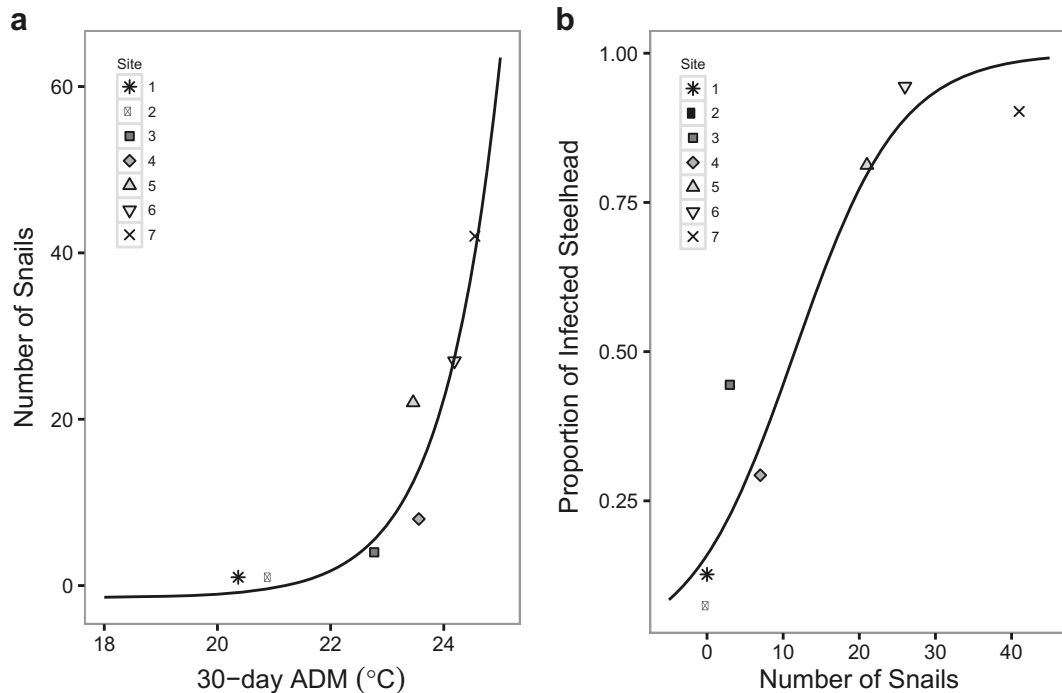


Fig. 6 Relationship between snail counts and average daily maximum temperature (ADM) (panel a) calculated over 7-day windows, and between trout infection rate and snail counts (panel b)

We found that infected fish tended to be larger, a pattern that is commonly seen in other parasitized salmonids (e.g., *Salvelinus fontinalis*, Poulin et al. 1991; *O. kisutch*, Barndt and Stone 2003). One possible reason is that larger fish are usually older and, thus, have had a longer cumulative period of exposure to parasites compared to smaller, younger fish (Berra and Au 1978). In fact, metacercarial cysts can persist for up to 4.5 years in some fish species (Hoffman and Putz 1965), suggesting that some of the black spots detected on age-1 and older fish may reflect infections from previous years.

Spatial and temporal variation in infection rates

At the South Fork Eel River, trout captured from downstream sites had the highest infection rates throughout the summer (Table 1, Fig. 5). These same sites were associated with the highest abundance of intermediate-host ramshorn snails (Fig. 6). Since infectious trematode cercariae survive for less than 24 h (Pechenik and Fried 1995), they would likely not influence infection rates at downstream sites by being carried downstream in low flows. Thus, snail host abundance is the most obvious factor that would influence local infection. Increased abundance of intermediate host snails within certain stream reaches may encourage growth of the trematode population, increasing local black spot infection in fish. The proliferation of snails in downstream sites may be linked to features of these sites. Previous work has shown that the abundance of aquatic grazers like snails increases steadily with an increasing biomass of epiphytes and macroalgae (Cattaneo 1983). In the South Fork Eel River, increased solar radiation in wide, downstream channels can cause proliferations of macroalgae (Power et al. 2008, 2013), which may explain increased snail abundance at downstream sites.

