

RESEARCH ARTICLE

Rapid proliferation of the parasitic copepod, *Salmincola californiensis* (Dana), on kokanee salmon, *Oncorhynchus nerka* (Walbaum), in a large Colorado reservoir

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Abstract

Ecologically and economically valuable Pacific salmon and trout (*Oncorhynchus* spp.) are widespread and susceptible to the ectoparasite *Salmincola californiensis* (Dana). The range of this freshwater copepod has expanded, and in 2015, *S. californiensis* was observed in Blue Mesa Reservoir, Colorado, USA, an important kokanee salmon (*O. nerka*, Walbaum) egg source for sustaining fisheries. Few *S. californiensis* were detected on kokanee salmon in 2016 (<10% prevalence; 2 adult *S. californiensis* maximum). By 2020, age-3 kokanee salmon had 100% *S. californiensis* prevalence and mean intensity exceeding 50 adult copepods. Year and kokanee salmon age/maturity (older/mature) were consistently identified as significant predictors of *S. californiensis* prevalence/intensity. There was evidence that *S. californiensis* spread rapidly, but their population growth was maximized at the initiation (the first 2–3 years) of the invasion. Gills and heads of kokanee salmon carried the highest *S. californiensis* loads. *S. californiensis* population growth appears to be slowing, but *S. californiensis* expansion occurred concomitant with myriad environmental/biological factors. These factors and inherent variance in *S. californiensis* count data may have obscured patterns that continued monitoring of parasite–host dynamics, when *S. californiensis* abundance is more stable, might reveal. The rapid proliferation of *S. californiensis* indicates that in 5 years a system can go from a light infestation to supporting hosts carrying hundreds of parasites, and concern remains about the sustainability of this kokanee salmon population.

KEYWORDS

gill lice, intensity, invasion, maturity, prevalence

1 | INTRODUCTION

Salmon and trout of the genus *Oncorhynchus* are widely distributed throughout the Pacific Rim region, historically supporting large-scale fisheries in their native ranges. Managers have introduced *Oncorhynchus* salmon and trout widely in freshwater systems to create new recreational and sport fishing opportunities. For example, kokanee salmon (lacustrine sockeye salmon, *O. nerka*, Walbaum) have

become an important sport and food fish in many North American lakes (Biser, 1998; Nelson, 1968; Wydoski & Bennett, 1981). They have been introduced in systems as large as the Great Lakes and many smaller waterbodies in western states such as California, Colorado, Idaho, Montana, New Mexico, Utah and Wyoming, as well as in north-eastern states such as Connecticut, Maine and New York (United States Geological Survey, 2021). Kokanee salmon are often heavily managed in these systems as well as others in western

Canada (e.g. British Columbia) where egg collection and/or stocking takes place regularly in multiple waterbodies (pers. comm. BC, CO, ID, NM, WY management personnel). Although kokanee salmon represent valuable sport fish and management tools, they (and other *Oncorhynchus* species) are susceptible to the freshwater ectoparasitic copepod *Salmincola californiensis* (Dana), also known as “gill-lice” or “gill-maggots” (Kabata, 1969). Kokanee salmon and anadromous sockeye salmon interact with the ectoparasite *S. californiensis* within their native ranges on the Pacific Coast (Bailey & Margolis, 1987; Chigbu, 2001; Kabata, 1969). However, the distribution of *S. californiensis* has expanded eastward through the movement and stocking of infected fish (Kamerath et al., 2009; Ruiz et al., 2017; Sutherland and Whittrock, 1985) and now represents an emerging concern for some populations of kokanee salmon and other *Oncorhynchus* salmon and trout.

Despite wide distribution and potential impacts on valuable fish populations, relatively little is known about *S. californiensis* (Murphy et al., 2020). The adult stage of the ectoparasite is visible with the naked eye, appearing similar to a grain of rice. They attach to host fish in a variety of locations including the gill filaments, branchial cavity and fin rays (Kabata and Cousens, 1977). Although low-level infections are not thought to cause mortality directly, anaemia, reduced gas exchange and osmotic regulation, and poor swimming endurance are associated with severe infections (Herron et al., 2018; Pawaputanon, 1980). These sublethal effects may contribute to higher mortality under stressful environmental conditions (Hargis et al., 2014; Vaughn and Coble, 1975) or during critical life stages such as spawning or smoltification (Barnett et al., 2020; Herron et al., 2018). Further, high prevalence (proportion of individuals parasitized) and intensity (number of adult parasites per fish) of *S. californiensis* infestations may impede recovery efforts for threatened species like Chinook salmon (*O. tshawytscha*, Walbaum) and have been implicated in the deterioration of some recreational fisheries concomitant with other stressors (Hargis et al., 2014; Monzyk et al., 2015; Vigil et al., 2016).

Prevalence and intensity of *S. californiensis* can be highly variable on their hosts, and multiple mechanisms may be contributing to this observed variability. For example, *S. californiensis* prevalence, intensity and rate of accumulation were higher in reservoirs versus streams, and disparate among species and life stage in Pacific salmon and trout including kokanee salmon, Chinook salmon, cutthroat trout *O. clarkia* (Richardson) and rainbow trout *O. mykiss* (Walbaum) (Monzyk et al., 2015). Additionally, water temperature in the laboratory has been shown to influence *S. californiensis* reproduction/fecundity rates, the length of time for free swimming parasites to successfully attach to a host and the intensity of infections on hosts (Murphy et al., 2020; Neal et al., 2021; Vigil et al., 2016). *S. californiensis* has been found to increase in prevalence and intensity on larger kokanee salmon (Barndt & Stone, 2003; Neal et al., 2021) and with increasing salmon age (Hargis et al., 2014). Further, host maturity or sex might play a role in host-parasite dynamics (Rolff, 2002; Sheldon & Verhulst, 1996). Although the mechanisms driving *S. californiensis* variability are

uncertain, deleterious impacts are evident (e.g. Herron et al., 2018) and could be detrimental to some reservoir populations of ecologically and/or economically important Pacific salmon and trout throughout their native and introduced ranges.

