Mechanisms underlying the correlation of parasite infection and host body condition: a case study in parasitic copepods of the genus *Salmincola* and their host salmonids

（寄生虫感染と宿主のボディコンディションの相関に見られるメカニズム：寄生性カイアシ類サルミンコーラ属とその宿主サケ科魚類における実証研究）

長谷川稜太

Ryota Hasegawa

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Abstract

Parasites are ubiquitous in natural ecosystems and play pivotal roles in many biological interactions. Since parasites are defined as organisms causing negative impacts on host health, many studies evaluated such negative impacts using host body condition calculated from host body length-weight relationships. However, these studies mostly examined mere correlations within specific time periods, and lacked explanations for the specific mechanisms of how these negative correlations were produced. In this dissertation, I examined mechanisms underlying negative correlations between body condition and parasite infections using ectoparasitic copepods *Salmincola* spp. and their host freshwater salmonids. Since the copepods infect the mouth cavity or gill rakers of host fish, I expected strong negative effects by impeding foraging activities. In addition, they are easy to detect in the wild without sacrificing the host, allowing us to monitor their impacts longitudinally.

Firstly, I systematically reviewed previous studies investigating host body condition and parasite infection relationships in fish host and parasite systems. Based on 215 publications with 966 data points, I found (1) many studies examined correlations between body condition and parasite infections only within limited time frames, (2) as much as 70% of the studies reported no significant relationships, (3) nearly 90% of the studies concluded that parasites are causes of host body condition reduction.

Then, I examined if negative correlations between host body condition and copepod infections were found in two *Salmincola*-salmonid systems in Hokkaido, Japan (chapters 2 and 3). I found clear negative correlations between copepod infections and host conditions in both systems, and such correlations were consistently found in all four examined seasons in mouth-dwelling copepod *S. markewitschi* and white-spotted charr system (chapter 3).

In chapter 5 and 6, I further examined ecological mechanisms causing condition reduction by parasites in the *S. markewitschi* and white-spotted charr system, paying special attention to host foraging changes. I found infected fish were less vulnerable to angling when they had poor body condition. Stomach contents analysis revealed that infected fish frequently foraged aquatic invertebrates, but again only when they had smaller body size. These results suggest that infected hosts changed foraging tactics dependent on body condition and body size.

While I found consistent negative correlations between parasite infections and host body condition in my replicated systems, such mere correlations do not necessarily mean causality given alternative possibilities: (1) parasites reduce host condition; and (2) parasites are likely to infect individuals with poor body condition. Additionally, positive feedback is also expected: infected hosts with reduced body condition can be vulnerable to further infections. This positive feedback may create heavily-infected hosts that could show low survival rate, resulting in driving population dynamics. Therefore, I tested these hypotheses using mark-recapture survey in the *S. markewitschi* and white-spotted charr system (chapter 6). I found that copepods reduced host conditions, and hosts with poor conditions were likely to be infected. These results indicate that positive feedback can occur in the wild. Most importantly, fish with smaller body size, poorer body condition and higher parasite loads showed lower apparent survival rate, suggesting that positive feedback could significantly affect host population dynamics via reduction of host survival.

Together, this dissertation revealed complex ecological mechanisms hidden under correlations between host body condition and parasite infections. Previous studies that simply analyzed and discussed these correlative relationships should have overlooked the real host-parasite relationships. I also discussed physiological and genetic mechanisms

inducing these correlations in wild populations, and why consistent negative correlations were maintained in my systems but not others in general discussion (chapter 7).

1. General Introduction

1.1. Ecological significance of parasites and its effects on host health

Host-parasite relationships are one of the most ubiquitous biological associations in natural systems (Begon et al. 1986; Combes 2001; Kuris et al. 2008). Parasites are defined as organisms living in or on another organism, and causing negative impacts on host health (Begon et al. 1986; Combes 2001). Such negative impacts by parasites could significantly affect host evolutionary changes (Combes 2001; Decaestecker et al. 2007; Paterson et al. 2010), host population dynamics (Hudson et al. 1998; Finley & Forrester 2003), community compositional changes (Friesen et al. 2020) and energy flow within and among ecosystems (Lafferty et al. 2008; Sato et al. 2012; Wood & Johnson 2015). Thus, assessing negative impacts by parasites is fundamental steps for understanding many ecological and evolutionary phenomena (Wood & Johnson 2015; Johnson et al. 2019).

Although parasites have been defined as organisms causing harmful effects on host health, the outcome of previous studies highly varied; while some studies found strong negative impacts of parasites (e.g. Rohr et al. 2008; Ferguson et al. 2011), others concluded that the effects of parasite are negligible (e.g. Carrassón & Cribb 2014; Shanebeck et al. 2022). Surprisingly, certain studies have even reported positive relationships between parasite infections and host health (Budischak et al. 2018; Paterson & Blouin-Demers 2020). These varying degrees of negative impacts could result in different ecological and evolutionary consequences. These inconsistent results can occur because some theory predicts that negative impacts are dependent on host and parasite traits, evolving toward maximum fitness of parasites (Anderson & May 1978), but it is also likely that many studies have failed to accurately evaluate negative impacts.

I suggested that three important aspects have been overlooked in previous studies. Firstly, the parasite negative impacts could vary among study timing and periods. This is because such impacts could be dependent on host-related (e.g. host health status, resource availability, host density) and parasite-related factors (e.g. infection levels, per-parasite negative impacts), all of which fluctuate among seasons, years, and host-parasite-developmental stages (Klemme et al. 2021; McNew et al. 2019; Ramsay & Rohr 2023b). Negative impacts by parasites, in some cases, become apparent after long periods (Treasurer et al. 2006; Ooue et al. 2017), even after parasite's detachments (Ooue et al. 2017). Despite the importance of these temporal aspects, many previous studies have been cross-sectional and conducted within short periods.

Secondly, there are many mechanisms underlying the reduction of host condition by parasites. In fact, negative impacts of parasites arise from various non-independent and non-mutually exclusive mechanisms, broadly categorized into two types; parasite-derived and host-derived mechanisms (Poulin 2011). Parasites, especially intestinal parasites, exploit host energy directly as resources to maximize their fitness, often depleting essential nutrition and resources from the hosts (Iaria et al. 2021). Many parasites also cause physical damages to host tissues to firmly attach to host individuals and/or exploit host tissues as resources (Shariff et al. 2008; Cardon et al. 2011; Fast 2014; Katahira et al. 2021). Parasites, especially ectoparasites those attaching to the host body surfaces, induce additional moving costs due to increased friction drag along the fish's body, leading to increased energy expenditure (Binning et al. 2013). In response to these parasite infections, host changes some physiological and behavioral functions that could subsequently reduce host health. Parasite infections also trigger host biological defenses including immunity, incurring significant costs for the maintenance and developments (Sheldon & Verhulst 1996). Immunity often causes

autoimmune-pathology that also cause host health reduction (Sheldon & Verhulst 1996; Weber et al. 2022). All processes induced by parasites ultimately lead to changes in host behavior (Barber et al. 2010; Chrétien et al. 2023). For instance, parasite infections alter host foraging, dispersal, competitive abilities, and social behaviors (Barber et al. 2010; Ezenwa et al. 2016). All these changes strongly affect host health status (Barber et al. 2010). Given that these different mechanisms result in different consequences for host health, it is crucial to identify the specific mechanisms responsible for the reduction in host health. Nevertheless, many previous parasitological studies mostly limited in pathological observations, and studies examining behavioral mechanisms on host performance are relatively scarce despite its importance (Chrétien et al. 2022).

Thirdly, most studies have overlooked hidden causalities underlying host health and parasite infection relationships. Although I so far treated parasites as negative components reducing host health, parasite infections also occur as consequences of reduced host health (Beldomenico et al. 2008; Blanchet et al. 2009a, b; Beldomenico & Begon 2010). Compromised host health generally leads to lower resource allocations into immunity (Becker et al. 2020), resulting in higher susceptibility to subsequent infections (Beldomenico & Begon 2010). This phenomenon is instinctively pervasive among medical areas as “opportunistic infection”, but surprisingly, only a few studies addressed this topic in wild populations (Beldomenico et al. 2008; Beldomenico & Begon 2010). These limited studies suggested that bidirectional causalities are also possible, which results in positive feedback. Thus, considering both aspects are highly necessary to predict host-parasite dynamics in the wild.

While exploring above three aspects is necessary to evaluate and interpret the results of body condition and parasite infections, there are several difficulties, and hence studies comprehensively examined all these aspects are critically lacking. One of the

challenging points is assessing host health status in the field because it requires longitudinal monitoring for each host individual, even though parasitological studies need host dissections in most cases (Poulin 2019). Many host species such as birds and mammals have high dispersal abilities, and only appear within short period (i.e. specific seasons; e.g. Liedvogel et al. 2011; Abrahms et al. 2019) that could also impede the individual tracking. Another obstacle derives from parasite's typical biology; many parasites are small, cryptic and easily drop off from the hosts. Additionally, many parasites show low infection levels in the wild (prevalence is under 10 %; e.g., Nagasawa 1984; Katahira et al. 2017) and only appear restricted areas during the specific seasons (Katahira et al. 2017; Ostfeld et al. 2005). These typical characteristics of parasites make it difficult to collect reliable data. Appropriate model systems that could overcome these difficulties are required to understand host-parasite associations in the wild.

Here, I examined above aspects in host-parasite associations using the *Salmincola* spp. and their host salmonids systems. The genus *Salmincola* is an ectoparasitic copepod groups that generally infect to branchial and buccal cavities of freshwater salmonids (Kabata 1969). While males are dwarf forms, attaching to female bodies (Kabata & Cousens 1973), females have large bodies (2mm <; Kabata 1969), firmly holds host tissues using their organs called "bulla" (Kabata 1969). These characteristics enables us to track both hosts and parasites without sacrificing them (see chapter 1.3 for detailed life-cycle of the genus *Salmincola*) and quantify the parasite impacts longitudinally (i.e. not cross-sectionally), and among different time periods. Their hosts, freshwater salmonids, generally show high residency (e.g. Nakamura et al. 2002), and the infection levels of *Salmincola* spp. are relatively high (prevalence is generally 10-30 %; Amundsen et al. 1997; Monzyk et al. 2015). Thus, we could monitor both host

and parasite dynamics in the field. Finally, 24 copepod species have been recorded from the genus *Salmincola* (Walter & Boxshall 2020) and many of which have circumpolar distribution, associating with host salmonid species in each region (Kabata 1969). This globally spreading host-parasite associations could be an ideal model system that might provide the opportunity to test general insights of host health and parasite infections.

To assess host health in wild populations, I focused host body condition index. Evaluating body condition index as host health or fitness components are general and pervasive approach to assess parasite negative impacts in the wild due to the simpleness and robustness (Lemly & Esch 1984; Lagrue & Poulin 2015; Sanchez et al. 2018; Comas 2020). So far, numerous body condition indices have been developed (Sanchez et al. 2018; Peig & Green 2009), and several body condition indices are known to correlate with some major host fitness components such as growth, survival and reproduction (Reimers et al. 1993; Neff & Cargnelli 2004; Burton et al. 2013). Since most indices are calculated from host body weight-length relationships (Bolger & Connolly 1989; Sanchez et al. 2018), they are easily calculated without sacrificing host individuals. Consequently, this method is useful to assess host health status in wild populations (Lagrue & Poulin 2015).

In this dissertation, I firstly conducted a systematically review on previous studies examining fish host body condition and parasite infections to identify the overall patterns and knowledge gaps in this research field (chapter 1.2). While many studies used host body condition index to quantify the negative impacts of parasites, no consensus of the associations between host condition and parasite infection have been available. Only recently, Sanchez et al. (2018) and Shanebeck et al. (2022) systematically reviewed these relationships, but the datasets in these studies were mostly focused on birds and mammals, and largely lacked many case studies of

fish-parasite systems, even though body condition indices have been widely used in fish ecology and fisheries (Lemly & Esch 1984; Bolger & Connolly 1989; Cone 1989). Secondly, I examined if negative correlations between host body condition and infections can be observed in two *Salmincola* – salmonid systems (chapters 2 and 3). In particular, I investigated if such negative correlations are consistent and/or variable among four different seasons in chapter 3. In chapter 4 and 5, I explored several behavioral mechanisms of host condition reduction by parasites. Here, I focused host foraging behavior because this behavior could strongly correlate with host health status (Godwin et al. 2018; Mrugała et al. 2023). Our main target species, *S. markewitschi*, attach to mouth cavities of host fish, and I could easily predict that their infections impede host foraging. In chapter 4, I evaluated host foraging motivation using angling vulnerability, a potential indicator for host foraging. For detailed examinations, I further investigated stomach contents of fish to see if host change prey items and amounts in chapter 5. In chapter 6, I examined causes and consequences of parasite infections and host condition, taking advantage of our mouth infecting large copepod systems that allowed me to track both hosts and parasites. Here, I particularly focused a positive feedback and resultant reduction of host survival. In chapter 7, I discussed further points of view and future research perspectives based on my findings. Consequently, this dissertation became one of a few studies that extensively examined the mechanisms underlying the negative correlations between fish host body condition and parasite infections in the wild host populations.

1.2. Systematic review on the correlation of parasite infection and fish host body condition

Introduction

Evaluating parasite's negative impacts using body condition has been one of the main topics in fisheries and fish biology (Lemly & Esch 1984; Cone 1989; Neff & Cargnelli 2004), and the number of such studies are still growing (Sanchez et al. 2018). However, no quantitative assessments have been conducted. Thus, I quantitatively evaluated the studies examining the relationships between host body condition and infections to identify the knowledge gaps in this fields.

Materials and methods

I extracted literatures examining fish body condition and parasite infections through Web search using ISI Web of Science. I used following key words; "fish*" AND ("parasit*" OR "infect*" OR "helminth*" OR "trematod*" OR "digenea*" OR "nematod*" OR "cestod*" OR "tapeworm*" OR "acanthocephala*" OR "monogenea*" OR "copepod*" OR "isopod*" OR "hirudin*" OR "branchiura*" OR "glochid*") AND ("condition ind*" OR "body condition*" OR "host condition*" OR "physiological condition*" OR "fish condition*" OR "condition factor*") on October 26, 2022.

A total of 809 studies were hit through web search above, of which 243 publications were retained after the title, abstract and key words screening (Figure 1). In this process, I only extracted publications apparently examined the relationships between infections of parasites and host body condition. Since I focused macroparasites throughout my dissertation, I removed publications examined the relationships between microparasites such as protozoa and virus. After the full text screening, I retained 215

publications for quantitative analysis. These 215 publications include 966 data points (Figure 1).

I recorded study types as (1) field study: studies conducted under natural environments, (2) aquaculture study: studies conducted under aquaculture facility such as fish farms and (3) experimental study: studies conducted under laboratory rearing conditions including experimental infections.

I categorized each data point into "correlative data point" and "comparative data point" to understand overall patterns of condition-infection studies. Correlational data points were studies that used correlative analysis such as Pearson's correlational test and simple regression (e.g. correlation between host body condition and infections). Comparative data points were studies that used comparative methods such as Student's *t*-test and Wilcox rank sum test (e.g. comparison of host body condition among infection status or infection categories).

I recorded reported statistical results for each data point. The correlative data points were categorized into "significant negative correlation", "significant positive correlation", and "no significant correlation". For comparative data point, I similarly categorized into "significant negative differences", "significant positive differences", and "no significant differences". The significant negative differences mean that one group that was infected (or heavily infected) by parasites showed poorer (lower) body condition compared to those of non-infected (or lightly infected) group. A statistical significance was considered based on reported *p*-value; the results were considered as significant if *p*-value was ≤ 0.05.

I also recorded "parasite taxonomic group" for each data points as following: Trematodes, Cestodes, Monogeneans, Copepods, Acanthocephalans, Isopods, mixed parasite groups (data points mixed with several species of parasites) and others (I combined several parasite groups such as Branchiura and Hirudinea due to the small

sample size). Further, these parasite groups were specifically summarized as “parasite types” as following; ectoparasites (parasites attaching on host surface), endoparasites (parasites infecting to host internal organs), and mixed parasite groups.

Additionally, I recorded “parasite transmission modes” for each data point. The category was divided into three following groups: trophically transmitted parasites (e.g. some cestodes and nematodes), directly attaching parasites (e.g. some copepods and trematodes), and others.

To understand the general patterns in field studies, I compared frequency of each type of results (no significant, significantly positive, and significantly negative) among parasite taxonomic groups (e.g. Trematodes, Nematodes, Copepods), parasite type (i.e. ectoparasite, endoparasite, others) and parasite transmission modes (i.e. trophically transmitted, directly attached, and others) using Fisher’s multiple comparisons. I conducted the statistical test with datasets combined with both correlative and comparative datasets to increase sample size.

Finally, I recorded how author(s) expected and interpreted the results of body condition and infection relationships based on full text reviewing; that is, I categorized each study into (1) authors treated parasites as causes of poor host body condition, (2) authors treated parasites as consequences of poor host body condition, (3) authors treated parasites as both causes and consequences of poor host body condition. Although several publications reported both negative and positive relationships between host body condition and infections, and some of them found no significant relationship between them, I estimated author’s intention for treating parasites from the whole texts, especially from introductions and discussions. Since authors in several publications did not mention or discuss the results of body condition and infection relationships, these publications are treated as (4) no discussion.

Results & Discussions

Publications I collected were mostly carried out in wild populations (N = 189, 88%). Other studies were carried out under aquaculture environments (N = 13, 6%) and experimental setups (N = 13, 6%). The total obtained data points were 966 (Field N = 848, 87.8%; Aquaculture N = 58, 6.0%; Experiment N = 60, 6.2%).

Other than experimental studies, correlative data points were more common compared to comparative data points (Figure 2). Strikingly, the category "no significant results" occupied the largest proportion (55-78%) of all datapoints in both correlative and comparable datapoints of all study types (Figure 2). Other trend was also similar among study and data point types; the category "significantly negative results" occupied the secondly largest proportion (14-39%) of all categories, then "significantly positive results" followed (0-11%) (Figure 2).

Of all data points in field study (N = 848), Trematodes represents the largest proportion (N = 209, 24.7%), followed by Cestodes (N=209, 24.7%), mixed parasite groups (data points combined with numerous parasites, N=129, 15.2%), Nematodes (N = 126, 14.9%), Copepods (N = 75, 8.9%), Acanthocephalans (N = 57, 6.7%), Monogeneans (N = 52, 6.1%), Isopods (N = 38, 4.5%) and Others (such as hirudinea, mussel's glochidium and branchiura N = 32, 3.8%).

Significant differences of frequencies of the results were found among Trematodes and other parasites. While the frequency of no significant result of Trematodes was significantly higher compared to those of Nematodes, Cestodes and Copepods, significantly negative results was tended to be higher in these three types of parasites (Fisher's multiple comparisons; $p < 0.05$). In the same analysis, there were no significant differences of frequency of each result among parasite types (all $p > 0.05$).

Parasites directly attaching to hosts showed higher frequency of no significant results compared to that of trophically transmitted parasites ($p < 0.05$). On the contrary,

trophically transmitted parasites showed higher frequency of negative results ($p < 0.05$). When comparing trophically transmitted parasites with other types, the frequency of both no significant results and significant positive results of trophically transmitted parasites were marginally higher than those of other groups ($p = 0.08$).

I found most authors assumed and/or concluded that parasites are causes of poor body condition ($N = 187$, 87 %), and surprisingly only 4 studies (2 %) concluded that parasite infections occur as consequences of poor host body condition. Most of the authors lacked the bidirectional relationships between host body condition and infections; only 7 publications (4 %) expected that parasites as both causes and consequences of poor host condition. 15 publications (7%) did not mention and discuss the relationships.

Surprisingly, majority of the studies (55-78%) I systematically reviewed did not detect significant negative relationships, despite that majority of definitions in these studies treating parasites as "negative components" that reduce host health (Combes 2001; Poulin 2011). However, this is rather consistent with a previous systematic review in mammals' and birds' literature, reporting that only about 30% of datasets found significant negative relationships (Sanchez et al. 2018). Therefore, this may be a general pattern across vertebrate hosts and parasite systems.

Why were many studies unable to detect negative correlations between host body condition and infection? One possible reason is the limited time frame in previous studies. Many parasites are not expected to cause a rapid decline in host health but rather gradually reduce host health for a long span (Dobson & May 1987; Shanebeck et al. 2022). Additionally, some parasites may negatively affect host health within a specific period or under particular situation, such as during host breeding timing and in the environments with resource shortages (McNew et al. 2019). Therefore, studies that evaluated host health within a short period using cross-sectional assessments might have

missed the negative impacts of parasites. Another reason that may have contributed to these results is the failures to accurately evaluate host body condition. Although body condition indices serve as good surrogates for assessing host health status (Sanchez et al. 2012), some indices may not be sufficiently sensitive to detect negative impacts of parasites in certain host-parasite systems (Parker & Booth 2013). In particular, many studies still tend to use the famous indices such as Fulton's body condition index, although that index is known to correlate with host body size (Cone 1989). Therefore, there are possible methodological failures, and researchers should carefully consider which indices are appropriate in focused host-parasite relationships.

Several studies have identified the positive relationships between host body condition and infection. These patterns were detected not only with trophically transmitted parasites but also with parasites directly attaching to the host body. Although most studies in my datasets concluded these relationships as "no negative impacts by parasites" without deep discussions, there are plausible and logical reasons why several studies detected this pattern. For instance, fish with better body condition could tolerate negative impacts by parasites (Råberg et al. 2007, 2009; Boots 2008). When hosts are in a better body condition, they could cure the damages caused by parasites themselves, and consequently, they could harbor many parasite individuals (Råberg et al. 2007, 2009; Paterson & Blouin-Demers 2020). Similar relationships have been documented in other vertebrate host and parasite systems (Råberg et al. 2007, 2009; Kutzer & Armitage 2016; Paterson & Blouin-Demers 2020). Furthermore, hosts with better condition tend to occupy better positions for foraging (Carrascal et al. 1998; Sanchez et al. 2018), frequently disperse among habitats (Terui et al. 2017) and socially interact with many individuals (Sauter & Morris 1995). These interactions increase the likelihood of acquiring infections, contributing to positive correlations between

condition and infections (Folstad & Karter 1992; Sauter & Morris 1995; Sanchez et al. 2018).

Most importantly, many studies treated negative correlations between body condition and infections as “results of parasite infections”, and overlooked the opposite causal links, “parasite infections occur as consequences of poor host body condition”, and “bidirectional relationships between such relationships”. Consequently, many studies concluded that focal parasite species does not cause harmful impacts on host health when they detected no significant relationships. This may have led researchers not to additionally examine mechanisms of condition reduction in those systems.

Figure 1. PRISMA diagram showing the flow of inclusion and exclusion of publications identified during the literature search is shown. The sample size of publications (shown as *N*) were provided.

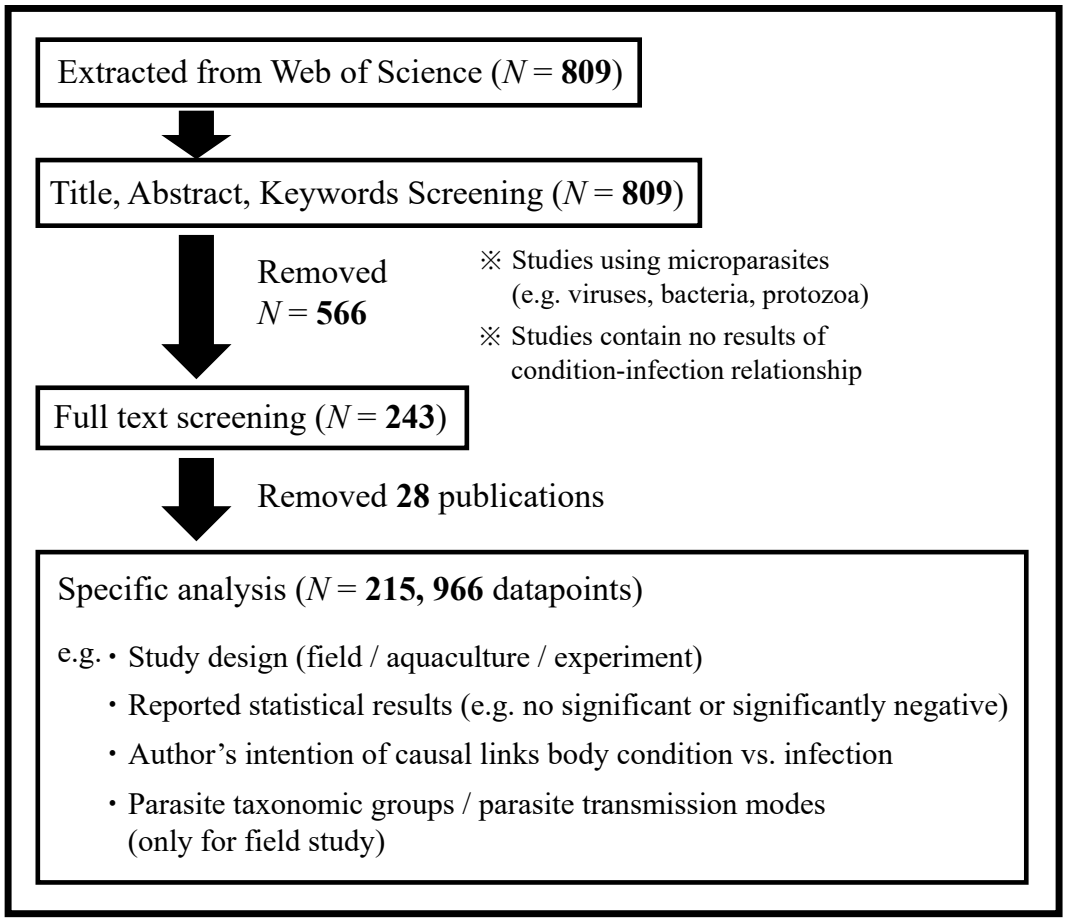
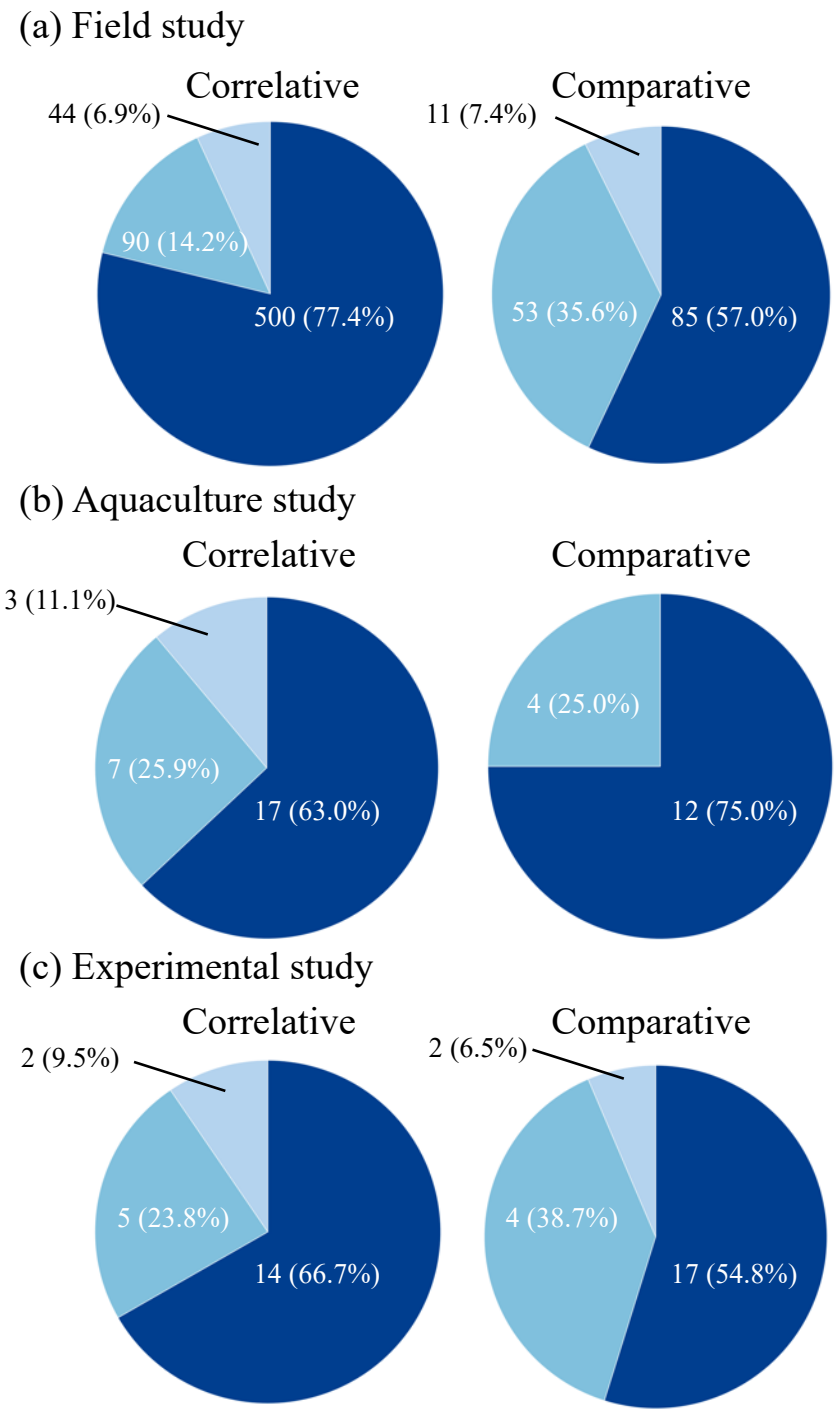


Figure 2. The proportion of reported statistical results for each data point. (a) field study, (b) aquaculture study, (c) experimental study. Dark blue, light blue and pale light blue indicates the proportion of "no significant results", "significantly negative results" and "significantly positive results", respectively. Left and right pie chart indicates the results of correlative and comparative data points, respectively.



Chapter 2

Negative correlation of parasite infection and host body condition: A case of *Salmincola edwardsii* parasitic on Southern Asian Dolly Varden *Salvelinus curilus*

Abstract

The genus *Salmincola* is an ectoparasitic copepod group commonly infesting the branchial and buccal cavities of salmonids. While negative impacts on hatchery fishes have been reported, their impacts on wild fish populations and distribution patterns are critically understudied. In the Shiretoko Peninsula, Hokkaido, Japan, I found parasites belonging to this genus on the branchial cavity of a stream salmonid, Southern Asian Dolly Varden *Salvelinus curilus*. All parasites recovered were identified as *Salmincola edwardsii* based on morphological characteristics and partial 28S rDNA sequences. Prevalence was highly heterogeneous even among neighboring streams (0–54.8%, < 10 km) with the mean intensity among streams being generally low (2.19 parasites/infeted fish). Despite the low intensity, quantile regression analysis showed negative trends between parasite intensity and host condition, suggesting that the infestation of *S. edwardsii* has a potential negative impact on the host salmonid. In addition, a single copepod was found from an anadromous fish, which could indicate some salinity tolerance of the copepods. It is important to evaluate the effects of *Salmincola* spp. on host species and determine the limiting factors on the parasite's distribution for proper management.

1. Introduction

The genus *Salmincola* (Family Lernaeopodidae), an ectoparasitic copepod group, mainly parasitizes freshwater salmonids (Kabata 1969). Most species in this genus have a circumpolar distribution like their salmonid hosts (Kabata 1969). They generally attach to the branchial cavity, buccal cavity, and fins (Kabata 1969; Black et al. 1982), with each species possessing preferred attachment sites and demonstrating strong host specificity, especially at the host genus level (Kabata 1969). For instance, adult female *S. californiensis* generally attach to the branchial cavity of *Oncorhynchus* spp. (Kabata & Cousens 1973), while *S. carpionis* commonly attach to the buccal cavity of *Salvelinus* spp. (Nagasawa et al. 1995). *Salmincola* spp. have been regarded as serious pests in hatcheries (Gall et al. 1972; Piasecki et al. 2004; Roberts et al. 2004). Heavy infestations can cause mechanical damage to gill tissue, which may affect the host's oxygen uptake, swimming performance, and resistance to environmental stressors (Kabata & Cousens 1973; Pawaputanon 1980; Sutherland & Wittrock 1985; Herron et al. 2018). Some studies suggested that their infestations can also cause a decrease in fecundity (Gall et al. 1972) and is lethal for fries, or even adult fishes (Kabata & Cousens 1977; Hiramatsu et al. 2001).

While some negative impacts have been reported on hatchery or experimental fishes (Gall et al. 1972; Piasecki et al. 2004; Roberts et al. 2004), their impacts on wild fish populations have been less understood. Only a few studies have suggested negative impacts on wild host salmonids, such as reduced recruitment (Mitro 2016) or condition (Kusterle et al. 2012), whereas many others found no apparent effects (Chigbu, 2001; Nagasawa & Urawa, 2002; Kusterle et al., 2012; Boone & Quinlan, 2019; Ayer et al., 2022). Some authors have concluded that the impacts of *Salmincola* spp. on host fishes are negligible in the wild because the prevalence and intensity are generally low

compared to hatchery fishes (Chigbu, 2001; Amundsen et al. 1997; Kusterle et al., 2012; Ayer et al., 2022). However, there are some cases where *Salmincola* spp. might have significantly affected or even eliminated local populations of stream salmonids (Mitro 2016; Mitro & Griffin 2018).

Detailed distributional records of *Salmincola* spp. have also been limited, even though such basic information is important for pest management. The duration of their life cycle and attachment to the host is affected by numerous environmental factors, such as host behavior (Poulin et al. 1991), host density (Hasegawa & Koizumi 2021), water temperature (McGladdery et al. 1988; Conley & Cutis 1993; Vigil et al. 2016), and water flow (Hasegawa & Koizumi 2021; Monzyk et al. 2015). Thus, the local environment should affect the parasites' infestation parameters (i.e. prevalence and intensity). However, there are only a few studies that examined their distribution at the regional scale (Mitro & Griffin 2018, Hasegawa & Koizumi 2021; White et al. 2020). Such distribution studies are needed, especially in East Asia, the southernmost edge of the distribution for both these copepods and their salmonid hosts (Nagasawa 2020a).

During a survey of Southern Asian Dolly Varden *Salvelinus curilus* (previous studies refered to the same species as *Salvelinus malma* (Umatani et al. 2018), *Salvelinus malma kraschenninnikovi* (Katahira 2017) or *Salvelinus malma kraschenninnikova* (Nagasawa 2020a), but I used this name following Sahashi & Morita (2021) in the Shiretoko Peninsula in eastern Hokkaido, Japan (Umatani et al. 2018), I found ectoparasites identical to *Salmincola edwardsii* on the branchial cavities of Southern Asian Dolly Varden. I recovered these parasite specimens from the hosts and examined their morphology and partial sequences of the 28S ribosomal RNA gene. In this study, I focused particularly on the host use and the regional distribution pattern of this parasite. I also examined if *S. edwardsii* was found from anadromous (i.e. sea-run)

host fish, which could be a possible indication of salinity tolerance in this parasite species.

2. Material and methods

2.1. Host collection and inspection

Fish samples used in this study were originally aimed for investigating the anadromy of Southern Asian Dolly Varden in the Shiretoko Peninsula using otolith Sr:Ca ratio (Umatani et al. 2018). Most Southern Asian Dolly Varden are fluvial (stream resident) in Hokkaido Island, but anadromous (sea-run) fish have been found in some streams in the Shiretoko Peninsula (Morita et al. 2005; Umatani et al. 2008; Umatani et al. 2018). Southern Asian Dolly Varden were collected by backpack-electrofisher (Smith-Root, Inc., Vancouver, Washington) and cast-net at 14 streams in the Shiretoko Peninsula, eastern Hokkaido, Japan (Table 1, Figure 1). For the purpose of the original study (Umatani et al. 2018), Southern Asian Dolly Varden larger than 17 cm were mainly collected. Sampling reaches were around 100–200 m from the mouth of the streams. Field surveys were conducted from October 2006 to November 2006. A total of 218 fish were brought to the laboratory for analyzing otolith Sr:Ca ratios to examine the anadromy of Southern Asian Dolly Varden (Umatani et al. 2018). I had frozen 215 Southern Asian Dolly Varden samples after the initial study (Umatani et al. 2018), and kept them in storage until the examination of the genus *Salmincola* in 2017.

In 2017, fish body length (fork length: FL) and weight (somatic weight: SW) were measured to the nearest 1 mm and 0.1 g, respectively in the laboratory. I used somatic weight (excluding internal organs) instead of total body weight because some Southern Asian Dolly Varden might have released eggs or sperm (samples were collected during

breeding season), which could cause potential bias when assessing body condition. Although some fish exhibited fork length shrinkages due to the long term freezing, fork lengths taken at collection (in 2006) were highly correlated with those measured in 2017 (Pearson's $r = 0.986$). In addition, since all sampled fish had been frozen in the same way, the potential biases due to freezing should be minimal.

The branchial cavity, buccal cavity, body surface, fins and fin bases were examined for the presence of the parasites. Since it was difficult to confirm the presence of copepods on the branchial and buccal cavity, I dissected the head area of all fish for a more comprehensive examination. When I found parasitic copepods, I recorded their attachment sites following two categories: gills (gill filaments and gill arches) and inner opercula. All copepods found were removed and preserved in 90 % ethanol. As one individual had no tail fin, I excluded this-individual from the statistical analysis (though retained it for the calculation of prevalence and mean intensity, see below).