Infection rates in cool tributaries were considerably lower than in mainstem sites (<1% versus 44.8%). A similar pattern - of lower infection rates in cooler waters - was also observed in other salmonid species (Cairns et al. 2005; Ferguson et al. 2010, 2012) and a cyprinid (Mahon et al. 1979). The tributary sites are shaded and cooler due to groundwater inputs, and lower productivity may limit algal growth (Power et al. 2013) and snail establishment, both of which would impede parasite transmission to trout residing in tributary habitat. Shaded tributary habitats may represent critical refugia for salmonids at the southern end of their range, both because of the cooler temperatures but also by providing

escape from warm-water parasites and predators, both of which are more common in the warmer mainstem waters of the South Fork Eel River. Moreover, models predict that such tributary sites are less likely to warm as a result of climate change (Daigle et al. 2015) possibly due to their tendency to be shaded and fed by cool groundwater, which further highlights their importance for conserving salmonid fishes in a warming world.

Black spot infection rates increased with sample date at our South Fork Eel River sampling sites, suggesting that infection increases with exposure history. That both time and temperature are significant in our best-supported model suggests that both are important. However, the large variation in infection rates among sites early in the summer suggests that other (unmeasured) variables could also be important for explaining spatiotemporal variation in infection patterns – e.g., water flow, parasite dispersal, and abundance of other fish hosts.

Water temperature and trematode activity

We found that black spot infection rates increased with water temperature (Fig. 4) in the mainstem South Fork Eel River, as did black spot infection in coho salmon from an Oregon watershed (Cairns et al. 2005; Rodnick et al. 2008). Previous studies have revealed that warm temperatures trigger the growth and release of trematode cercariae from snails (Ataev 1991; Poulin 2006). In one trematode (*Ribeiroia ondatrae*), development was four times faster at 26 °C than 17 °C (Paull and Johnson 2011). In a trematode that commonly infects *O. mykiss* (*Diplostomum spathaceum*), cercariae were shed from snails more than five times faster at 20 °C than 10 °C (Lyholt and Buchmann 1996). We postulate that infection rates increased over the summer because rising temperatures in June and July accelerated trematode development and cercarial releases. Another possibility is that the cumulative period of exposure increases over the summer.

Fitness and health effects of black spot disease

Previous studies on black spot disease have revealed assorted information about the extent to which the disease can negatively impact infected fish. Research by Ferguson et al. (2010) revealed lower overwinter survival in coho salmon heavily infected with *Apophallus* sp. and neascus-type metacercariae. Other freshwater

fish species show increased mortality when infected with neascus-type metacercariae, possibly due to increased predation susceptibility (*Esox lucius*, Harrison and Hadley 1982; *Chasmistes brevirostris*, Markle et al. 2014). Reported sub-lethal impacts of neascus-type infection vary greatly from no effect on salmonid metabolism and growth (Ferguson et al. 2012) to significant weight loss at high levels of parasite load in Centrarchids (e.g., *Micropterus dolomieu*, Hunter and Hamilton 1941; *Lepomis macrochirus*, Lemly and Esch 1984). In our study, heavily parasitized steelhead felt rough to the touch (CJS, personal observation), suggesting a possible loss of streamlining and increased swimming burden. Increased swimming burden due to ectoparasites has been observed previously for marine fish (Binning et al. 2012). Future studies on the fitness effects of the parasitic condition in steelhead are warranted given the potential sub-lethal and lethal effects of black spot disease reported in other species. However, Ferguson et al. (2012) stress the importance of examining more than one type of parasite when assessing fitness impacts and mortality.