In 2015, *S. californiensis* were confirmed in Blue Mesa Reservoir (Gunnison County, Colorado) with an observation of an adult *S. californiensis* on a rainbow trout that was captured in a gill net during a survey in May (C. Gunn, Colorado Parks and Wildlife, Aquatic Animal Health Laboratory Case # 15-102, pers. comm.). There is a lack of information about early *S. californiensis* invasion dynamics in reservoirs, with evaluations of infestations largely focused on established populations (e.g. Hargis et al., 2014; Monzyk et al., 2015; Murphy, Gerth, Pauk, et al., 2020). We describe the rapid proliferation of *S. californiensis* in the Blue Mesa Reservoir kokanee salmon population. We hypothesized 1) the spread (increase in prevalence and intensity) of *S. californiensis* infestation in Blue Mesa Reservoir would occur, and be most evident in older, mature kokanee salmon, 2) there are differences in *S. californiensis* dynamics on male and female kokanee salmon, 3) there are seasonal components to *S. californiensis* infestations, 4) mature kokanee salmon would be more susceptible to *S. californiensis* relative to others, and 5) accumulation of *S. californiensis* will be highest on the gills relative to other tissues of kokanee salmon. We describe our findings and the potential implications of this infestation and others, highlighting the need to limit further spread of *S. californiensis*.

2 | METHODS

2.1 | Study system

Blue Mesa Reservoir (38°28'30" N and 107°12'30" W) is a mesotrophic, 3,793-ha impoundment with a maximum depth of 101 m, mean depth of 28 m and an approximate hydraulic retention time of 0.8 years (the reservoir can fluctuate widely; see Results). The reservoir thermally stratifies, generally between May and October, with peak surface temperatures reaching 20–22°C. Densities of *Daphnia* spp and typically peak in June at ~10–30 individuals/L in surface waters (Hansen et al., 2019). Kokanee salmon are stocked annually in Blue Mesa Reservoir, and overall, feeding and growth conditions in the reservoir support high kokanee salmon growth rates (Stockwell & Johnson, 1997). Kokanee salmon dominate angler catch and harvest (averaged 64.3% of total catch between 1993 and 2012), and Blue Mesa Reservoir fish often provide a large portion of the kokanee salmon eggs needed to meet the stocking objectives for Colorado. There is also a trophy lake trout *Salvelinus namaycush* (Walbaum) fishery sustained by a naturalized population supported in large part by energetically dense kokanee salmon that serve as prey (Johnson et al., 2017; Pate et al., 2014). Large individual lake trout exhibit some of the fastest growth rates measured in North America (Martinez et al., 2009). These trophy fish are an important component of the fishery overall, and the presence of *S. californiensis* adds even more complexity to successfully balancing a hatchery-sustained kokanee

salmon population and a naturalized lake trout population in Blue Mesa Reservoir (Pate et al., 2014).

2.2 | Kokanee salmon collection

Kokanee salmon were collected with three different methods during this study, all in accordance with required Colorado Parks and Wildlife animal care protocols. Approximately 100 kokanee salmon per week (~ 1:1 male and female fish) were collected upstream from Blue Mesa Reservoir at the Roaring Judy Hatchery along the East River (Gunnison County, Colorado, USA) during the fall spawning runs of salmon from 2016 through 2020 ($n = 2,329$). In fall 2020, a floating trap net called a Merwin trap (Hubert et al., 2012) was deployed nearshore and retrieved on 19 October and 26 October to collect mature kokanee salmon ($n = 146$) in addition to those collected during the salmon run to Roaring Judy Hatchery. Finally, from 2018 to 2020, vertical gill nets, a standard suite of six multi-mesh nets described in Hansen (2019), were deployed overnight in offshore regions of Blue Mesa Reservoir in the spring (May/June), summer (July/August) and fall (November) to collect both mature and immature kokanee salmon ($n = 580$).

2.3 | Kokanee salmon processing

Kokanee salmon collected at the hatchery were measured for total length (TL; to the nearest millimetre), and these fish were assumed to be mature. Sex was determined visually and confirmed with gamete expression. Age was estimated (due to large sample size) with a machine-learning technique using fish length, sex, as well as the weights of sagittal otoliths extracted from each individual as predictor variables (Lepak et al., 2012). Kokanee salmon collected in the Merwin trap were processed in the same way as those collected in the hatchery. Kokanee salmon collected from vertical gill net sampling were put in ice water and transported to the laboratory where they were measured for TL and wet weight (WW; nearest gram). Reproductive condition (immature versus mature, or maturing) was assessed across all seasons for these fish and confirmed based on gamete presence/production during dissection. Ages were assessed in these individuals by extracting sagittal otoliths and visually inspecting their surfaces for annuli under a dissecting microscope.

2.4 | *Salmincola californiensis* counts

In 2016 and 2017, kokanee salmon (collected at the Roaring Judy Hatchery) were examined for the total number of adult *S. californiensis* attached. Adult *S. californiensis* were assessed because they could be seen relatively easily with the naked eye, though we acknowledge juveniles may have been present and impacting kokanee salmon. In 2018, 2019 and 2020, total counts of adult *S. californiensis* and the

locations where they were attached to kokanee salmon were documented. Attachment points were categorized as adipose fin, anal fin, body, cleithra, dorsal fin, gills, gill arches, isthmus, mouth, opercles, pectoral fins, pelvic fins and vent. These categories were condensed into three: 1) gills (gills and gill arches); 2) head (cleithra, isthmus, mouth and opercles); and 3) body/fins (adipose fin, anal fin, body, pectoral fins, pelvic fins and vent). All *S. californiensis* were counted in the laboratory setting. The ice water holding kokanee salmon captured in vertical gill nets and transported to the laboratory was filtered occasionally after laboratory processing was complete to assess whether any adult *S. californiensis* were shed, but none were observed.