2.2. Morphological identification of the copepod specimens

Since the parasite specimens recovered in 2017 were relatively low quality due to being frozen for a long time (i.e. about 11 years), I could not confidently identify them. Thus, I conducted additional sampling at the Pereke Stream, Shiretoko Peninsula on 26 July 2020. Cast net fishing was performed in four pools of the stream and a total of 30 Southern Asian Dolly Varden were captured. I visually checked the branchial cavity of each collected fish in the field. When infestation of the copepods was confirmed, the infested fish was immediately frozen (i.e. about a week) and sent to the laboratory of Azabu University, Kanagawa prefecture. In the laboratory, I carefully removed the copepods by forceps and preserved them in 70% ethanol for morphological and molecular identification.

Morphological examination was carried out using a light microscope (BX53, Olympus Inc., Japan) and a stereo microscope (SZX16, Olympus Inc., Japan). Five copepods were soaked in lactophenol, then dissected under the stereo microscope using the wooden slide method described by Humes & Gooding (1976). Morphological descriptions were made with the aid of a drawing scope equipped to the light microscope. The morphological terminology followed Kabata (1969). As males of the genus *Salmincola* are a dwarf form (Kabata & Cousens 1973), only females were subject to the morphological examinations. The specimens examined were deposited in the Invertebrates collection of the Hokkaido University Museum (ICHUM 6259, 6260, 6261, 6262, 6263), Sapporo, Japan.

2.3. Molecular analysis

Twenty-three specimens, i.e. five from Horobetsu Stream (No. 1 in Figure 1), seven from Funbe Stream (No. 2), one from Oshobaomabu (No. 6), two from Kamoiunbe Stream (No. 7), three from Chienbetsu (No. 11), two from Okkabake (No. 13) and one from Mosekarubetsu Stream (No. 12) in 2017, and two specimens from Pereke Stream in 2020 were used in the following molecular analysis for species identification. Total genomic DNA was extracted from whole parasites using a PureGene DNA isolation kit (Applied Biosystems) for the former twenty-one samples. For the latter two specimens, a part of the egg sac was used for DNA extraction, lysed in 20 μL of 0.02 N NaOH at 98 ℃ for 30 min (Nakao et al. 2018). I amplified a partial sequence of 28S rDNA region, which is known to be useful for identifying *Salmincola* spp. (Ruiz et al. 2017). The region was amplified with PCR using primers D1a (5'-CCC(C/G)CGTAA(T/C)TTAAGCATAT-3') and D3b (5'-TCCGGAAGGAACCAGCTACTA-3') (von Reumont et al. 2009). The PCR

reactions were performed in 10 μL and 25 μL volumes for the former and latter specimens, respectively, with thermocycling protocol for gene amplification as follows: initial denaturation at 95 ºC for 2 min, 35 cycles of 95 ºC for 30 s, annealing at 55 ºC for 40 s and extension at 72 ºC for 90 s, followed by a further extension at 72 °C for 8 min. Purified products were cycle sequenced with both the forward and reverse primers (i.e. D1a and D3b). The obtained sequences were analyzed with the software MEGA ver. 10.0.4 (Kumar et al. 2018), and compared with known sequences of *S. edwardsii* from Norway (DQ180346) and North America (KY113080, KY113081) and *S. californiensis* from North America (KY113082, KY113083; Ruiz et al. 2017) from the GenBank database.

2.4. Statistical analysis

I used the infestation parameters described in Bush et al. (1997); those were prevalence (percentage of individuals infested), intensity (the number of individual parasites in a single infested fish), and mean intensity (the average intensity among the infested fish).

To assess the effect of the parasite on Southern Asian Dolly Varden, I evaluated if the condition factor (CF) of the fish negatively correlated with the parasite number. CF was calculated as $CF = 10^5 \times SW/FL^3$, where SW is somatic weight (g) and FL is fork length (mm). CF was highly heterogenous among individuals specially within uninfested fish, and the variance decreased with increasing the parasite number (see Results). Therefore, I used quantile regression analysis instead of normal regression analysis (e.g. least squares regression analysis). Quantile regression analysis estimates any conditional quantiles of a response variable independently (instead of conditional mean) and is robust for the data with unequal variance (Cade & Noon 2003; Das et al.

2019). I calculated focal quantiles in steps of 0.1 from tau = 0.1 to tau = 0.9. The response variable was the CF and explanatory variables were the number of parasites. I first analyzed all host individuals except for one fish with 13 parasites, which was considered to be an outlier (total $n = 211$). I then performed the same analysis focused only on infested fish ($n = 52$) because the CF of uninfested fish had large variance and skewed the distribution of the data points (with an excess of zero). I used the package quantreg (Koenker et al. 2018) for quantile regression analysis. All the statistical analyses were conducted using R.3.5.2 (R Core team 2017). Differences were considered significant at $p < 0.05$.

3. Results

3.1. Morphological details of the parasite

Each individual body consisted of three major components: cephalothorax, second maxilla, and trunk (Figure 2A). From the dorsal view, the cephalothorax was tapered from posterior to anterior, and had weak constriction around the middle (Figure 2B). It was slightly shorter than its trunk (2.19–2.58 mm, mean = 2.40 mm, $n = 5$) and was separated by slight constriction from the trunk. Second maxilla was extended from each side of the cephalothorax (Figure 2A), and the distal end was fused forming the base of the bulla. The distal surface of the bulla was convex. Trunk was almost ovoid (2.15–2.98 mm, mean = 2.50 mm, $n = 5$). Two egg sacs were attached at its posterior end (Figure 2A), though one specimen had only one egg sac. Total body length (excluding egg sacs) was 3.54–4.70 mm (mean = 4.15 mm, $n = 5$).

First antenna, devoid of segmentation, with generally three short setae at its apex (Figure 2M, N). Some were well developed and slender (Figure 2M), but others were short and thick (Figure 2N). Second antenna was located at anterior part of the

cephalothorax. The tips of the biramous sympod had a large spiny pad on the basal surface and were composed of an endopod with two segments and an unsegmented exopod (Figure 2C). Large, protruding spiny pads were also present on the lateral side of the basal segment of the endopod. The distal segment of the endopod was usually covered by five apical armatures; those were 1) dorsal hook, 2) spine, 3) tubercle, 4) and 5) processes, with fourth armature, i.e. 4) process, being much bigger than the others (Figure 2D); tubercle was not observed in some specimens. The exopod was highly inflated, and their distal surface was covered by many large spines (Figure 2E). Two palps were projected laterally, with one-two small spines around each (Figure 2E).

The mandible usually had six teeth, but some specimens had seven. The distal four teeth were noticeably larger than the proximal two (Figure 2F). One pair of maxillipeds was located on the anterior part of the cephalothorax (Figure 2A). A short and curved claw was present on the distal end of the subchela with a small protrusion near its base (Figure 2G). One auxiliary papilla (shown as “auxiliary palp” in Ruiz et al. 2017) projected from near the posterior part of the claw (Figure 2H, I). There were some variations in the number of small spines distributed around the auxiliary papilla (Figure 2H, I). Some specimens had only 3–4 spines (Figure 2H), whereas others had many (generally more than 15) (Figure 2I). Prominent palp, also with some variations, positioned at the medial margin of the corpus (Figure 2G). Some were biramous (Figure 2J), while others had three-branched outgrowths, but the middle one was moderate (Figure 2K). The first maxilla with three subequal papillae at the distal end, had a small exopod near its base (Figure 2L). Each papilla had short seta at its tip.

3.2. Molecular analysis

The 708 bp partial 28S rDNA region sequences including gaps were obtained from all twenty-three specimens from fish caught in the Shiretoko Peninsula. Only a single haplotype was detected (under the process for the deposition of GenBank), which showed a 99.72% identity with *S. edwardsii* collected in Norway (2 bp difference with no gap; GenBank accession numbers is DQ180346) and 99.57 and 99.43% identity with the same species caught in North America (3 bp difference with 0-1 gap; GenBank accession numbers are KY113080 and KY113081; Ruiz et al. 2017). On the other hand, identities with *S. californiensis* from North America were 98.72 and 98.58% (8 bp difference with 1-3 gap; GenBank accession numbers are KY113082 and KY113083; Ruiz et al. 2017).

3.3. Distribution and effects on the host

A total of 215 Southern Asian Dolly Varden (112 males, 100 females, and 3 undetermined) were examined from the 14 streams (Table 1). The fish ranged from 114 mm to 275 mm (mean 189 mm) in fork length and 10 g to 199 g (mean 67 g) in somatic weight. Condition factor ranged from 0.44 to 1.38 (mean 0.97). Among the 98 Southern Asian Dolly Varden examined for otolith Sr:Ca ratios, 83 were stream resident and 15 were anadromous. Only a single copepod had infested an anadromous fish (sampled at Funbe), whereas all of the other copepods were found from resident fish. All *S. edwardsii* were found in the branchial cavity. Of the total 116 copepods detected, 104 (89.7%) were found from the gills (gill filaments and gill arches), whereas 12 (10.3%) were found from the inner opercula. Some of the attachment sites of the gill filaments turned white (Figure 3) as reported in previous studies (Sutherland & Wittrock 1985; Ruiz et al. 2017).

S. edwardsii was present in 10 streams and absent in 4 streams (Table 1). Of the 215 Southern Asian Dolly Varden, 53 individuals were infested. The mean prevalence among the streams with the parasites presence was 52.4%, whereas the prevalence among the streams was markedly different (Table 1, Figure 1). The highest value was 54.8% (Funbe, No. 2 in Figure 1 and Table 1) and the lowest value was 0% (No. 3, Opekepu and No. 10, Kennebetsu) among the streams where enough samples were collected (> 30 individuals) (Table 1, Figure 1): these streams are separated by <30 km. The mean intensity of *S. edwardsii* on Southern Asian Dolly Varden among streams was 2.19 with the maximum intensity was 13 (No. 1 Horobetsu, fish with FL 210 mm, SW 97 g).

Condition factor showed negative trends with the number of *S. edwardsii*, although the correlation was statistically significant or marginally significant only for the 0.2th, 0.3th, and 0.9th quantiles (Table 2a; Figure 4a). After excluding the uninfested fish from the analysis, however, a significant negative effect was detected for most of the focal quantiles (Table 2b; Figure 4b). In both analysis, the variance of CF became smaller with increasing the parasite number and the upper bound decreased with increasing the parasites (e.g., tau = 0.9, Table 2; Figure 4).

4. Discussion

4.1 The parasite identification and attachment sites

So far, five species of the genus *Salmincola* have been recorded from Japan; *S. californiensis* (reported as *S. yamame* in Hoshina & Suenaga 1954, Nagasawa & Urawa 2002; Hoshina & Nishimura 1976), *S. carpionis* (reported as *S. falculata* in Yamaguti 1939, Nagasawa et al. 1995; Nagasawa & Urawa 2002), *S. stellata* (Nagasawa & Urawa 1991, Nagasawa et al. 1994; Hiramatsu et al. 2001), *S. edwardsii* (Nagasawa 2020a,b;

Nagasawa & Kawai 2020) and *S. markewitschi* (Shedko & Shedko 2002; Nagasawa 2021; Nagasawa 2020c). Of these five species, *S. edwardsii* is distinguished from the other species according to the following characteristics; process 4 was the most prominent component of all five armatures at the distal segment of the second antenna endopod; huge and inflated spiny pads on basal segment of the second antenna endopod and sympod; bulla was not stellate, but round in shape (Kabata 1969). Almost all morphology of the copepod specimens in the present study were consistent with *S. edwardsii* specimens in other studies (Kabata 1969; Shedko & Shedko 2002; Ruiz et al. 2017; Nagasawa 2020a). Thus, I morphologically identified these specimens as *S. edwardsii*. Additionally, *S. edwardsii* was also recently found from rivers in eastern Hokkaido (Nagasawa 2020a, b, Nagasawa & Urawa 2002) and the Kuril Islands (Shedko & Shedko 2002).

It is well noted that the members of the genus *Salmincola* showed morphological variations in some body parts among regions, particularly between the Palearctic and the Nearctic regions (Kabata 1969). In the present study, some specimens had numerous spines on the ventral side of the maxilliped tip (Figure 2I), whereas others had few (Figure 2H) even in the same population. Kabata (1969) reported that numerous spines on these parts were one of the characteristics of specimens from Eurasia, and spines were few or absent in those of North America (Kabata 1969). However, Ruiz et al. (2017) also found similar spines from North American specimens. Russian and Japanese specimens also had greater or fewer numbers of spines (Shedko & Shedko 2002; Nagasawa 2020a). Trunk length in the present study was 2.15–2.98 mm (mean = 2.50 mm), which was consistent with the previous reports that specimens from the Palearctic region had a longer trunk length (2.96–3.00 mm) than those from the Nearctic (1.60–2.00 mm; Kabata 1969). Although it was not simply concluded that there are

differences in trunk length between the two regions (Palearctic vs. Nearctic), because other reports showed shorter trunk length even though such specimens were recovered from the same or an adjecent area to the present study (Shedko & Shedko 2002; Nagasawa 2020a).

Overall, considering the high morphological variations despite the small geographic scales, these differences were possibly derived from phenotypic plasticity. Parasitic copepods often change their morphology depending on the ambient environment, such as attachment sites (Hogans 1987; Abaunza et al. 2001). However, in many cases, the sample size in each population or area was so small that the authors could not refer to the mechanisms producing the variation. Future studies with larger geographic scales and sample sizes will reveal these mechanisms. Another cause of morphological variation was artifacts, as some previous reports have shown that the method of storage or handling of specimens may cause shrinking or loss of specimen body parts (Kabata 1969; Nagasawa 2020a).

While I detected all the copepods from the branchial cavities of host fish, some previous studies found that infestation of *S. edwardsii* occured on body surfaces such as the fins and fin bases (Black 1982; White et al. 2020). Their attachment sites are also affected by host body size and environmental factors like flow velocity (Black 1982). Although copepods were likely to infest the fins and fin bases on small hosts (Kabata & Cousens 1977; Black 1982), I could not confirm if the smaller fish could be infested on other body parts, because of the lack of small fish samples (Umatani et al. 2018). However, the main attachment site for *S. edwardsii* seemed to be branchial cavities in my study area, as previous studies reported (Shedko & Shedko 2002; Nagasawa 2020a).

4.2. Effects of *S. edwardsii* on host fish

To date, while several studies have examined the effects of *Salmincola* spp. on host body condition in the wild, many of them did not find any effects of the copepod infestation (Amundsen et al. 1997; Chigbu 2001; Kusterle et al. 2012; Ayer et al. 2022) or found significant negative effects only in the cases where the infestation intensity was very high (>100 copepods per host, Kusterle et al. 2012). Some researchers, therefore, concluded that *Salmincola* spp. have negligible effects on host fishes in the wild because their infestation level was generally low in natural conditions (Black et al. 1983; Amundsen et al. 1997). However, the present study detected negative trends between host fish condition and the infestation of copepods even at low-intensities (max intensity = 7 for statistical analysis). In addition, because the upper bound of condition factor decreased with increasing the numbers of the copepods, the parasite might be a limiting factor for the host condition. These results suggest that a low-intensity of copepods can also reduce the host's body condition in the wild. Previous studies showed that the infestation of copepods can have serious histopathological effects on host tissues such as gills (Kabata & Cousens 1977; Herron et al. 2018; Nagasawa 2020a) and body surfaces, even at a low-infestation level (White et al. 2020). I also observed whitened attachment sites, suggesting that the copepods' attachment caused gill lesions. Such damage can severely drain host energy (White et al. 2020), and negatively affect host condition as a consequence. This histopathological effect of infestation might be the reason for the negative relationship between host fish condition and intensity in the present study. Further pathological studies are required to understand the histopathological effects of copepods on host fish condition in this region.

My results, however, should be viewed with some cautions. First, the negative relationship between host fish condition and the parasite number became obscure when

including uninfested fish. This is because the condition factor of uninfested fish is highly variable. Uninfested fish with low body condition might have experienced copepod infestations in the past, which may have led to the high variability of condition among hosts. Such a large variance of uninfested host was also observed in other host-parasite system (e.g. Lemly & Esch 1984; O'Connell-Milne et al. 2016). One of the reasons why past studies have not detected the effects of *Salmincola* spp. may be the inclusion of uninfested host fishes that have high variations in body condition. Thus, it is worth analyzing the data both including and excluding uninfested individuals when assessing parasite effects accurately.

Second, the sample size was skewed to low intensity individuals: about 70% of infested fish had only 1 or 2 parasites. Although my sample size was not small (> 200 host individuals) and this system naturally had relatively low prevalence and intensity, data on heavily infested fishes are crucial to further understanding the effects of *Salmincola* spp. on host fish condition in wild populations. Nevertheless, the quantile regression analysis clearly showed overall trends for the negative relationship and decrease the variability in host condition. Because the quantile regression analysis can handle the data with unequal variance (Cade & Noon 2003; Das et al. 2019), this will be effective to analyze the complex effects of parasites.

Finally, the negative correlation between parasite number and host condition does not necessarily mean a causal link. An alternative mechanism is that the hosts with lower body conditions are more susceptible to parasites (Beldomenico et al. 2008; Beldomenico & Begon 2010). To reveal the causal relationship of my results, a mark-recapture study and/or lab experiments are required in a future study (Ayer et al. 2022).

4.3. Regional distribution pattern and infestation of anadromous fish

My results showed that the distribution of *S. edwardsii* was highly heterogeneous even within a relatively small geographic scale. Previous studies also reported similar results on *Salmincola* spp. and discussed the heterogeneous distribution in terms of habitat connectivity, host extinction and reintroduction (Mitro & Griffin 2018; Hasegawa & Koizumi 2021). In particular, since the genus *Salmincola* is host-specific (Kabata 1969), once their host populations go locally extinct, they will also go extinct with their hosts. Thus, local population dynamics and extinction of hosts could be a major factor determining the local abundance of parasites. However, in my system, Southern Asian Dolly Varden populations in the Shiretoko Peninsula are generally healthy, with no recent record of population extirpation or artificial reintroduction (except for a very small population that was significantly influenced by non-native rainbow trout *Oncorhynchus mykiss* (Morita et al. 2003). Therefore, local population dynamics of the host may not be the primary cause for the high variation in parasite abundance.

Local environmental differences may explain the heterogeneous distribution. In particular, different levels of artificial modification occurred in the studied streams, such as construction of dams and logging, which increases water temperature (Kishi et al. 2009). Because the development and life-history of *Salmincola* spp. are strongly affected by water temperature (McGladdery & Johnston 1988; Conley & Cutis 1993; Vigil et al. 2016), such habitat modifications mediate parasite life cycles and, hence, affect parasite load. In addition, construction of dams can change the water current pattern. In general, large pools or glides were often created below or above the dams, which would reduce water current velocity. It is suggested that the copepodids of the genus *Salmincola* can attach to hosts more easily under lower current conditions

(Hasegawa & Koizumi 2021; Monzyk et al. 2015). Furthermore, not only physical but also biological characteristics could affect the distribution and abundance of *S. edwardsii*. For example, the density of hosts generally plays an important role in the sustainability of parasite populations (e.g. Anderson & May 1978; Arnebert 2002), including *Salmincola* (Hasegawa & Koizumi 2021). In the future, I should consider multiple variables in identifying the limiting factors of distribution.

Since I recovered a copepod from an anadromous individual, it is possible for dispersal of the copepod between streams via anadromous host fish. Indeed, though *S. edwardsii* are a freshwater species, living individuals were recovered from hosts captured in the sea or brackish water, suggesting that this species has salinity tolerance (Black et al. 1983; Shedko & Shedko 2002). Nagasawa (2020a) investigated the distribution of *S. edwardsii* from 9 rivers on Hokkaido Island, and found them only from the eastern side of the island, where some fish show anadromy (Umatani et al. 2018; Morita et al. 2005; Umatani et al. 2008). The author concluded that the anadromy of the hosts may play an important role in its distribution expansion (Nagasawa 2020a). However, this possibility is limited to the regional scale because the degree of anadromy was low in the Shiretoko Peninsula (Morita et al. 2005). If the introduction of this parasite by migrants frequently occurs, the infestation level should be similar among neighboring streams because dispersal of migrants would occur in neighboring streams. However, in the present study, no such pattern was observed. Therefore, dispersal should be insufficient to homogenize the abundance or distribution of *S. edwardsii*. In fact, the probability of dispersal on anadromous fish is possibly very low, because I could find only one copepod from an anadromous form. However, the sample size was very small (anadromous host, $n = 15$), and I cannot rule out the possibility that the infestion of the copepod on the migrant occurred after returning to the stream from the

sea. It is necessary to confirm if this population of copepods can survive in saline conditions.

4.4. Conclusion

Infestation of *S. edwardsii* may affect host health and they have a heterogeneous distribution pattern, even on very small geographic scales like that of the Shiretoko Peninsula in Hokkaido, Japan. However, I know very little about the limiting factors affecting the distribution, prevalence, and intensity of *Salmincola* spp., which could be critical to proper population management. In particular, Hokkaido Island is the southernmost margin of the Southern Asian Dolly Varden's native range, and populations in the area are thought to be the most vulnerable to climate change (Nakano et al. 1996). Some southern populations of *S. edwardsii*, in Wisconsin, North America for example, have undergone outbreaks and significantly affected brook trout *S. fontinalis*, which may be exacerbated by global warming (Mitro 2016). Additional studies and monitoring are needed to evaluate the effects of *S. edwardsii* to better understand the epizootics of these ectoparasites.

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Table 1. Prevalence and mean intensity of *Salmincola edwardsii* and characteristics of Southern Asian Dolly Varden *Salvelinus curilus* in each stream in the Shiretoko Peninsula, Hokkaido, Japan.

No. in Fig. 1	Stream	Number of the fish inspected	Number of the fish infected	Fork length range (mean ± SD)	Prevalence (%)	Mean intensity (range)
1	Horobetsu	12	3	164–214 (188 ± 16)	25.0	5.33 (1–13)
2	Funbe	31	17	173–235 (196 ± 19)	54.8	2.71 (1–7)
3	Opekepu	30	0	158–238 (193 ± 21)	0	-
4	Shariki	1	1	234	100	4
5	Kanayama	1	0	209	0	-
6	Oshobaomabu	1	1	228	100	1
7	Kamoiunbe	30	9	143–204 (176 ± 16)	30.0	1.56 (1–3)
8	Aidomari	1	0	198	0	-
9	Kikiribetsu	2	2	194–197 (196 ± 2)	100	1.5 (1–2)
10	Kennebetsu	31	0	114–243 (191 ± 28)	0	-
11	Chienbetsu	31	5	153–219 (192 ± 15)	16.1	1.2 (1–2)
12	Mosekarubetsu	30	10	147–243 (177 ± 17)	33.3	1.8 (1–5)
13	Okkabake	9	4	161–205 (184 ± 15)	44.4	1.75 (1–3)
14	Ponhoromoi	5	1	173–275 (213 ± 39)	20.0	1
	Total	215	53	114–275 (189 ± 22)	24.7	2.19

Table 2. Results of the quantile regression analysis of host condition factor and the number of *S. edwardsii*. (a) including uninfested fish and (b) excluding uninfested fish.

(a)

tau = 0.1

	Estimate	SE	*t*-value	*p*-value
Intercept	0.826	0.017	48.042	<0.01
Parasite number	−0.005	0.008	−0.604	0.546

tau = 0.2

	Estimate	SE	*t*-value	*p*-value
Intercept	0.884	0.014	65.397	<0.01
Parasite number	−0.013	0.007	−2.042	0.042

tau = 0.3

	Estimate	SE	*t*-value	*p*-value
Intercept	0.915	0.011	81.051	<0.01
Parasite number	−0.018	0.008	−2.121	0.035

tau = 0.4

	Estimate	SE	*t*-value	*p*-value
Intercept	0.945	0.011	85.874	<0.01
Parasite number	−0.012	0.011	−1.112	0.267

tau = 0.5

	Estimate	SE	*t*-value	*p*-value
Intercept	0.974	0.012	80.680	<0.01
Parasite number	−0.010	0.014	−0.710	0.478

tau = 0.6

	Estimate	SE	*t*-value	*p*-value
Intercept	1.009	0.012	84.095	<0.01
Parasite number	−0.014	0.012	−1.167	0.245

tau = 0.7

	Estimate	SE	*t*-value	*p*-value
Intercept	1.040	0.013	83.090	<0.01
Parasite number	−0.005	0.008	−0.581	0.562

tau = 0.8

	Estimate	SE	*t*-value	*p*-value
Intercept	1.072	0.014	78.269	<0.01
Parasite number	−0.012	0.008	−1.408	0.161

tau = 0.9

	Estimate	SE	*t*-value	*p*-value
Intercept	1.127	0.015	73.245	<0.01
Parasite number	−0.030	0.017	−1.744	0.083

(b)

tau = 0.1

	Estimate	SE	*t*-value	*p*-value
Intercept	0.874	0.0542	16.132	<0.01
Parasite number	−0.015	0.014	−1.092	0.280

tau = 0.2

	Estimate	SE	*t*-value	*p*-value
Intercept	0.918	0.045	20.218	<0.01
Parasite number	−0.020	0.014	−1.367	0.178

tau = 0.3

	Estimate	SE	*t*-value	*p*-value
Intercept	0.98	0.034	29.228	<0.01
Parasite number	−0.027	0.007	−3.634	<0.01

tau = 0.4

	Estimate	SE	*t*-value	*p*-value
Intercept	1.014	0.034	30.160	<0.01
Parasite number	−0.032	0.010	−3.106	<0.01

tau = 0.5

	Estimate	SE	*t*-value	*p*-value
Intercept	1.057	0.038	27.812	<0.01
Parasite number	−0.038	0.014	−2.653	0.011

tau = 0.6

	Estimate	SE	*t*-value	*p*-value
Intercept	1.091	0.030	35.924	<0.01
Parasite number	−0.041	0.012	−3.317	<0.01

tau = 0.7

	Estimate	SE	*t*-value	*p*-value
Intercept	1.091	0.030	35.924	<0.01
Parasite number	−0.041	0.012	−3.317	<0.01

tau = 0.8

	Estimate	SE	*t*-value	*p*-value
Intercept	1.115	0.056	20.015	<0.01
Parasite number	−0.030	0.018	−1.665	0.102

tau = 0.9

	Estimate	SE	*t*-value	*p*-value
Intercept	1.19	0.090	13.279	<0.01
Parasite number	−0.046	0.018	−2.516	0.020

Figure 1. Sampled streams and prevalence of *Salmincola edwardsii* on Southern Asian Dolly Varden *Salvelinus curilus* in the Shiretoko Peninsula, Hokkaido, Japan. Refer to Table 1 for detail information on the streams. Size of each pie-chart represents sample size of Southern Asian Dolly Varden: Small: 1–2, Medium: 5–12, Large: > 12. The numbers in each pie-chart represents prevalence (%) calculated by all the fish inspected in each stream.

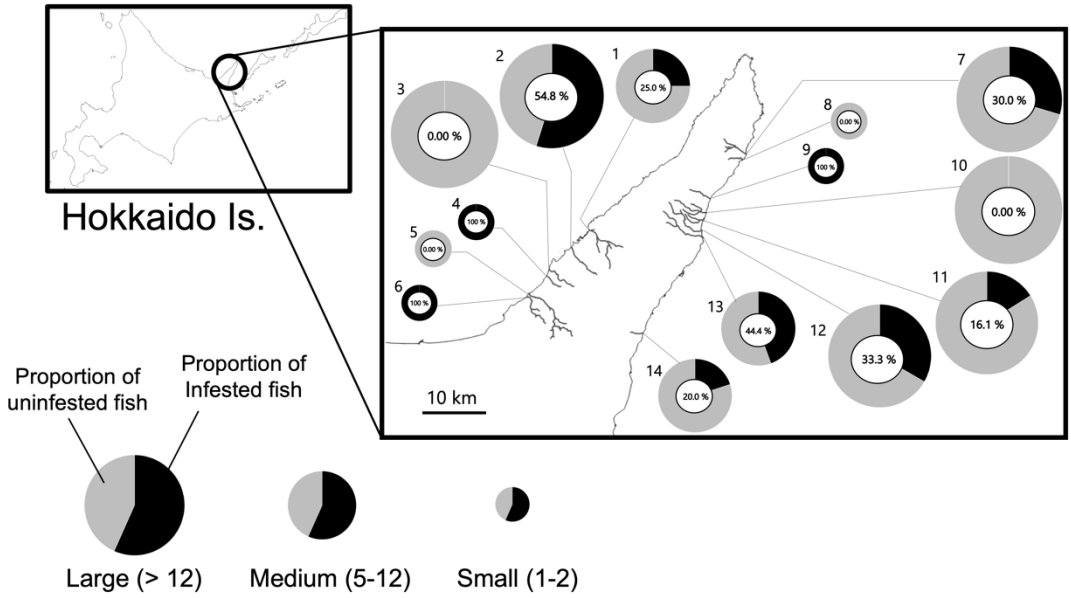
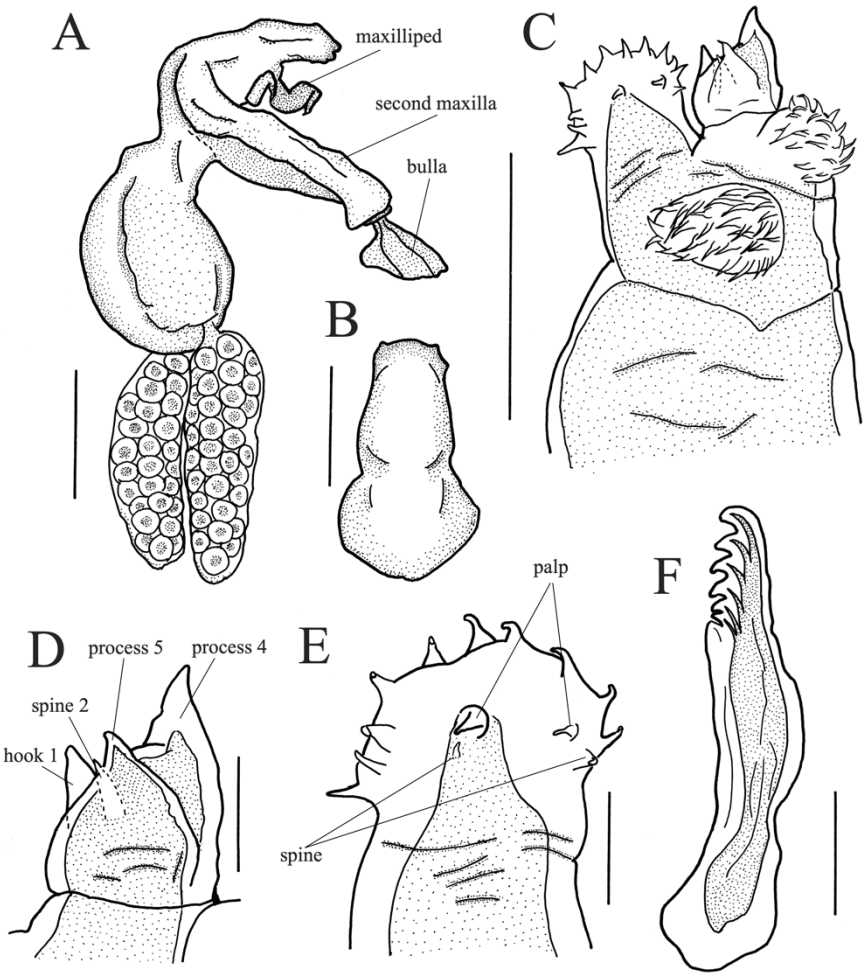


Figure 2. Adult female *Salmincola edwardsii* (Copepoda: Lernaeopodidae) from Southern Asian Dolly varden *Salvelinus curilus,* from the Shiretoko Peninsula, Hokkaido, Japan. ID indicates the specimen's ID. A. Entire (lateral view, ID4); B. cephalothorax (dorsal view, ID5); C. second antenna, entire (lateral view, ID2); D. same, tip of endopod (lateral view, ID2); E. same, tip of exopod (lateral view, ID2); F. mandible (lateral view, ID5); G. maxilliped, entire (ventral view, ID3); H. same, maxilliped tip (ventral view, ID3); I. maxilliped tip (ventral view, ID1); J. same, maxilliped palp (ventral view, ID3); K. maxilliped palp (ventral view, ID1); L. first maxilla (lateral view, ID1); M. first antenna (lateral view, ID5); N. first antenna (lateral view, ID2).
Scale bars: A–B, 1 mm; C, 150 μm; D–F, H–N, 30 μm; G, 40 μm.



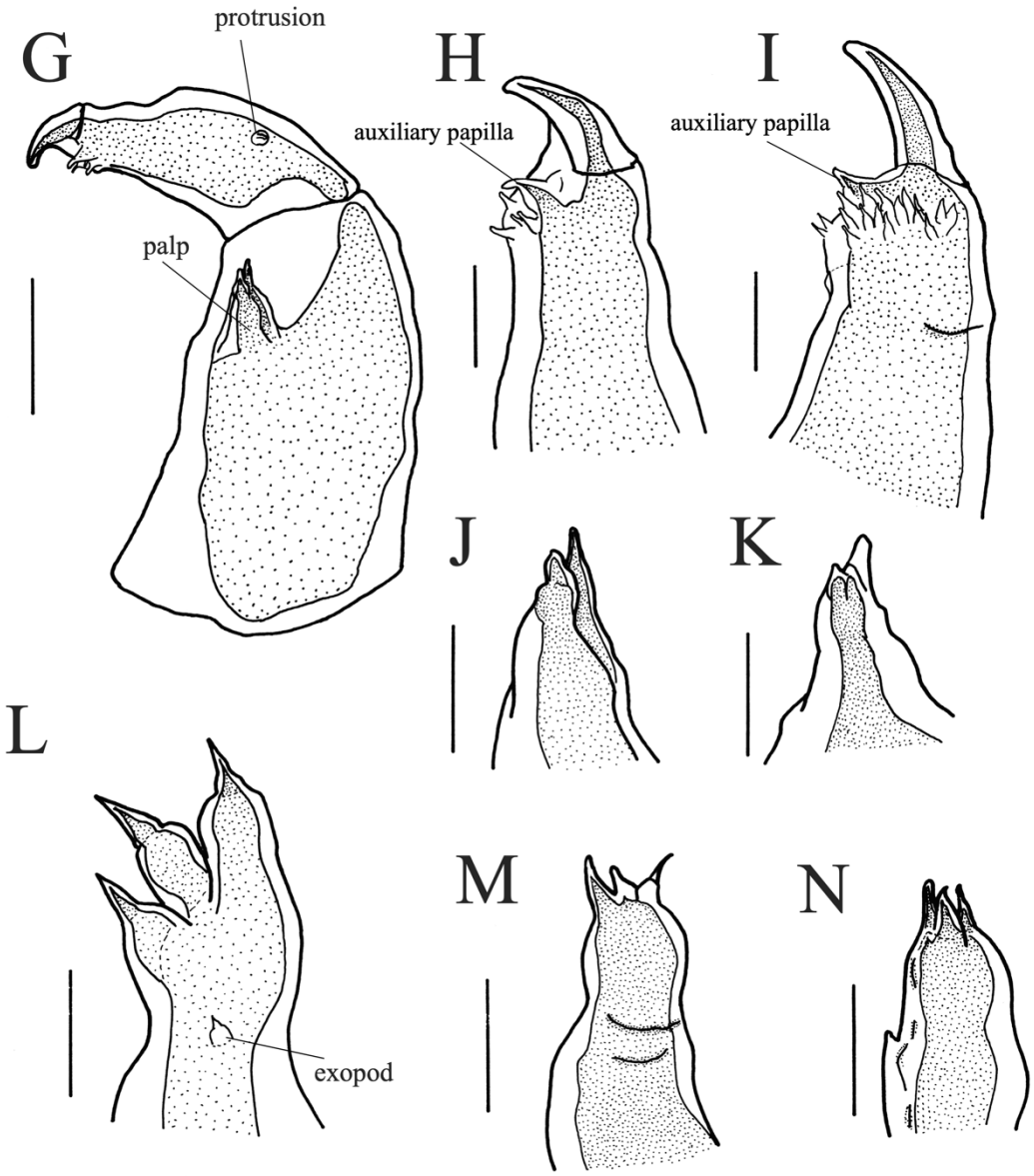

G
protrusion
palp
H
auxiliary papilla
I
auxiliary papilla
J
K
L
exopod
M
N

Figure 3. Infestations of *Salmincola edwardsii* (Copepoda: Lernaeopodidae) on the gill filaments of Southern Asian Dolly Varden *Salvelinus curilus* and gill lesions at their attachment sites.