Temperature thresholds

As in our study, black spot infection rates also increased with stream temperature in coho salmon in the West Fork Smith River, Oregon, USA (Cairns et al. 2005; Rodnick et al. 2008). However, one important difference emerged between the two studies: the threshold temperature at which infection rates increased was *lower* for the Oregon (West Fork Smith River) population compared to the California (South Fork Eel River) population. Specifically, Cairns et al. report over 50% infection in coho salmon from Oregon at 21 °C ADM, while we found that the 50% threshold was not reached until 23.3 °C ADM for steelhead trout in California (Fig. 4).

There are several non-mutually exclusive explanations for the observed geographical differences in temperature thresholds for black spot infection rates in salmonid fishes. One possibility is that the threshold for infection differs for different species of salmonid fishes, due to differences in their physiology or habitat use. Another intriguing possibility is local adaptation to warmer conditions at more southern latitudes. Overall, the South Fork Eel River, California experiences warmer temperatures than the West Fork Smith River, Oregon (average seven-day maximum of 22.8 °C in the SFER (this study) vs 19.3 °C (Rodnick et al. 2008)),

suggesting that the more southern salmonids experience warmer temperatures overall may have adjusted to their thermal habitat through local adaptation and increased resistance to parasites. Such local adaptation in salmonids to warm waters is not without precedent (e.g., Verhile et al. 2016). This possibility of local adaptation to thermal regime could provide another reason to conserve salmonids at the southern end of their range as a valuable genetic reservoir for persisting in a changing world (Katz et al. 2013).

Uncertainty regarding the neascus-type trematode in our study

Although we did not collect and taxonomically identify the black spot-causing trematode in our study from any host (fish or snail), we believe that infection was likely caused by a neascus-type trematode. The parasitic cysts were small, fibrous and raised off the skin upon tactile examination (Fig. 2), characteristics of neascus-type trematodes, rather than other black spot-inducing trematodes (e.g., *Apophallus* sp.) (Vaughn 1962; Ferguson et al. 2010; V. Tkach, pers. comm.; J. Ferguson pers. comm.). Two possible trematodes include *Uvulifer ambloplitis* and *Crassiphilia bulboglossa*, both of which use *P. trivolvis*, the snail that is very abundant in our study sites, as an intermediate host (Hoffman and Putz 1965; Berra and Au 1978; V. Tkach, pers. comm.).

However, it is possible that non-neascus-type trematodes were responsible for some of the observed infections, since observation of metacercarial cysts under a microscope is the only true way to identify trematode-specific infections (Ferguson et al. 2010). Further investigation into the exact trematode species and corresponding intermediate snail host involved in the infection cycle is needed to confirm linkages between snail and infection data, and would greatly improve our understanding of the causes of black spot infection in fishes, including imperiled salmonid fishes.

Broader implications and conclusions

In summary, we found that the prevalence of black spot disease in juvenile steelhead trout can be high in a large northern California river, but that disease prevalence varies through space and time. Importantly, we found that infection rates increased rapidly once water temperatures exceeded 23 °C ADM, a threshold most likely to be exceeded in sunny, warm mainstem reaches. As

climate changes and stream temperatures warm, it is possible that other populations inhabiting sunny river reaches may experience increased vulnerability to black spot disease, highlighting the importance of shaded and cool tributary habitats for sustaining salmonids in a changing climate.

Acknowledgements We thank the National Science Foundation for the Eel River Critical Zone Observatory financial support (CZP EAR-1331940), including an REU that supported CJZ (EAR-1443164), and a Graduate Research Fellowship to SJK (NSF 201517964). Additionally, we thank Tina Mendez, Kurt Spreyer, and Mary Power for feedback on an earlier version of the manuscript, and Katie Kobayashi for assistance in the field. We also thank Vasyi Tkach and Jayde Ferguson for providing expertise in parasite identification. The research activities presented in this manuscript were carried out with approval from the University of California Berkeley Animal Care and Use Committee.

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