2.5 | Statistical analyses

All statistical analyses were conducted using R 4.0.5 (R Core Team, 2021).

We applied a suite of generalized linear models (GLMs) to draw inference about patterns in the prevalence and intensity of *S. californiensis* on kokanee salmon through time and to evaluate the importance of key biological factors. Based on the distribution of adult *S. californiensis* count data, we accounted for overdispersion in each analysis of prevalence and intensity on hosts by using logistic regression for prevalence analysis, and the more appropriate negative binomial distribution (versus the Poisson) for intensity analyses. We used Akaike's information criterion (corrected for small sample size; AIC_c) to determine the most parsimonious (GLM) characterizing each dataset described below (Burnham & Anderson, 2002) and interpreted results from the reduced GLMs.

For prevalence analyses, we used information from kokanee salmon collected from 2016 through 2020 during the fall spawning run upstream from Blue Mesa Reservoir at the Roaring Judy Hatchery. Year of collection, length, estimated age (Lepak et al., 2012), sex, and the presence or absence of adult *S. californiensis* were noted for each individual. A strong correlation between length and estimated age precluded the inclusion of both variables (note: the age estimate calculation incorporates kokanee salmon length; Lepak et al., 2012) in the same models. Thus, kokanee salmon length was removed and estimated age was retained. We used logistic regression to evaluate the additive effects of year of collection, estimated age, a quadratic year term and sex on the probability of adult *S. californiensis* presence on these fish.

Information collected from 2016 through 2020 during the fall spawning run at Roaring Judy Hatchery (as described in the prevalence analysis) was used to quantify how the intensity of *S. californiensis* infection on mature kokanee salmon changed over the study period. We fit our models to the adult *S. californiensis* total counts (y_i for $i = 1, \dots, n$; $n =$ total count) on individual kokanee salmon using several covariates, including year of collection, estimated age (Lepak et al., 2012), a quadratic year term and sex. A reduced negative binomial GLM was fit to draw inference about covariates with significant explanatory value.

To evaluate whether the progression of *S. californiensis* infection intensity varied seasonally and whether the maturation process modified dynamics, we used data collected from kokanee salmon captured in Blue Mesa Reservoir from 2018 to 2020. These data arose from fish collected using vertical gill nets (2018–2020) and supplemented with fish captured in fall 2020 using the Merwin trap (set nearshore in the reservoir proper) since no mature kokanee salmon were captured in vertical gill nets in fall 2020. The Merwin trap was only used in 2020 and precluded a relevant comparison of fish collected in the Merwin trap to others; however, lice counts on fish collected with the Merwin trap were not significantly different than those collected at the hatchery in 2020. Information was available for each individual kokanee salmon about the year of collection, length, estimated (or interpreted in the case of individuals from the gill nets) age, sex, maturity and season of capture. Length and estimated (or interpreted) age were correlated (as expected) with maturity. Because kokanee salmon maturity was of interest, it was retained in analyses while length and estimated (or interpreted) age were excluded. We fit these data by modelling the adult *S. californiensis* total counts (y_i for $i = 1, \dots, n$) on individual kokanee salmon using several covariates, including year of collection, maturity, a quadratic year term, sex and season of capture. A reduced negative binomial GLM was fit to draw inference about covariates with significant explanatory value. Because age and maturity could not be included in the same model, we parsed and plotted data from 2018 to 2020 by estimated (or interpreted in the case of individuals from the gill nets) age and maturity to visualize differences in the effects of these correlated covariates.

From 2018 to 2020, adult *S. californiensis* on kokanee salmon sampled were categorized as being located on the gills, head and body/fins because there was increasing concern about sublethal effects and the importance of parasitism on the gills in particular. This was in contrast to data from 2016 and 2017 (used in the analyses described above), which were total counts and not location-specific. Kokanee salmon collected at the hatchery, in vertical gill nets, and the Merwin trap were included in analyses to evaluate *S. californiensis* intensity at these locations. We evaluated the influence of the year of collection, kokanee salmon maturity (versus correlated length and age covariates which were excluded from the model set), a quadratic year term and kokanee salmon sex, on the counts of adult *S. californiensis* on each of the three locations (gills, head and body/fins). A reduced negative binomial GLM was fit to draw inference about covariates with significant explanatory value.

3 | RESULTS

3.1 | *Salmincola californiensis* prevalence and intensity

The prevalence of adult *S. californiensis* infections increased significantly on mature Blue Mesa Reservoir kokanee salmon collected during the egg-take operation at the hatchery from 2016 to 2020.

Prevalence reached 100% for all age classes sampled 4 years after initial detection in 2015 (Figure 1). The most parsimonious logistic regression model indicated that year of collection, estimated age