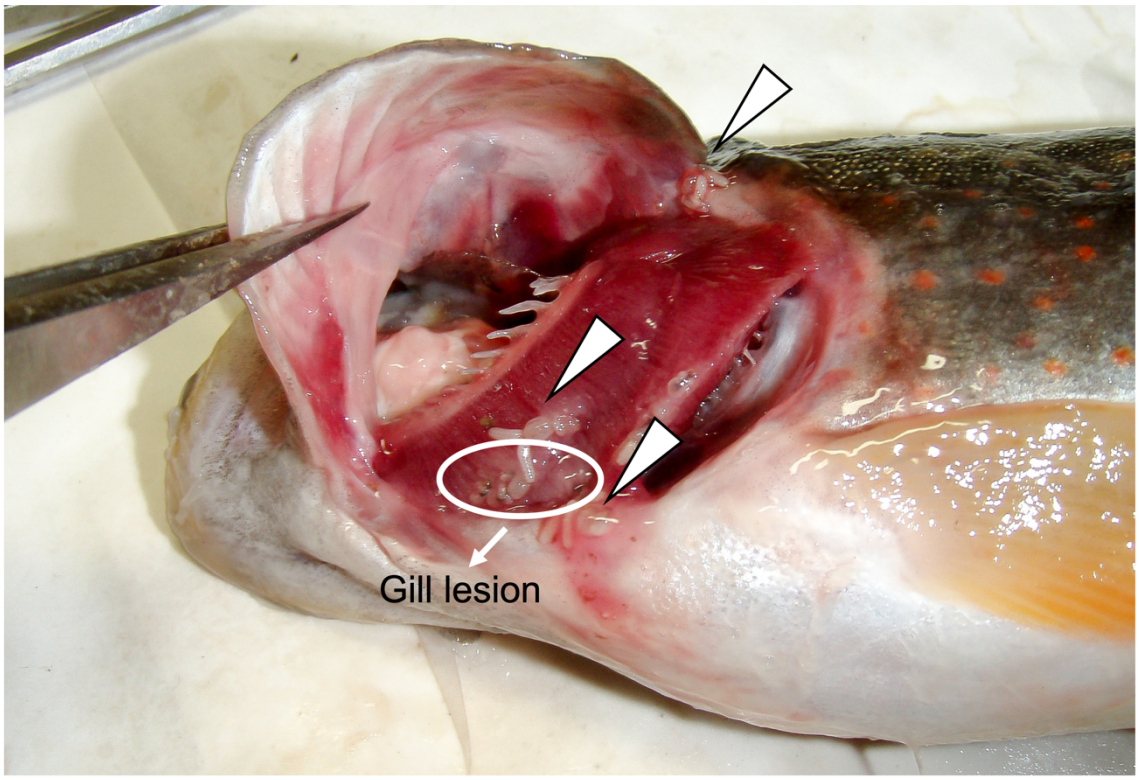
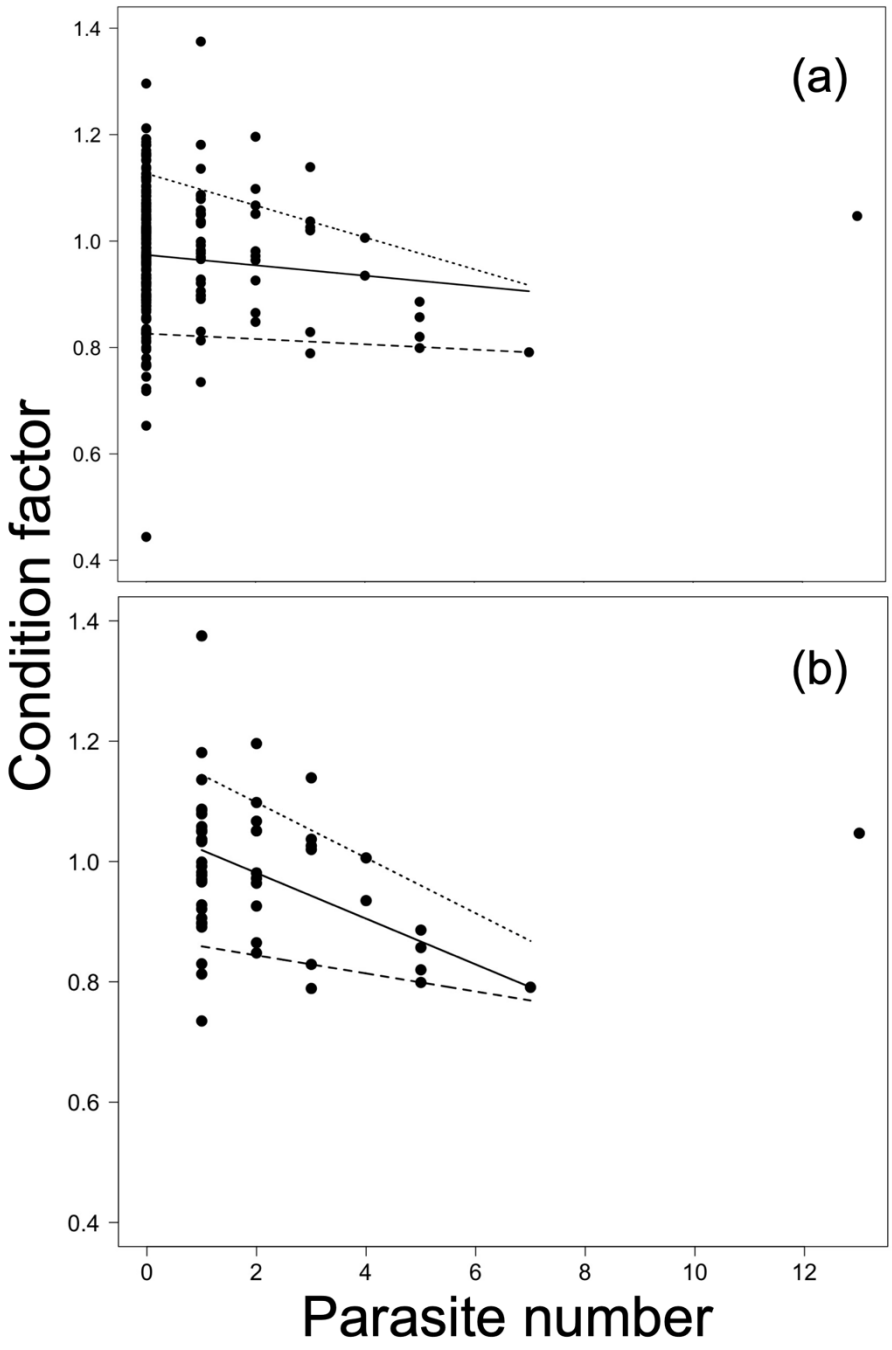


Figure 4. The relationship between the condition factor of Southern Asian Dolly Varden *Salvelinus curilus* and the number of *Salmincola edwardsii*, analyzed by a quantile regression. (a) the results analyzed with all fish (except for the individual with 13 parasites) and (b) the results analyzed with only infested fish. Dashed, solid and dotted line indicate 0.1, 0.5 and 0.9 quantile, respectively.



Chapter 3

Negative correlation of parasite infection and host body condition: A case of *Salmincola markewitschi* parasitic on white-spotted charr, *Salvelinus leucomaenis*

Abstract

Assessing the impacts of parasites on wild fish populations is a fundamental and challenging aspect of the study of host–parasite relationships. *Salmincola*, a genus of ectoparasitic copepods, mainly infects salmonid species. This genus, which is notorious in aquaculture, damages host fishes, but its impacts under natural conditions remain largely unknown or are often considered negligible. In this study, I investigated the potential impacts of mouth-attaching *Salmincola markewitschi* on white-spotted charr (*Salvelinus leucomaenis*) through intensive field surveys across four seasons using host body condition as an indicator of harmful effects. The prevalence and parasite abundance were highest in winter and gradually decreased in summer and autumn, which might be due to host breeding and/or wintering aggregations that help parasite transmissions. Despite seasonal differences in prevalence and parasite abundance, consistent negative correlations between parasite abundance and host body condition were observed across all seasons, indicating that the mouth-attaching copepods could reduce the body condition of the host fish. This provides field evidence suggesting that *S. markewitschi* has a potential negative impact on wild white-spotted charr.

1. Introduction

Parasites negatively affect host fitness components and can ultimately regulate host population dynamics and even cause local extinctions (Hudson et al. 1998; Krkošek et al. 2007; Rohr et al. 2008; Costello 2009). In particular, recent climate change effects and the expansion of aquaculture industries may facilitate parasite epizootics (Krkošek et al. 2007; Rohr et al. 2008; Marcogliese 2008; Costello 2009; Altizer et al. 2013; Mitro 2016). Understanding parasite impacts on host species in the wild is fundamental and necessary for elucidating host–parasite relationships.

The assessment of parasite impacts on host individuals is generally challenging in field studies. First, detecting parasites is not easy because parasite prevalence is often low in the wild, and parasites are usually small and cryptic (Poulin 2011; Kennedy 2012). Second, sacrificing host individuals is often necessary, especially when investigating endoparasites, and it is an undesirable practice for endangered host species (Kwak et al. 2020). Third, evaluating the fitness components of host fishes requires long-term individual monitoring (Beldomenico et al. 2008; Wilber et al. 2016), which may not be feasible for mobile species in open habitats, such as oceans. Fourth, the impacts of parasites may be context dependent and may vary across host individuals, host developmental stages, seasons, and ambient environments (Cardon et al. 2011; Klemme et al. 2021). Overall, detailed case studies or model systems are needed to quantify the effects of parasites—that is, when, where, how, and to whom they become harmful.

Ectoparasitic copepods *Salmincola* spp. commonly infect the branchial and buccal cavities of freshwater salmonids (Kabata 1969), which makes quantitative field assessment possible (White et al. 2020). *Salmincola* spp. are known to be harmful parasites in aquaculture, causing gill destructions, swellings (Kabata & Cousens 1977; Sutherland & Wittrock 1985; Nagasawa et al. 1995; Herron et al. 2018; White et al.

2020), and even mortality (Kabata & Cousens 1977; Hiramatsu et al. 2001; Neal et al. 2021) in fish. On the other hand, many field studies have reported little or no impact on salmonid hosts (Chigbu 2001; Nagasawa & Urawa 2002; Kusterle et al. 2012; Boone & Quinlan 2019; Ayer et al. 2022; Hasegawa et al. 2022a), which may be due to low prevalence and intensity in the wild (Black et al. 1983; Bowen & Stedman 1990; Amundsen et al. 1997). Recently, however, growing evidence suggests that outbreaks of *Salmincola* spp., especially *S. californiensis* and *S. edwardsii*, have occurred in wild salmonid populations and that their infections deleteriously reduce wild salmon stocks (Monzyk et al. 2015; Mitro 2016; Mitro & Griffin 2018; Lepak et al. 2022). Therefore, evaluating whether these *Salmincola* species are harmful and should be considered in wild salmon management contexts is necessary.

In this study, I examined the effects of ectoparasitic copepod, *Salmincola markewitschi*, on a stream-dwelling salmonid, white-spotted charr *Salvelinus leucomaenis*, through intensive field surveys across four seasons using host body condition as an indicator of host fitness components. As fish body condition is directly related to growth, survival, and reproductive success (Bolger & Connolly 1989; Gabelhouse 1991; Nicoletto 1995; Schloesser & Fabrizio 2017), it can be a good surrogate for host fitness components. In addition, body condition is easy to measure in the field without sacrificing the host individuals. Many studies have used body condition indices to assess the effects of parasites (Neff & Cargnelli 2004; Morton & Routledge 2006; Kusterle et al. 2012; Welicky et al. 2018; Kawanishi et al. 2019). I also compared the seasonal patterns of infection levels and potential parasite impacts to determine whether the effects of parasite infections on host condition are consistent or variable across seasons. This information would be valuable for understanding whether and when parasite impacts should be considered for the conservation or resource management of salmonid fishes.

2. Materials and methods

2.1. Study species, study area, and field surveys

A series of field surveys was carried out at the Shiodomari River system, southern Hokkaido, Japan (Figure 1). In this river system, white-spotted charr have a wide distribution and reproduce naturally in most of their tributaries. Similar to other charr populations in Hokkaido (Morita, 2001; Morita & Morita 2002), white-spotted charr breed from September to October in the upstream reaches of tributaries (Hasegawa & Koizumi 2021).

In the Shiodomari River, white-spotted charr are frequently infected with parasitic copepods in their buccal cavities (Hasegawa & Koizumi 2021; Ayer et al. 2022; Hasegawa et al. 2022b; Hasegawa & Koizumi 2023). These copepod species were morphologically and genetically identified as *Salmincola markewitschi* (Hasegawa et al. 2022b; Shedko et al. 2023). Although no detailed information is available on the life history of the target species, the congeneric species, *S. californiensis* and *S. edwardsii*, have direct life cycles with seven separate stages: nauplius, free-living copepodid, chalimus 1–4, and mature individuals (Kabata & Cousens 1973; Stankowska-Radziun & Radziun 1993; Conley & Cutis 1994; Murphy et al. 2020). Free-living copepodids can generally live for up to a few days, but this depends on the species and the external temperature (Kabata & Cousens 1973; McGladdery & Johnston 1988; Conley & Cutis 1993). After attaching to suitable hosts, females generally take a few months to mature and produce egg sacs (Kabata & Cousens 1973).

In total, fish were captured at 19 sites in the Shiodomari River across four seasons (i.e., May 28 to June 1, 2019, hereafter *spring*; July 24 to July 31, 2019, hereafter *summer*; October 19 to October 23, 2019, hereafter *autumn*; and February 10 to February 13, 2020, hereafter *winter*) through electrofishing (Table 1, Figure 1). I captured fish within 100–300 m of study reach, aiming to capture at least 30 individuals

at each site in order to calculate reliable prevalence. In winter, additional fish were collected from outside the study reach because some study reaches were covered with thick ice, and, thus, capturing enough samples was difficult. In these study reaches, I broke the surface ice with hammers and pickaxes before electrofishing. Captured fish were anesthetized using the anesthetic agent FA100 (DS Pharma Animal Health Co., Ltd., Osaka, Japan), and body length (fork length: FL) and body weight (BW) were measured to the nearest 1 mm and 0.1 g, respectively. The body surfaces (including buccal cavities) of the fish were visually checked in the field for the presence of copepods. When parasitic copepods were found, I counted their numbers and recorded the attachment locations on each fish. I only counted female copepods because the males of several species of *Salmincola* are dwarf forms that attach to female bodies (Kabata & Cousens 1973; Conley & Cutis 1994).

The captured fish were categorized into three groups (migrants, residents, and age 0 fish) based on coloration and body size (Ishigaki 1984; Yamamoto et al. 1999). Migrants can easily be distinguished from residents due to their silver body color with relatively large white spots on the sides of the body (Ishigaki 1984; Yamamoto et al. 1999; Morita 2001). I only used residents consisted of age 1 and older fish for subsequent analyses because migrants have higher infection levels, which might cause bias in the analyses, and age 0 fish are rarely infected by *S. markewitschi* (Hasegawa & Koizumi 2021). During the October 2019 survey, I examined the maturity of each fish by gently pressing its ventral area and confirming the presence of sperm or egg, and then I categorized the fish into male, female, or an immature individual.

As mature females in October 2019 might have already released eggs, which skew the fish body condition, I removed these fish ($N = 20$) from the analyses of body condition (see Section 2.2., but I retained them in the analyses of seasonal comparison of prevalence and parasite abundance). I also removed 12 fish (mostly over 330 mm in

FL) from the body condition analyses (see Section 2.2); I could not measure their BWs because of a weight scale error, and I could not calculate the residual index described in the next section (but I retained them in the other analyses).

2.2. Data analysis

I followed Bush et al. (1997) for the definition of infection indices (e.g., prevalence, abundance, and intensity). For the accurate evaluation of host body condition, I used the residual index (Jakob et al. 1996); ln (BW) was regressed on ln (FL), and the residual distances of individual points from the regression line served as the estimators of host body condition. This index, which is widely used in many fish–parasite systems (e.g., Bagamian et al. 2004; Lagrue & Poulin 2015; Perrot-Minnot et al. 2020), allowed me to assess the impacts of parasite infection on host condition regardless of host body length (Jakob et al. 1996). To examine the differences in body conditions across the four seasons, I calculated the residual index using all datasets (i.e., including all seasons).

All statistical analyses were performed using R 4.3.1. (R Core Team 2021). Infection levels of *Salmincola* spp. (i.e., prevalence and parasite abundance) are highly heterogeneous within and across watersheds (Mitro & Griffin 2018; Hasegawa et al. 2022a), and this pattern holds true in this system (Hasegawa & Koizumi 2021). Thus, I analyzed the seasonality of prevalence and parasite abundance using only the sites where all seasons' data were available (see Results). To elucidate the seasonality of infection prevalence, I constructed a generalized linear mixed model (GLMM) with a binomial error distribution and a logit link function using the *lme4* package (version 1.1-33; Bates et al. 2011). The candidate full model was also constructed, in which the response variable was a binary variable that indicated infection or non-infection (infected = 1, uninfected = 0), and the explanatory variables were FL, season (i.e.,

winter, spring, summer, and autumn), and their interaction terms. The models were selected on the basis of Akaike's information criterion (AIC; Akaike 1983); I considered models with ΔAIC (the difference between a focused model and the best model with the lowest AIC) < 2 to be meaningful models (Burnham & Anderson 2002). To compare the differences in prevalence across all possible seasonal combinations, I conducted Tukey's honest multiple comparison tests using the *multcomp* package (version 1.4-25; Hothorn et al. 2016).

I also constructed a GLMM using the *glmmADMB* package (version 0.8.5; Fournier et al. 2012) to compare parasite abundance across seasons. The response variable was parasite abundance, and the explanatory variables were FL, season (i.e., winter, spring, summer, and autumn), and their interaction terms. The study sites were treated as random effects. In the model constructions, I considered two error distribution patterns because the variables exhibited overdispersion and zero excess: negative binomial (NB) and zero-inflated negative binomial (ZINB). I selected the models according to AIC, as described above. The differences across seasons were examined with Tukey's honest multiple comparison tests using the same package described above.

To elucidate whether infection by *S. markewitschi* decreased the host residual index, I constructed a GLMM. Because the residual index did not meet the normality in the preliminary analysis (Kolmogorov–Smirnov test; $P < 0.001$), I developed the model with Gamma distribution and a log link function using the *lme4* package (version 1.1-33; Bates et al. 2011). The response variable was the residual index, and the explanatory variables were the abundance of *S. markewitschi* and the season (i.e., winter, spring, summer, and autumn). To meet the assumption of Gamma distribution (i.e., numbers should be positive), I added 1.5 to the residual index. Adding constant values to data sets to meet the assumption of Gamma distribution is common among ecological studies (e.g. Rojas et al. 2019; von Königslöw et al. 2022). Nonetheless, to evaluate the

sensitivity of the results caused by this data treatment, I repeated the same analysis adding two other small values (1.1 and 2). These results qualitatively the same (Supplementary material 1), and thus I only presented the results adding 1.5 in the main text. As there were no significant effects of interaction terms on parasite abundance and seasons in the preliminary analysis (GLMM, parasite abundance × spring: $t = 1.043$, $P = 0.297$; parasite abundance × summer: $t = 0.906$, $P = 0.365$; parasite abundance × autumn: $t = 0.363$, $P = 0.717$), I did not include the interaction terms. The study sites were treated as random effects.

3. Results

A total of 1,791 age 1 and older resident white-spotted charr were captured across the four seasons (Table 1, Supplementary material 2). All seasons' data were available at six sites ($N = 825$ in total; Figure 1), and these were used for the seasonal comparison of parasite abundance and prevalence (see below). All copepods were found in the buccal cavities.

The prevalence of *Salmincola markewitschi* across seasons was 22.3% (Table 1). The best model (Table 2a) showed that the prevalence in winter was significantly higher than that in autumn (GLMM, $z = -6.038$, $P < 0.001$), but it was not significantly different from the prevalence in spring or summer (winter vs. spring, $z = -1.268$, $P = 0.582$; winter vs. summer, $z = -1.908$, $P = 0.223$). The prevalence in spring was also significantly higher than that in autumn (spring vs. autumn, $z = -4.968$, $P < 0.001$), but it was not significantly different from the prevalence in summer (spring vs. summer, $z = -0.692$, $P = 0.900$). Fork length was positively correlated with prevalence ($z = 9.509$, $P < 0.001$).

According to the best model with NB error distribution (Table 2b), parasite abundance in winter was significantly higher than that in autumn (GLMM, $z = -6.184$,

$P < 0.001$) and marginally higher than that in summer ($z = -2.227$, $P = 0.096$), whereas no significant difference was found between winter and spring ($z = -1.456$, $P = 0.412$). Parasite abundance was lowest in autumn (spring vs. autumn, $z = -4.968$, $P < 0.001$; summer vs. autumn, $z = -4.336$, $P < 0.001$). There were no significant differences between spring and summer ($z = -0.692$, $P = 0.900$). Fork length was positively correlated with parasite abundance ($z = 13.54$, $P < 0.001$; Figure 2).

Despite the seasonal differences in prevalence and parasite abundance, host body condition (i.e., residual index) significantly decreased with an increase in the abundance of copepods in all four seasons (Table 3, Figure 3).

4. Discussion

4.1. Consistent negative correlations between parasite number and residual index across seasons

This is the first study to demonstrate the consistent negative correlations between *Salmincola* infections and host body condition across seasons, suggesting the potential negative impacts of these infections on the host fish. The use of body condition indices is a powerful method for estimating the impacts on host fitness components, and many studies have found that body condition can be a good surrogate for host growth, survival, and reproduction (Bolger & Connolly 1989; Gabelhouse 1991; Nicoletto 1995; Schloesser & Fabrizio 2017). In fact, several studies have examined the relationships between host body condition and infections of *Salmincola* spp. in the wild; these have focused on, for example, *S. salmoneus* infections in Atlantic salmon *Salmo salar* (Kusterle et al. 2012), *S. californiensis* infections in sockeye salmon *Oncorhynchus nerka* (Chigbu 2001) and masu salmon *O. masou masou* (Nagasawa & Urawa 2002), *S. edwardsii* infections in southern Asian Dolly Varden *S. curilus* (Hasegawa et al. 2022a), *Salmincola* sp. infections in brook trout *S. fortinalis* (Boone & Quinlan 2019), and

Salmincola sp. (the authors speculated either *S. carpionis* or *S. markewitschi*) infections in white-spotted charr (Ayer et al. 2022), although most of these studies have reported negligible *Salmincola* spp. impacts on wild salmonid populations (Black et al. 1983; Bowen & Stedman 1990; Amundsen et al. 1997; Chigbu 2001; Nagasawa & Urawa 2002; Kusterle et al. 2012; Boone & Quinlan 2019; Ayer et al. 2022). Among these studies, only Kusterle et al. (2012) and Hasegawa et al. (2022a) found significant negative correlations between parasite infection and host body condition, although the authors focused on specific periods or a particular analysis. Therefore, it is rather surprising that I observed clear negative impacts, although abundance in this system was generally much lower (maximum of 5–10 parasites) than that reported in previous studies (up to 100 parasites, e.g., Kusterle et al. 2012).

The differences in the results may depend on the attachment site of the *Salmincola* species. While most previous studies have examined species infecting gill cavities, such as *S. salmoneus* and *S. californiensis* (Chigbu 2001; Nagasawa & Urawa 2002; Kustele et al. 2012), my target species, *S. markewitschi*, infects buccal cavities. Even with a low infection intensity level, mouth-attaching copepods may directly inhibit the host's feeding and thus reduce its condition, growth, and survival. In fact, *S. stellata* have been observed to infect the buccal cavities of Sakhalin taimen (*Parahucho perryi*), thus decreasing feeding appetite and finally killing the host fish in the experimental tanks (Hiramatsu et al. 2001). To confirm this hypothesis, further research should focus on buccal-cavity-attaching *Salmincola* spp., such as *S. stellata* (Nagasawa & Urawa 1991).

The differences in the study results could also be related to interactions with stream environments. Streams in Japan are in the southernmost ranges for salmonids and possibly *Salmincola* spp. as well (Nagasawa 2020a). Because salmonids, especially *Salvelinus* spp., are highly adapted to cold water environments and are stressed at high water temperatures, salmonid populations in these relatively warmer areas of Japan may

be more vulnerable to climate change (Nakano et al. 1996; Takegawa et al. 2017). While host salmonids suffer under warm conditions, parasites generally have wider optimal thermal ranges compared with host species (Cohen et al. 2017; Gsell et al. 2023). In fact, *Salmincola* spp. grow faster and reproduce more eggs under warm conditions (Murphy et al. 2020; Neal et al. 2021), suggesting that their optimal thermal ranges are wider than those of salmonids and that this species could have higher resistance against warm temperatures. Copepod's feeding activities, which cause physical damage to the hosts, increase under warm temperatures because their activity levels are temperature dependent (Conley & Cutis 1993). *Salmincola* infections also decrease the tolerance of hosts to warm water temperatures (Vaughan & Coble 1975). Given the differential resistance to warmer temperatures between hosts and copepods and the reduction in host resistance as a result of copepod infections, the effects of infections on host conditions would be stronger in the southernmost range of *Salmincola* spp.'s distribution. This could also explain the epizootics of *S.* cf. *edwardsii* and the subsequent decline in host populations of brook trout *S. fontinalis* in Wisconsin, USA, possibly related to global warming (Mitro 2016; Mitro & Griffin 2018). These results or predictions do not contradict the fact that the effects of *Salmincola* infections are not observed further north (Chigbu 2001; Kusterle et al. 2012; Boone & Quinlan 2019).

My findings, on the other hand, were inconsistent with those of Ayer et al. (2022), who suggested the negligible effects of the same parasite, the same host, and the same river system. One possible explanation for the inconsistency is the lower infection levels in the previous study. Ayer et al. (2022) reported apparently low infection prevalence and mean intensity for each study season (June 2016: prevalence 15.5%, mean intensity 1.24; October 2017: prevalence 13.1%, mean intensity 1.30) compared with the present study (prevalence 22.9%, mean intensity 1.88). Furthermore, they found a maximum

intensity of 3–4, and only 3% of 119 infected hosts were parasitized by more than three copepods. Considering these low infection levels, the authors might have failed to detect the actual relationships between parasite infection and host body condition. In fact, I reanalyzed the datasets used by Ayer et al. (2022) in a way similar to what I did in the present study (i.e., GLM), but I found no significant negative effects of parasite numbers (GLM, June 2016: $t = 0.709$, $P = 0.479$, October 2017: $t = -0.709$, $P = 0.449$), suggesting that a small number of infected hosts, especially fish having three or more copepods, might not be enough to detect the negative correlation between parasite infections and host body condition. Moreover, while my study captured fish from various sites in the river system, Ayer et al. (2022) examined only one population in the upstream river system, where a constantly low water temperature was observed compared with other study sites (R. Hasegawa et al., unpublished data). Under these cold environments, white-spotted charr could tolerate the effects of parasites. Boone & Quinlan (2019) hypothesized that brook trout could tolerate gill-attaching *Salmincola* sp. under constantly low water temperatures.

S. markewitschi infections could potentially affect the survival and reproduction of white-spotted charr in the Shiodomari River because salmonids' survival and reproduction are explained, at least partially, by body condition (Reimers et al. 1993; Robinson et al. 2008; Burton et al. 2013). For instance, winter is a harsh season for salmonids, and a lack of energy storage (i.e., poor body condition) may be the major cause of high mortality during this period (Huusko et al. 2007). Salmonids also expend considerable energy during breeding migrations, gonad maturation, and fighting with other fish to find suitable locations for redds (Jonsson et al. 1991; Hinch & Rand 1998; Hendry & Beall 2004). In these situations, infected fish with poor body conditions are forced to compromise resource allocations. Therefore, as *S. markewitschi* infections

could lead to declines in wild white-spotted charr populations, body condition indices are useful for assessing the impacts of the parasite on host fitness in natural conditions.

4.2. Infection patterns of *S. markewitschi* across seasons

Infection levels by *S. markewitschi* fluctuated across seasons, with the prevalence and parasite abundance increasing in winter (February) and decreasing in summer (July) and autumn (October). These results were largely consistent with those of previous studies (Amundsen et al. 1997; Monzyk et al. 2015). As suggested by Amundsen et al. (1997), seasonal host aggregation may contribute to the high infection levels in winter. Salmonids, including white-spotted charr, often show extreme aggregation in pools or small tributaries during winter (Cunjak & Power 1986; Huusko et al. 2007; Koizumi et al. 2017). These high aggregations can allow free-swimming copepodids to attach easily to the host fish. The breeding aggregation of the hosts (Nakamura 1999) may also create conditions for the variable seasonal prevalence pattern of infections. Given the life cycles of other *Salmincola* species (*S. californiensis* take a few months to produce eggs after their attachments; Kabata & Cousens 1973), there should be a time lag during the copepodid attachment (breeding season) and the prevalence increments (winter). Future studies are necessary to confirm whether copepodid recruitments occur during the period when hosts aggregate.

Host body size (FL) was significantly and positively correlated with both prevalence and parasite abundance, a pattern observed in many other *Salmincola*–salmonid systems (Bowen & Stedman 1990; Nagasawa & Urawa 2002; Barndt & Stone 2003; Monzyk et al. 2015; Hasegawa & Koizumi 2021). Because small infectious copepodids may have poor swimming ability, a larger body size may allow copepodids to infect easily (Poulin et al. 1991; Monzyk et al. 2015). Ayer et al. (2022) found a similar pattern in the same system, and they discussed that larger hosts are

dominant in the current, and thus the chance of copepodid attachments is likely to increase in these hosts. Fish age can also be an important predictor; larger fish are generally older, so a longer exposure time against parasites can create greater infection levels on larger fish (Nagasawa & Urawa 2002; Monzyk et al. 2015; Nagasawa & Urawa 2022).

In conclusion, *S. markewitschi* infections could potentially reduce host body condition, and their impacts can be consistent across seasons, suggesting that *S. markewitschi* should be considered harmful parasites in the conservation or management of native white-spotted charr. Outbreaks of *Salmincola* spp., such as *S. edwardsii* and *S. californiensis*, have been emerging problems in some areas, possibly because of the high water temperature and drought conditions caused by climate change (Mitro 2016; Mitro & Griffin 2018). As Japanese populations are in the southernmost margin of their host native ranges, these populations are vulnerable to parasite outbreaks. Field knowledge of *Salmincola* spp., including host usage, distribution, and effects on the hosts, has recently increased in Japan (Nagasawa 2020a, b; Hasegawa & Koizumi 2021; Ayer et al. 2022; Hasegawa et al. 2022a, b; Nagasawa & Urawa 2022; Hasegawa & Koizumi 2023), but I still do not know the ecology of the parasites in the wild, such as their population dynamics. In particular, the infection levels of *Salmincola* spp. possibly fluctuate significantly over the years (Mitro 2016; Ayer et al. 2022; Lepak et al. 2022), and, thus, their host–parasite relationships could change across years. Future long-term monitoring is necessary to establish proper control or management strategies.

Table 1. Summary of field surveys for each season. Individual fish over 330 mm were not measured in May and February 2019 for logistical reasons; they were excluded from the calculation of the mean FL but were retained for the calculation of other metrics. Prevalence was shown as %.

Study period	Spring	Summer	Autumn	Winter
Date	28 May to 1 June, 2019	24 July to 31 July, 2019	19 Oct. to 23 Oct., 2019	10 Feb. to 13 Feb., 2020
Number of studied sites	17	15	14	7
Number of fish inspected (Number of infected)	567 (143)	606 (119)	439 (66)	179 (71)
Host FL range (mean)	81–322 (154.7)	78–401 (147.4)	89–343 (161.3)	100–323 (183.0)
Prevalence range (mean)	0–53.6 (26.4)	0–38.9 (19.4)	0–46.7 (14.3)	16.7–60.0 (38.8)
Intensity range (mean)	1–9 (1.99)	1–13 (1.57)	1–5 (1.59)	1–17 (2.51)

Table 2. Results of the top three models selected on the basis of Akaike's information criterion (AIC). (a) Generalized linear mixed models (GLMMs) with a binomial error distribution with a logit link function analyzing whether prevalence differs across seasons. (b) GLMMs with a negative binomial (NB) and zero-inflated negative binomial (ZINB) error distribution with a log link function analyzing whether parasite abundance differs across seasons.

(a) Whether prevalence differs among seasons (N = 825)

Model	Intercept	FL	seasons	FL × seasons	*df*	AIC	ΔAIC
1	**−4.040**	**0.019**	**+**		**6**	**718.4**	**0.00**
2	−3.434	0.016	+	+	9	720.7	2.32
3	−4.474	0.018			3	759.3	40.9

(b) Whether parasite abundance differs among seasons (N = 825)

Model	Intercept	FL	seasons	FL × seasons	*df*	AIC	ΔAIC	Error distribution
1	**−3.375**	**0.015**	**+**		**7**	**1154.8**	**0.00**	**NB**
2	−3.575	0.015	+		8	1156.8	2.00	ZINB
3	−3.319	0.015		+	10	1160.8	5.97	NB

The models are arranged according to AIC. *Df* and ΔAIC indicate the degree of freedom and the AIC difference between the best and focal models, respectively. The "+" symbol indicates statistically significant parameter effects. The most supported model (ΔAIC < 2) is in bold.

Table 3. Relationships between the residual index of white-spotted charr *Salvelinus leucomaenis* and abundance of *Salmincola markewitschi* across seasons, analyzed using a generalized linear mixed model (GLMM) with a Gamma error distribution with log link function (February = winter, May = spring, July = summer, and October = autumn).

	Coefficient	Standard Error	*t*-value	*P*-value
Intercept	0.016	0.017	0.964	0.335
Parasite number	−0.027	0.003	−8.979	**<0.001**
February vs. May	0.096	0.012	7.739	**<0.001**
February vs. July	0.074	0.013	5.941	**<0.001**
February vs. October	0.077	0.013	6.082	**<0.001**

Figure 1. Map of the sampled tributaries in the Shiodomari River system, southern Hokkaido, Japan. Open and closed circles indicate the sites where I captured fish in all seasons (i.e., winter, spring, summer, and autumn) and the sites where I did not capture in all seasons, respectively.

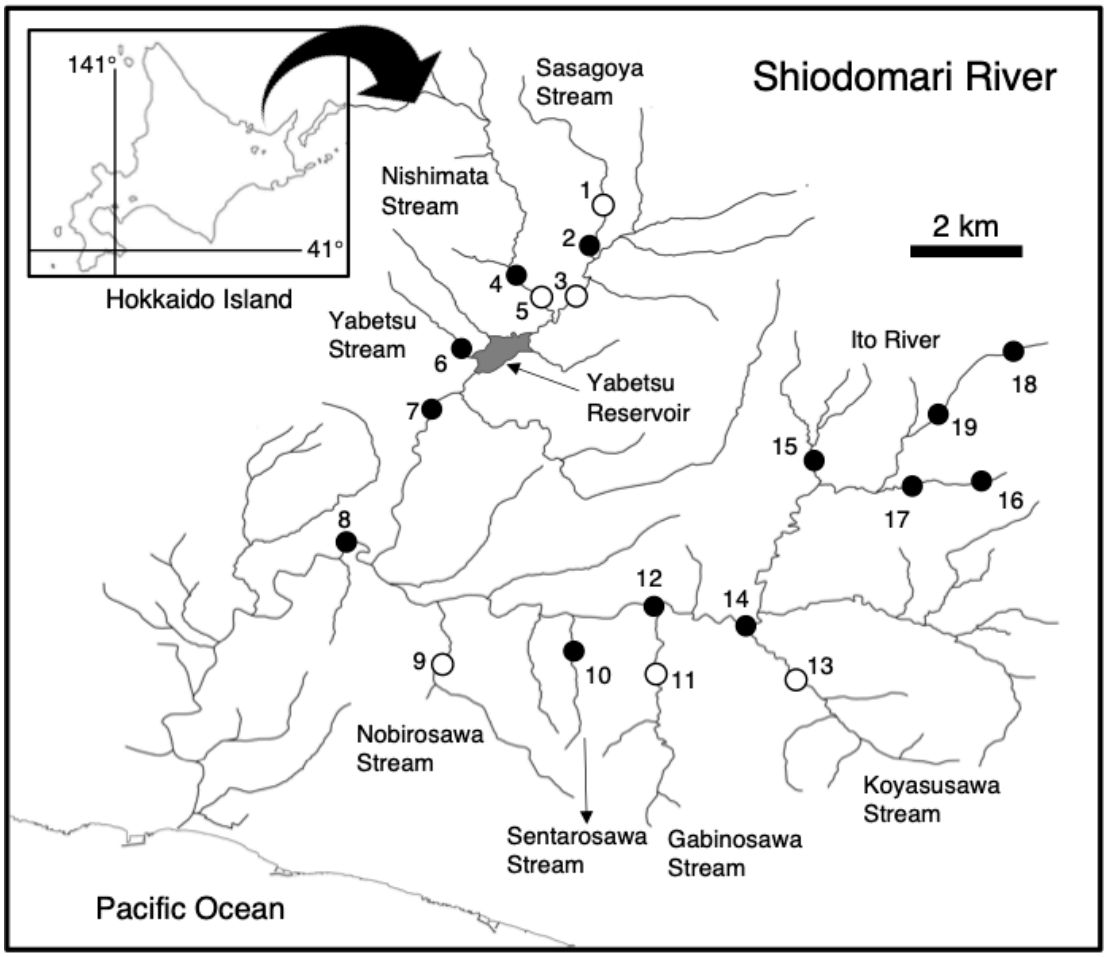


Figure 2. Relationship between the body size (FL) of white-spotted charr *Salvelinus leucomaenis* and the abundance of the copepods *Salmincola markewitschi* in each season (winter, spring, summer, and autumn) in the Shiodomari River system, southern Hokkaido, Japan. The 95% confidence interval is indicated by gray areas.