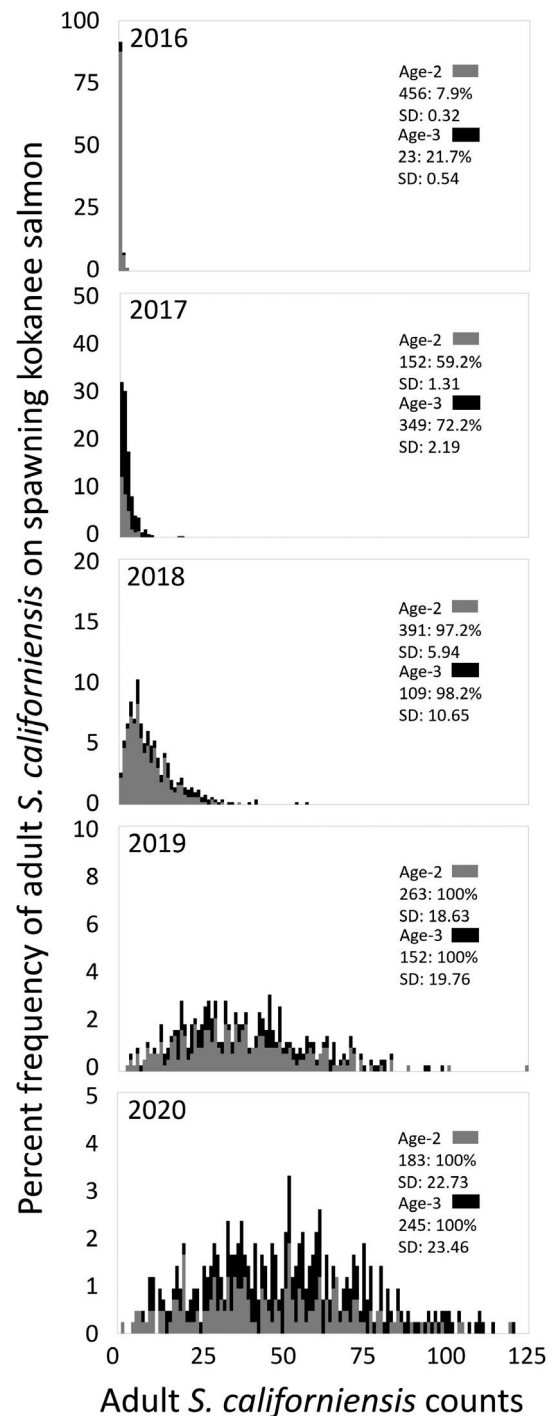


FIGURE 1 Prevalence and intensity of adult *S. californiensis* on kokanee salmon collected during the Blue Mesa Reservoir spawning run at the Roaring Judy Hatchery from 2016 to 2020. Essentially all kokanee salmon were estimated to be age-2 (light grey bars) or age-3 (black bars). Note the difference in scales on the x-axes for per cent frequency of adult *S. californiensis* counts on kokanee salmon. In the upper right of each panel, sample sizes are provided by age class, followed by the prevalence of adult *S. californiensis* infections on kokanee salmon, and the standard deviation (SD) of adult *S. californiensis* counts each year (upper left)

and sex were significant factors characterizing prevalence of adult *S. californiensis* (z -values = 20.3, 3.24 and 2.65, and p -values <.01, respectively), with estimated regression coefficients of 2.95 (± 0.15 , S.E.), 0.43 (± 0.16 , S.E.) and 0.53 (± 0.16 , S.E.), while the quadratic year term was not retained as a significant predictor of *S. californiensis* prevalence after accounting for the other factors. Based on these results, for every year that passed from 2016 to 2020, the log odds of *S. californiensis* being present on hosts increased by 2.95, female fish had significantly higher log odds of having *S. californiensis* present 0.43, and as fish increased a year in age, their log odds of having *S. californiensis* present increased 0.53.

The intensity of adult *S. californiensis* infections increased significantly on Blue Mesa Reservoir kokanee salmon collected during the egg-take operation at the hatchery from 2016 to 2020 (Figure 1). The variation in *S. californiensis* counts also increased significantly throughout the study period (Figure 1). We evaluated the influence of year of collection, kokanee salmon estimated age, a quadratic year term and kokanee salmon sex on the intensity of adult *S. californiensis*. The most parsimonious negative binomial GLM included the effect of year of collection (z -value = 27.4 and p -value <.01), estimated age (z -value = 9.1 and p -value <.01) and the quadratic year term (z -value = -27.4 and p -value <.01), with estimated regression coefficients of 1647 (± 61.1 S.E.), 0.30 (± 0.03 S.E.) and -0.41 (± 0.02 S.E.), respectively, while kokanee salmon sex was not retained as a significant predictor of *S. californiensis* intensity after accounting for the other factors. Based on these results, for every year that passed from 2016 to 2020, the log mean *S. californiensis* intensity increased 1,647, and as fish increased a year in age, their log mean *S. californiensis* intensity increased 0.30. The negative quadratic year term indicates a slowing in the increase in *S. californiensis* intensity over time. Indeed, unadjusted, mean (\pm S.D.), yearly intensities of *S. californiensis* on mature kokanee salmon collected at the hatchery were (0.1 \pm 0.3), (1.6 \pm 2.0), (9.2 \pm 7.7), (36.9 \pm 19.1), (48.1 \pm 23.7) in 2016–2020, respectively. This corresponds to increases in over an order of magnitude, nearly an order of magnitude, 4.0-fold and 1.3-fold, each year, respectively. Thus, *S. californiensis* intensity should be nearing an asymptote.

3.2 | The influence of season and kokanee salmon maturity on *S. californiensis*

The negative binomial GLM demonstrated that the intensity of adult *S. californiensis* infections increased significantly on Blue Mesa Reservoir kokanee salmon collected from the reservoir from 2018 to 2020 (Figure 2). We evaluated the influence of year of collection, kokanee salmon maturity, a quadratic year term, kokanee salmon sex and season of capture (spring, summer or fall) on the intensity of adult *S. californiensis*. The most parsimonious negative binomial GLM included the effect of year of collection (z -value = 7.6 and p -value <.01), maturity (z -value = 20.7 and p -value <.01) and the quadratic year term (z -value = -7.5 and p -value <.01), with estimated regression coefficients of 2,671 (± 353 S.E.), 1.55 (± 0.08 S.E.) and -0.66

(± 0.09 S.E.), respectively, while kokanee salmon sex and season of capture were not retained as significant predictors of *S. californiensis* intensity after accounting for the other factors. Based on these results, for every year that passed from 2018 to 2020, the log mean *S. californiensis* intensity increased 2,671, and mature fish had log mean *S. californiensis* intensities that were 1.55 higher than immature fish. The negative quadratic year term indicates again that there is a slowing in the increase in *S. californiensis* intensity over time (2018–2020 in this case). This adds further support to the idea that *S. californiensis* intensity is nearing an asymptote in Blue Mesa Reservoir.

Kokanee salmon age and maturity were positively correlated and could not be included in the same models. However, we were interested in the relative importance of each factor for determining *S. californiensis* intensity. Thus, Figure 3 was created showing mean adult *S. californiensis* counts by year, age and maturity to qualitatively visualize the effects of these factors from 2018 to 2020 using fish collected from the reservoir (vertical gill nets and the Merwin trap). In some cases, it was challenging to obtain rare samples (e.g. age-1 mature fish and age-3 immature fish), and sample sizes were limited for some age-maturity combinations (Figure 3). However, the mean infection intensity of mature fish generally exceeded those of their similarly aged counterparts over the 2018–2020 period, suggesting that the effects of being mature (or maturing) can increase levels of host infection beyond those expected by age alone. Mean age-0 and age-1 (immature) fish infection intensities remained relatively low and stable from 2018 through 2020. Conversely, mean infection intensities for older, mature fish increased over the study period and remained elevated above the immature members or their same cohort. One exception to this pattern was age-2 mature fish captured in vertical gill nets in 2020, but mature fish were rare in the catch that year and sample size was small ($n = 2$). However, numerous age-2 mature kokanee salmon were captured in the Merwin trap and these fish had an elevated mean infection intensity (Figure 3). Interestingly, age-4 kokanee salmon ($n = 4$) were captured in 2018, and these exhibited relatively low *S. californiensis* counts (1, 2, 7 and 24), while a single mature age-1 fish documented in 2020 had a count of 16. We acknowledge the limited sample sizes reflected in some of these comparisons as well as multiple interacting factors potentially influencing the observations (e.g. *S. californiensis* population dynamics and kokanee salmon ageing and maturation), but the representation provides some indication for the potential effect of maturation after accounting for age.

3.3 | *Salmincola californiensis* attachment location

The intensity of adult *S. californiensis* infections increased significantly on kokanee salmon gills, heads and fins/body collected from the reservoir, and at the hatchery, from 2018 to 2020, and focused largely around the head and gills (Figure 4). The negative binomial GLMs describing *S. californiensis* intensity on gills, heads and fins/bodies included year of collection, kokanee salmon maturity, a quadratic year term and kokanee salmon sex on the intensity of adult

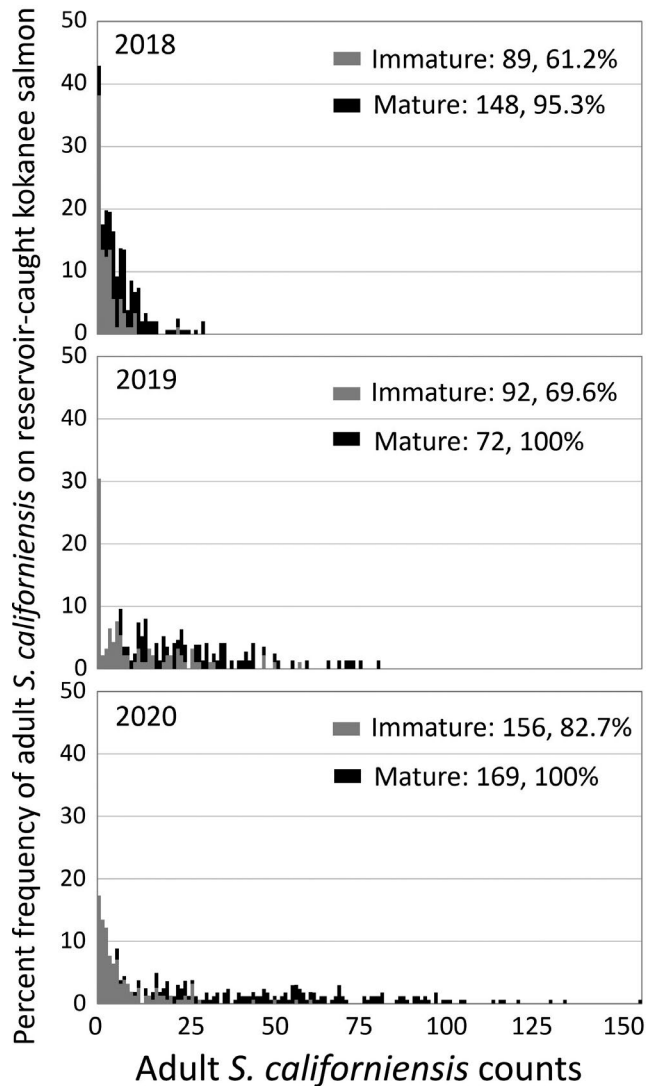


FIGURE 2 Intensity of adult *S. californiensis* on immature (light grey bars) and mature (black bars) kokanee salmon collected in Blue Mesa Reservoir from 2018 to 2020. Sample sizes are provided for immature and mature kokanee salmon followed by adult *S. californiensis* prevalence for each year (upper left), respectively

S. californiensis and showed that the same factors found to be important predictors of *S. californiensis* intensity in the previous analysis (year of collection, kokanee salmon maturity and a quadratic year term) were also important within these data. After accounting for these effects, we found that *S. californiensis* attachment locations were not independent, and characterizing *S. californiensis* at one attachment location provides information for predicting intensities at other locations. For example, *S. californiensis* log mean intensity on host gills increased 0.05 (± 0.002 , S.E.) and 0.03 (± 0.008 , S.E.) with increases in *S. californiensis* intensities on host heads (z -value = 23.6 and p -value $< .01$) and fins (z -value = 3.6 and p -value $< .01$), respectively. Similarly, *S. californiensis* log mean intensity on host heads increased 0.02 (± 0.001 , S.E.) and 0.05 (± 0.006 , S.E.) with increases in *S. californiensis* on host gills (z -value = 17.8 and p -value $< .01$) and fins (z -value = 8.2 and p -value $< .01$), respectively. Finally, *S. californiensis*

log mean intensity on host fins increased 0.007 (± 0.002 , S.E.) and 0.03 (± 0.003 , S.E.) with increases in *S. californiensis* on host gills (z -value = 3.6, and p -value $< .01$) and head (z -value = 12.0 and p -value $< .01$), respectively. This suggests that if individual fish are prone to high infections, those infections will likely be high across multiple attachment locations.