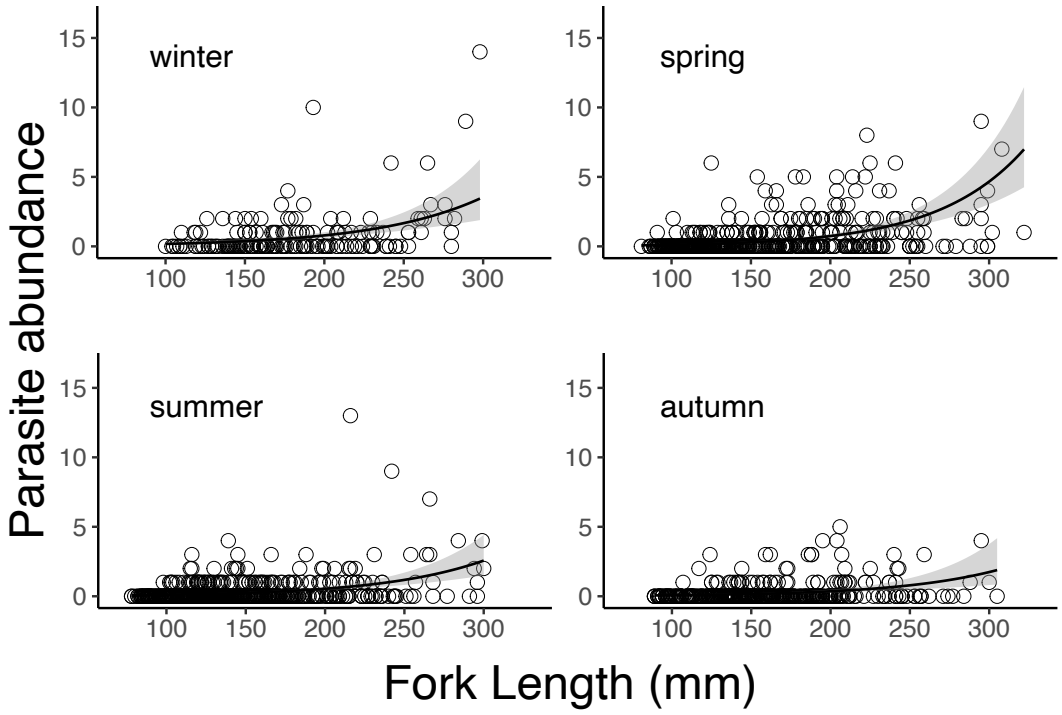
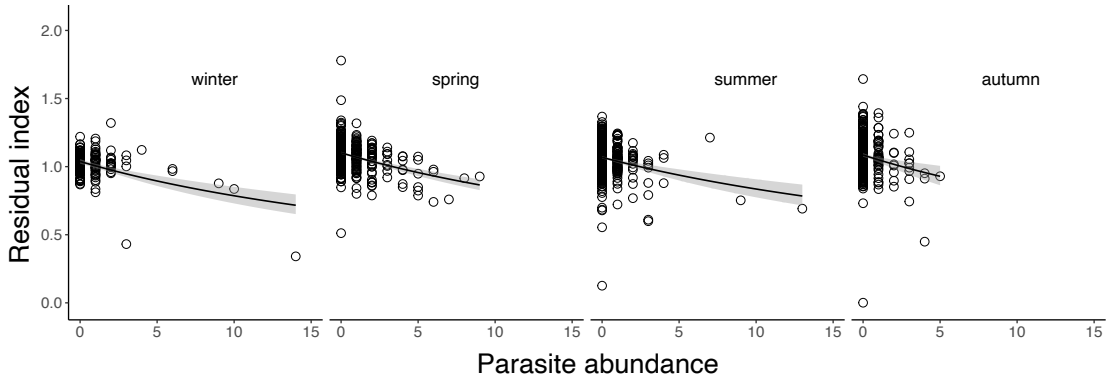


Figure 3. Relationship between the body conditions (residual index) of white-spotted charr *Salvelinus leucomaenis* and the abundance of copepods *Salmincola markewitschi* in each season (winter, spring, summer, and autumn) in the Shiodomari River system, southern Hokkaido, Japan. The 95% confidence interval is indicated by gray areas.



Supplementary material 1. Results of the additional GLMM analysis to examine the relationships between the residual index of white-spotted charr *Salvelinus leucomaenis* and abundance of *Salmincola markewitschi* across seasons. To ensure the assumption of Gamma distribution, I had to add small values to all data before the analysis. These results were calculated from the analysis when adding (a) 1.1, (b) 1.5, (c) 2 to all residual index. In the main text, I presented the results of the analysis when adding the value of 1.5 (see Table 3).

(a) 1.1

	Coefficient	Standard Error	*t*-value	*P*-value
Intercept	0.047	0.015	3.088	**<0.002**
Parasite number	−0.026	0.003	−10.273	**<0.001**
February vs. May	0.094	0.010	8.954	**<0.001**
February vs. July	0.072	0.011	6.886	**<0.001**
February vs. October	0.076	0.011	7.037	**<0.001**

(b) 1.5

	Coefficient	Standard Error	*t*-value	*P*-value
Intercept	0.370	0.011	34.224	**<0.001**
Parasite number	−0.019	0.002	−11.183	**<0.001**
February vs. May	0.069	0.007	10.106	**<0.001**
February vs. July	0.053	0.007	7.800	**<0.001**
February vs. October	0.056	0.007	7.985	**<0.001**

(c) 2

	Coefficient	Standard Error	*t*-value	*P*-value
Intercept	0.667	0.008	82.616	**<0.001**
Parasite number	−0.014	0.001	−11.264	**<0.001**
February vs. May	0.052	0.005	10.401	**<0.001**
February vs. July	0.040	0.005	8.044	**<0.001**
February vs. October	0.042	0.005	8.247	**<0.001**

Supplementary material 2. Prevalence (%) and mean intensity of *Salmincola markewitschi* on white-spotted charr *Salvelinus leucomaenis* in each study site and season in the Shiodomari River system.

	Spring			Summer			Autumn			Winter		
Site	*N*	Prevalence (%)	Mean Intensity	*N*	Prevalence (%)	Mean Intensity	*N*	Prevalence (%)	Mean Intensity	*N*	Prevalence (%)	Mean Intensity
1	50	4.0	1.00	33	0.0	NA	31	0	NA	29	17.2	1.20
2	59	22.0	1.46	39	25.6	1.50	33	15.2	1.00	30	30.0	1.44
3	50	24.0	1.17	34	17.6	1.33	33	12.1	1.75	12	16.7	2.00
4	59	0.0	NA	44	0.0	NA	33	0	NA	NA	NA	NA
5	28	53.6	1.93	36	38.9	1.71	40	10	1.00	5	60.0	2.67
6	NA	NA	NA	11	27.3	1.33	NA	NA	NA	NA	NA	NA
7	2	0.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
8	4	0.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
9	23	34.8	2.38	25	16.0	1.00	20	5.0	1.00	47	57.4	2.63
10	NA	NA	NA	8	0.0	NA	16	0	NA	NA	NA	NA
11	4	50.0	1.50	18	27.8	1.00	22	4.5	4.00	31	41.9	4.38
12	4	25.0	1.00	NA	NA	NA	NA	NA	NA	NA	NA	NA
13	25	28.0	2.29	22	27.3	2.00	26	26.9	1.71	25	48.0	1.58
14	5	40.0	2.00	NA	NA	NA	NA	NA	NA	NA	NA	NA
15	29	48.3	2.21	30	26.7	2.50	30	46.7	1.50	NA	NA	NA
16	55	16.4	1.44	89	27.0	1.25	31	16.1	1.20	NA	NA	NA
17	54	24.1	4.46	51	21.6	1.18	43	25.6	1.64	NA	NA	NA
18	63	33.3	2.10	120	15.8	1.89	51	11.8	2.17	NA	NA	NA
19	53	45.3	2.42	46	19.6	1.78	30	26.7	1.75	NA	NA	NA

Prevalence (%) and mean intensity are given as the proportion of fish infected and the average number of parasites among infected fish in each population, respectively. “N” indicates the sample size of all inspected fish at each site. “NA” indicates that the variable was not applicable.

Chapter 4

Does parasite infection affect host feeding behavior ? A test with angling vulnerability

Abstract

Parasites generally increase host vulnerability to predators via host manipulation for trophic transmission and reduction of host activities. Predators also select prey depending on the parasite infection status. Despite such parasites' roles in prey–predator interactions in wild animals, how parasites affect human hunting probability and resource consumption remains unknown. I examined the effects of the ectoparasitic copepod *Salmincola markewitschi* on fish vulnerability to angling. I found that infected fish were less vulnerable compared with non-infected fish when the fish body condition was low, which was probably due to reduced foraging activity. On the contrary, infected fish were more vulnerable when the host body condition was high, probably due to the compensation of parasites' negative effects. A twitter analysis also suggested that people avoided eating fish with parasites and that anglers' satisfaction decreased when captured fish were parasitised. Thus, I should consider how animal hunting is affected by parasites not only for catchability but also for avoiding parasite infection sources in many local regions.

1. Introduction

Parasites play critical roles in predator–prey interactions in natural systems (Hatcher et al. 2006; Lafferty et al. 2006; Wood & Johnson 2015). Whereas some parasites are known to manipulate host behaviour to increase their transmission probability (reviewed by Poulin & Maure 2015), other parasites undermine host health and activities, resulting in increased vulnerability to prey (Brassard et al. 1982; Temple 1987; Hudson et al. 1992). Predators also select prey, depending on infection status. Predators disproportionally capture parasitised hosts because of their reduced mobility (Brassard et al. 1982; Temple 1987; Hudson et al. 1992), whereas some predators avoid parasitised prey with intermediate-stage parasites to reduce infection risks, especially when they can be the definitive hosts for the parasites (Norris 1999; Bustnes & Galaktionov 2004).

Parasites can either increase or decrease susceptibility to prey in the prey–predator context. However, the roles of parasites in human hunting have been critically lacking, even though humans have classically hunted wildlife, such as mammals, birds and fish (Fujita et al. 2016; Shchelinsky 2020). Fragmented evidence suggests that parasites may increase or decrease the catchability of wildlife (Rau & Caron 1979; Wilson et al. 2011). Rau & Caron (1979) found that heavily infected mooses are predisposed to human hunting, probably due to the disruption of normal breathing by parasites. Wilson et al. (2011) found that bluegill sunfish caught by angling have fewer ectoparasites, but specific causalities have never been examined or discussed. Humans' resource consumption rates may also be affected by parasites. Some parasites are harmful for human health and have been the major natural selection forces on humans since ancient times (Prokop & Fedor 2013; Curtis 2014), resulting in the evolution of 'disgust' towards parasites and somethings associated with parasites (Curtis 2014; Kupfer & Le

2018; Buck et al. 2018) and the evolution of avoidance against some organisms or places with high infection risks (Curtis 2014; Buck et al. 2018).

Fishing or angling is one of the most common human hunting activities, dating back to at least 40,000 years ago (O'Connor et al. 2011; Fujita et al. 2016). Currently, approximately seven million people or one in ten people across the world engage in recreational fishing (Cooke & Cowx 2004; Arlinghaus et al. 2019). Although anglers often encounter parasites in the fish they catch and parasite outbreaks are emerging problems in some fishing areas (Bartholomew et al. 2005; Mitro & Griffin 2018), no studies have specifically examined how parasites affect vulnerability to recreational fishing. Given that fish parasites generally reduce host feeding activities (Giles 1987; Tierney et al. 1994; Barber et al. 2000; Finley & Forrester 2003), I can predict that fish parasites may dampen angling susceptibility. Alternatively, angling susceptibility may increase in parasitised fish because some infected hosts increase the feeding rate or growth rate to regain the resources exploited by parasites (Arnott et al. 2000; Barber et al. 2000; Voutilainen et al. 2008).

Here, I examined the effects of parasitism on host vulnerability to angling using the mouth-infecting copepod *Salmincola markewitschi* on the stream-dwelling salmonid white-spotted charr *Salvelinus leucomaenis.* Copepods of the genus *Salmincola* are common ectoparasites on salmonids, and their main attachment sites are the gill or mouth cavity (Kabata 1969). These infections cause damage to host organisms and can induce host fitness or body condition loss (Nagasawa et al. 1998; Hasegawa et al. 2022a). Therefore, I predicted that copepod infection would reduce angling vulnerability. In addition, I also predicted that angling vulnerability depends on host body condition (i.e. health status), because an infected host with good health may compensate for the negative effect by exploiting more resources (Arnott et al. 2000; Barber et al. 2000; Voutilainen et al. 2008). I further examined whether parasite

infections reduce people's fish consumption rates by using social media (i.e. Twitter). Humans have historically consumed fish as natural resources (O'Connor et al. 2011; Fujita et al. 2016), and some fish parasites, such as *Anisakis* spp. and *Kudoa* spp., are harmful to human health (Arizono et al. 2012; Iwashita et al. 2013; Mattiucci et al. 2018). Therefore, people should feel disgusted towards fish parasites, leading to the reduction of satisfaction and consumption rate when they find parasites (Cooke et al. 2018). Some reports suggest that people make complaints, return or discard fish when they find parasites at markets (Ichihara 1983; Tokyo Metropolitan Market Sanitation Inspection Center 1990), and it is anecdotally observed that anglers also tend to release parasitised fish (Mitro & Griffin 2018) and also parasites might decrease angler's satisfaction (Cooke et al. 2018). However, quantitative research has never been conducted. Twitter is a common and efficient tool for collecting data from citizens (Hart et al. 2018). I quantitatively examined people's reactions against fish parasites using three years of Twitter data.

2. Materials and methods

2.1. Field survey

I conducted a field survey at a small tributary of Ito River in the Shiodomari River system, southern Hokkaido, Japan (41°50′N 140°58′E), where white-spotted charr are frequently infected with *Salmincola markewitschi* on mouth cavities (Hasegawa & Koizumi 2021; Ayer et al. 2022; Hasegawa et al. 2022b). The study reach (536 m in total) was located between two waterfalls (both are approximately 2 m in high). Given that the waterfalls prevent upstream migration of most fishes (Hasegawa & Koizumi 2021), the charr population was mainly composed of resident form (Morita et al. 2009), and no other fish species occurred in the study reach (Hasegawa unpublished data). The study reach was divided into 22 sections (i.e. 25 m section × 21 + 11 m section × 1).

Block nets were installed at both ends of each section to prevent fish movement between sections. The river system has been designated as a protected freshwater area year-round (Tsuboi & Morita 2004), so all white-spotted charrs in this system did not experience any angling events.

To evaluate vulnerability to angling, I first captured fish by bait angling and subsequently captured the remaining fish by electrofishing. Bait angling was carried out by one person (R. Hasegawa) in daytime (5:00–16:00) using the following gears and baits: carbon fishing rod (4.5 m), 3 lb nylon line, a 1/64 oz sinker, single barbed hook (gape width: 7.7 mm) and live bait waxmoth larva *Galleria mellonella*, as in Tsuboi & Morita (2004). Angling was conducted at all pools and riffles until no fish were caught. After the angling, I conducted two-pass electrofishing at each study section and captured the remaining fish (see Supplementary material 1). All captured fish were anesthetised by FA100 (DS Pharma Animal Health Co., Ltd.) and measured for body length (fork length; FL) and body weight (BW) to the nearest 1 mm and 0.1 g, respectively. The mouth cavities and body surfaces of the fish were macroscopically checked for the presence of copepods, as in previous studies (Hasegawa & Koizumi 2021; Ayer et al. 2022; Hasegawa et al. 2022b). All parasitic copepods found were counted.

2.2. Twitter analysis

I analysed people's reactions to fish parasites using Twitter. I first extracted tweets using the Japanese tags 'Sakana' (meaning fish) and 'Kisei-tyu' (meaning parasites) and selected specific tweets with images or videos posted from July 2019 to July 2022 (i.e. 3 years). Based on the uploader's description, selected tweets were classified into four categories: negative reaction, neutral reaction, positive reaction and reaction undetermined. For instance, if uploaders left comments implying 'fear',

‘disgust’ or equivalent words, I classified them into negative reactions. If uploaders released and/or discarded parasitised fish or if they removed parasites before they ate, they apparently treated parasites as negative things, and these tweets were therefore classified into negative reactions. On the contrary, when uploaders treated parasites as ‘cute’, ‘happy’ or equivalent words, I classified them into positive reactions. Neutral reactions were defined as tweets in which uploaders did not care about parasites. Tweets without the above reactions were classified into ‘reaction undetermined’ and removed from further analysis. From tweets and posted images, I identified the means of fish collection (i.e. angling, buy, other, unidentified), the parasite group (e.g. helminth, copepod, isopod, trematode) and host species at least at the order level. Given the consistency of the classification of tweets, a single person (R. Hasegawa) classified people’s reactions. Consistency was confirmed through blind tests by two volunteers by providing 50 random tweets and conducting the same evaluation: few observer biases were found (90% and 88% matched the classification categories).

2.3. Data analysis

All statistical analyses were conducted using R 4.1.2 (R Core Team 2021). To predict the factors affecting vulnerability to angling, I constructed a generalised linear mixed model using the R package ‘*lme4*’ version 1.1 (Bates et al. 2015). The response variable was vulnerability to angling (captured by angling = 1, captured by electrofishing = 0). The explanatory variables were body length (FL), body condition, infection status (infected = 1, not infected = 0) and their interactions (i.e. body length × infection status, body condition × infection status). The studied sections were included as random effects. I used the residual index calculated from ln (BW)−ln (FL) relationships as the host body condition (Jakob et al. 1996). Because fish less than 100 mm were rarely infected and captured by angling, which may have resulted in biased

results, I removed these fish from the analysis. I also removed data from one section (Section 7) because I did not conduct angling for practical reasons. All explanatory variables without infection status were standardised before the analysis.

3. Results

In total, 124 (prevalence: 37.10%, intensity: 1–6, mean intensity: 1.50) and 188 fish (prevalence: 34.04%, intensity: 1–7, mean intensity: 1.56) were captured by angling and electrofishing, respectively. The prevalence and mean intensity in each section are summarised in the Supplementary material 1. The interaction term of body condition and infection status was marginally significant, suggesting that vulnerability to angling changed depending on body condition and infection status (Table 1, Figure 1). Whereas infected fish with high body conditions showed higher vulnerability to angling, uninfected fish with lower body conditions were vulnerable to angling (Table 1, Figure 1). Larger fish were more vulnerable to angling (Table 1).

In total, 230 tweets were collected from three years of Twitter data, in which 122 (53%), 6 (3%), 11 (5%) and 91 (40%) tweets were categorised into negative, neutral, positive and reaction undetermined, respectively (Table 2). After removing the 'reaction undetermined' (i.e. 139 tweets), 35 (25%) and 66 (47%) were posted when uploaders found parasites at angling and markets (Table 2). Most anglers' satisfaction apparently decreased when the captured fish were infected by parasites (negative reaction, 94%). Most people who bought infected fish from markets also negatively treated parasites (negative reaction, 86%).

4. Discussion

Humans have hunted wildlife for resource consumption since ancient times (Fujita et al. 2016; Shchelinsky 2020), and parasites may have strong effects on catchability

and resource consumption rates by humans. Despite their potentially important roles, only two studies have examined the effects of parasites on human hunting (Rau & Caron 1979; Wilson et al. 2011). I provided the first evidence showing that parasite infections affect the angling vulnerability of host fish. Twitter analysis also showed that people's satisfaction significantly decreased when they found parasites on the fish they caught/bought. Therefore, parasite infections not only affect catchability but also affect human's fish consumption rates.

As I predicted, infected fish were less vulnerable to angling but only when they had poor body conditions, suggesting that parasites reduce the feeding rates of host fish. Similarly, several studies have found that parasitised hosts show reduced feeding activities, mostly due to physiological stress caused by parasites (Tierney 1994; Arneberg et al. 1996; Hiramatsu et al. 2001; Crane et al. 2011; Slavík et al. 2017). As in other parasites, *Salmincola* spp. infections can reduce host body conditions via physiological stress and physical damage to attachment sites (White et al. 2020; Hasegawa et al. 2022a). Reduced feeding would be particularly evident on my target parasite because *S. markewitschi* occupies mouth cavities, which mechanically hampers feeding (Nagasawa et al. 1998). Therefore, given the body condition index representing energy allocations (Sanchez et al. 2018), host with poor condition may not have enough resources to allocate for feeding.

Another possible explanation for the reduced vulnerability to angling is the loss of the competitive abilities of infected hosts with poor conditions. Stream salmonids generally form a dominance hierarchy, and individuals at higher hierarchy occupy better feeding habitats (Fausch 1984; Hughes 1992), resulting in higher vulnerability to angling (Tsuboi & Morita 2004). Given that parasite infections generally reduce the competitive ability of hosts (Barber et al. 2000), infected fish, especially those with poor conditions, are outcompeted by other fish, resulting in low vulnerability to angling.

In contrast, infected fish with higher body conditions are vulnerable to angling. In some host–parasite systems, infected hosts show longer foraging times (Giles 1987; Voutilainen et al. 2008) and higher growth rates (Arnott et al. 2000), possibly because they try to regain the resource shortage when they have enough energy to allocate to feeding behaviour. Similarly, fish infected by copepods with higher body conditions may have increased foraging behaviours to try to compensate for exploited resources, resulting in higher angling rates. This may also explain the higher vulnerability of uninfected fish with poor conditions to angling. The fact that salmonids having poorer body condition are susceptible to angling is also reported by other studies (Tsuboi et al. 2021).

So far, many studies have demonstrated the evolution of fish behaviour by angling; since angling generally select bolder, more aggressive and more explorative individuals (Biro & Post 2008; Härkönen et al. 2014; Wilson et al. 2015; Koeck et al. 2019), resulting in higher proportion of more shy or timid individuals in the original populations (Arlinghaus et al. 2017). In addition, such behavioural traits are often correlated with fish body condition (Kanno et al. 2023). In these contexts, what does my study add new perspectives on these timely topics? If parasite infection does not depend on host genotypes, parasite inflections and resulting susceptibility to angling will not cause evolution of fish behaviour. In addition, body condition is also highly plastic, depending on how much foods individuals eat. Thus, the condition-dependent vulnerability of infected fish, demonstrated by this study, will not significantly affect evolutionary change of fish behavior. In reality, however, host genotype affects the susceptibility to parasites, for example individuals showing specific heritable behaviours such as boldness tend to have more parasites (Wilson et al. 1993; Boyer et al. 2010). Fatness or body condition are also genetically determined (Merila 1996). Therefore, behavioural evolution induced by human angling activities are rather

complex, and parasite infections and body condition should also be considered in the context.

My Twitter analysis clearly revealed that people have negative impressions of fish parasites: angler's satisfaction generally decreased when their captured fish were parasitised, and people also felt disgusted when they bought parasitised fish. More importantly, some people discarded or released fish when they found parasites, suggesting that parasites reduced the fish consumption rate. People in particular feel fear when they find parasites that harm human health, such as *Anisakis* spp. (Supplementary material 2). People are also uncomfortable with parasites with no effects on human health, such as isopods, copepods and other helminths, which is probably due to their appearance, ignorance for their biology and actual effects. These results suggest that all types of parasites can potentially reduce human's fish consumption rates. Parasites have been a strong agent for natural selection on humans, and the feeling of disgust has evolved (Prokop & Fedor 2013; Curtis 2014). Consequently, people might have begun to feel disgusted towards all parasites, even to those with no harmful effects on human health. If most parasites decrease angler's satisfaction and increased angler's release motivation (Mitro & Griffin 2018), parasite infection might result in the increase of both host and parasite survival. Further studies examining the global patterns of people's reactions against parasites sheds light on the new insight into the roles of parasites in human resource consumption and natural selection on hosts and parasites.

Table 1. Results of the generalised linear mixed model examining the predictors affecting the angling vulnerability of white-spotted charr.

	Coefficient	Standard Error	*t*-value	*p*-value
Intercept	−0.503	0.239	−2.106	**0.035**
Residual	−0.236	0.239	−0.985	0.325
Infection status	−0.004	0.269	−0.016	0.988
Fork Length	0.441	0.182	2.420	**0.016**
Residual × infection status	0.571	0.311	1.839	**0.066**
Fork Length × infection status	−0.112	0.267	−0.419	0.675

Table 2. People's reactions against fish parasites as analysed using three-year Twitter posts.

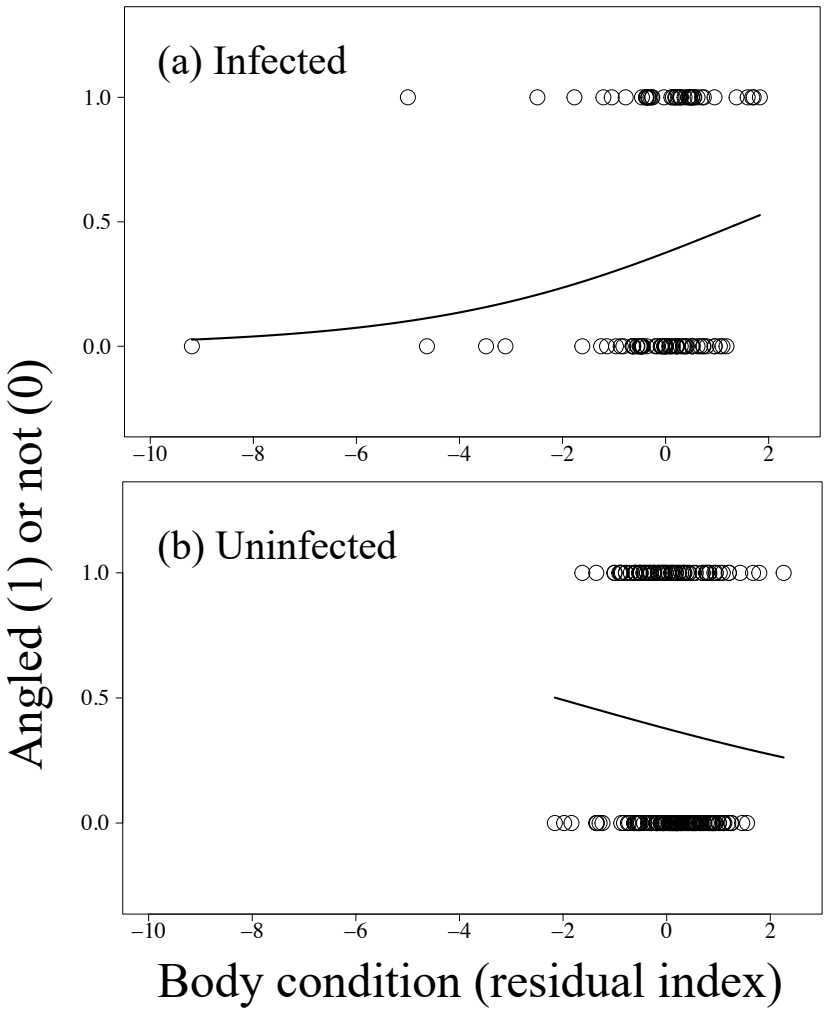
(a) including 'reaction undetermined'

	Angling	Buy from markets	Others	Unidentified	Total
Negative reaction	33 (42%)	57 (68%)	4 (40%)	28 (48%)	122 (53%)
Neutral reaction	1 (1%)	4 (5%)	0	1 (2%)	6 (3%)
Positive reaction	1 (1%)	5 (6%)	0	5 (9%)	11 (5%)
Reaction undetermined	43 (55%)	18 (21%)	6 (60%)	24 (41%)	91 (40%)
Total	78	84	10	58	230

(b) after removing 'reaction undetermined'

	Angling	Buy from markets	Others	Unidentified	Total
Negative reaction	33 (94%)	57 (86%)	4 (100%)	28 (82%)	122 (88%)
Neutral reaction	1 (3%)	4 (6%)	0	1 (3%)	6 (4%)
Positive reaction	1 (3%)	5 (8%)	0	5 (15%)	11 (8%)
Total	35	66	4	34	139

Figure 1. Relationships between the angling probability and body condition (residual index) of white-spotted charr. (a) Fish infected by mouth-attaching copepod *Salmincola markewitschi*, (b) Fish uninfected by the copepod.



Supplementary material 1. Summary of field surveys in each section.

Day/time of angling		Fish caught by angling (infected)	Fish caught by shocker (infected)	Total
July 5th (11:41-11:53)	Sec1	0	3 (0)	3 (0)
July 5th (11:56-12:32)	Sec2	3 (1)	9 (2)	12 (3)
July 5th (12:35-12:54)	Sec3	3 (2)	4 (2)	7 (4)
July 5th (15:50-16:23)	Sec4	3 (1)	6 (0)	9 (1)
July 6th (5:54-6:23)	Sec5	3 (0)	6 (2)	9 (2)
July 6th (6:24-7:27)	Sec6	14 (5)	22 (5)	36 (10)
NA	Sec7	NA	NA	NA
July 6th (10:20-11:01)	Sec8	7 (3)	12 (1)	19 (4)
July 6th (11:01-11:19)	Sec9	0	12 (4)	12 (4)
July 6th (15:24-15:53)	Sec10	1	11 (3)	12 (3)
July 6th (15:53-16:28)	Sec11	2 (1)	17 (8)	19 (9)
July 7th (6:25-7:16)	Sec12	9 (4)	6 (2)	15 (6)
July 7th (7:16-7:51)	Sec13	7 (5)	7 (3)	14 (8)
July 7th (7:51-8:05)	Sec14	1 (0)	15 (4)	16 (4)
July 7th (9:05-9:25)	Sec15	5 (1)	7 (3)	12 (4)
July 7th (9:30-10:12)	Sec16	7 (2)	3 (1)	10 (3)
July 7th (13:36-14:18)	Sec17	8 (1)	6 (3)	14 (4)
July 7th (14:18-14:45-15:04)	Sec18	9 (3)	7 (4)	16 (7)
July 8th (7:17-8:02)	Sec19	11 (6)	9 (4)	20 (10)
July 8th (8:02-8:40)	Sec20	8 (3)	7 (1)	15 (4)
July 8th (8:42-9:13)	Sec21	6 (2)	9 (6)	15 (8)
July 8th (9:16-9:22-9:32-10:08)	Sec22	17 (6)	10 (6)	27 (12)
	Total	124 (46)	188 (64)	312 (110)

Chapter 5

Does parasite infection affect host feeding behavior ? A test with stomach contents analysis

Abstract

Many parasites infect fish host mouth cavities, and these parasites are expected to physically impede host foraging. Despite their potential impacts on host foraging ecology, only a few studies evaluated host foraging activities and provided unclear evidence. Here, I examined the effects of mouth-infecting copepods, *Salmincola markewitschi,* on foraging of wild white-spotted charr *Salvelinus leucomaenis,* using stomach contents analysis. Contrary to my predictions, stomach fullness and total prey abundance were not significantly different between infected and uninfected fish. However, I found that smaller infected hosts foraged on a lower proportion of terrestrial invertebrates compared to uninfected counterparts. These results suggest that small infected fish rather increased foraging activities to compensate their energetic loss induced by infections, whereas they shifted main diets from large terrestrial to small aquatic invertebrates, possibly due to physical inhibitation and reduced competitive abilities by copepod infection.

1. Introduction

Parasites substantially induce host behavioral changes and ultimately affect host fitness (Barber et al. 2000; Binning et al. 2017; Mrugała et al. 2023). One such behavior that is strongly affected by parasite infection is host foraging (Barber et al. 2000; Mrugała et al. 2023). Parasites generally decrease host foraging activities by exploitation of host's energy (Crane et al. 2011), reduction of prey searching and handling efficiency (Cunningham et al. 1994; Österling et al. 2014; Souza et al. 2019; Vivas Muñoz et al. 2019) and host competitive abilities over food resources (Finley & Forrester 2003; Filipsson et al. 2018; Godwin et al. 2018). On the other hand, infected hosts occasionally increase foraging activities to try to regain resources that are exploited by parasites (Arnott et al. 2000; Hasegawa & Koizumi 2023). In addition, some parasites, especially trophically transmitted parasites, could increase host foraging activities via host manipulation to increase the chance to transmit to definitive hosts (Bernot & Lamberti 2008). Since foraging are tightly linked to host fitness (Werner & Hall 1974; Waite & Field 2007; Hintz & Lonzarich 2018) and these host behavioral changes also spill over into host population dynamics, community changes and ecosystem level consequences (Minchella & Scott 1991; Wood et al. 2007; Reisinger & Lodge 2016; Morton & Silliman 2020), understanding the parasite's roles in host foraging is fundamental, but central issues in ecology and evolution (Born-Torrijos et al. 2023; Mrugała et al. 2023).

Many parasites, particularly parasitic isopods and copepods are known to infect on mouth cavity of fish hosts (Kabata 1969; Weinstein & Heck 1977; Smit et al. 2014; Vigneshwaran et al. 2019). Since these parasites often occupy large proportion of the mouth cavity space, they could physically inhibit host foraging and subsequently reduce host body condition and growth rate (Weinstein & Heck 1977; Vigneshwaran et al. 2019). Researchers have long been curious about if and how such infected fish forage

for preys (Lanzing & O'Connor 1975; Weinstein & Heck 1977; Kimmel & Arneson 1978; Brusca & Gilligan 1983). On the other hand, only a few studies have evaluated host foraging ecology, employing specific methods such as stomach contents and stable isotope analyses (Parker & Booth 2013; Carrassón & Cribb 2014; but see Vigneshwaran et al. 2019). And surprisingly, these studies provided unclear evidence of serious impacts of these parasites (Parker & Booth 2013; Carrassón & Cribb 2014; but see Vigneshwaran et al. 2019). For instance, Carrassón & Cribb (2014) assessed the impacts of mouth-infecting isopods *Ceratothoa* cf. *imbricataon* on the banded scat *Selenotoca multifasciata*, but found no evidence of reduced stomach fullness and body condition indices. Parker & Booth (2013) more specifically explored negative impacts of similar isopods *Cymothoa borbonicaon* on large spot pompano *Trachinotus botla* using stomach contents and stable isotope analyses. They identified a decline in the growth rate of infected fish, but other indices such as stomach fullness, diet compositions and stable isotope levels were similar between infection status. Only Vigneshwaran et al. (2019) reported significant changes of dietary compositions, stomach fullness and body condition of Black Pomfret *Parastromateus niger* infected by isopod *C. eremita*. Consequently, several studies assumed that the impacts of mouth-infecting parasite on host body condition and growth are benign or negligible in the field (refs; Carrassón & Cribb 2014). Nonetheless, all these previous studies predominantly focused mouth-infecting isopod systems, and further examinations on different types of systems with specific evaluations of diet compositions, are still required to draw conclusions.

In this study, I evaluated the effects of a mouth-infecting copepod *Salmincola markewitschi* on foraging ecology of their hosts white-spotted charr *Salvelinus leucomaenis* in a natural stream of northern Japan (Figure 1). The genus *Salmincola* is an ectoparasitic copepod group that mainly utilize freshwater salmonids as hosts (Kabata 1969). Their infections generally occur at mouth and gill cavities, and cause

physical damages to attachment sites (Kabata & Cousens 1973; White et al. 2020). Fragmented observations suggest that infections of mouth attaching *Salmincola* spp. such as *S. carpionis* and *S. stellata* reduce host appetites in the aquarium (Nagasawa et al. 1994; Nagasawa et al. 1998; Hiramatsu et al. 2001), which might lead loss of host body condition (Nagasawa et al. 1998) and host death (Hiramatsu et al. 2001). In fact, I found consistent negative correlations between the numbers of *S. markewitschi* and their host body condition throughout the year, suggesting that this parasite could strongly reduce foraging activities and cause subsequent reduction of host body condition (Hasegawa & Koizumi 2024). On the contrary, host vulnerability to angling, a potential indicator for host foraging activities, was not significantly different among infection status, and differences became clearer only when I considered host body condition (Hasegawa & Koizumi 2023). Nonetheless, these studies evaluated the impacts of mouth-infecting copepods on host foraging using indirect method (i.e. body condition and vulnerability to angling), and hence more direct and detailed evaluation is necessary.

For assessing detailed impacts of the parasites on host foraging ecology, I quantified host diet compositions using stomach contents analysis, a traditional method for evaluating foraging quantity and quality of wild fish populations (Braga et al. 2012; Amundsen & Sánchez-Hernández 2019). In this study, I tested two specific predictions. Firstly, I predicted that infected hosts would show lower levels of stomach fullness and prey abundance because the mouth-infecting copepods could physically and directly inhibit host foraging and reduce the host's prey capturing success. Secondly, I predicted that infected hosts would change diet compositions, showing a lower proportion of terrestrial invertebrates. Salmonids, especially charrs (i.e. fishes in the genus *Salvelinus*), are generalist feeders and forage aquatic and terrestrial organisms (Yamamoto 1991; Morita & Suzuki 1999; Goto et al. 2023). Terrestrial invertebrates are generally larger

than aquatic ones, and thus infected hosts should struggle to prey on these larger terrestrial invertebrates due to physical impediment caused by mouth-infecting copepods. In addition, reduced proportion of terrestrial invertebrates is expected from the consequences of intra- and inter-specific competitions. It is well known that salmonids generally have strong dominance hierarchy and dominant fish tend to stay in the middle of the flow and forage on terrestrial organisms, whereas subordinates tend to prey aquatic organisms (Nagoshi & Sakai 1980; Miyasaka et al. 2003; Nakano et al. 2020; Fausch et al. 2021). Due to reduced health condition and a loss of handling efficiency caused by parasites, infected hosts would be easily outcompeted by conspecifics (Barber et al. 2000; Filipsson et al. 2016; Hasegawa & Koizumi 2023). Consequently, these hosts are forced to prey aquatic invertebrates.

2. Materials and methods

A field survey was carried out at the two sites of Ito-River, one of the main tributaries of Shiodomari River system, southern Hokkaido, Japan (hereafter, site A and B, the same sites of ID 19 and 17 in Hasegawa & Koizumi 2021). In this river system and tributary, white-spotted charr widely distribute and are frequently infected by parasitic copepod *Salmincola markewitschi* (Hasegawa & Koizumi 2021, 2023, *in press*; Hasegawa et al. 2022b). Other than white-spotted charr, masu salmon *Oncorhynchus masou masou* and fluvial sculpin *Cottus nozawae* occur in both study sites, but stone loach *Barbatula oreas* only occur in site B. The density of masu salmon was high in site B (Supplementary material 1).