We visualized means and standard deviations of the *S. californiensis* count data for immature and mature kokanee salmon separately by year and by attachment location to provide an indication of the shape of the quadratic term suggesting that *S. californiensis* intensity increase is slowing/declining (Figure 5). At all attachment locations evaluated for immature individuals, mean *S. californiensis* intensities appeared similar or lower in 2020 than in 2019. Conversely, in mature kokanee salmon, mean *S. californiensis* intensities at all locations were higher in 2020 than in 2019, but with a lower proportional increase from 2019 to 2020 relative to the period from 2018 to 2019 (Figure 5).

4 | DISCUSSION

To our knowledge, this work represents the first rigorous documentation and extensive monitoring of the early invasion dynamics of *S. californiensis* on kokanee salmon in a large inland reservoir. In less than 5 years, Blue Mesa Reservoir went from having kokanee salmon with very few *S. californiensis* to supporting heavy parasite loads similar to those found in reservoirs with established *S. californiensis* populations (Hargis et al., 2014). As expected, the most infected fish tended to be older, mature individuals that carried over 100 individual adult parasites, largely focused around the head and gills. Though older, mature individuals tended to carry more parasites, there was also high variation in infection intensity among individuals. Higher intensities of *S. californiensis* were observed on older, mature kokanee salmon, but the increase in *S. californiensis* abundance can largely be described as a function of time, and it appears that the *S. californiensis* population is nearing an asymptote in Blue Mesa Reservoir under current conditions. These findings suggest that when *S. californiensis* first invade a system, there may be only a few years for managers and biologists to react before they can become well-established on host fish populations.

Based on our observations from Blue Mesa Reservoir, there was a rapid increase of *S. californiensis* on spawning kokanee salmon from 2016 to 2020. As hypothesized, year of collection was a significant predictor of prevalence and/or intensity of *S. californiensis* on kokanee salmon in all cases evaluated, indicating a strong effect of *S. californiensis* population growth on the results presented here. In 2016, relatively few *S. californiensis* were detected, and by 2020, age-2 through age-4 individuals had 100% prevalence, and in 2020, age-3 spawning kokanee salmon had a mean adult *S. californiensis* intensity exceeding 50, relative to a mean of less than one in 2016 when data were first being collected.

The relatively large variance and broad distribution of *S. californiensis* count data from hosts were indicative of a parasite

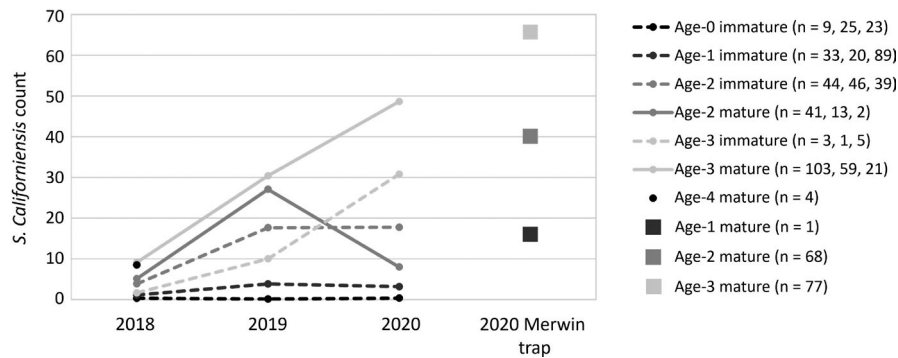


FIGURE 3 Mean intensity of adult *S. californiensis* on kokanee salmon collected in Blue Mesa Reservoir from 2018 to 2020. Mean *S. californiensis* counts from fish collected in the Merwin trap in 2020 are shown at the right and represented by solid boxes corresponding in colour and symbol to the age, maturity status and sample size listed in the legend. Standard deviations (when calculable) from left to right and top to bottom corresponding to parenthetical sample sizes listed in the legend are 0.5, 0.6, 0.7, 1.5, 2.0, 2.5, 4.1, 13.3, 10.9, 3.8, 11.7, NA, 1.5, NA, 18.6, 6.6, 19.6, 26.0, 10.7, NA, 21.6, 29.7, respectively. Mean *S. californiensis* counts from fish collected with vertical gill nets in 2018–2020 are represented with circles and solid lines (mature fish) and broken lines (immature fish) corresponding in colour and symbol to the age, maturity status and sample size listed in the legend. Age-4 mature fish are a single circle as they were only captured in 2018

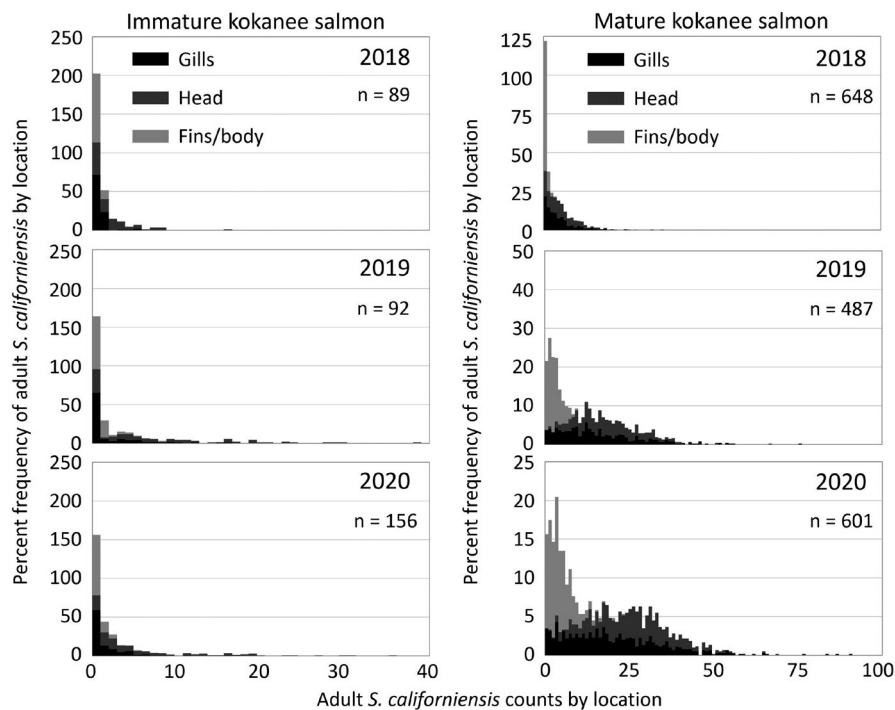


FIGURE 4 Attachment location of adult *S. californiensis* on immature (left) and mature (right) kokanee salmon by year. Adult *S. californiensis* attached to kokanee salmon gills (black bars) were those found on gills and gill arches of hosts. Adult *S. californiensis* attached to kokanee salmon heads (dark grey bars) were those found on the cleithra, isthmus, mouth and opercles of hosts. Adult *S. californiensis* attached to kokanee salmon fins/body (light grey bars) were those found on the adipose fin, anal fin, body, pectoral fins, pelvic fins and vent of hosts. Sample sizes are provided for each year (upper right of each panel). Note that the per cent frequencies (calculated location specifically) are presented cumulatively across locations and can therefore exceed 100% when multiple locations are considered simultaneously for a given adult *S. californiensis* count. Also, note the difference in scales on the x-axes for per cent frequency of adult *S. californiensis* counts on kokanee salmon by location

with relatively high host specificity (Combes, 1997; Poulin, 1998). Based on the findings of others (i.e. Barndt & Stone, 2003; Hargis et al., 2014), we expected that the oldest (and generally largest) hosts had the potential to carry the highest numbers of *S. californiensis*. Indeed, numbers of *S. californiensis* increased with kokanee salmon estimated age and when hosts were mature. However, we

note that kokanee salmon maturity is strongly correlated to length and age, and *S. californiensis* prevalence and intensity have been reported to increase with length as well as age (Barndt & Stone, 2003; Hargis et al., 2014; Neal et al., 2021). Given the inherent variability in the data, it is not surprising that even larger, older fish had the potential to carry light infections in Blue Mesa Reservoir.

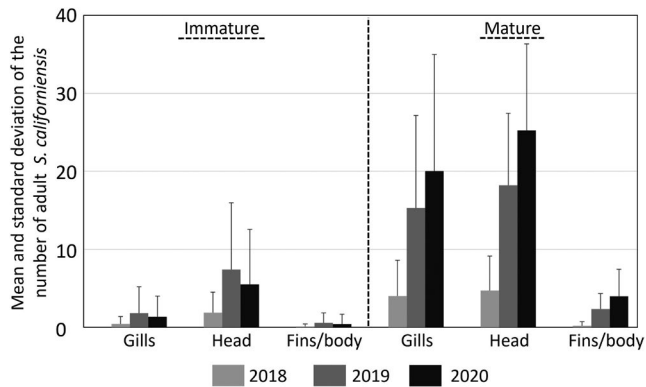


FIGURE 5 Adult *S. californiensis* attachment location: immature kokanee salmon sample sizes were 89, 92 and 156 in 2018 (light grey bars), 2019 (dark grey bars) and 2020 (black bars), respectively. Mature kokanee salmon sample sizes were 648, 487 and 601 in 2018, 2019 and 2020, respectively. Means and standard deviations of adult *S. californiensis* counts on kokanee salmon gills (those found on gills and gill arches of hosts), heads (those found on the cleithra, isthmus, mouth and opercles of hosts) and fins/body (those found on the adipose fin, anal fin, body, pectoral fins, pelvic fins and vent of hosts) are shown

Kokanee salmon maturity was a significant predictor of adult *S. californiensis* intensity, with mature individuals carrying higher parasite loads. Blue Mesa Reservoir salmon and trout do not transition from fresh to saltwater and back, but they do divert energy to maturation, reproductive processes/behaviours, as well as gamete production. As they mature, kokanee salmon change morphologically and also congregate prior to spawning and running upstream. The physiological and behavioural characteristics associated with the process of maturation could be contributing mechanistically to this observation (Sheldon & Verhulst, 1996). We note that maturity is correlated with age (and length), and host–parasite relationships influenced by these factors could be confounding. For example, one could reasonably assume that physically larger hosts (presumably with more parasite habitat) would have higher intensities of *S. californiensis* (Neal et al., 2021). If there are behavioural or physiological differences related to age/maturity that could influence *S. californiensis* intensity, those mechanistic effects could be obscured by larger fish having higher surface area and parasite habitat. We evaluated kokanee salmon age and maturity separately, and also attempted to represent these effects simultaneously, and found evidence that both are likely influencing *S. californiensis* counts despite low sample size in some cases for direct comparison, and we note that correlations between host size, age and maturity may confound the interpretation of results.