In July 2019, I captured white-spotted charr by electrofishing within 100–300 m study reaches at each study site ($N = 227$). July is the main foraging and growing period for white-spotted charr (Ishigaki 1984; Morita & Suzuki 1999). Captured fish were anesthetized using the anesthetic agent FA100 (DS Pharma Animal Health Co., Ltd.,

Osaka, Japan), and body length (fork length: hereafter FL) and body weight (hereafter BW) were measured to the nearest 1 mm and 0.1 g, respectively. The body surfaces (including buccal cavities) of fish were macroscopically checked for the presence of copepods. When parasitic copepods were found, I counted their numbers and recorded the attachment locations on each fish. I only counted female copepods because male *Salmincola* spp. are dwarf forms that attach to female bodies (Kabata & Cousens 1973). All captured fish were visually categorized into age 0 and age 1 and older fish. Since age 0 fish were rarely infected by *S. markewitschi* (Hasegawa & Koizumi 2021, 2024), age-1 and older fish were subjected to stomach collections as described below.

The stomach contents of almost all captured age-1 and older fish ($N = 223$) were quickly collected by stomach flushing method with a 500-mL wash bottle (Giles 1980; Sato et al. 2012). Collected stomach contents were preserved in 70% ethanol until analyzed in the laboratory (see below). All captured fish were released at sites where they were collected.

Preserved stomach contents were analyzed under a stereo microscope (SZ10, Olympus Inc., Japan). Stomach contents of most fish individuals were composed by terrestrial and aquatic arthropods, mainly Insecta (Table 1). These arthropods were identified at least order levels. Other taxon such as snails, earth worm, and gammaridean amphipod were identified at least class levels. I measured wet weight for each category to the nearest 0.01 mg after blotting for about 10 s (Nakano et al. 1999; Miyasaka et al. 2003). I also counted numbers for each prey category from digested body parts as total prey abundance (e.g. Goto et al. 2023).

All statistical analyses were performed using R 4.1.2 (R Core Team 2021). All the fish larger than 227mm ($N = 10$) showed apparent low amounts of stomach contents and developed gonad, and some fish may be migrant ascended form that sea to the streams for spawning. They possibly ceased foraging due to the preparation for

reproduction after September (Yamamoto 1991). Another fish's stomach ($N = 1$) was filled with unidentified organisms, possibly small mammals, and it was highly difficult to identify other specific prey types in the stomach. Therefore, these fish ($N = 11$) were removed from the subsequent analyses but retained the calculation of infection indices such as prevalence (see below).

Infection indices (i.e. prevalence, intensity, mean intensity) were calculated for each study site following the methodology defined by Bush et al. (1997). To evaluate a host body condition, I calculated stomach excluded weight (SEW; BW – total amount of stomach contents weight) for each fish.

A body condition ("residual index" defined by Jakob et al. 1996) was calculated from the regression of ln (SEW) and ln (FL): residuals from the regression lines were served as a relative body condition for each fish (Jakob et al. 1996). This index allows to quantify fish body condition or overall health status regardless of host body length (Jakob et al. 1996), and thus it has been widely used in parasitology and fish ecology (e.g. Bagamian et al. 2004; Lagrue & Poulin 2015; Perrot-Minnot et al. 2020).

Like other studies of white-spotted charr (e.g. Morita & Suzuki 1999), two-age classes, age 1 and age 2 and older, could be visually distinguished from the FL-frequency histogram. Using R package"*mclust*" (Fraley et al. 2012), I statistically decided thresholds of upper and lower side of each size-distribution. In this analysis, age-1 class and age-2 and older class were successfully distinguished as ≦ 127 mm and > 127 mm FL, and therefore I used these categories in the following analysis (see below).

I compared the frequency of each prey category in the stomach contents among infection status (i.e. infected and uninfected) and fish age classes (i.e. age-1, age 2 and older) using Fisher's exact test.

To test whether stomach fullness differed between infection status, I constructed a generalized linear mixed model (GLMM) using a quasibinomial distribution with a logit link function to account for the over-dispersion of response variable (e.g. Larios et al. 2023). The model was constructed by penalized quasi-likelihood function (i.e. glmmPQL) in R package "*MASS*" (Venables & Ripley 2002). I calculated stomach fullness for each fish using following equation: Stomach fullness = 100 × weight of stomach content / SEW (e.g. Godwin et al. 2018). The response variable was stomach fullness and explanatory variables were infection status (infected = 1, uninfected = 0), FL, body condition, study sites (site A and B), and interaction terms (i.e. FL × infection status, body condition × infection status). Due to the possible significant effects of these two interaction terms as I found in my previous study (Hasegawa & Koizumi 2023), I included these in the model. I also included study sites as an explanatory variable to account for the heterogeneity of physical and biological characteristics between two study sites (Supplemental material 1), I included sample's ID as random effects to ensure the high variance of response variables. Due to the logistical reasons in glmmPQL, I removed three fish with empty stomach (total $N = 206$ fish).

To test if infections decrease the total prey abundance, I also constructed GLMM with negative binomial distribution using R package *lme4* (Bates et al. 2015). The response variable was total prey abundance and the explanatory variables were infection status (infected = 1, uninfected = 0), FL, body condition, study sites (site A and B) and interaction terms (i.e. FL × infection status, body condition × infection status). I also included sample's ID as random effects in this analysis.

Finally, to test if copepod infection decreases the proportion of terrestrial invertebrates, I also constructed GLMM using a quasibinomial distribution with logit link by glmmPQL function described above. The response variables were proportion of terrestrial invertebrates, that was defined as follows: cbind (weight of terrestrial

invertebrates, weight of aquatic invertebrates). The explanatory variables were FL, body condition, infection status (infected = 1, uninfected = 0), study sites (site A and B) and interaction terms (i.e. FL × infection status, body condition × infection status). I included sample's ID as random effects. I used 206 fish data as the same reasons as described above.

3. Results

In total, 227 age 1 and older resident white-spotted charr were captured at two sites in Ito River, Shiodomari River system (Site A: *N*=104, Site B: *N*=123). FL range were 86–294 (mean 153.25 mm) and 89–300 (mean 145.78 mm) for Site A and B, respectively. All copepods were found in buccal cavities. The infection prevalence and mean intensity were 25.0 % and 2.19 (range: 1–6) at site A and 24.4 % and 1.4 (range 1–4) at site B, respectively.

The four phylums and six classes were identified in the stomach contents of 212 fish (Table 1). Of which, arthropod is the main prey types for most fish and 14 orders were recorded. The majorities of stomach contents were composed with Trichoptera's larvae (61.3 %), followed by Ephemeroptera's larvae (58.0%), Hymenoptera (mainly ants, 50.0 %), Coleoptera (49.5 %), Lepidoptera (all larvae, 46.2 %), Diptera (40.6 %) and Orthoptera (22.2 %) (Table 1).

In age 1 class, frequency of Lepidoptera was significantly different among infection status (Figure 2). While 9.1 % of infected fish foraged Lepidoptera, 44.0 % of uninfected fish foraged the same prey item (Fisher's exact test; $p = 0.04$; Figure 2). In age 2 and older class, uninfected fish showed significantly higher foraging rate of Diptera's adult (47.8 %, $p = 0.04$; Figure 2) compared to infected counterparts (27.8 %; Figure 2). Infected fish in age 2 and older class foraged marginally higher rate for

Ephemeroptera's adult (5.4 %, $p = 0.08$; Figure 2), whereas no uninfected fish foraged them (0 %; Figure 2).

No significant differences of stomach fullness were found among infection status (Table 2a). Body condition and FL was positively and negatively correlated with stomach fullness, respectively (Table 2a). Stomach fullness of fish captured in site A was significantly lower than that of site B (Table 2a).

In the analysis for total prey abundance, the interaction term of FL and infection status was marginally significant (Table 2b); while total prey abundance were positively correlated with FL in uninfected hosts, these were negatively correlated with FL in infected hosts. Body condition had significant positive effects (Table 2b). Total prey abundance was significantly higher in site A (Table 2b).

In GLMM analysis for proportion of terrestrial invertebrates, interaction terms of FL and infection status was significant (Table 2c; Figure 3). This means that the proportion of terrestrial invertebrates of infected hosts were low when FL was small, whereas the proportion was high when their FL was large (Table 2c; Figure 3). Body condition did not have significant effects on proportion of terrestrial invertebrates (Table 2c). Proportion of terrestrial invertebrates was higher in site B (Table 2c).

4. Discussion

To date, many researchers have suspected that mouth-attaching parasites could strongly hinder and reduce host foraging activities and efficiency (Smit et al. 2014; Vigneshwaran et al. 2019). However, only a few studies specifically evaluated the dietary compositions of fish infected by these parasites, and most of them reported no or minor effects (Parker & Booth 2013; Carrassón & Cribb 2014; Vigneshwaran et al. 2019). Through intensive evaluations of host stomach contents, I successfully detected host foraging shifts from terrestrial to aquatic invertebrates which may be induced by

physical inhabitation of feeding by the mouth attaching parasite *Salmincola markewitschi*. Contrary to this feeding shifts, I found no significant differences of stomach fullness and total prey abundance, suggesting that fish may have increased foraging to compensate their energetic loss by parasites.

As predicted, I found infected fish showed lower proportion of terrestrial invertebrates but only when they had smaller body size. Additional analysis comparing the diet category in different size class supported this result; age 1 infected fish (younger and smaller size group) showed lower frequency of Lepidoptera (terrestrial insects). These results could have been derived from several mechanisms. First, copepods physically inhabit host feeding, and subsequently reduce host prey handling efficiency. Most teleost fishes employ suction feeding, the ability to utilize a strong pressure gradient inside the oral cavity to suck preys with water into the mouth (Wainwright et al. 2015; Dearden et al. 2023). When parasitic copepods attach to the mouth, copepods block the sucked preys, and hence fish could not effectively swallow preys. This is particular the case when the fish have a smaller body size, generally characterized by a smaller mouth cavity space, and when such fish capture large prey, such as grasshoppers and earthworms. Since these large prey items are commonly terrestrial invertebrates, the stomach contents of small infected hosts consequently consisted of relatively small-sized benthic invertebrates. Significantly lower frequency of Lepidoptera in age-1 infected hosts well supported this explanation because Lepidoptera has relatively larger size among stomach contents in the study area (Hasegawa personal observation).

Second, smaller infected hosts shifted their main diets to minimize energy expenditure. While terrestrial invertebrates flow around the surface of the river, aquatic invertebrates flow around the riverbed (Furukawa-Tanaka 1992). Thus, capturing aquatic invertebrates around the riverbed (i.e. bottom) does not require much energy

compared to those for terrestrial invertebrates. Given that copepod infections reduce host body condition (Hasegawa et al. 2022a; Hasegawa & Koizumi 2023, 2024) and such parasite impacts tend to be larger in small hosts (Spitzer et al. 2022), smaller infected hosts with low energy reserves shifted their foraging behaviors to selectively capture aquatic invertebrates. In other host-parasite systems, fish infected by parasites similarly exhibited selective foraging on preys with low mobility and activity because capturing these preys does not require a huge amount of energy (Vivas Muñoz et al. 2019).

Third, infected fish, especially having smaller body size, shifted foraging behavior because of possible reduced competitive abilities by parasites (Barber et al. 2000; Tompkins et al. 2003). Salmonids have strong dominance hierarchy and small fish tend to lose in competitions (Nakano 1995; Fausch et al. 2021). Infected hosts particularly could have poor competitive abilities because of their reduced body condition and induced stress (Barber et al. 2000; Tompkins et al. 2003). In fact, several studies found that parasitized fish tend to be outcompeted by intra-and inter specific competitions (Barber & Huntingford 1995; Barber et al. 2000; Filipsson et al. 2018). In my study sites, density of white-spotted charr was relatively higher than other sites in the river system (Hasegawa & Koizumi 2021), and thus intra-specific competitions could have occurred frequently. Further, masu salmon, a strong competitor for white-spotted charr (Furukawa-Tanaka 1988; Nakano 1995; Morita & Suzuki 1999; Miyasaka et al. 2003) inhabits both study sites as well. Masu salmon is generally superior to white-spotted charr in inter-specific competitions and preyed terrestrial invertebrates a lot (Morita & Suzuki 1999; Miyasaka et al. 2003). Under these situations, it is highly possible that intra- and inter-specific competitions, infected small fish were forced to shift their prey to aquatic insects such as Trichoptera. On the contrary, larger

fish could be dominant even when they are infected by parasites because of their large body size and large amount of holding resources.

Strikingly, there were no significant differences in stomach fullness and total prey abundance among infection statuses. I also found that body condition correlated with both stomach fullness and total prey abundance. These findings are consistent with previous studies showing no differences of stomach fullness and subsequent body condition indices among infection status (Parker & Booth 2013; Carrassón & Cribb 2014). These results may be explained by increments of feeding to compensate for energetic demands in infected hosts. Parasites reduce host energy reserves through several pathways such as the direct exploitation of host resources, increments in metabolism and the activation of costly immunity (Haye & Ojeda 1998; Arnott et al. 2000; Östlund-Nilsson et al. 2005). To counter this, hosts often increase foraging activities to regain energy to cover their reduced energy reserves (Arnott et al. 2000; Östlund-Nilsson et al. 2005). In infected hosts, this trend must be evident in fish with high body condition because they can afford to allocate their resources into foraging activities (Hasegawa & Koizumi 2023). Thus, compensative food intakes might have masked the differences in stomach fullness and total prey abundance among infection statuses in the present and previous studies (Parker & Booth 2013; Carrassón & Cribb 2014). Then, it is also predicted that parasite negative impacts become apparent in situations where food resources are limited and/or host's energetic demands are high (Östlund-Nilsson et al. 2005). Several studies supported these predictions; for instance, negative impacts by parasites were restricted under high-stress and low-food conditions (Östlund-Nilsson et al. 2005; Sala-bozano et al. 2012; Kawanishi et al. 2016).

There are several limitations in my studies. My results were not only reflected by host behavioral differences, but also could be derived from differences of host digestive abilities among infection status (Born-Torrijos et al. 2023). Several previous

studies pointed out that infected hosts showed different metabolisms and consequent digestive abilities due to limited resource allocations (Toscano et al. 2014; Born-Torrijos et al. 2023). Furthermore, while stomach contents analysis provides us insightful information of host foraging ecology (Giles 1980; Amundsen & Sánchez-Hernández 2019), these results represent mere snapshots, and thus generality of my findings to different timing of capture is not known. For instance, given the seasonal fluctuations in fish foraging activities (Yamamoto 1991; Railsback et al. 2005), body condition (Morita et al. 2011; Spangenberg et al. 2023) and resource availability (Kawaguchi & Nakano 2001; Armstrong et al. 2016), my results could differ among seasons. Future studies such as laboratory experiments and stable isotope analysis which enables us to detect long-span patterns of host foraging (Layman et al. 2007), are necessary to determine whether mouth-infecting copepods induce host foraging at other times.

In summary, I found that mouth-infecting copepod *S. markewitschi* induced dietary shifts in small host fish, but infection does not alter the overall stomach fullness and total prey abundance. Our results indicate that mouth-infecting parasites affect host foraging ecology, but that impacts are not stronger than many researchers expected despite the obvious occupancy of these parasites in host mouth cavity. Nonetheless, my target species is relatively smaller compared to the focal species in previous studies (Barkenhaster et al. 2006; Sala-bozano et al. 2012; Carrassón & Cribb 2014), and hence, dietary shifts induced by mouth infecting parasites may be prevalent among other host-parasite associations, and previous studies that did not specifically examine the stomach contents of fish might have overlooked such host behavioral changes. These host foraging shifts could partially explain poor body condition of infected hosts (Hasegawa & Koizumi 2024), potentially leading to the loss of fitness components such as growth and survival (Hasegawa & Koizumi 2024). Furthermore, the foraging shifts

induced by parasite infections also trigger trophic cascades (Sato et al. 2012; Mrugała et al. 2023). In my study system, since small infected fish selectively foraged upon aquatic invertebrates, this behavioral shifts might affect algae production and nutrient uptake (Sato et al. 2012; Nakano et al. 1999). To these end, further studies focusing other host – mouth-attaching parasite systems are highly necessary.

Table 1. Summary of prey taxonomic categories found from stomach contents of white-spotted charr in the Shiodomari River system.

Taxonomic groups		Infected		Uninfected	
		age-1 (N = 11)	age-2 and older (N = 36)	age-1(N = 75)	age-2 and older (N = 90)
Aquatic organisms					
Arthropod					
–Malacostraca	Amphipoda	0 (0 %)	2 (5.6 %)	6 (8.0 %)	4 (4.4 %)
–Insecta	Ephemeroptera	9 (81.8 %)	16 (44.4 %)	46 (61.3 %)	52 (57.8 %)
	Plecoptera	1 (9.1 %)	2 (5.6 %)	2 (2.7 %)	6 (6.7 %)
	Trichoptera	7 (63.6 %)	17 (47.2 %)	54 (72.0 %)	52 (57.8 %)
	Diptera	1 (9.1 %)	0 (0 %)	3 (4.0 %)	7 (7.8 %)
Vertebrata					
–Osteichthys		0 (0 %)	3 (8.3 %)	0 (0 %)	4 (4.4 %)
Terrestrial organisms					
Annelida					
–Oligochaeta		0 (0 %)	0 (0 %)	0 (0 %)	1 (1.1 %)
Mollusca					
–Gastropoda		0 (0 %)	1 (2.8 %)	1 (1.3 %)	5 (5.6 %)
Arthropod					
–Malacostraca	Isopoda	0 (0 %)	1 (2.8 %)	2 (2.7 %)	1 (1.1 %)
–Arachnida	Araneae	2 (18.2 %)	8 (22.2 %)	17 (22.7 %)	25 (27.8 %)
	Opiliones	0 (0 %)	0 (0 %)	1 (1.3 %)	1 (1.1 %)
–Insecta	Ephemeroptera	0 (0 %)	2 (5.6 %)	0 (0 %)	0 (0 %)
	Plecoptera	0 (0 %)	0 (0 %)	0 (0 %)	1 (1.1 %)
	Trichoptera	0 (0 %)	0 (0 %)	2 (2.7 %)	1 (1.1 %)
	Dermaptera	0 (0 %)	3 (8.3 %)	3 (4.0 %)	4 (4.4 %)
	Orthoptera	0 (0 %)	13 (36.1 %)	8 (10.7 %)	26 (28.9 %)
	Hemiptera	1 (9.1 %)	3 (8.3 %)	5 (6.7 %)	8 (8.9 %)
	Hymenoptera	5 (45.5 %)	25 (69.4 %)	28 (37.3 %)	48 (53.3 %)
	Coleoptera	6 (54.5 %)	20 (55.6 %)	27 (36.0 %)	52 (57.8 %)
	Lepidoptera	1 (9.1 %)	20 (55.6 %)	33 (44.0 %)	44 (48.9 %)
	Diptera	5 (45.5 %)	10 (27.8 %)	28 (37.3 %)	43 (47.8 %)

Table 2. Results of the generalized linear mixed models (GLMMs) examining the predictors affecting the (a) stomach fullness, (b) total prey abundance, (c) proportion of terrestrial invertebrates of white-spotted charr in the Shiodomari River system.

(a) Stomach fullness

Variables	Coefficient	Standard Error	*t*-value	*p*-value
Intercept	−5.172	0.108	−48.082	**<0.001**
Fork Length	−0.268	0.093	−2.887	**0.010**
Body condition	0.298	0.088	3.436	**0.001**
Infection status	0.160	0.208	0.767	0.445
Study sites	−0.274	0.127	−2.150	**0.035**
Fork Length × infection status	−0.178	0.178	0.224	0.823
Body condition × infection status	0.040	0.145	−1.232	0.221

(a) Total prey abundance

Variables	Coefficient	Standard Error	*z*-value	*p*-value
Intercept	2.018	0.068	29.527	**<0.001**
Fork Length	0.062	0.050	1.233	0.218
Body condition	0.134	0.050	2.688	**0.010**
Infection status	0.020	0.129	0.158	0.874
Study sites	0.718	0.081	8.852	**<0.001**
Fork Length × infection status	−0.200	0.115	−1.735	0.083
Body condition × infection status	−0.063	0.102	−0.617	0.537

(c) Proportion of terrestrial invertebrates

Variables	Coefficient	Standard Error	*t*-value	*p*-value
Intercept	0.777	0.154	5.056	**<0.001**
Fork Length	0.400	0.120	3.340	**<0.01**
Body condition	0.165	0.108	1.530	0.130
Infection status	−0.510	0.280	−1.822	**0.072**
Study sites	−1.093	0.162	−6.736	**<0.001**
Fork Length × infection status	0.641	0.240	2.674	**<0.01**
Body condition × infection status	−1.093	0.203	−0.467	0.641

Figure 1. *Salmincola markewitschi* (arrow heads) attaching to mouth cavity of white-spotted charr *Salvelinus leucomaenis.*

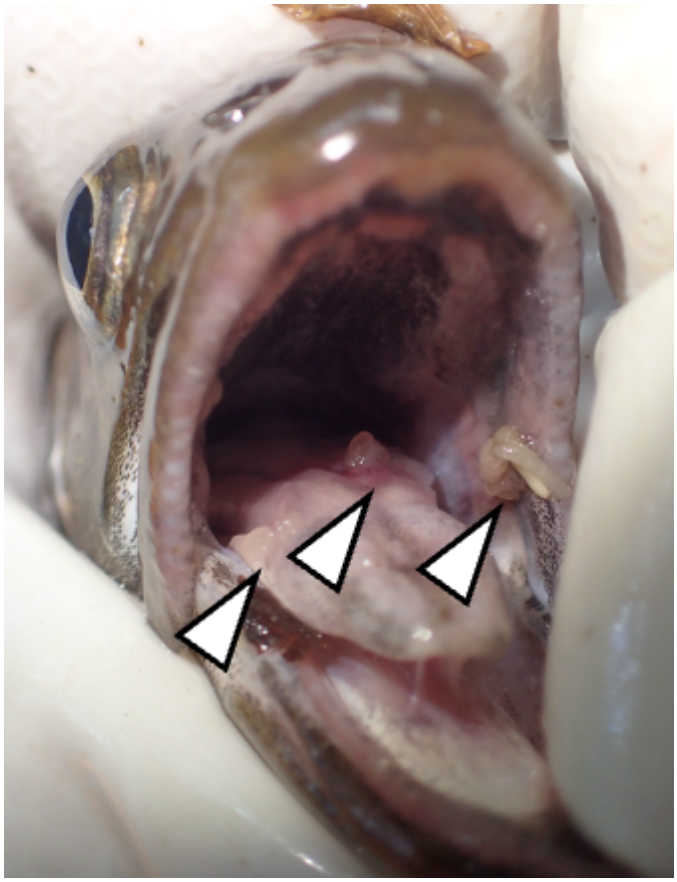


Figure 2. Proportion of each prey category in the stomach contents of white-spotted charr collected in the Shiodomari River. Figures were shown for each FL class (each 30 mm) and infection status (a. infected; b. uninfected). Only top 8 prey categories were shown.

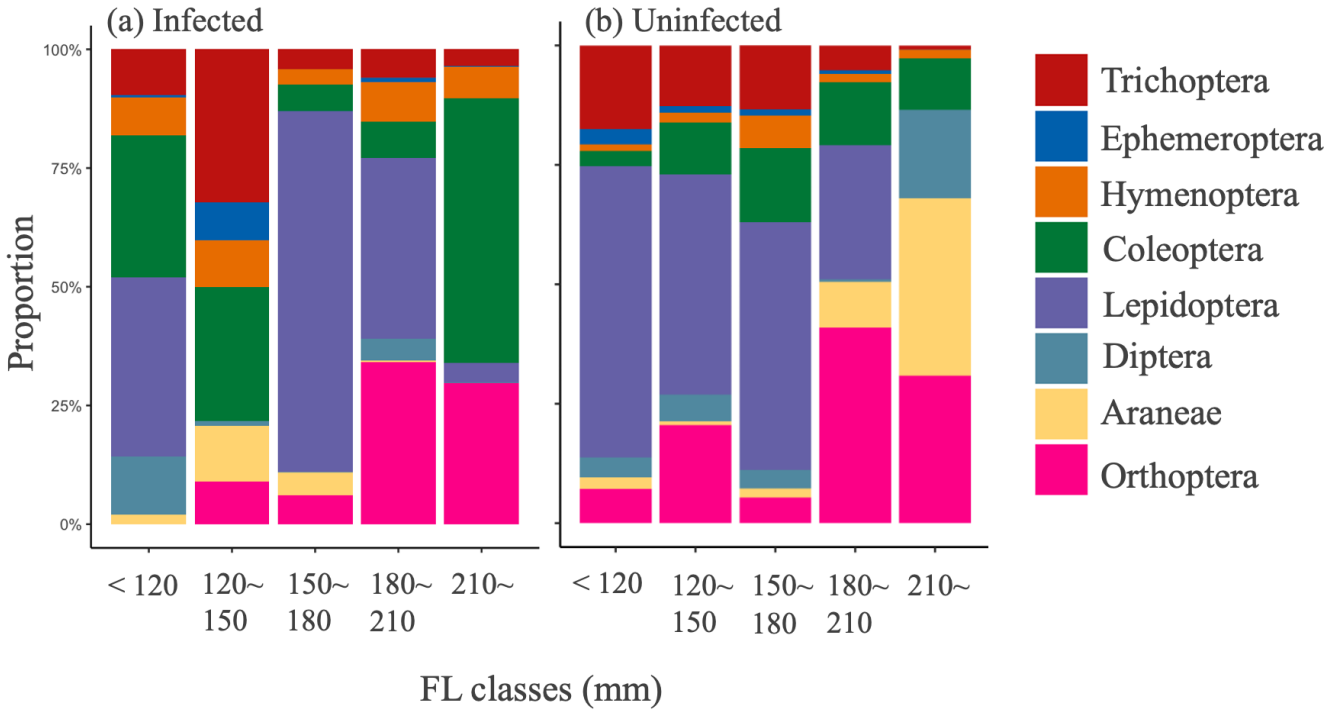
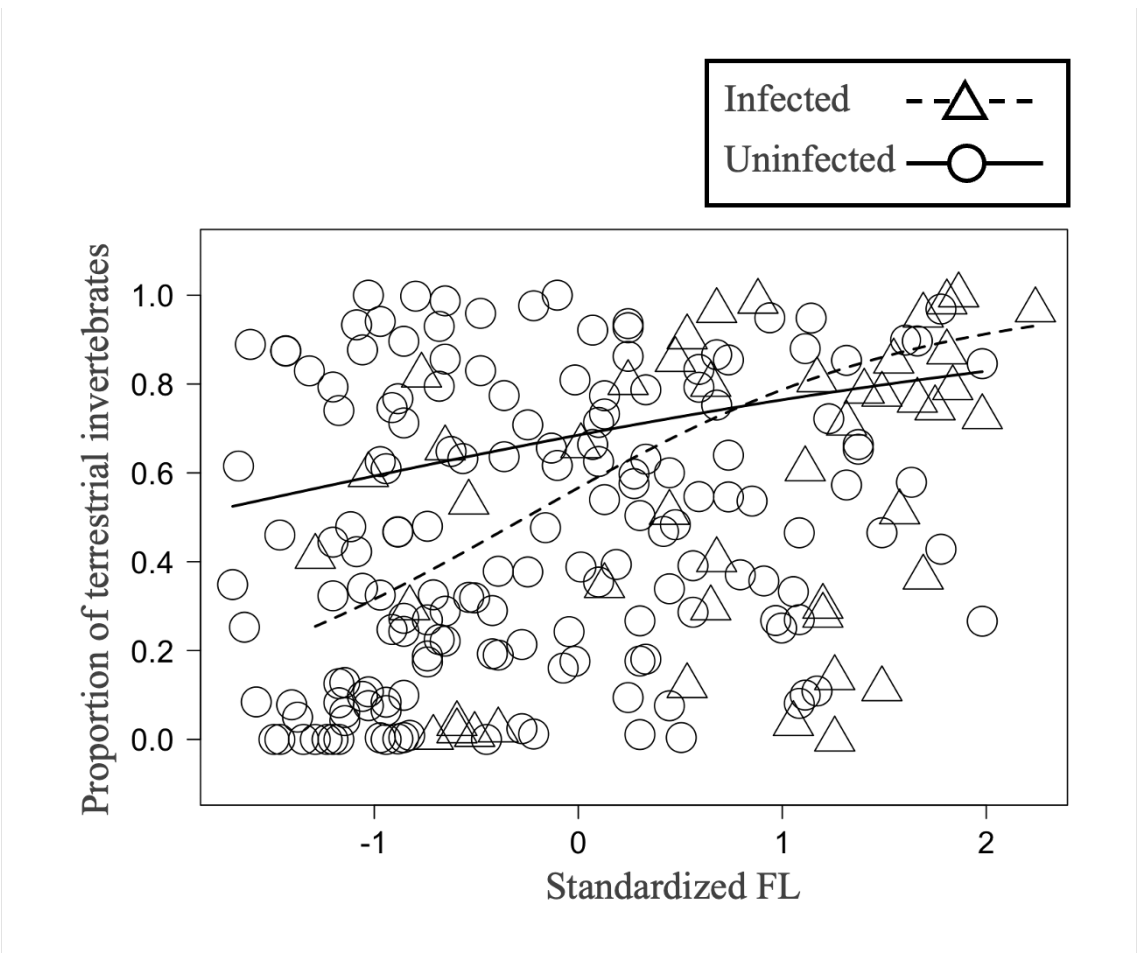


Figure 3. The relationships between standardized FL of white-spotted charr and proportion of terrestrial invertebrates against all stomach contents. Regression curves were estimated by generalized linear mixed models (GLMM). Fitted lines and plots are shown for each infection status (i.e. infected and uninfected fish).



Chapter 6

Disentangling the causality between parasite infections and poor host condition in the wild population

Abstract

Host-parasite relationships are ubiquitous on Earth. Although parasites reduce host health, parasite infections also occur as a consequence of compromised host health. Both causalities could induce positive feedback, in which infected hosts with poor body conditions may suffer further infection. Such positive feedback could increase host mortality and may finally affect host population dynamics. However, both causalities and how positive feedback affect host population dynamics has rarely been demonstrated in the wild, possibly due to methodological difficulties. Here, I used a mark-recapture survey combined with structural equation modelling (SEM) to examine whether both causalities and positive feedback occurred in stream salmonid and parasitic copepod systems. I also examined the factors affecting the apparent survival of hosts during the mark-recapture period using Cormack Jolly Seber (CJS) model. I found that parasitic copepods reduced host conditions and hosts with poor conditions were likely to be infected, suggesting that positive feedback can occur in the wild. Importantly, both body condition and parasite abundance significantly affect for host survival, suggesting that positive feedback reduce host survival in the wild. My findings provide robust evidence showing host condition–parasite infection dynamics, offering novel insights into how positive feedback could undermine the wild host population via reduction of host survival.

1. Introduction

Parasites account for more than one-third of species on Earth and a great deal of biomass in ecosystems (Lafferty et al. 2006; Dobson et al. 2008; Kuris et al. 2008); hence, host–parasite relationships are one of the most common biotic associations in nature (Hudson et al. 2006; Dobson et al. 2008; Kuris et al. 2008). Parasites damage host health via directly exploiting resources from hosts or indirectly causing physiological burdens (Poulin 2011; Sheldon & Verhulst 1996) and can be major drivers of host evolutionary changes (Paterson et al. 2010) and host population dynamics (Hudson et al. 1998; Poulin 2011). Parasite infections also occur as a consequence of poor host health (Lochmiller 1996; Pederson & Greives 2008; Beldomenico et al. 2008). Given that gaining or maintaining immunity is nutritionally costly for hosts (Lochmiller 1996; Sheldon & Verhulst 1996), host individuals without enough available food resources can be easily predisposed to higher parasite loads (Tadiri et al. 2013; Forbes et al. 2016). These opportunistic infections in epidemiology may cause parasite outbreaks and finally crush wild populations (Lochmiller 1996). Therefore, parasite infections and host health synergistically affect wild host and parasite dynamics.

Elucidating the synergy of parasite infections and host body conditions in the wild can advance our understanding of wild host population dynamics. However, most field studies have only examined cross-sectional correlations and have discussed one-sided causalities (Beldomenico et al. 2008). Field studies suggest a negative correlation between host body condition and infection parameters generally indicate that parasites are causes of poor host condition (Harper et al. 1999; Vicente et al. 2004; Sala-Bozano et al. 2012; Hasegawa et al. 2022a), although some showed poor condition situations such as food limitations may increase parasite prevalence and intensity because hosts compromise their immune functions under such situations (Forbes et al. 2016).

Most importantly, when parasite infections are both the cause and consequence of a poor host condition, I can also expect positive feedback: an infected host with a poor body condition due to the parasite infection will be more susceptible to further infection (Beldomenico et al. 2008; Beldomenico et al. 2009 a, b; Beldomenico & Begon 2010). This positive feedback is particularly important for understanding wild host population dynamics because this may create heavily infected hosts, which have low survival rate and eventually undermine the host population (Beldomenico & Begon 2010). Heavily infected hosts could be "super spreaders" among the populations (Beldomenico & Begon 2010), and thus this concept can also be important for parasites including disease dynamics (Beldomenico & Begon 2010).

Both causalities and positive feedback are likely to occur but have rarely been demonstrated in natural populations. This is probably because tracking small and cryptic parasite infections is usually difficult without sacrificing host individuals, although longitudinal studies are one of the best ways to estimate the causalities in natural systems (Beldomenico et al. 2008; Telfer et al. 2010). Only a few studies have overcome these problems and specifically tested their causalities in wild conditions. A series of studies by Beldomenico et al. (2008, 2009a, 2009b) successfully detected parasite infections on field voles *Microtus agrestis* in the field using a haematological method, and they monitored the infection status and host body condition combined with mark-recapture analysis of the host, clearly demonstrating positive feedback. A haemogram can be a useful indicator of infection; however, the authors did not observe parasites directly in the blood, and specific changes in infection intensity were not clarified. Blanchet et al. (2009a) also demonstrated the causal relationships between parasite infections and host growth rates by estimating the growth of two host fishes from scales and otoliths, although these methods have potential estimation errors (e.g. Neilson 1992), and the duration, frequency, and intensity of parasites before sampling

were unknown. Thus, previous findings have not sufficiently demonstrated the existence of condition–infection causality and positive feedback, necessitating more rigorous empirical evidence. Moreover, these previous studies failed to evaluate host survival rate, even though positive feedback could likely cause host death in natural populations and, hence, affect wild host population dynamics (Beldomenico & Begon 2010). Consequently, evidence for how positive feedback drive host population dynamics in the wild have never been available.

Here, I provide the first rigorous evidence of both causalities and positive feedback in wild populations by using a mark-recapture survey combined with structural equation modelling (SEM) in a wild stream fish–parasitic copepod system. SEM analysis is a powerful method for estimating the causalities in longitudinal datasets because of its simplicity and robustness (Fan et al. 2016). In fact, several studies have applied this approach to longitudinal studies and have revealed complex natural interactions (Almaraz 2005; Byrnes et al. 2011). My focused ectoparasitic copepod, *Salmincola markewitschi*, is ideal for examining the causality between host body condition and parasite abundance because of their relatively large body size (2–5 mm; Kabata 1969) and characteristic of attaching to the mouth cavities of host salmonid, white-spotted charr (Kabata 1969), enabling me to track the change in infection intensity and host body condition longitudinally without sacrificing host fish. Further, previous studies have suggested that *Salmincola* spp. have negative impacts on host fitness components under rearing conditions, such as decline of fecundity (Gall et al. 1972), appetite (Nagasawa et al. 1994; Hiramatsu et al. 2001), and body condition (Nagasawa et al. 1998). My previous studies also showed clear negative correlations between *S. markewitschi* loads and fish conditions in natural streams (Figure 1; Hasegawa & Koizumi 2024), but I still do not know the causalities hidden under these correlations. I conducted a mark-recapture survey of white-spotted charr *Salvelinus*

leucomaenis and *S. markewitschi* infecting the host mouth cavity in the Shiodomari River in southern Hokkaido. I also evaluated the apparent survival rate of host fish during the mark-recapture period to assess whether positive feedback reduces host survival in the wild.

2. Materials and Methods

2.1. Study species

White-spotted charr (Figure 1) is a common salmonid fish inhabiting mountain streams in the Japanese archipelago (Hosoya 2013). Like many other salmonids, they have two types of life history in Hokkaido Island: some individuals remain and reproduce in their natal river throughout their lives (i.e. stream residents), whereas others migrate to the sea or lakes and later come back to the natal rivers for reproduction (i.e. migrants) (Morita 2001; Morita et al. 2009). Above natural waterfalls or man-made dams, most individuals mature as residents (Morita et al. 2009). White-spotted charr are assumed to live up to 10 years in the wild condition (Morita & Morita 2007).

In my study systems, white-spotted charr have frequent infections by parasitic copepods in their mouth cavities (Hasegawa & Koizumi 2021). These copepods were identified as *Salmincola markewitschi* based on morphological observations and molecular analysis (Figure 1; Hasegawa et al. 2022b; Shedko et al. 2023). Although no detailed information about the life-history of the target species is available, their relative species such as *S. californiensis* and *S. edwardsii* have direct life cycles with seven separate stages; nauplius, free-living copepodid (less than 1mm; Kabata 1969), chalimus 1–4 and mature adults (Kabata & Cousens 1973; Stankowska-Radziun & Radziun 1993; Conley & Cutis 1994; Murphy et al. 2020). Infectious free-living copepodids live up to a few weeks (Kabata & Cousens 1973; Conley & Cutis 1994). During this short period, they find and attach to suitable hosts using shock waves and shadows produced by hosts

as cues (Poulin et al. 1991). After the attachments, adult females infect to hosts at least a few months, and longevity of adult females were estimated longer than a few months (Kabata & Cousens 1973; Murphy et al. 2020). Because all male copepods are dwarf form attaching to female's body (Kabata & Cousens 1973), I only counted females.

2.2. Study area

Mark-recapture surveys were conducted at the headwater tributary of the Ito River, Shiodomari River system, southern Hokkaido, Japan. The study reach was located between two waterfalls (both are about 2 m high). Since the waterfall prevents the upstream migration of most, but not all, sea-run migrants (Hasegawa unpublished data), the population was mainly composed of residents. Nonetheless, migrants can be easily distinguished from stream residents in the field due to is silvery colour, large body size and large white spots on the body surface (Ishigaki 1984). Since migrants only appeared after October in this tributary (Hasegawa unpublished data) and they have extremely high infection levels compared to residents (Hasegawa & Koizumi 2021), I only captured and marked residents.

The study reach was 536 m, and it was divided into 22 sections (i.e. 25 m section × 21 + 11 m section × 1). The water temperature was measured hourly with a HOBO data logger (Onset Computer Corporation, Bourne, MA) from 2 June 2020 to 7 July 2021, and the average water temperature in the study reach was 8.4 °C (min 0.0 °C–max 18.9 °C). No other fish species were observed in the study reach, apart from white-spotted charr (Hasegawa unpublished data).

2.3. Mark-recapture survey

During the study period of 2 and 6 June 2020 (hereafter the period called "June 2020"), I captured white-spotted charr by two-pass electrofishing using a backpack Electrofisher unit (300 V DC, Model 12-B, Smith-Root Inc., Vancouver, WA, USA) and a dip net (2-mm mesh) at each section to estimate the charr abundance by the removal method (e.g. Riley & Fausch 1992). Block nets were set at the start and end points of each section to prevent fish from entering or leaving during electrofishing. Captured fish were anesthetised using FA100 (DS Pharma Animal Health Co., Ltd.), and body length (fork length; FL) and body weight (BW) were measured to the nearest 1 mm and 0.1 g, respectively. I checked the presence and number of copepods by observing the fins, body surface, and mouth cavity of each fish. Fluorescent elastomer tags (North-west Marine Technology Inc., Shaw Island, WA, U.S.A.) were injected with a unique combination of six colours at four landmarks in each fish (four landmarks at the head and posterior points to each eye). Photographs of all individuals were taken on the left side with a digital camera (TG4, Olympus, Tokyo, Japan) to double-check the individual identification based on the variation of the white-spot pattern (Watz et al. 2019). After fish recovery, I gently released the fish into the middle of each section from which they were captured. As age-0 fish were rarely infected by the copepod in previous studies (Hasegawa & Koizumi 2021), I only captured and marked age-1 and older fish individuals.

Recapture sessions were conducted three times, given the parasite's life history described above (see 2.1) and due to some logistical seasons: July 2020 (4–9 July 2020), October 2020 (31 September–3 October 2020), and July 2021 (5–9 July). Fish were recaptured and treated in the same manner as the marking session (i.e. June 2020), except for the July 2021 survey, in which I captured marked fish by angling and

two-pass electrofishing (Hasegawa & Koizumi 2023). For individuals with partly fading elastomer colours or exhibiting body length shrinkage, I confirmed and identified them by checking the photographs. Given the relatively long interval between October 2020 and July 2021 (i.e. 9 months), I did not use the data of July 2021 in the estimation of causality by SEM; however, they were used for the survival rate estimation (see Section 2.4.3). All capture histories are represented in Figure 2.

2.4. Statistical analyses

2.4.1. Calculating variables

The infection level (prevalence, intensity, and mean intensity) was calculated following Bush et al.'s (1997) method. The estimated charr abundance in each section was calculated using the removal method implemented in the program CAPTURE (White et al. 1978; available at http://www.mbr-pwrc.usgs.gov/software/index.html). The host density in each section was calculated from estimated charr abundance, and each section area (m^2) calculated from mean stream width and section length (i.e. 25 m or 11 m). For the accurate evaluation of the host body condition, I used the residual index (Jakob et al. 1996): I calculated residual distances of individual points from the regression of ln (BW) with ln (FL). This body condition index is widely used in fish-parasite system (Bagamian et al. 2004; Lagrue & Poulin 2015; Perrot-Minnot et al. 2020). As fish body condition is assumed to be different among seasons in salmonids (e.g. Morita et al. 2011), I calculated residuals in each capture session (i.e. June 2020, July 2020, October 2020, and July 2021). I also calculated the growth rate (mm / day) for each host individual for each capture-recapture interval.

2.4.2. Structural Equation Modelling

All statistical analyses were performed using R 4.1.2 (R Core Team 2021). To estimate the causalities among host body conditions, parasite infections, and other possible factors, I used piecewise structural equation modelling using package "*piecewise SEM*" version 2.1.2 (Lefcheck 2016) based on the hypothetical scheme shown in Figure 3. Piecewise SEM allowed me to test the effects of parasite abundance and host body conditions on several parameters in subsequent months simultaneously and to use mixed effects. The whole model is composed of several generalised linear (mixed) models (Shipley 2009; Lefcheck 2016). The goodness of fit of the whole model was evaluated by Shipley's test of direct separation using Fisher's C value (Shipley 2009; Lefcheck 2016). If that value did not fall below a significant level ($p < 0.05$), the model was fitted and explained my datasets well. Since unexpected correlations, such as temporal correlations between parasite abundance and body conditions among months (Figure 3), severely reduced the model fitting due to collinearity, I treated these correlations as correlated errors (Shipley 2009). All linear mixed models in piecewise SEM were constructed using the R package "*lme4*" version 1.1 (Bates et al. 2015), and all responses and explanatory variables were standardised before the analysis to ensure the normality. I analysed my datasets for each of two separate seasons (i.e. from June 2020 to July 2020 and from July 2020 to Oct. 2020).

Based on the hypothetical schema (Figure 3), I constructed three linear mixed models. I expected that parasite abundance in the pre month decrease host body condition in the post month (i.e. parasite are a cause of poor condition; Figure 3). Fish with higher growth rates may show higher body conditions in the post month, and high host density also decrease host body condition in the post month (Figure 3). Therefore, in the first model, the response variable is host body condition in the post month, and

explanatory variables are parasite abundance, host density and host growth rate in the pre month. Parasite infections are likely to occur due to poor body condition (i.e. parasites are a consequence of poor condition Figure 3). Parasite abundance could also be affected by fish body size (FL) and host density in the pre month; larger fish may be susceptible to infections due to their large body surface areas (e.g. Poulin et al. 1991) and higher host density can contribute to parasite transmissions (Anderson & May 1982). Thus, in the second model, the response variable is parasite abundance in the post month, and the explanatory variables are body condition, host body size and host density in the pre month (Figure 3). Host body size (FL) and body condition in the pre month can affect host growth rate (Gabelhouse 1991; Morita 2001; Figure 3). Therefore, I constructed another model that included growth rate as the response variable, with its explanatory variables being body condition and body size in the pre month. The study sections and fish individual ID were included as random effects in all constructed models.

2.4.3. Cormack–Jolly–Seber model

Survival probability of white-spotted charr between sampling occasions was estimated by Cormack-Jolly-Seber (CJS) models (Lebreton et al. 1992) using the Bayesian hierarchical approach (Kéry & Schaub 2012). I assumed that individual i survived from occasion t to occasion t+1 with a survival probability that differed by occasion and individual:

$$z_{i,t+1} | \, z_{i,t} \sim Bernoulli \, (z_{i,t} \, \phi_{i,t})$$

$$logit(\phi_{i,t}) = \alpha 0_t + \alpha 1_t * ForkLength_{i,t} + \alpha 2 * BodyCondition_{i,t} + \alpha 3 * \ Parasite \ abundance_{i,t},$$

The latent state variable was binary, where $z_{i,t} = 1$ if individual *i* was alive on occasion *t*, and 0 if dead. I modeled individual- and interval-specific survival probability, $\phi_{i,t}$, as a function of fork length, body condition, and parasite abundance of individual *i* on occasion *t*. The covariates were standardized by mean divided by standard deviation on each occasion, so that the intercept, $\alpha 0_t$, was the predicted survival probability of an individual with average values of these covariates on occasion *t* on the logit scale. Missing values of the covariates were not allowed in this model, although data could not be collected on sampling occasions when individuals could not be collected. Missing fork length values were imputed by developing a simple linear regression model using fork length values of the individuals captured between two consecutive sampling occasions. Missing values of body condition and parasite abundance were imputed with their mean values on each occasion (i.e., 0) because strong predictive relationships between the two consecutive occasions did not exist for the two covariates. I let the fork length effect on survival to vary by occasion ($\alpha 1t$) but the effects of body condition and parasite abundance to be time constant ($\alpha 2$ and $\alpha 3$) because their posterior distributions overlapped greatly among sampling occasions (Appendix 1). Three individuals suffered handling mortality on the second occasion (July 2020) and two additional individuals on the third occasion (October 2020), and these individuals were excluded from survival estimation after their known mortality events.

Because electrofishing and angling cannot capture all individuals present in the study area, I modeled capture probability ($p_{i,t}$) of individual *i* on occasion *t* using fork length as a covariate:

$$y_{i,t} | \, z_{i,t} \sim Bernoulli \, (z_{i,t} \, p_{i,t})$$

$$logit \, (p_{i,t}) = \beta 0_t + \beta 1 * ForkLength_{i,t}$$

where $y_{i,t}$ is the capture-history data (1 if captured, 0 if not) of individual i on occasion t, $\beta 0_t$ is an occasion-specific intercept, and $\beta 1$ is a time-constant effect of fork length on capture probability because their posterior distributions overlapped greatly among sampling occasions in a different model with time-varying effects of fork length (Appendix 1). Fork length was standardized by mean, so that $\beta 0_t$ is the capture probability of average-sized individuals on occasion t on the logit scale. Parameters of survival and recapture probabilities were modeled as fixed effects, and survival and capture probabilities cannot be independently estimated in the last sampling interval in the CJS model (Kéry & Schaub 2012). Because additional information on capture probabilities was available from two-pass removal electrofishing on each sampling occasion, I used it to constrain the capture probability of white-spotted charr on the last sampling occasion (July 2021). Specifically, I assumed that the capture probability was similar between the two preceding occasions (July and October 2020; $\beta 0_1 = \beta 0_2$), when only electrofishing was conducted. I further assumed that capture probability was higher in July 2021, when angling occurred prior to electrofishing ($\beta 0_3 > \beta 0_1 = \beta 0_2$). I estimated capture probabilities of white-spotted charr using a removal method (Zippin 1958) and found that the capture probability was approximately 7% higher in July 2021 relative to July and October 2020 ($\beta 0_3 = \beta 0_1 \times 1.07$). I incorporated this information in the CJS model to estimate the survival and capture probabilities individually in the last sampling interval.

I fit CJS models using a Markov chain Monte Carlo (MCMC) method in Program JAGS (Plummer 2017) called from R Program (R Core Team 2023) with the jagsUI package. Diffuse priors were used throughout in the Bayesian approach. Posterior distributions of model parameters were characterized by taking every 10th sample from 20,000 iterations of four chains after a burn-in period of 10,000 iterations. Model convergence was assumed to have converged when the R-hat statistic was < 1.1

for all model parameters (Gelman & Hill 2007). I report 95% credible intervals (CRI) and the proportion of posterior samples with the same sign as the posterior mean (i.e., f value in JAGS output) and use both metrics to evaluate covariate effects on survival and capture probabilities.

3. Results

The total marked and recaptured fish, infection parameters, and infection patterns of *Salmincola markewitschi* during the mark-recapture session are summarised in Figure 2 and Table 1.

From June 2020 to July 2020, the hypothesised model constructed by piecewise SEM fitted my datasets well (Fisher's C = 4.80, p = 0.09; Figure 4a). Both body condition and parasite abundance in the previous month negatively correlated with parasite abundance and body condition in the next month, respectively (Figure 4a). Host body size also had significant positive effects on parasite abundance (Figure 4a). There were no significant effects of host density on both body condition and parasite abundance (Figure 4a). Fish with higher body conditions exhibited higher growth rates, and there was significant negative correlation between growth rates and body size (Figure 4a).

From July 2020 to October 2020, the model marginally fitted my datasets (Fisher's C = 6.11, p = 0.05; Figure 4b). Individual fish that exhibited poor conditions were likely to gain further parasite infections in the post month, but opposite causality was not detected (Figure 4b). As the previous period, host body size had significant positive effects on parasite abundance (Figure 4b). There was negative correlation between the growth rate and body size in the previous month, and positive correlation between host body condition (Figure 4b). Fish that showed higher growth rates had

higher body conditions in the next month (Figure 4b). Host density did not have significant effects on other variables (Figure 4b).

Survival probability of white-spotted charr changed over time to align with sampling interval lengths. Posterior mean probability of survival was 0.91 (95% CRI: 0.85, 0.97) from June to July 2020, 0.81 (0.74, 0.88) from July to October 2020, and 0.46 (0.39, 0.53) from October 2020 to July 2021. Mean capture probability was estimated to be 0.73 (0.69, 0.78) in July and October 2020 when only electrofishing was conducted, and 0.79 (0.74, 0.83) when both angling and electrofishing were used.

Survival differed among individuals based on their covariates (Figure 5). Larger charr were more likely to survive than smaller charr from June to July 2020 (Figure 5; $\alpha 1_1 = 1.55$ [95% CRI: 0.75, 2.63, and $f = 100\%$]), but body size effect was much weaker from July to October 2020 (Figure 5; $\alpha 1_2 = -0.27$ [95% CRI: -0.68, 0.11, and $f = 91\%$]) and from October 2020 to July 2021 (Figure 5; $\alpha 1_3 = 0.13$ [95% CRI: -0.15, 0.42, and $f = 83\%$]). Individuals with better body condition were more likely to survive (Figure 5; $\alpha 2 = 0.21$; $f = 97\%$), although their 95% CRI just overlapped with 0 (0, 0.44). Survival decreased with higher parasite abundance (Figure 5; $\alpha 3 = -0.24$ [95% CRI: -0.46, -0.02, and $f = 98\%$]). Larger charr were more catchable than smaller charr (Figure 5; $\beta 1 = 0.28$ [95% CRI: 0.05, 0.51, and $f = 99\%$]).

4. Discussion

Although many studies have pointed out the negative impacts of parasites on wild host populations, most reported simple correlations and hence overlooked causal relationships (Harper et al. 1999; Vicente et al. 2004; Sala-Bozano et al. 2012; Hasegawa et al. 2022a). This is probably due to methodological difficulties in the long-term tracking of host individual and parasite infections. Only two studies have explicitly tested the causality using unique methods, such as haematological inspection

and otolith/scale back-calculation (Beldomenico et al. 2008, 2009 a, b; Blanchet et al. 2009a). My study, by contrast, directly monitored the changes in infection intensity, host body condition, growth rate, and survival by the mark-recapture method, and therefore, serves more rigorous evidence of more probable causal relationships and positive feedback in wild populations.

Strikingly, my study showed that both causalities (i.e. parasite reduced host condition and poor host condition increased parasite infections) were possible in wild populations, suggesting that positive feedback could occur in wild conditions; parasite infections reduced host conditions, and reduced conditions caused further parasite infections, and so on. The body condition index, including the index I used calculated from body weight-length relationships, generally represents the host's overall health status, energy budget, and immune functions (Wilder et al. 2016; Sánchez et al. 2018). Although hosts commonly cope with parasite infections using innate and adaptive immune systems (Graham et al. 2010, 2011; Fast 2014), developing and maintaining these systems are very costly (Lochmiller 1996; Sheldon & Verhulst 1996), and therefore hosts with poor conditions, mainly due to parasite infections, cannot allocate their resources to immunity, resulting in higher parasite intensity. My study suggests this trend.

Behavioural differences dependent on host body condition also explain positive feedback. Animals often show anti-parasite tactics such as dispersal from infection sources (Brown et al. 2016; Terui et al. 2017) and "parasite-removing behaviours" such as substrate rubbing (Kabata & Cousens 1977; Atkinson et al. 2018). However, these behaviours are commonly considered energy dependent (Krohn & Boisclair 1994; Bonte et al. 2012; Terui et al. 2017); thus, hosts with poor conditions cannot employ these tactics.

How did the copepods in my study cause positive feedback? *Salmincola* spp. cause tissue damage, such as gill destructions and mouth cavity swellings (Kabata & Cousens 1977; Nagasawa et al. 1998; Hasegawa et al. 2022a). These infections also induce the immune response of hosts (Hiramatsu et al. 2001). Beyond developing immune systems, repairing damaged tissues also requires much energy (White et al. 2020), eventually leading to loss of host body condition (Hasegawa et al. 2022a). Physical attachment itself could induce body condition loss. In particular, since my focused copepods mainly attach to the mouth cavity, their infections reduce host foraging activity and strongly reduce host body conditions (Nagasawa et al. 1994). Further, intraspecific competition may play an important role in susceptibility to infections. Poor condition fish are commonly outcompeted by other conspecifics in intra-specific competitions, especially among salmonids with a strong dominance hierarchy (Nakano 1995). Whereas fish with a high hierarchy dominate at the centre of the flow (Nakano 1995), outcompeted fish may be forced to move outside of the flow, where free-swimming copepodids may easily attach to the hosts under such low-flow environments (Monzyk et al. 2015). Under these mechanisms, positive feedback can easily occur, as demonstrated in my system.

Most importantly, I found that both body condition and parasite abundance significantly predicted host apparent survival rates, suggesting that positive feedback could ultimately undermine the host population through the reduction of host survival (Beldomenico & Begon 2010). Therefore, to the best of my knowledge, my results first supported the Beldomenico & Begon (2010)'s hypothesis that positive feedback drive wild host populations. Given that body condition is continuously reduced as positive feedback occurs, host body condition eventually fails to meet the threshold for maintaining critical physiological and physical functions such as metabolism. Further, heavily infected hosts, generally in poor condition, are likely to be preyed by predators

(Temple 1987) and outcompeted by conspecifics (Barber et al. 2000; Filipsson et al. 2018). These biological interactions indirectly reduce the host survival rate.

Finally, positive feedback should be carefully taken into account when considering host–parasite dynamics because this concept may also work at the population level (Beldomenico & Begon 2010). Beldomenico & Begon (2010) predicted that populations with a large proportion of individuals in poor conditions are likely to have a higher prevalence and infection intensity, and this also increases the risk of further infections. Since average body condition and immune ability vary among populations (Cornet et al. 2009; Becker et al. 2020), such predictions are likely to occur in natural systems. Further, positive feedback may eventually cause host death, as discussed above, so this may affect host population dynamics. In this context, the southern salmonid populations, as in the present case, will be threatened by positive feedback. Such populations will especially be vulnerable to increasing water temperature induced by climate change (Nakano et al. 1996) because temperature increment would be stressful for cold water-adopted salmonids and would ultimately decrease their body condition (Peterson et al. 1979; Larsson 2005). Under such a scenario, the proportion of fish individuals with poor conditions will increase, and parasites will expand more rapidly there. More case studies and monitoring are needed to verify this prediction.

Figure 1. (A) *Salmincola markewitschi* (arrowhead) infecting the mouth cavity of white-spotted charr *Salvelinus leucomaenis*. (B) White-spotted charr not infected by copepods. (C) White-spotted charr infected by several copepods.

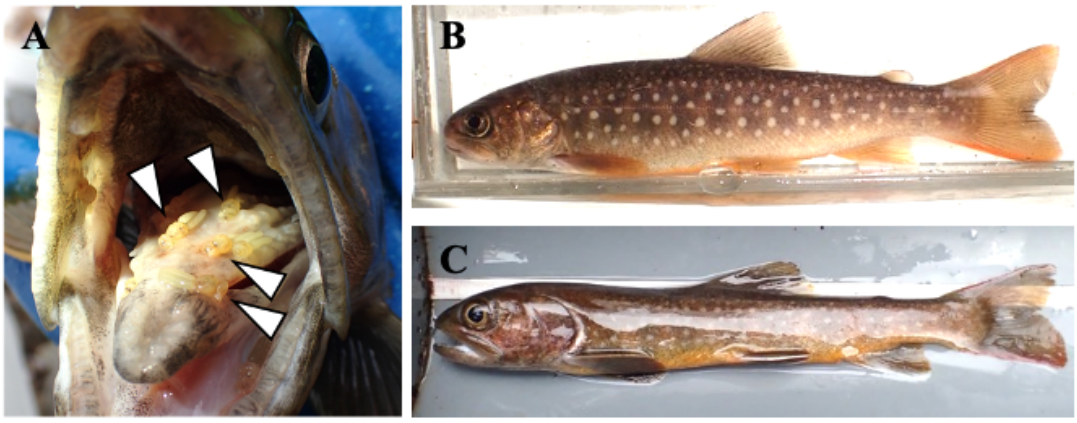


Figure 2. Summary of mark-recapture survey conducted from June 2020 until July 2021.

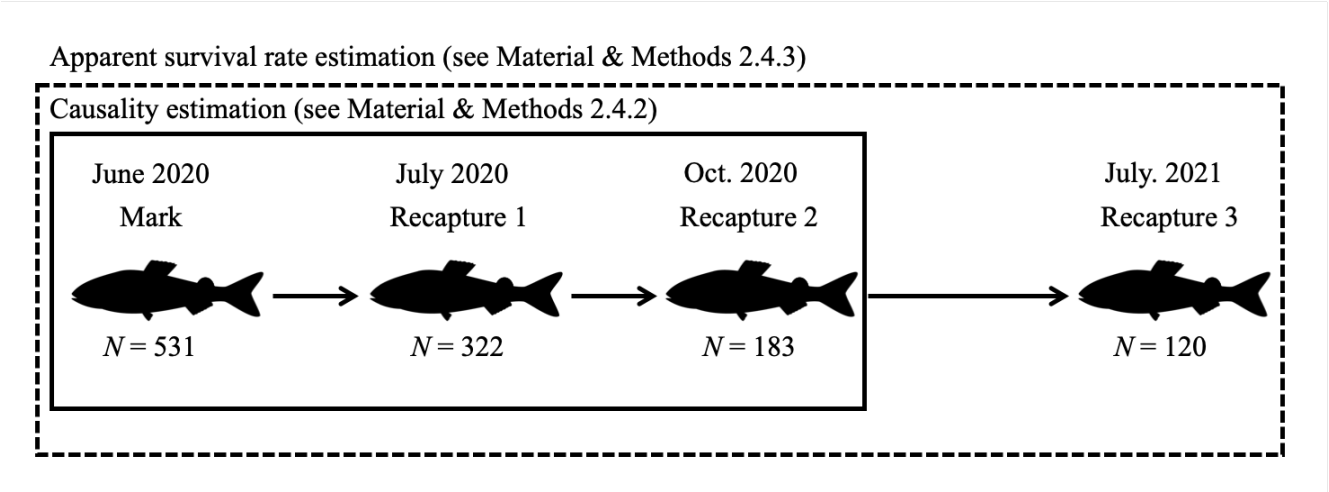


Figure 3. Hypothetical causal relationships among host body condition, parasite infections, and other potentially related factors of both host and parasite.

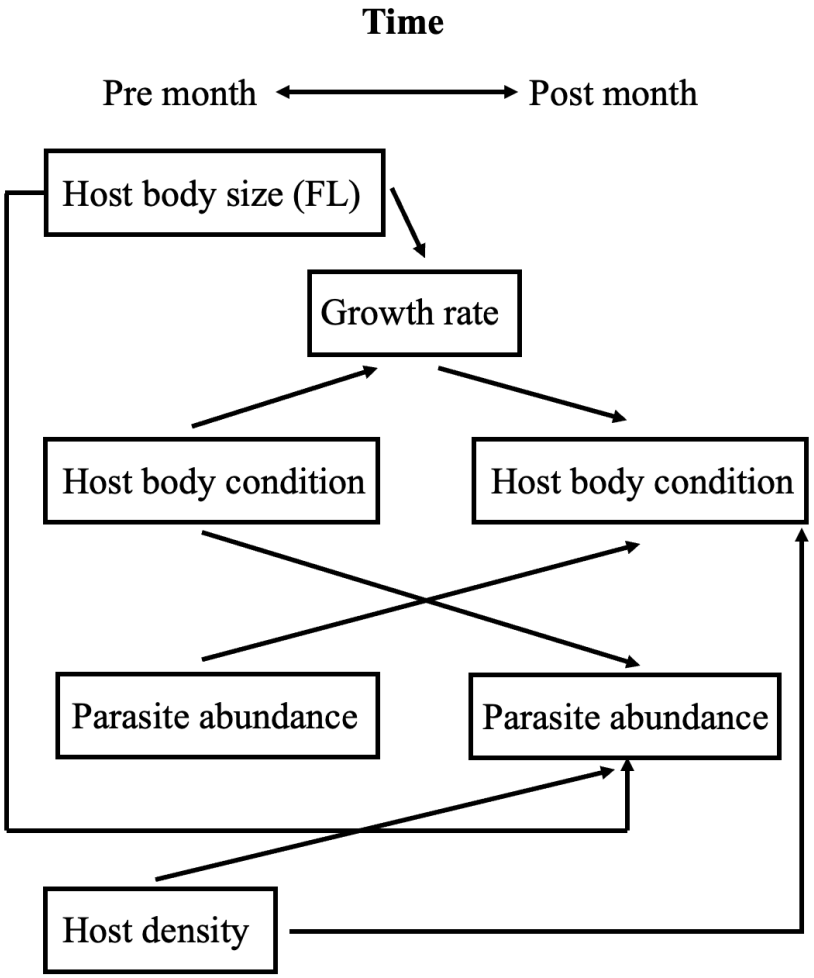
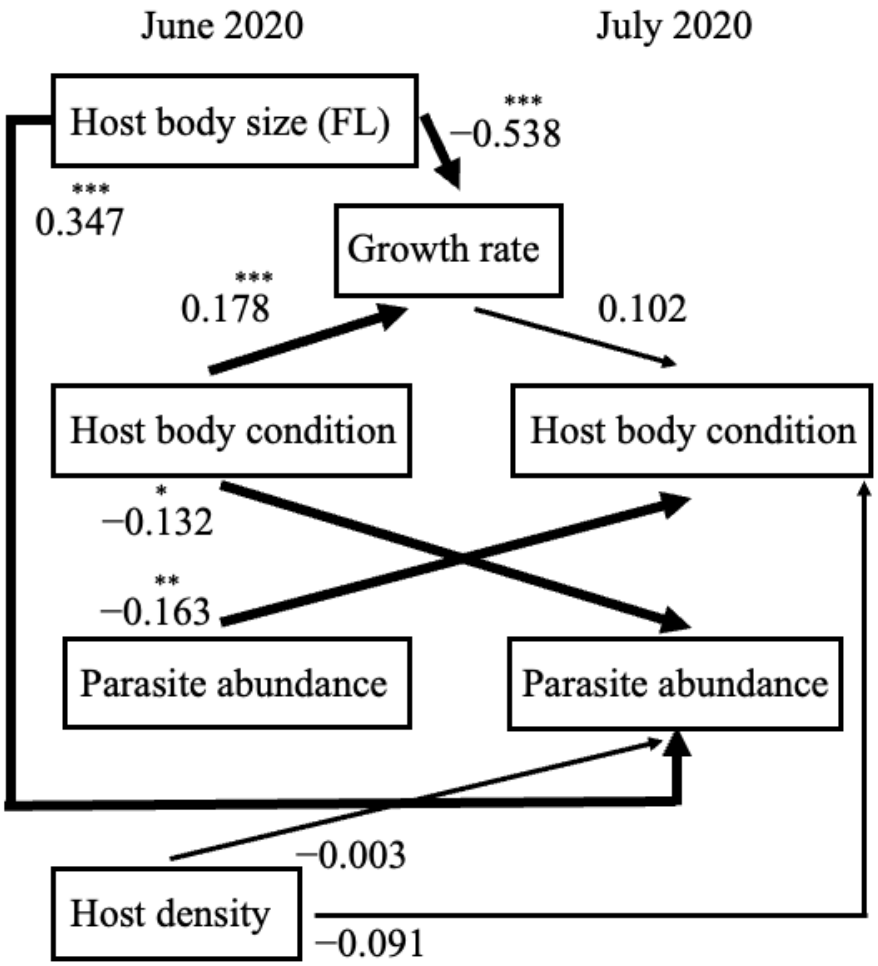


Figure 4. Causal relationships among factors of hosts and parasites inferred by piecewise SEM analysis. (a) Results from June 2020 to July 2020. (b) Results from July 2020 to October 2020. Bold arrows indicate statistical significant effects (***$p < 0.001$; ** $p < 0.01$; * $p < 0.05$).

(a)



(b)

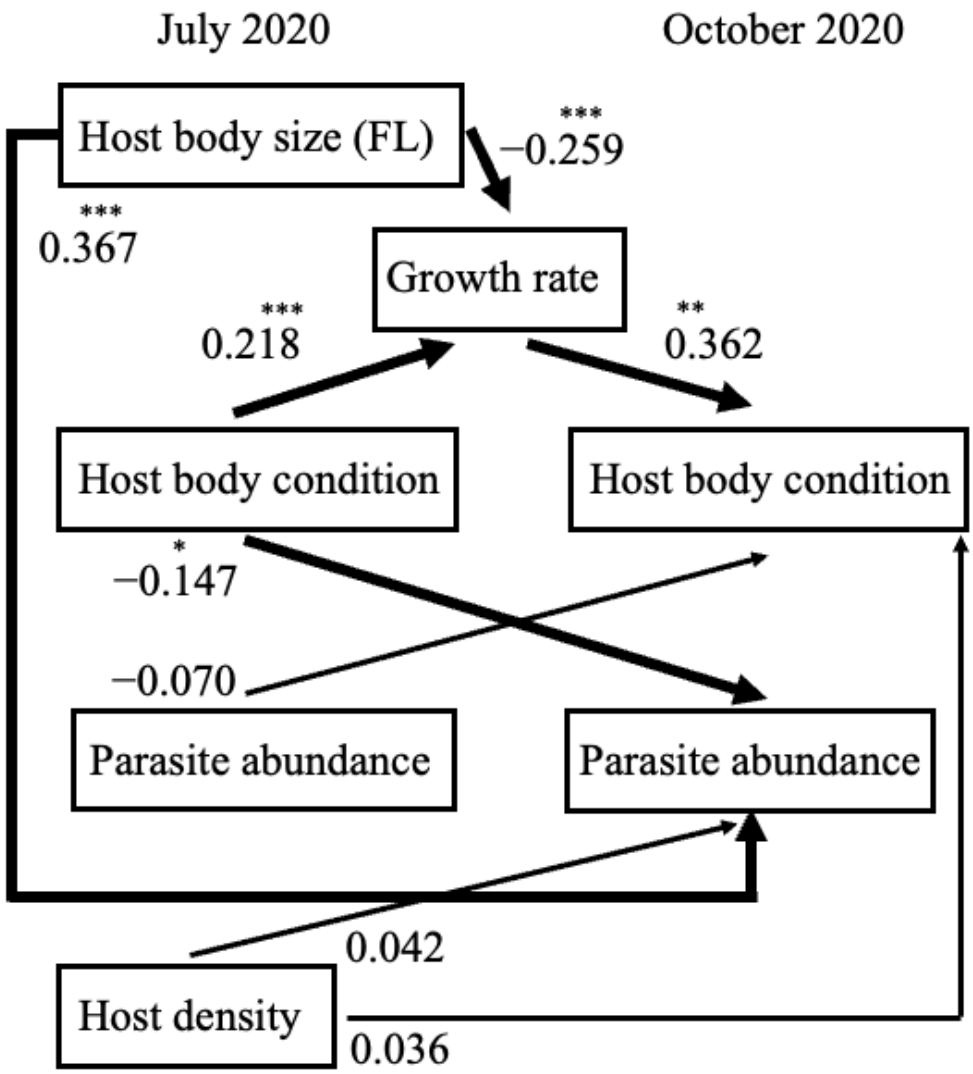

July 2020
October 2020
Host body size (FL)
***
−0.259
***
0.367
Growth rate
***
0.218
**
0.362
Host body condition
Host body condition
*
−0.147
−0.070
Parasite abundance
Parasite abundance
0.042
Host density
0.036

Figure 5. Covariate effects on survival and capture probability of white-spotted charr in the Cormack-Jolly-Seber model. Posterior mean values are shown by dots with 50% (thick lines) and 95% (thin lines) credible intervals. A vertical dotted line is drawn at 0 (i.e., no effect).

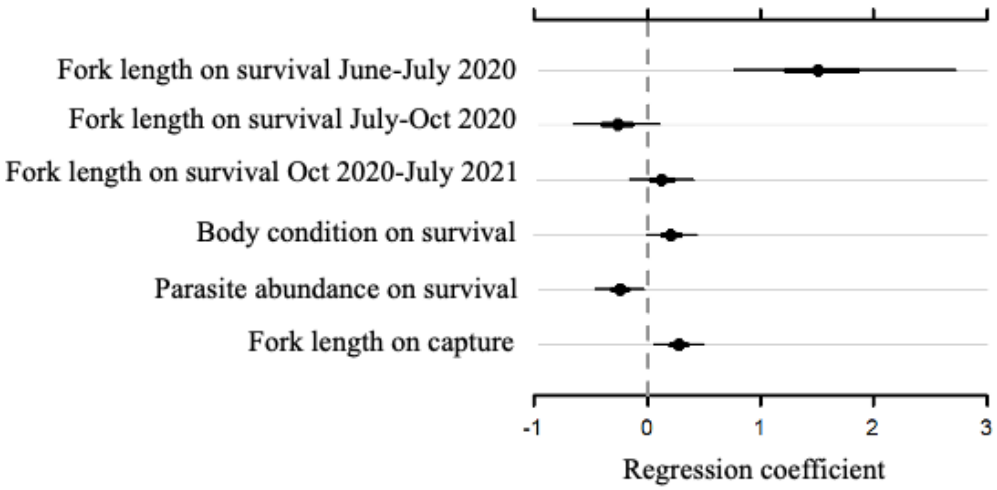


Table 1. Summary of host abundance, infection parameters, and infection history at each capture-recapture event. Note that prevalence and mean intensity were calculated from marked fish.

	June 2020 (Mark)	July 2020 (Recapture 1)	Oct. 2020 (Recapture 2)	July 2021 (Recapture 3)
Total host numbers (recapture rate)	531	322 (60.6 %)	183 (34.5 %)	120 (22.6 %)
Prevalence (%)	35.8%	38.2%	31.1%	35.8%
Intensity (mean)	1-9 (1.59)	1-11 (1.67)	1-6 (1.47)	1-6 (1.49)
Fish gained new parasite infections (%)	*NA*	41 (12.7 %)	31 (16.9 %)	22 (18.3 %)
Fish experience parasite detachments (%)	*NA*	59 (18.3 %)	45 (24.6 %)	15 (12.5 %)

Supplementary file 1. An additional Cormack-Jolly-Seber model.

In addition to the Cormack-Jolly-Seber model presented in the main text, I fit a model in which all individual covariates on survival and capture probabilities were time-varying. Following the notation in the main text, this additional model can be shown below, and it differs because all covariates ($\alpha 1t$, $\alpha 2t$, $\alpha 3t$, and $\beta 1_t$) were assumed to have time-varying effects:

・Survival

$$z_{i,t+1} | \, z_{i,t} \sim Bernoulli\,(z_{i,t}\,\phi_{i,t})$$

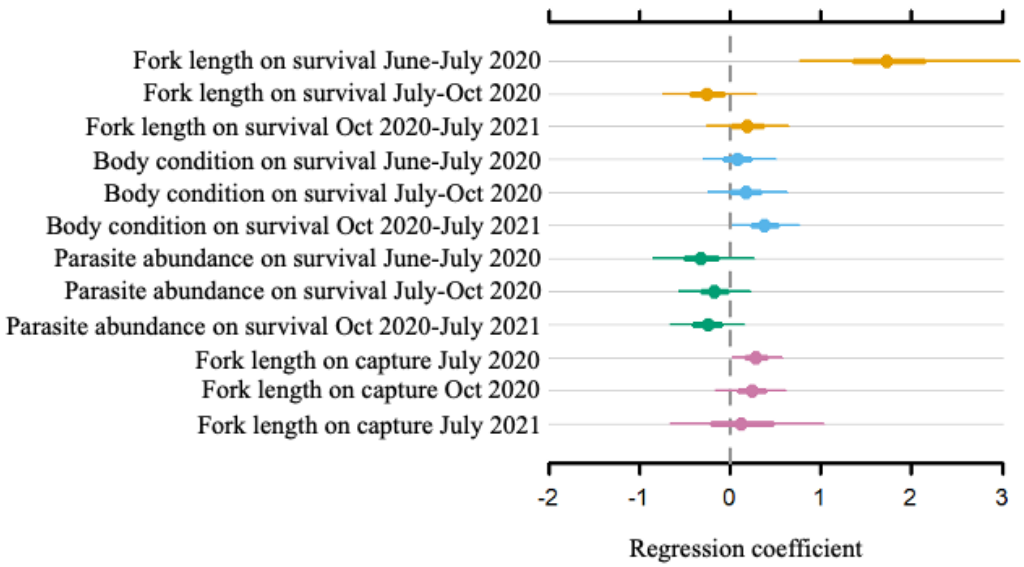
$$logit(\phi_{i,t}) = \alpha 0_t + \alpha 1_t * ForkLength_{i,t} + \alpha 2_t * BodyCondition_{i,t} + \alpha 3_t * Parasiteabundance_{i,t}$$

・Capture

$$y_{i,t} | \, z_{i,t} \sim Bernoulli\,(z_{i,t}\,p_{i,t})$$

$$logit\,(p_{i,t}) = \beta 0_t + \beta 1_t * ForkLength_{i,t}$$

Covariate effects of this model are shown below. Posterior mean values are shown by dots with 50% (thick lines) and 95% (thin lines) credible intervals. A vertical dotted line is drawn at 0 (i.e., no effect). For each covariate, shown by different color, posterior distributions overlapped greatly among sampling intervals, except fork length effects on survival probability. Thus, I report in the main text the model where only this parameter was time-varying ($\alpha 1_t$) and the effects of other covariates did not vary by time ($\alpha 2$, $\alpha 3$, and $\beta 1$).