Currently, external (fins, body and vent) attachments of *S. californiensis* on kokanee salmon in Blue Mesa Reservoir appear to reach a maximum of about 15–20 adult individuals. By comparison, head (cleithra, isthmus, mouth and opercles) and gill (gills and gill arches) tissues of kokanee salmon supported higher numbers of *S. californiensis*, in some cases exceeding 50 adult individuals. Based on surface area alone, the adult *S. californiensis* attached on the fins/

body (adipose fin, anal fin, body, pectoral fins, pelvic fins and vent) of kokanee salmon had more habitat (albeit of unknown suitability) to colonize on individual hosts. This was also the case (but to a lesser extent) for kokanee salmon gills and heads, and there may be some upper limit to the number of adult *S. californiensis* that can occupy a host in a single area. However, it appears that something (perhaps physiological/biological/behavioural factors at the host and/or parasite level) is limiting (or enhancing) larger build-ups of adult *S. californiensis* at some locations versus others on kokanee salmon.

We were interested in potential differences in adult *S. californiensis* on male and female kokanee salmon (e.g. Rolff, 2002), and also potential seasonal patterns of adult *S. californiensis* intensity. Kokanee salmon sex was a significant predictor of adult *S. californiensis* prevalence, with females having increased prevalence compared to males. However, sex was not found to be a significant predictor of *S. californiensis* intensity in any analysis. Thus, we are cautious about drawing inference from these results. There may be mechanism(s) influencing these data beyond the covariates evaluated, and we also acknowledge that the population of *S. californiensis* (as indicated by adults on kokanee salmon) was growing rapidly during this time period, and this effect may be obscuring more subtle processes related to kokanee salmon sex. Seasonal patterns were not evident after accounting for the effects of year, kokanee salmon maturity and the quadratic year term on adult *S. californiensis* intensity data. If seasonal effects like increased water temperatures driving increased *S. californiensis* intensities were present, they were likely being obscured by *S. californiensis* population growth, and perhaps they will become evident as the *S. californiensis* population reaches a more stable state.

The expansion of *S. californiensis* to new, available habitat patches (hosts), was expected, though the expansion was relatively rapid. The growth of the *S. californiensis* population in Blue Mesa Reservoir occurred concomitant with several factors that may have influenced some of our observations. Stocking of kokanee salmon was conducted throughout this period, adding potential hosts to the system. In 2018, the estimate of Blue Mesa Reservoir volume above deadpool was the lowest in the data series (reduced by over half) based on data available from the US Department of Interior, Bureau of Reclamation unpublished data (K. Rogers, Colorado Parks and Wildlife, pers. comm.). This likely resulted in warmer overall water temperatures (Johnson & Martinez, 2012) and higher host and parasite densities within the water column.

These factors likely created conditions that were more ideal for *S. californiensis* and exacerbated their spread. But the combination of a lack of some data, correlations between many of the metrics used to indicate the data available, and the rapid population growth of *S. californiensis*, made it challenging to determine the importance of factors like water temperature and reservoir volume on *S. californiensis* spread. Thus, continued monitoring of evolving host–predator dynamics could provide more insight on factors exacerbating negative impacts from *S. californiensis*.

In 2020, the highest intensities of adult *S. californiensis* were observed on mature kokanee salmon during the egg-take operation

at Blue Mesa Reservoir. The kokanee salmon run in 2020 produced the lowest number of eggs since 1984 because of a lack of spawning adults (D. Brauch, Colorado Parks and Wildlife, pers. comm.). As in the past in Colorado, the *S. californiensis* infestation in Blue Mesa Reservoir occurred concomitant with a variety of potentially influential factors. Angling pressure and entrainment are factors to consider in Blue Mesa Reservoir, as well as a dynamic predatory lake trout population that consumes kokanee salmon (Pate et al., 2014). Further, heavily infected individuals may be more vulnerable to some of these stressors and predation, and it has been shown that once a host is infected with *S. californiensis*, they may be more susceptible to repeated infection (Neal et al., 2021), though these factors were outside of the scope of this study. These complexities/stressors can contribute to uncertainty/variation associated with *S. californiensis* infestations, and concern remains high about potential population-level impacts on kokanee salmon experiencing these conditions.

The Blue Mesa Reservoir kokanee salmon population is particularly important for sustaining approximately two dozen kokanee salmon fisheries throughout Colorado and demonstrates the potential for wide-reaching impacts of *S. californiensis* infestation. Conditions in Blue Mesa Reservoir are such that managers may have selective breeding options to promote *S. californiensis* resistance. Blue Mesa Reservoir is also a highly managed, non-native fishery, so some management options might be considered more feasible there relative to other systems. However, managers may face challenges at larger scales associated with changing climate conditions like warmer temperatures and elongated growing seasons (potentially favourable for *S. californiensis* life history; Murphy, Gerth, & Arismendi, 2020; Neal et al., 2021; Vigil et al., 2016) that may exacerbate the spread and ultimate impacts of *S. californiensis*. Further, Marcogliese (2001) described how warmer temperatures from climate change could intensify fish crowding, negatively influence fish immune response and increase parasite transmission. The combination of pre-existing/recurring stressors and the new threat from introduced *S. californiensis* could prove detrimental to the Blue Mesa kokanee salmon population (and others) in the future. Due to the continued spread of *S. californiensis* outside their native range (Kamerath et al., 2009; Ruiz et al., 2017; Sutherland and Whittrock, 1985), there is a need to better understand (through observation and experimentation) host-*S. californiensis* dynamics to avoid negative outcomes associated with infestations.

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CONFLICT OF INTEREST

To our knowledge, there are no conflicts of interest with any of the authors.

DATA AVAILABILITY STATEMENT

Data were not made accessible publicly for this manuscript.

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