Fork length on survival June-July 2020
Fork length on survival July-Oct 2020
Fork length on survival Oct 2020-July 2021
Body condition on survival June-July 2020
Body condition on survival July-Oct 2020
Body condition on survival Oct 2020-July 2021
Parasite abundance on survival June-July 2020
Parasite abundance on survival July-Oct 2020
Parasite abundance on survival Oct 2020-July 2021
Fork length on capture July 2020
Fork length on capture Oct 2020
Fork length on capture July 2021
-2
-1
0
1
2
3
Regression coefficient

Chapter 7. General discussion

To date, many studies have examined parasite's negative impacts on host health status using host body condition (reviewed by Sanchez et al. 2012). However, most of these only examined correlations between host body condition and infection parameters during the specific periods and did not examine possible specific causalities and mechanisms of body condition reduction by parasites (chapter 1.2). Here, I intensively examined host body condition and infection relationships using *Salmincola* spp. and their salmonid systems. I found clear negative correlations between body condition and infections in two different study systems (chapters 2 and 3). Additionally in chapter 3, I found such negative correlations were consistent among all four examined seasons. In chapter 4 and 5, I examined how copepod infections reduce host condition, with a focus on changes in foraging behaviors of hosts induced by mouth-infecting copepods *S. markewitschi.* I found copepods reduce host vulnerability to angling, but only when host had poor body condition. Further, I found parasites affect host dietary compositions; infected fish with smaller body size showed low proportion of terrestrial invertebrates compared to uninfected counterparts. These results suggest that infected hosts changed foraging tactics dependent on body condition and body size. Finally in chapter 6, I examined two causalities underlying these negative correlations; parasites reduce host condition and poor body condition increase new infections. My mark-recapture study successfully detected both causalities, as well as decreased survival due to the positive feedback of reduced condition and further infection. Overall, my dissertation contributed to the understanding of complex causalities hidden under simple correlations and several mechanisms of body condition loss, which has been lacked in previous studies. I summarized all my findings as a schematic diagram in Figure 1. In

this section, I discuss these results in more detail and provide future perspectives that should be considered in this study area.

How parasites reduce host condition? other potential mechanisms

Although I predicted that parasites reduce host body condition via reduction of host foraging activities because these parasites apparently cover mouth cavities, I found similar levels of stomach fullness and total prey abundance among infection status (chapters 4 and 5). On the contrary, I found infected individuals changed dietary compositions but these foraging shifts were restricted within fish with poor body condition or small body size (chapters 4 and 5). Therefore, foraging behaviors alone do not fully account for the loss of host body condition, and it is essential to consider other potential mechanisms.

Increased foraging activities tying to compensate for their energy intake (chapters 4 and 5) might actually have reduced host body condition in infected hosts. While stomach fullness and total prey abundance did not significantly differ among infection status, small infected individuals shifted their main diets from terrestrial to aquatic invertebrates (chapters 4 and 5). I also found that stomach fullness positively correlated with body condition in both infection status (chapter 4). These results suggest that infected hosts may have increased foraging activities to compensate their energic loss (chapters 4 and 5). Such foraging compensation is generally known as “making the best of a bad job (Dawkins 2019)” for subordinate individuals and may be the benefits in the long run (Nakano 1995). However, under the parasitism, increased foraging activities comes with energetic costs, and it could potentially reduce host body condition, especially when the costs of increased feeding activities outweigh the benefits gained from resources through behavioral shifts. This can explain body condition decline in my

study systems. Increased foraging activity might be large costs because hosts may be required to move frequently within- and among habitats to gain resources. Despite such high energic costs, they could not gain sufficient energy to maintain body condition through foraging because these fish are forced to shift from large- high nutrient terrestrial invertebrates to small-low nutrient aquatic invertebrates. Therefore, the costs of increased activities outweigh the energetic benefits through obtaining by foraging, resulting in a further decline in their body condition.

This increased foraging activities may also contribute to the high mortality in infected hosts observed in chapter 6. Several studies have shown that increased activities could increase the risk of predation (Östlund-Nilsson et al. 2014). In my study case, there are several bird species and foxes that potentially prey on white-spotted charr. Additionally, I found that small white-spotted charr were preyed upon by larger white-spotted charr in this river system (i.e. cannibalism, Hasegawa unpublished data), suggesting that such predations could often occur in our system. Individuals with high activity may be also vulnerable to flooding events (Yamada & Wada 2021, 2023), which often result in fish mortality (Seegrist & Gard 1972; Weese et al. 2011). A specific behavioral observation including feeding activity is required to understand if and how these copepods induce host activity level and subsequent high mortality.

Another behavior that could contribute to the loss of body condition may be parasite removing behaviors (Atkinson et al. 2018; Thompson & Meeuwig 2022). Many animals including fish exhibit specific behaviors such as grooming, rubbing, and leaping, as potential mechanisms to remove parasites from their bodies (Villa et al. 2016; Kabata & Cousens 1977; Atkinson et al. 2018; Thompson & Meeuwig 2022). Such behaviors come with energetic costs (Krohn & Boisclair 1994, Costello 2006), and in the worst case, may cause additional physical damages to hosts (Kabata & Cousens 1977; Costello 2006). For instance, Kabata & Couses (1977) found that damages to the

fins of fish infected by copepods might be induced by rubbing behavior against solid objects, triggered by irritation from parasite infections. Such damages can also lead to secondary infections, resulting in additional costs for tissue repair and immune responses, ultimately leading to the loss of body condition (see discussion below). Further, these behaviors might often attract predators, potentially resulting in high mortality as well (Costello 2006).

Not only behavioral shifts but also physiological costs should be taken into accounts in this context. Host immune systems could be the major physiological aspects and may explain poor body condition of infected hosts. All vertebrates have both innate and adaptive (or acquired) immunity, and both requires a lot of energy in developments and maintenance (Sheldon & Verhulst 1996; Lochmiller 1996; Lochmiller & Deerenberg 2000). The endo-and ectoparasites are known to induce both types of immunity (Wikel et al. 1996; Yang & Foster 2005), and the latter adaptive immunity particularly incurs high cost for hosts because it is maintained at least a few months to few years after the developments (Treasurer et al. 2006; Fast 2014). In fact, many studies have shown that immunized hosts reduced body condition, growth, and reproduction due to the trade-off between immunity and other life-history traits (Knowles et al. 2009; Vijendravarma et al. 2009; Graham et al. 2010, 2011; van der Most et al. 2011). Moreover, adaptive immunity does not always provide benefits to hosts, but rather cause damages, known as autoimmune pathology (Sheldon & Verhulst 1996; Weber et al. 2022), which might lead loss of body condition. In my study case, adaptive immunity could explain poor body condition of infected hosts. Although only one study investigated the immunological aspects of *Salmincola*-salmonid systems (Hiramatsu et al. 2001), this clearly showed that fish infected by mouth-attaching *Salmincola* produced Immunoglobulin M, suggesting that infected hosts developed acquired immune system after experiencing parasite infections (Hiramatsu et al. 2001). I

also found that some individuals did not get any copepod infections during field surveys and many fish experienced detachment of copepods. This suggests that they might have gained adaptive immunity before and during the field survey period. As discussed in chapter 6, fish with poor body condition might not get adaptive immunity because they could not allocate enough resources into developing adaptive immunity.

Damaged tissue repairments, which are characterized as innate immunity, could also explain poor body condition of infected hosts because it needs much costs for hosts (Allen & Sutherland 2014; White et al. 2016; Binning et al. 2017). Many parasites are known to cause physical tissue damages through attaching, feeding, and host attacking processes (Rajput et al. 2006; Johnson & Hoverman 2012; Neal et al. 2021). Hosts try to cope with these damages using several costly tactics such as inflammation and creating fibrosis (Fuess et al. 2021). Further, these damages often lead secondary infections by bacteria and viruses (Kotob et al. 2017) that trigger different types of immunity working for parasites (Yazdanbakhsh et al. 2002; Ramsey & Rohr 2021), resulting in rendering additional physiological costs on hosts. In my study system, I frequently found that sites of attachments were heavily wounded and/or whitened, suggesting that *Salmincola* infections severely damaged host tissues, and potentially cause secondary bacterial infections as is suggested in other *Salmincola* spp. (Nagasawa et al. 1995; White et al. 2020).

Future infection experiments under laboratory condition are necessary to understand the specific mechanisms of reduced host condition. Such experiments allow me to monitor foraging behaviors directly and take blood samples for immunological and physiological surveys. As I described above, all vertebrates have immune and tissue repairing systems, and partly shares the same mechanisms such as cell types and genetic basis (Riera Romo et al. 2016). Therefore, I could identify the commonality and generality in my findings of immunological/physiological mechanisms through

laboratory experiments. Eco-immunology, a study field examining the costs and benefits of immunity in organisms, has recently gathered attentions in ecology and evolution (Sasser & Weber 2023), and developed techniques in these areas can be applicable to my systems.

How poor host body condition induce parasite infections?

Although I focused the behavioral mechanisms of how parasites reduce host body condition in my dissertation, I also found parasite infections occurred as a consequences of poor host condition (see chapter 6). In this section, I discussed other possible mechanisms that induce additional parasite infections (see chapter 6).

As discussed in chapter 6, the most plausible mechanism is lower resource allocation into immune systems; hosts with poor body condition could not allocate enough resources into host immunity (Sanchez et al. 2018). Among animal kingdom, body condition index well represents host overall health status and the amount of holding resources (Peig & Green 2009; Sanchez et al. 2018). In fact, many studies suggest that body condition index correlated with lipid contents (Sutton et al. 2000; Wilder et al. 2016; Warner et al. 2016) and several immune functions (Gleeson et al. 2005; Gilot-Fromont et al. 2012). Given these facts, immune systems of hosts with poor body condition do not work effectively, leading to higher susceptibility to parasites. Specifically, in my cases, hosts with poor body condition could not secrete mucus and produce antibodies such as immunoglobulin M, which plays pivotal roles in first and adaptive defense lines, respectively (Fast 2014).

Several behavioral traits could also explain high host susceptibility since animal's behavioral traits are well correlated with body condition (Sih et al. 2015; Kanno et al.

2023). One such behavior is escaping dispersion from infection sources; some host species try to escape from infected individuals that could be sources of parasite infections (Terui et al. 2017; Weinstein et al. 2018; Buck et al. 2019; Baines et al. 2020) as defined as “ecological and evolutionary disgusting” (Weinstein et al. 2018; Buck et al. 2019). This theory can be applicable to ectoparasite – host systems because surrounding host individuals could notice visible ectoparasites (Baines et al. 2020). When infected hosts have higher body condition, they could allocate their resources into dispersal (Terui et al. 2017). However, even if hosts with poor body condition identify which individuals are infected, they could not escape from that hosts because of the reduced energy by parasites, resulting in high susceptibility to further parasite infections. Another host behavior that could affect susceptibility is overall host activity. Hosts with poor body condition generally show low activity and mobility (Sih et al. 2015; Kanno et al. 2023). Because infectious stages of parasite, which are small, commonly have low moving ability (Pietrock & Marcogliese 2003), poor conditioned immobile hosts can be main targets for infections in the wild.

Finally, since host susceptibility is partly determined by genetic basis (Lysne & Skorping 2002; Lazzaro & Little 2009; Bolnick et al. 2014), introducing genetic perspectives and analysis in my systems should advance our understanding for host-parasite associations. Several empirical evidence has been accumulated that host susceptibility against ectoparasites like copepods are genetically determined (Lysne & Skorping 2002; Fast 2014; Sallinen et al. 2020). In fact, I found that many fish never got infections and experienced parasite detachments during the field survey (chapter 6). I also found that parasite numbers increased in some individuals due to positive feedback, whereas other escaped from the positive feedback loop (chapter 6). This could be explained by acquired immunity (see above), but probably there must be genetic basis

partly controlling immunity. For instance, genes in the major histocompatibility complex (MHC) play important roles in biological defenses (Piertney & Oliver 2006; Kamiya et al. 2014), and these genes are a priority for investigation. MHC II class gene family is particularly important because this region contains many genes working toward extracellular antigens (Inaba et al. 1998; Hepworth et al. 2013). Moreover, as body condition itself is also partially determined by genetics (Merila 1996), genes controlling body condition can also be a key for understanding host-parasite associations.

Why consistent negative correlations found between *Salmincola* infections and host body condition, but not others?

While many studies did not find any negative correlations between host body condition and infections, others found consistent negative correlations (see chapter 1.2 and Sanchez et al. 2018). In particular, my study system *S. markewitschi* infecting to mouth cavity of hosts showed highly consistent negative correlations between host body condition and infections. I additionally analyzed my datasets collected in 2019-2022 and found that these trends were still clear at any spatial and temporal scales (Supplementary file 1). Since these correlations were created by both directional causalities (chapter 6), we could interpret this strong-consistent negative correlations as (1) parasites strongly and consistently reduce host body condition and (2) susceptibility to parasites strongly increases in hosts when they have poor body condition. From both aspects, I discussed why negative and consistent correlations are maintained in some but not other host-parasite systems.

These differences may be explained from two different host strategies, that is resistance and tolerance (Råberg et al. 2007, 2009; Boots 2008; Figure 2). Resistance is defined as the ability to reduce the parasite infections (Råberg et al. 2007, 2009) through behavioral avoidance and immune systems as examples (Råberg 2014; Kutzer & Armitage 2016). On the other hand, tolerance is defined as the ability to limit the damages caused by per-parasite individual (Råberg et al. 2007, 2009) through increasing wounds repairments and decreasing immune-pathology (Blanchet et al. 2010; Råberg 2014; Jackson et al. 2014). In general, hosts adopt either one of the strategies to counter parasite infections, and several studies showed trade-offs between resistance and tolerance (Råberg et al. 2007, 2009; Arriero et al. 2018; Klemme et al. 2020). That trade-off may be governed by either genetic trade off (tolerance and resistance are genetically fixed, respectively) or by resource-based trade-offs (resistance and tolerance strategy is generally costly). Since tolerance is defined as slopes of regression between host health status (e.g., body condition and fitness components; y-axis) and parasite burdens (e.g., intensity and abundance, x-axis), and a lower slope indicates that hosts is more tolerant to parasite negative impacts (Figure 2b; Råberg et al. 2007), my result in the systematic review indicates that most host species employ tolerance as strategy to minimize parasite impacts. In other words, most hosts allow parasite infection without actively removing them (i.e. without resisting against parasites). This is likely because most parasites do not strongly and rapidly reduce host fitness, and resistance strategy such as acquiring immunity rather incur higher costs for the hosts. Tolerance strategy, such as repairing damages caused by parasites (see discussion above) is also costly, but the costs of resistance may outweigh the costs of tolerance. Consequently, tolerance might have become common strategy in most host-parasite systems.

On the contrary, my results consistently revealed negative slopes between body condition and infections in *Salmincola* systems. This suggests that salmonids have adopted resistance strategy to the parasites. On the other hand, once they get infected, they could not tolerate the negative impacts. As demonstrated in my study, copepods can directly affect host fitness by impeding feeding (see chapters 4 and 5) and causing physical damages that incur substantial costs for repairing (see discussion above). Thus, once hosts allow copepod infection, they may experience deleterious fitness reduction, and these impacts are too strong to tolerate. Therefore, resistance appear to be more adaptive than tolerance in our study case and hosts actively attempt to remove and kill the parasite infections using resistance strategies such as immunity and behavioral parasite removals (Råberg et al. 2007, 2009; Boots 2008). Relatively low prevalence in our study systems (generally < 30 %; Hasegawa & Koizumi 2021, 2024) compared to many helminth systems (e.g. 70-100 %; Ogura & Hasegawa 2023) is one of the possible supporting evidence for the high resistance of salmonids hosts to the copepods (Figure 2).

These discussions on resistance and tolerance also contribute to explaining opposite causal links underlying the negative correlations (i.e. poor body condition increase parasite infections). When interpreting the negative correlations from an opposite causality, skewed slope in my study system may suggest that host with high body condition could limit parasite infections (i.e. low parasite loads) but not when they have poor body condition (i.e. high parasite loads). This indicates the existence of resistance such as immunity.

My results may provide important insights into the (co) evolution of host-parasite interactions. Although I found consistent negative correlations between host body condition and infections in my study system (i.e. resistance: Hasegawa & Koizumi

2024), other studies focusing on *Salmincola* spp. did not find significant relationships (i.e. indication of tolerance; Råberg et al. 2007, 2009; Boots 2008). This pattern persists even when I focus on a single parasite species. For instance, while I found significant correlations in Japanese populations of *S. edwardsii* (Hasegawa et al. 2022a), researchers did not find such correlations in other regions and concluded that this copepod does not cause harmful effects on host health (Amundsen et al. 1997; Boone & Quinlan 2018). Additionally, I found substantial differences in infection levels of *Salmincola* spp. within and among river systems (Hasegawa & Koizumi 2021; Hasegawa et al. 2022a), potentially indicating different levels of resistance among populations (Råberg et al. 2007, 2009; Boots 2008). Because variations in negative correlations and parasite infection levels could be driven by ecological mechanisms such as environmental factors (Hasegawa & Koizumi 2021), I could not conclude that these are caused by evolutionary processes. Nevertheless, these results suggest that tolerance and resistance have evolved in each host salmonid and *Salmincola* copepod systems, repeatedly. Evolutionary theory predicts that the benefits of evolving resistance must be balanced against the costs of resistance (i.e. immunity & behavior) (Graham et al. 2022). Therefore, it would not be surprising if one species and populations gained resistance, while others evolved tolerance. However, studies found such resistance / tolerance evolution for each population or closely related species are still limited (Blanchet et al. 2010; Weber et al. 2017, 2022). *Salmincola* -salmonids systems can be a good model system for understanding evolution of tolerance and resistance.

Different levels of resistance among populations may lead to create different types of parasite communities. I still do not know the specific mechanisms of resistance in my study systems, but it is likely to occur cross-resistance against other parasites and pathogens. In terms of immunity, macro parasites like *Salmincola* spp. inhibit other

macro parasite infections, such as nematodes and trematodes because these macro parasites activate the same T-helper 2 (Th2) types of immunity (Ezenwa et al. 2010; Ramsay & Rohr 2021). Conversely, since micro parasites like viruses and bacteria activate Th1 types of immunity and there are trade-offs between the two types, macro parasites may facilitate micro parasites infections (Ezenwa et al. 2010; Ramsay & Rohr 2021). Thus, populations showing higher levels of resistance may show lower infection levels of other macro parasites but higher infection levels of micro parasites. Unraveling these infection dynamics are important to predict outbreaks of harmful parasites and diseases (Ramsay & Rohr 2023a).

Which populations or species are likely to evolve resistance and tolerance? A theoretical study predicts that resistance is likely to evolve when hosts frequently encounter with parasites, these with high virulence, significantly reducing host fitness (Boots & Haraguchi 1999). Because encounter rates and virulence often correlate with other ecological traits such as transmission modes, attachment sites, and infection periods (Poulin 2011), we could estimate which species and populations show either resistance or tolerance. For instance, hosts may be likely to evolve tolerance against intestinal helminth, as these parasites typically do not strongly reduce host fitness and they infect to hosts for a long period (Shanebeck et al. 2022). Many of these parasites transmit through trophic transmission, with the success rate of such transmission commonly being low. In other words, the frequency of infection may be comparatively low compared to other types of parasites (Pietrock & Marcogliese 2003). In such situations, maintaining costly resistance may rather reduce host fitness, making the evolving of tolerance more adaptive. On the other hand, many ectoparasites have shorter infection periods than those of intestinal helminth and directly consume host resources, leading to fitness reduction. In particular in aquatic ecosystems, where many of these

parasites release eggs or infectious larvae to external environments (e.g. Neal et al. 2021) and hosts often experience high frequency of infections. Given these circumstances, hosts should evolve resistance when confront with ectoparasites. Although there are many exceptions in these examples, and several other factors should be considered such as host and parasite phylogenetic relationships and parasite's co-evolving periods with their hosts, there are likely general rules in the evolution of resistance and tolerance. Further empirical evidence examining correlations between host fitness and infections should be required to understand the general pattern of resistance and tolerance evolution.

Although the simple correlation between host body condition and infections, as observed in my study, provides valuable clues for identifying host strategies, several cautions should be necessary to interpret this analysis. For instance, it is possible that some studies in my systematic review could not detect dead individuals with poor body condition. In fact, I found poor body condition could lead low survival rate (chapter 6), and these individuals dropped off from the figure. Such missing datapoints apparently create moderate slopes, optimistically leading researchers to conclude high tolerance of hosts. Additionally, although body condition indices are good surrogates for assessing host health (Sanchez et al. 2012), some parasites reduce host life-time fitness without reducing body condition. For instance, some castrator parasites reduce host reproduction by destroying reproductive organs (Webb & Hurd 1999; Miura et al. 2006). Others manipulate host behaviors to increase their host susceptibility to predators, facilitating successful transmission (Lafferty & Morris 1996; Poulin & Latham 2002). Therefore, researchers could not detect negative correlations between body condition and infections in these cases, and other alternative indicators of host health must be required. Finally, as I insisted throughout my dissertation, uncovering mechanisms of body

condition (health) reduction by parasites and causalities hidden under negative correlations is necessary for assessing host resistance and tolerance strategies.

Figure 1. Schematic overview of my dissertation. Red and blue parts indicate what I found in a series of my chapters and what I did not examine, respectively.

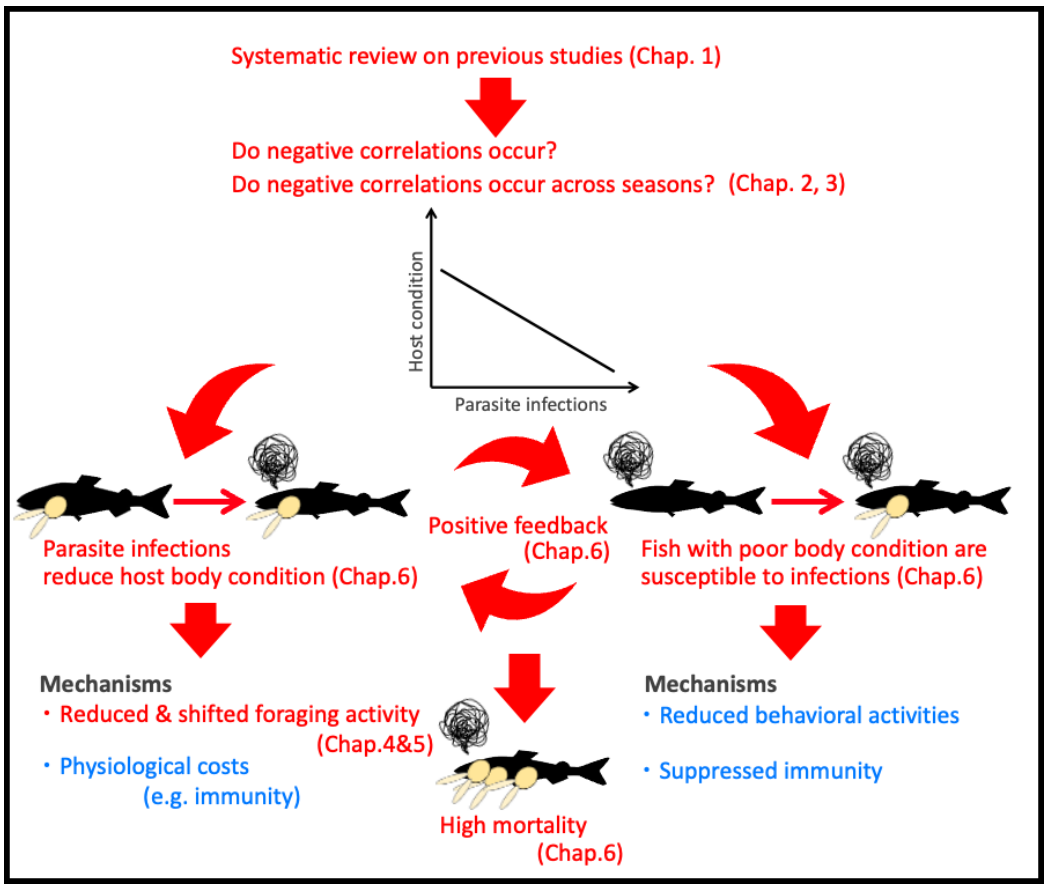
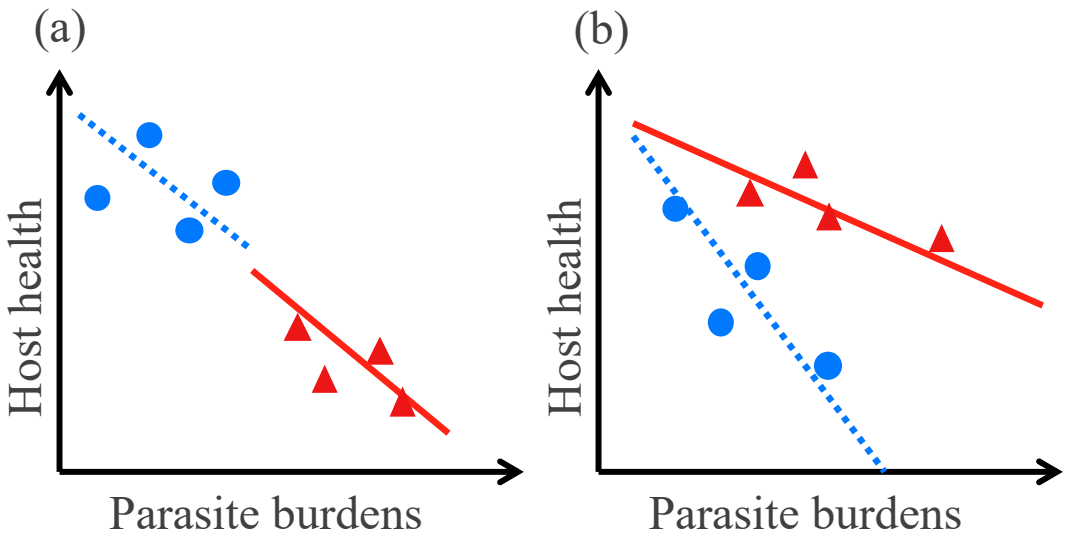


Figure 2. Schematic plots for identifying resistance and tolerance strategies. Each point indicates each infected individual. Different colors mean different genotypes or populations showing different strategies.

(a) The blue group shows lower parasite burdens due to a higher resistance compared to the red group.

(b) The slope of blue group is larger than that of red group, suggesting that individuals in the blue group are less tolerant to the negative impacts of parasite, while individuals in the red group successfully tolerate to these effects.



Supplementary file 1. Consistent negative correlations between body condition of white-spotted charr and *Salmincola markewitschi* infections in any spatial and temporal scales

We found highly consistent negative correlations between body condition of white-spotted charr and *Salmincola markewitschi* infections across four seasons (chapter 3). To confirm if these consistent patterns hold true in any spatial and temporal scales, I preliminary re-analyzed my datasets collected during 2019-2021 in the Shiodomari River system, southern Hokkaido, Japan (see Hasegawa & Koizumi 2021, 2024). In most of the tributaries in this river system, white-spotted charr are frequently infected with *S. markewitschi* on mouth cavities, though the infection levels such as prevalence are highly heterogeneous among study sites (Hasegawa and Koizumi 2021, 2024). In total, I collected fish from 19 study sites during the study period.

To test if negative correlations found in any study sites, I separately tested correlations for each study site where enough samples size were captured for the analysis (i.e. $N > 30$). I used datasets collected in spring (Number of study sites = 6) and winter (Number of study sites = 3) field surveys because of the high infection during the periods and low infection intensity might not allow us to detect negative impacts of parasites (see Hasegawa & Koizumi 2024). I constructed simple generalized linear models (GLMs) with gaussian error distribution for each study site. The response variable was body condition (i.e. residual index) and explanatory variable was parasite abundance. Study site number follows chapters 3 and appendix paper 2.

Additionally, to confirm if these negative correlations found in other areas within Hokkaido, I similarly analyzed other datasets collected at several river systems in eastern Hokkaido in June to July 2022 (Hasegawa unpublished data). This dataset consisted with 1,681 individuals of white-spotted charr collected at 55 sites in 12 river

systems. In these river systems, *S. markewitschi* were abundant and occured in the host mouth cavity. To consider the variations among study sites, I constructed a similar generalized linear mixed model (i.e. GLMM) with including study sites as random effects.

At site 18 in the Shiodomari River system (see chapter 3), I collected and measured fish for three consecutive years (i.e. 2019-2021; see Hasegawa & Koizumi 2023, chapter 6). Using this dataset, I examined if these negative correlations consistently found among years. I calculated residual index for each year, then ran the similar GLMs for each year as I described above.

In spring, I found significant negative effects of parasite abundance on body condition in three upstream sites of Itokawa, Shiodomari River system (GLM; Site 17, $t = -3.63$, $p < 0.01$; Site 18, $t = -3.35$, $p < 0.01$; Site 19, $t = -3.37$, $p < 0.01$). I also found marginally significant effects in upstream of mainstem (Site 3, $t = -1.98$, $p = 0.05$). No significant effects were found in any other study sites (Site 2, $t = -0.63$, $p = 0.53$; Site 16, $t = -0.97$, $p = 0.34$).

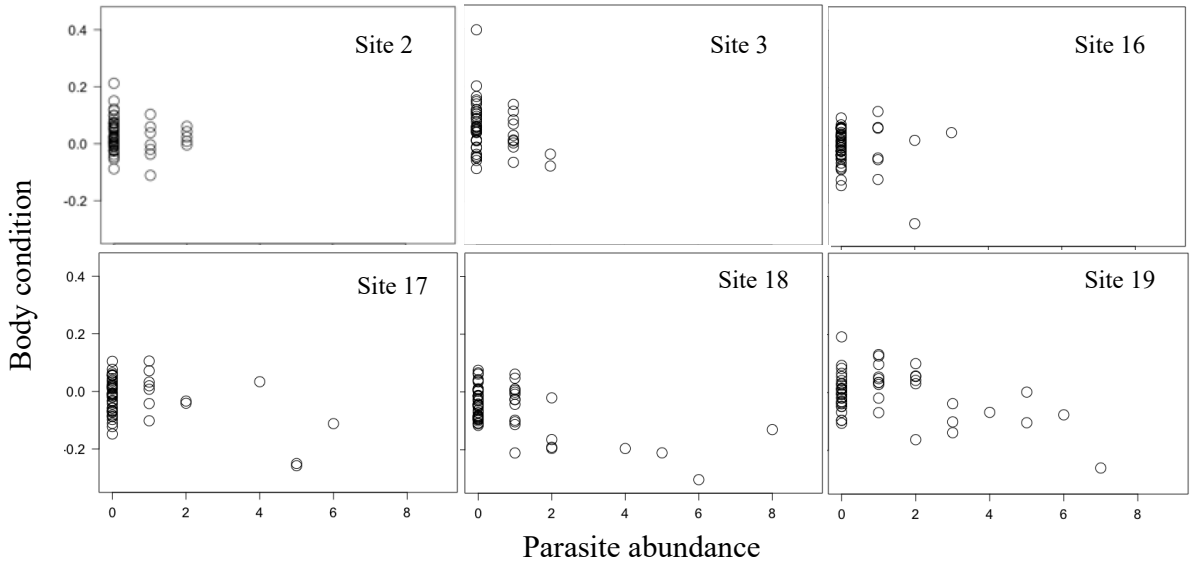
In winter, significant negative effects of parasite abundance were found in Nobirosawa stream (Site 9, $t = -7.71$, $p < 0.01$; Figure 1b), and marginal negative effects was found in Gabinosawa stream (Site 11, $t = -1.94$, $p = 0.06$; Figure 1b). No significant effect was detected in Sasagoya stream (Site 2, $t = -0.22$, $p = 0.83$ Figure 1d).

In eastern Hokkaido, I also found that significant negative correlations (GLMM; $t = -2.94$, $p < 0.01$; Figure 1d).

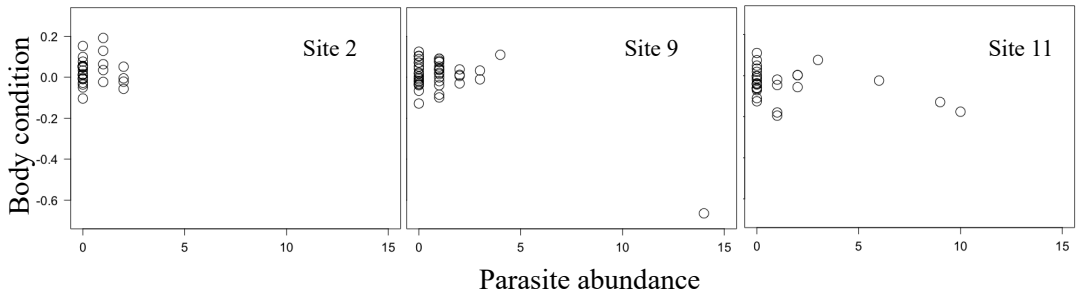
For the temporal patterns in site 18, I also found significant negative correlations in June 2020 ($t = -3.39$, $p < 0.01$; Figure 1c) and July 2021 ($t = -6.61$, $p < 0.01$; Figure 1c), as well as spring 2019 (see above).

Figure 1. Correlations between parasitic copepods *S. markewitschi* and body condition of their host white-spotted charr *Salvelinus leucomaenis.* (a) six sites in Spring, (b) three sites in Winter, (c) three consecutive sampling occasions at Site 18.

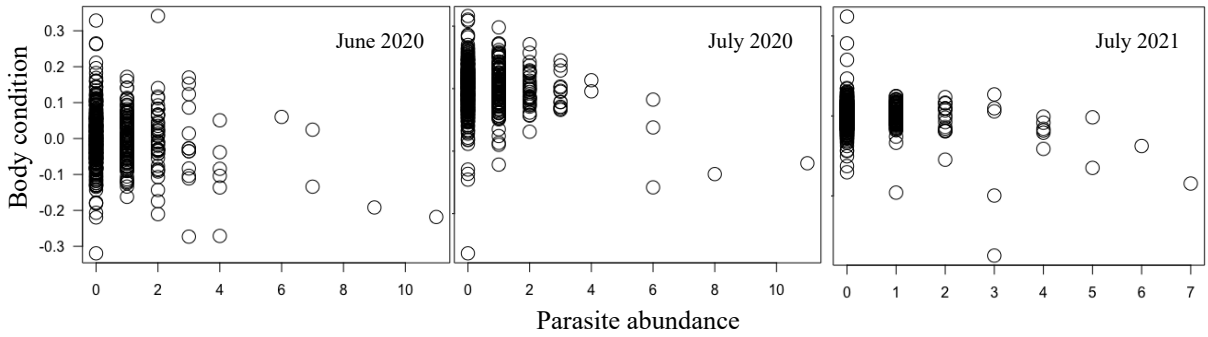
(a) Spring



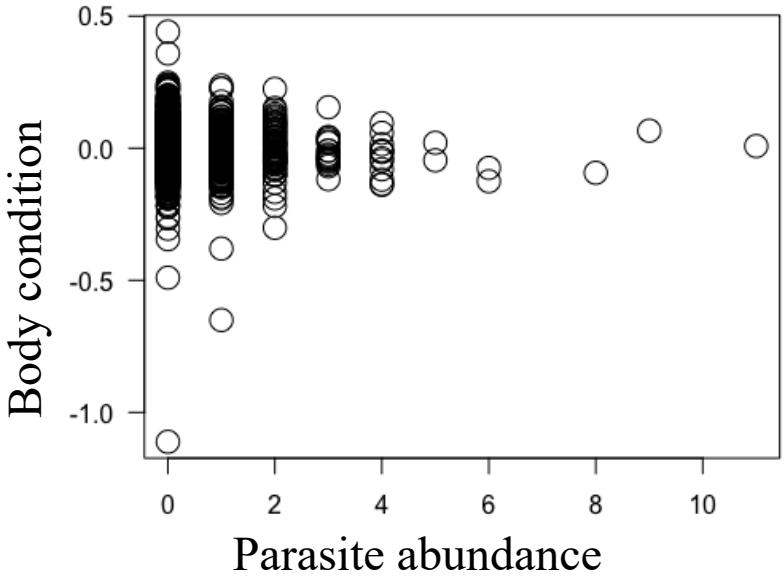
(b) Winter



(c) Three consecutive samplings at Site 18



(d) eastern Hokkaido



Appendix paper 1

Morphological variation of *Salmincola markewitschi*

Abstract

Salmincola markewitschi Shedko & Shedko, 2002 (Copepoda: Lernaeopodidae) is an ectoparasitic copepod mainly infecting the buccal cavities of white-spotted charr *Salvelinus leucomaenis* (Pallas, 1814) (Salmonidae). This species has only been recorded from Northeast Asia, where a morphologically similar congener *Salmincola carpionis* (Krøyer, 1837) is also distributed using the same host species. These copepods are hard to distinguish from each other because of their similarities. We thus examined the newly collected specimens morphologically and genetically from five populations of white-spotted charr in Japan. Most of the specimens were morphologically consistent with *S. markewitschi* but showed great variations in the numbers of spines on the exopods of the antennae, shape of the maxilliped myxal palps, and the bulla diameter. Consequently, some specimens shared characteristics with S. carpionis. In addition to the mophological continuities, genetic analyses of 28S rDNA and COI mitochondrial DNA confirmed that all specimens belong to a single species. Further taxonomic revisions are required to draw conclusions of whether *S. markewitschi* is a valid species different from *S. carpionis*, by collecting samples from across their wide distributional ranges, such as Europe, North America, and Northeast Asia.

1. Introduction

The genus *Salmincola* C. B. Wilson, 1915 is a group of ectoparasitic copepods commonly infecting salmonid fishes (Kabata 1969). Some of the species cause histopathological impact on their hosts and have been regarded as harmful parasites in hatcheries and fish farms (Gall et al. 1972; Sutherland & Wittrock 1985; Roberts et al. 2004; Ruiz et al. 2017; Neal et al. 2021). To date, 22 valid species have been recorded from the genus (Walter & Boxshall 2018) and the most members of the genus have circumpolar distribution (Kabata 1969). In Japan, the following five species have been recorded: *Salmincola californiensis* (Dana, 1852) (reported as *S. yamame* in Hoshina & Suenaga 1954; Hoshina & Nishimura 1976; Nagasawa & Urawa 2002), *S. carpionis* (Krøyer, 1837) (Nagasawa et al. 1995, 1998; Nagasawa & Sakaki 2019), *S. stellata* Markevich, 1936 (Nagasawa & Urawa 1991; Nagasawa et al. 1994; Hiramatsu et al. 2001; Nagasawa et al. 2021), *S. edwardsii* (Olsson, 1869) (Nagasawa 2020a, b; Nagasawa & Kawai 2020; Nagasawa 2021; Hasegawa et al. 2022a) and *S. markewitschi* Shedko & Shedko, 2002 (Shedko & Shedko 2002, Nagasawa 2020c, Nagasawa & Ishiyama 2021; Nagasawa 2021) (see key to the species of the genus *Salmincola* in Japan, provided in this study).

S. markewitschi was described as a new species in 2002 using the specimens recovered from the buccal cavities of white-spotted charr *Salvelinus leucomaenis* (Pallas, 1814) in the Kuril Islands, Northeast Asia (Shedko & Shedko 2002). This copepod has recently been found from the same host species in Japan (Nagasawa 2020b; Nagasawa 2021; Nagasawa & Ishiyama 2021), but the morphologically similar congener *S. carpionis*, which has a circumpolar distribution (Kabata 1969) attaching to the same infection site of the same host species, is also known to occur in this region (Nagasawa et al. 1995; Nagasawa 2020c). Due to their morphological and ecological similarities, these two species might have been mixed in past literature, as indicated by Nagasawa

(2020c). According to Shedko & Shedko (2002), *S. markewitschi* can be distinguished from *S. carpionis* and other congeners by three main characters; 1) the distal end of the exopod of the antenna (as second antenna in Kabata 1969) has numerous small spines in addition to the two large papillae, whereas *S. carpionis* has no spines, 2) maxilliped palp has two overhanging outgrowths, whereas *S. carpionis* has only a single outgrowth on the maxilliped palp, 3) bulla diameter is larger than those of other *Salmincola* spp. (Shedko & Shedko 2002). However, considering the high morphological variations in this genus (Kabata 1969; Fryer 1981), careful identification using a comprehensive approach with combining morphological and genetic analyses is required.

Here, we evaluated *S. markewitschi* and *S. carpionis* by examining morphological variations of newly collected specimens from various localities around Japan, together with a comparison of the nuclear 28S ribosomal DNA and mitochondrial cytochrome oxidase gene subunit I (COI) sequences. We also discuss the need for taxonomic reassessment of the genus using samples collected from all over the world to solve this taxonomic complexity, especially in the Northeast Asia.

2. Materials and methods

2.1. Fish and copepods collection

Host fish were caught from five sites (four rivers and one aquarium) from three prefectures (Hokkaido, Fukushima and Toyama) in Japan (Table 1, Figure 1). At the four rivers, wild fish were caught by angling and electrofishing. Found copepods were carefully removed by forceps and preserved in vials filled with 70% ethanol. The aquarium samples were collected from the Sapporo Salmon Museum (hereafter, SSM following Nagasawa 2021), Makomanai, Sapporo. In this aquarium, infections of *Salmincola markewitschi* in the buccal cavity of white-spotted charr have been reported

since 1985 (reported as *S. californiensis* in Anonymous 1989; Takayama et al. 1999; Nagasawa 2021). White-spotted charr originated from Toyohira River, Hokkaido and Miya River, Gifu (Anonymous 2006), and has been reared at SSM. The source populations of *S. markewitschi* have been unknown, but they were supposedly from some rivers in Hokkaido (Takayama et al. 1999; Nagasawa 2021). The buccal cavities of white-spotted charr were checked, and collected copepods were preserved in the same manner as at other sites.

2.2. Morphological description

Morphological examination of the parasite specimens was conducted using light microscopes (BX53 and BH2, Olympus Inc., Japan) and stereo microscopes (SZX16 and SZX10, Olympus Inc., Japan). The number of specimens examined from each site is as follows; Bekanbe-ushi (two), SSM (two), Shiodomari (four), Fukushima (two), Toyama (two). Before the morphological examination, specimens were soaked in lactophenol. Dissection and morphological examination was conducted using the wooden slide method following Humes & Gooding (1964). Drawings of each copepod specimen were made with the aid of drawing tubes attached to the light microscopes. All specimens we examined were deposited in the Invertebrates collection of the Hokkaido University Museum (ICHUM 8333–8337), Sapporo, Japan. The morphological terminologies were used following Huys & Boxshall (1991); antennule (as first antenna in Kabata 1969), antenna (as second antenna in Kabata 1969), maxillule (as first maxilla in Kabata 1969), maxilla (as second maxilla in Kabata 1969), maxilliped and mandible. For the armatures on the endopod of antenna (i.e., hook 1, spine 2, tubercle 3, process 4 and 5), we followed the terminologies used in Kabata

(1969). The morphological identifications were made by using Kabata (1969), Shedko & Shedko (2002) and Nagasawa (2020c).

2.3. Genetic analysis

A total of 13 and 12 specimens (Table 2) were used for the 28S rDNA and COI analyses, respectively. Total genomic DNA was extracted from whole parasites using a PureGene DNA isolation kit (Applied Biosystems). A part of the egg sac was used for DNA extraction, lysed in 20μL of 0.02 N NaOH at 98℃ for 30 min (Nakao et al. 2018). The PCR was performed using primers D1a (5'-CCC(C/G)CGTAA(T/C)TTAAGCATAT-3') and D3b (5'-TCCGGAAGGAACCAGCTACTA-3') for 28S rDNA (von Reumont et al. 2009) and the primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) for COI (Folmer et al. 1994). The PCR reactions for 28S rDNA were performed in 25μL volumes with thermocycling protocol for gene amplification as follows: initial denaturation at 95ºC for 2 min, 35 cycles of 95ºC for 30 sec, annealing at 55ºC for 40 sec and extension at 72ºC for 90 sec, followed by a further extension at 72°C for 8 min. For COI, the PCR reactions were carried out in 10μL volumes following protocol; 94°C for 5 min, 35 cycles of 94°C for 60 sec, 50°C for 60 sec and 72°C for 60 sec, and 5 min of final hold at 72°C. Purified products were cycle sequenced with the forward and reverse primer (i.e. D1a and D3b for 28S rDNA and LCO1490 and HCO2198 for COI). Sequence alignment and calculation of genetic distance were performed with the software MEGA ver. 10.0.4 (Kumar et al. 2018). The sequences of 28S rDNA were compared with known sequences of *S. edwardsii* from Norway and North America (DQ180346.2, KY113080.1, KY113081.1) and *S. californiensis* (KY113082.1, KY113083.1) (Ruiz et

al. 2017) from GenBank database. No inter-specific comparison was made for COI because of the lack of reference for genus *Salmincola*.

3. Results

The number of the copepod specimens and inspected fish are summarized in Table 1 and 2. All copepods were found from buccal cavities of fish examined.

3.1. Morphological description

The adult female, composed of three major body parts; cephalothorax, maxilla and trunk with egg sacs (Figure 2A). Oval-shaped cephalothorax, distinguished from the trunk by a deep constriction (Figure 2A). Cylindrical maxilla extending towards the ventral side distally with bulla (Figure 2A). Brownish bulla, mushroom shaped with short manubrium (Figure 2A). Ratio of bulla diameter / cephalothorax length 0.40–1.02 (mean 0.74, n = 12; Table 2), almost consistent with the original description of *S. markewitschi* (Shedko & Shedko 2002, Table 2). Trunk, almost ovoid, 2.46–4.82 long (mean 3.84 mm, n = 13). Two egg sacs, generally in equal size, attaching on posterior trunk (Figure 2A).

Antennule, no segmented, three to five short setae at their tips (Figures. 6F–J). Antenna, composed of biramous sympod; spiny pad generally with more than three spines on the lateral side of the sympod (Figure 2C). Biramous sympod, composed of two-segmented endopods with spiny pad on the basal segment and unsegmented exopods (Figure 2C). Five apical armatures on the distal end of the endopod: dorsal hook 1, spine 2, tubercle 3, process 4 and 5 (Figures 2D–I). The exopod, with large variations, generally having two papillae and numerous small spines (Figures. 3A–J); specimens from the Shiodomari River in Hokkaido (ID7, Figure 3B) and Fukushima

Prefecture (ID14, Figure 3H) possessing no spines, corresponding to the character of *S. carpionis*; specimens from SSM possessing more than 10 spines (ID8, Figure 3C), corresponding to the characteristics of *S. markewitschi*. Mandible in the buccal apparatus with seven teeth; the distal five teeth noticeably larger than the proximal two (Figures 4I–M).

Maxillule with three papillae extending ventrally from its tips and a small exopod near its basal area (Figures 6A–E). Maxilliped, two segmented, comprised with subchela with short curved claw and corpus (Figure 5A); claw positioned at the distal end of the subchela; subchela elongating from the distal end of the corpus; one short ventral seta and one auxiliary palp extending from basal and distal area of subchela; palp with two outgrowths (Figures 5G–K) positioned at the medial area of the corpus with variations in its shapes; most having two prominent outgrowths as reported from *S. markewitschi* (e.g. ID1, Figure 5G); others not prominent (e.g. ID13, Figure 5J) and some having humps or protrusions (e.g. ID8, Figure 5H).

3.2. Genetic analysis

A total of 884 bp of the partial 28S rDNA region showed a 100 % match amongst all specimens in the present study (n = 13). These sequences had a 99.55 % match with *S. edwardsii* from Norway (GenBank accession numbers is DQ180346.2) and a 99.43 and 99.32 match with *S. edwardsii* caught in North America (GenBank accession numbers are KY113080.1 and KY113081.1; Ruiz et al. 2017). All specimens also showed a 98.76 and 98.65 match with *S. californiensis* from North America (GenBank accession numbers are KY113082.1 and KY113083.1; Ruiz et al. 2017). For COI, a total of 601 bp was obtained. Four haplotypes were detected from different regions in Japan with only 0–4 base pair differences (mean genetic differences: 0.28%, range:

0–0.67 %, *n* = 12). GenBank accession numbers are LC713076–LC713088 for 28S rDNA and LC713314–LC713325 for COI.

4. Discussion

Our genetic analysis of both nuclear and mitochondrial DNA confirmed that the examined specimens from five sites in Japan contained only a single species of the genus *Salmincola*: 28S rDNA was monomorphic and genetic distance of COI fell within the range of intraspecific variation, as shown in other parasitic copepods (Montes et al. 2017). In addition, based on the morphological observations, most of the specimens were consistent with *Salmincola markewitschi* described by Shedko & Shedko (2002); 1) the distal end of the exopod had some small spines (mainly three to five) in addition to two papillae, 2) two outgrowths were present on the palp extending from the base of maxilliped, and 3) the ratio of the bulla diameter / cephalothorax length in the present study (range 0.40–1.02, mean 0.74) was similar to the original description (range 0.74–1.17, mean 0.91, Shedko & Shedko 2002).

However, morphological variations of our specimens were high, and some specimens partly had characteristics consistent with *S. carpionis*. While all 12 specimens had two outgrowths at the maxilliped palp, four (ID 1, 7, 9, 12, 14) had no spines at the distal end of the exopod of the antenna (Table 2). In addition, nine specimens (ID 4, 6, 7, 10, 12, 13, 14, 15, 16) had a small bulla diameter, which fits the range of *S. carpionis* (range 0.34–0.80, mean 0.56, Shedko & Shedko 2002). This makes a firm morphological species identification difficult.

To discriminate between the two species, we have to examine the validity of each morphological trait (i.e. spines at the distal end of the exopod of antenna, shapes of

maxilliped palp, bulla diameter). The number or shape of spines on the antenna's exopod has been widely used as a key character to discriminate *Salmincola* spp. (Kabata 1969). For instance, *S. californiensis* is distinguished by its cluster of very strong and large spines and this characteristic was consistently observed in all populations examined so far (Kabata 1969; Hoshina & Nishimura 1976; Ruiz et al. 2017). However, caution is still needed in using this feature, because of some variations. In *Salmincola thymalli* (Kessler, 1868), the specimens from Nearctic had long prominent spines, but those from the Palearctic had very small and scattered spines (Kabata 1969). As is this unreliable case, the present individuals represented both patterns with and without spines on the antenna's exopod even in the same site with identical 28S rDNA and COI sequences.

The shape of maxilliped palps is less reliable for discriminating *S. markewitschi* from *S. carpionis*. While some species such as *S. thymalli* are characterized by their long and slender palps, considerable variability was recognized in some species (Kabata 1969). For instance, *Salmincola salmoneus* (L.) and *S. californiensis* showed large intraspecific variations for the number and shape of the outgrowths on their palps. In our case, whereas most of the specimens had two outgrowths on the maxilliped palps, their length and shape showed high variation within and among populations as well.

The remaining key trait, the bulla diameter, is also a presumably unreliable character in our case. Although bulla shape is widely used for species identifications of the genus *Salmincola*, its diameter can be easily changed by its attachment sites and host characteristics (Kabata 1969). In other siphonostomatoid copepods, it is also reported that the attachment organs are affected by host body parts, as well as ambient environmental factors like temperature (Fryer 1961; Hogans 1987; Abaunza et al. 2001; Hua et al. 2019; Suyama et al. 2019; González et al. 2021). The ratio of the bulla

diameter / cephalothorax length of *S. markewitschi* was highly variable even within a small geographic range (Shedko & Shedko 2002; Nagasawa 2020c; Nagasawa & Ishiyama 2021) and the high variation may be due to host characteristics and/or physical environmental factors, which affect the parasite's development.

Taken together, while we can tentatively conclude our specimens as *S.* cf. *markewitschi* because of the overall morphological consistency, we should be careful of the possibility that *S. markewitschi* is a regional type of *S. carpionis* and the former is a synonym of the latter or a subspecies. Because *Salmincola* spp. often have large morphological variations (Kabata 1969), even the same species can show distinct morphological traits among local populations, especially considering the wide circumpolar distribution (Figure 1). Genetic analysis is particularly useful to delineate species with high morphological variations (Nadler & Pérez-Ponce de León 2011), but surprisingly very few studies have conducted genetic analysis for *Salmincola* spp. (Ruiz et al. 2017; Hasegawa et al. 2022a). It is necessary to compare specimens collected from throughout their distributional range, including the Northeast Asia, North America and Europe, using both genetic and morphological traits. In particular, Shedko & Shedko (2002) reported the morphological distinction between sympatric *S. markewitschi* and *S. carpionis* in Kuril Islands where the type collection of the former was determined. Genetic analysis in this population will be the priority.

Key to the species of the genus *Salmincola* in Japan based on the current publications

1. Ventral side of the basal segment of endopod of antenna with large smooth tapering outgrowth; bulla stellate; commonly parasitic on Sakhalin Taimen *Parahucho perryi*
……*Salmincola stellata* Markevich, 1936

Ventral side of the basal segment of endopod of antenna with spiny pad; bulla is not stellate (commonly round or mushroom shape); not commonly parasitic on Sakhalin Taimen *Parahucho perryi*
……2

2. One process as large as, or larger than dorsal hook and other much smaller processes are present at ventral side of the terminal segment of endopod of antenna; the exopod and sympod of antenna are highly inflated
……*Salmincola edwardsii* (Olsson, 1869)

Two processes generally smaller than dorsal hook and other much smaller process are present at ventral side of the terminal segment of endopod of antenna; the exopod and sympod of antenna are not inflated
……3

3. Maxilliped palp is large; the distal end of the exopod of antenna had more than five huge spines; commonly parasitic on the genus *Oncorhynchus*
……*Salmincola californiensis* (Dana, 1852)

Maxilliped palp is small; the distal end of the exopod of antenna had no or several small spines; commonly parasitic on the genus *Salvelinus*

·····4

4. Maxilliped palp have two outgrowths with no hump (protrusion) near its base; the distal end of the exopod of antenna have numerous small spines in addition to the two papillae; bulla diameter is large (the ratio of bulla diameter / cephalothorax length range 0.74–1.17, mean = 0.91 reported in original description by Shedko & Shedko 2002)

·····*Salmincola* cf. *markewitschi* Shedko & Shedko, 2002

Maxilliped palp have a single outgrowth with one hump (protrusion) near its base; the distal end of the exopod of antenna have only two papillae, no spines; bulla diameter is small (the ratio of bulla diameter / cephalothorax length range 0.34–0.80, mean = 0.56 reported in original description by Shedko & Shedko 2002)

·····*Salmincola carpionis* (Krøyer, 1837) (suspected as *Salmincola* cf. *markewitschi* Shedko & Shedko 2002)

Table 1. Sampling sites and date of the copepods parasitic on *Salvelinus leucomaenis* in Japan.

Sampling date	Prefecture	Sites	No. of fish inspected	Fork length range (mm)	Prevalence (%)	Intensity (mean)
September, 2020	Hokkaido Island	Toraibetsu brook, Bekanbe-ushi River, Akkeshi	13	141–347	30.8	1–2 (1.25)
July, 2020		Sapporo Salmon Museum, Sapppro, Makomanai, Sapporo	53	310–414	92.5	1–11 (4.71)
June, July and October 2020		Shiodomari River, Hakodate	754	69–528	33.6	1–11 (1.60)
May, 2020	Fukushima Pref.	Tagokura-high dam, Tadami River, Minamiaizu	1	501	100	4
June, 2020	Toyama Pref.	Jo-gan-zi River, Toyama	1	458	100	13

Table 2. Summary of previous identifications of *Salmincola markewitschi* and *Salmincola carpionis* and morphological variation of parasitic copepods recovered from *Salvelinus leucomaenis* in the present study. ID indicates the specimen's ID. (also shown in Figure 2–6).

Species & Sites	Specimen's ID	The ration of cephalothorax long / bulla diameter	The number of spines of exopod	The maxilliped palp	28S r analysis	COI analysis	Reference
Salmincola markewitschi							
Russian Far East	-	0.74–1.17 (n = 16, mean = 0.91)	4–5 (at least 3, n = 45)	two outgrowths, but some had three (n = 45)	-	-	Shedko and Shedko 2002
Magadan Region, Russia	-	0.58–1.32 (n = 86, mean = 0.84)	3–4 (n = 491)	two outgrowths, but some humps (n = 491)	-	-	Shedko et al. 2005a
Sakhalin Island		0.67–1.13 (n=22, mean = 0.84)	No description	No description	-	-	Shedko et al. 2005b
Nagano Prefecture, Japan	-	0.72–1.13 (n = 6, mean = 0.90)	3–4 (n = 1)	two outgrowths (n = 1)	-	-	Nagasawa 2020b
Ishikawa Prefecture, Japan	-	0.56–0.80 (n= 9, mean = 0.66)	several number (n = 1)	two outgrowths (n = 1)	-	-	Nagasawa and Ishiyama 2021
SSM, Hokkaido, Japan	-	No description	several number (n = 1)	two outgrowths (n = 1)	-	-	Nagasawa 2021
Salmincola carpionis							
Some countries in circumpolar region	-	No description	0 (n > 78)	quite irregular shape	-	-	Kabata 1969
Aquarium in Aomori Prefecture, Japan	-	No description	0 (n = 10)	quite irregular shape	-	-	Nagasawa et al. 1995
Russian Far East	-	0.34–0.80 (n = 31, mean = 0.56)	0 (n = 102)	one outgrowth (n = 102)	-	-	Shedko and Shedko 2002
Magadan Region, Russian	-	0.27–0.68 (n = 47, mean = 0.48)	0 (n = 110)	one outgrowth (n = 110)	-	-	Shedko et al. 2005a
Salmincola markewitschi							
Toraibetsu (Bekanbe-ushi)	ID11	Lost bulla	3	two outgrowths	-	-	-
	ID12	0.738	0	two outgrowths	○	○	-
							-
Sapporo Salmon museum (SSM)	ID8	0.747	14	two outgrowths	○	○	-
	ID15	0.670	7	two outgrowths and one hump near its base	○	○	-
							-
Shiodomari River	ID1	1.020	0	two outgrowths	○	○	-
	ID3	-	-	-	○	○	-
	ID4	0.722	3	two outgrowths	○	○	-
	ID5	-	-	-	○	○	-
	ID6	0.741	1	two outgrowths	○	-	-
	ID7	0.730	0	two outgrowths	○	○	-
	ID16	0.402	-	-	-	-	-
	ID17	-	-	-	-	-	-
							-
Fukushima Prefecture	ID13	0.707	4	two outgrowths	○	○	-
	ID14	0.786	0	two outgrowths	○	○	-
							-
Toyama Prefecture	ID9	0.924	0	two outgrowths	○	○	-
	ID10	0.683	2	two outgrowths	○	○	-

Figure 1. Map of the known localities of *Salmincola carpionis* and *Salmincola markewitschi*. 1–25; *S. carpionis* (References; Yamaguti 1939 (reported as *Salmincola faculata*), Kabata 1969, Kumagai 1985 (reported as *Salmincola* sp.), Nagasawa et al. 1995, 1997, 1998, Wakabayashi 1997, Yamamoto and Nagasawa 1999, 2001, Watanabe and Ishii 2000, Nagasawa and Urawa 2002, Shedko and Shedko 2002, Shedko et al. 2005a, b, Sokolov et al. 2012, Nagasawa and Ishikawa 2017, Nagasawa and Sakaki 2019, Kawanobe 2018, 2020). 26–42; *S. markewitschi* (References; Nishimura and Hoshina 1977 (reported as *Salmincola californiensis*), Shedko and Shedko 2002, Shedko et al. 2005a, b, Denda and Ogawa 2011 (reported as *Salmincola californiensis*), Sokolov et al. 2012, Nagasawa 2020c, Nagasawa and Ishiyama 2021, Nagasawa 2021, This study. See specific information in other studies (e.g., Kabata 1969, Shedko and Shedko 2002, Nagasawa 2020c).

Salmincola carpionis: 1. Greenland (type locality); 2. Hrúta Fjord (described as Hrutafjordara in Kabata 1969), Iceland; 3. Etah, Greenland; 4. Alitak Bay, Alaska; 5. Attu, Alaska; 6. Bering Island; 7. Lake Taymyr (described as Lake Taimyr in Kabata 1969); 8. Baffin Island; 9. Quebec; 10. Sakhalin; 11. Kamchatka Peninsula; 12. Shumushu Island; 13. Onekotan Island; 14. Shantar Islands; 15. Primorye; 16. Magadan region; 17. Toraibetsu brook, Bekanbe-ushi River, Hokkaido; 18. Lake Panke, Hokkaido; 19. Aomori Prefecture; 20. Iwate Prefecture; 21. Fukushima Prefecture; 22. Tochigi Prefecture; 23. Toyama Prefecture; 24. Yamanashi Prefecture; 25. Nagano Prefecture.
Salmincola markewitschi: 26. Shumshu Island (type locality); 27. Kamchatka Peninsula; 28. Sakhalin Island; 29. Primorye; 30. Shantar Islands; 31. Magadan region; 32. Iturup Island; 33. Kunashir Island; 34. Shikotan Island; 35. Toraibetsu brook, Bekanbe-ushi River, Hokkaido; 36 & 37. Sapporo Salmon Museum (SSM), Hokkaido; 38. Shiodomari River, Hokkaido; 39. Fukushima Prefecture; 40. Toyama Prefecture; 41. Ishikawa Prefecture; 42. Nagano Prefecture.

Salmincola carpionis
Type locality of Salmincola carpionis
Salmincola markewitschi
Type locality of Salmincola markewitschi
Salmincola cf. markewitschi in the present study

Figures 2–6. Female *Salmincola* cf. *markewitschi* from white-spotted charr *Salvelinus leucomaenis* at five sites in Japan. Abbreviations in parentheses represent places where specimens were collected as follows; Shiodomari River, southern Hokkaido (Shiodomari), Sapporo Salmon Museum, central Hokkaido (SSM), Bekanbe-ushi River, eastern Hokkaido (Bekanbe-ushi), Tadami River, Fukushima Prefecture (Fukushima), Jo-gan-zi River, Toyama Prefecture (Toyama).

Figure 2. A. Entire, lateral (Shiodomari, ID17); B. Cephalothorax, dorsal (Shiodomari, ID16); C. Antenna, entire, lateral (Shiodomari, ID7); D. Antenna, tip of endopod, lateral (Shiodomari, ID7); E. Same, lateral (Shiodomari, ID1); F. Same, lateral (SSM, ID15); G. Same, lateral (Bekanbe-ushi, ID12); H. Same, ventral (Fukushima, ID13); I. Same, lateral (Toyama, ID10). Scale bars: A. 1mm; B–C. 4μm; D–I. 3μm.

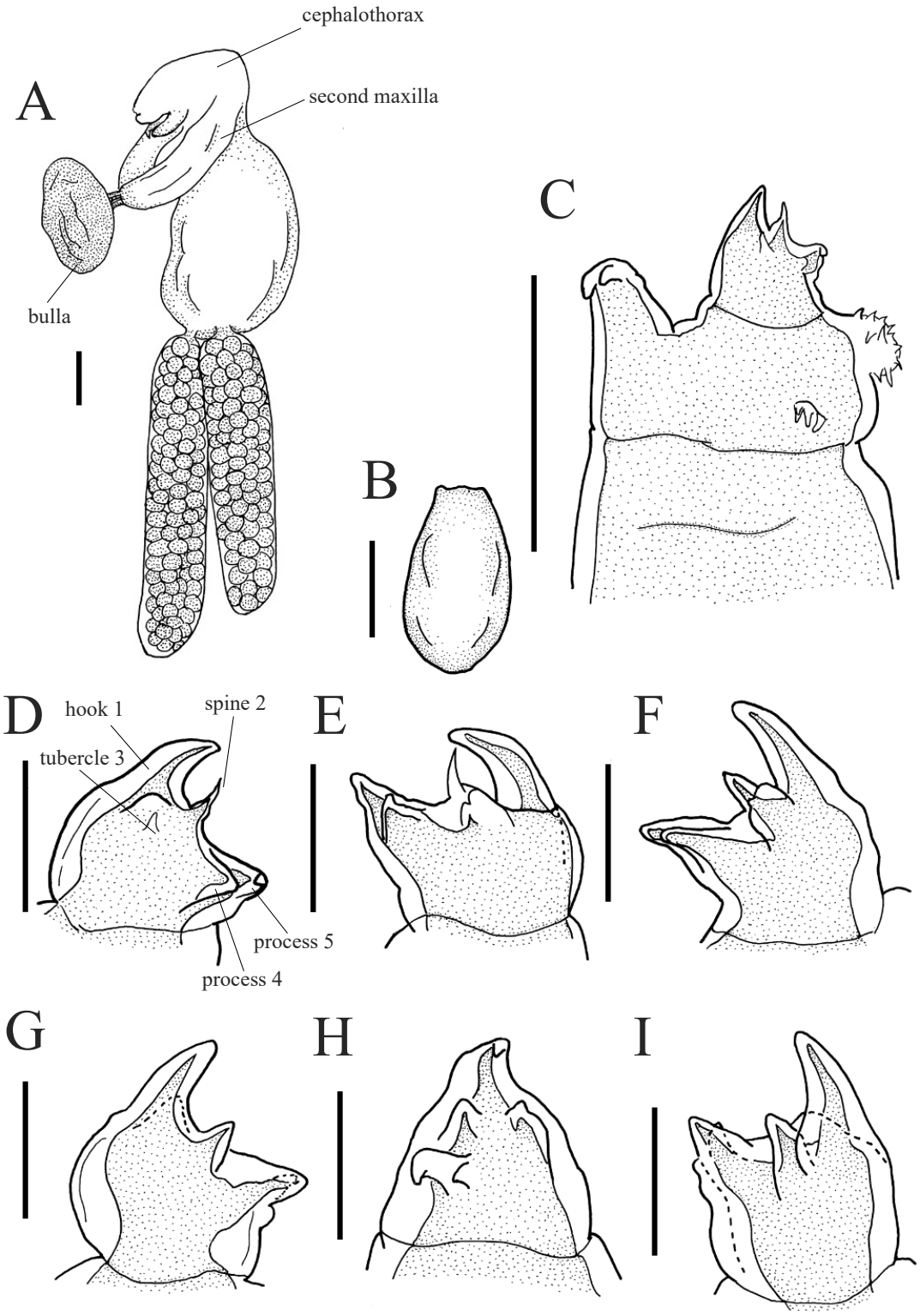

A
cephalothorax
second maxilla
bulla
B
C
D
hook 1
spine 2
tubercle 3
process 5
process 4
E
F
G
H
I

Figure 3. A. Antenna, tip of exopod, lateral (Shiodomari, ID4); B. Same, lateral (Shiodomari, ID7); C. Same, lateral (SSM, ID8); D. Same, lateral (SSM, ID15); E. Same, ventral (Bekanbe-ushi, ID12); F. Same, lateral (Bekanbe-ushi, ID11); G. Same, ventral (Fukushima, ID13); H. Same, lateral (Fukushima, ID14); I. Same, ventral (Toyama, ID9); J. Same, dorsal (Toyama, ID10); K. Spiny pad of endopod, lateral (Shiodomari, ID4); L. Same, lateral (SSM, ID15). Scale bars: A–J. 3μm; K–L. 1μm.

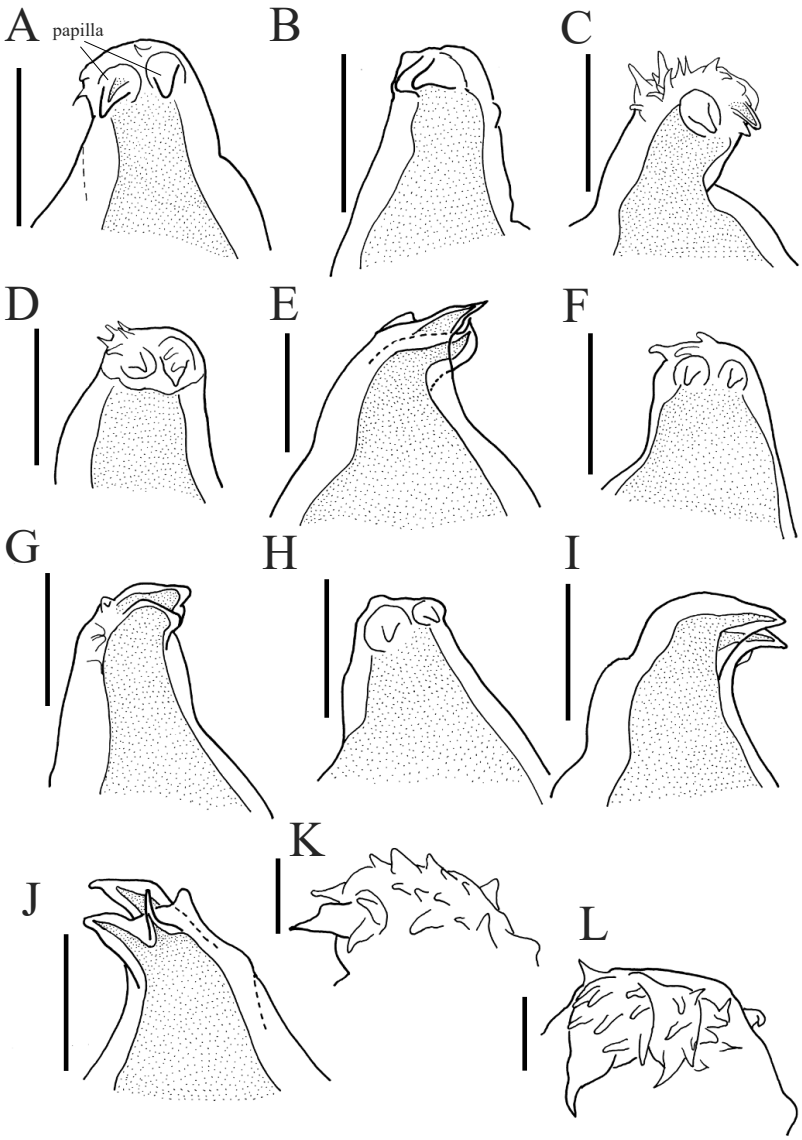


Figure 4. A. Same, lateral (Bekanbe-ushi, ID11); B. Same, lateral (Fukushima, ID13); C. Same, lateral (Toyama, ID10); D. Spiny pad of sympod, lateral (Shiodomari, ID4); E. Same, lateral (SSM, ID15); F. Same, lateral (Bekanbe-ushi, ID11); G. Same, lateral (Fukushima, ID13); H. Same, lateral (Toyama, ID10); I. Mandible, lateral (Shiodomari, ID1); J. Same, lateral (SSM, ID8); K. Same, lateral (Bekanbe-ushi, ID11); L. Same, lateral (Fukushima, ID14); M. Same, lateral (Toyama, ID9). Scale bars: A–H. 1μm; I–M. 3μm.

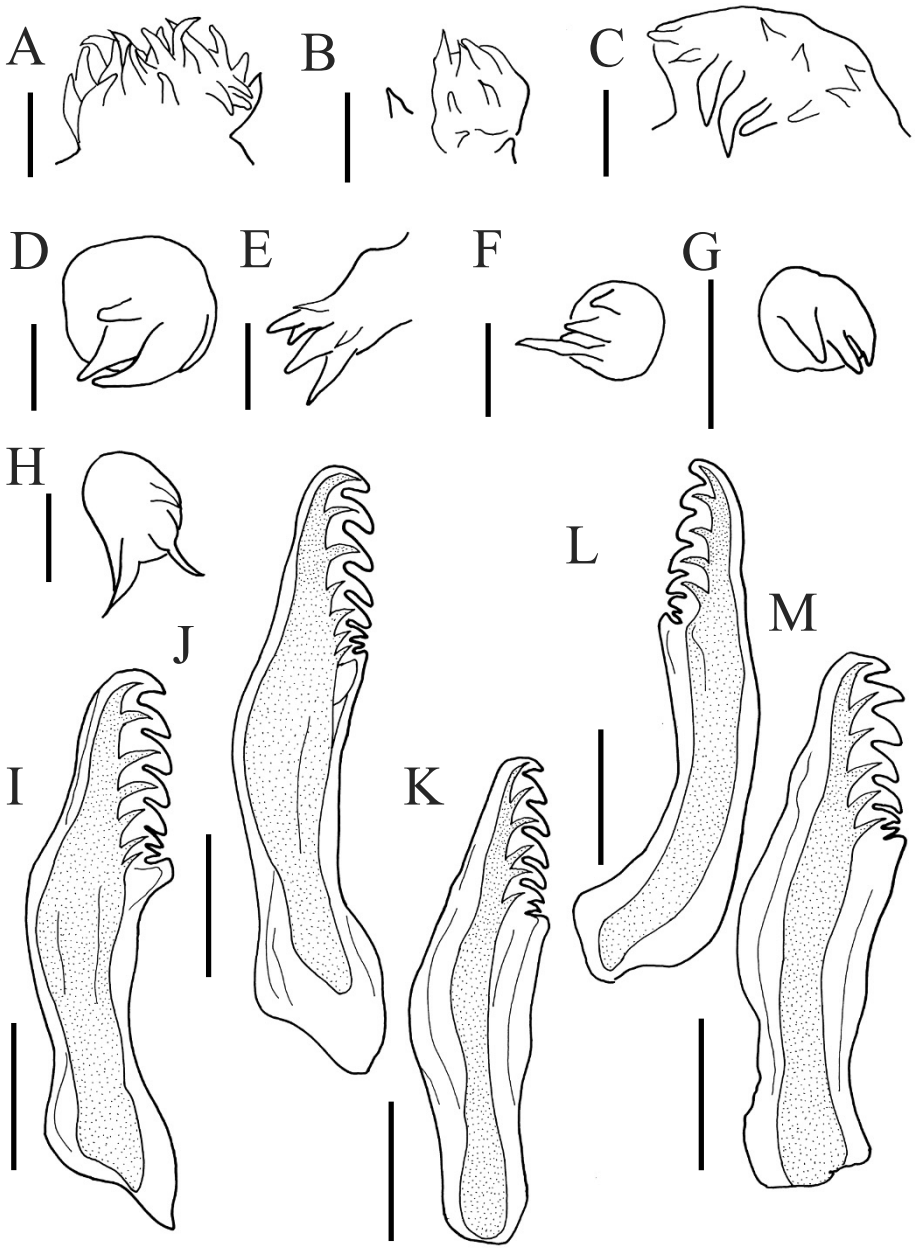


Figure 5. A. Maxilliped, entire, ventral (Shiodomari, ID1); B. Tip of maxilliped, ventral (Shiodomari, ID1); C. Same, ventral (SSM, ID8), D. Same, ventral (Bekanbe-ushi, ID11); E. Same, ventral (Fukushima, ID13); F. Same, ventral (Toyama, ID9); G. Maxilliped palp, ventral (Shiodomari, ID1); H. Same, ventral (SSM, ID8); I. Same, ventral (Bekanbe-ushi, ID11); J. Same, ventral (Fukushima, ID13); K. Same, ventral (Toyama, ID9). Scale bars: A. 4μm; B–K. 3μm.

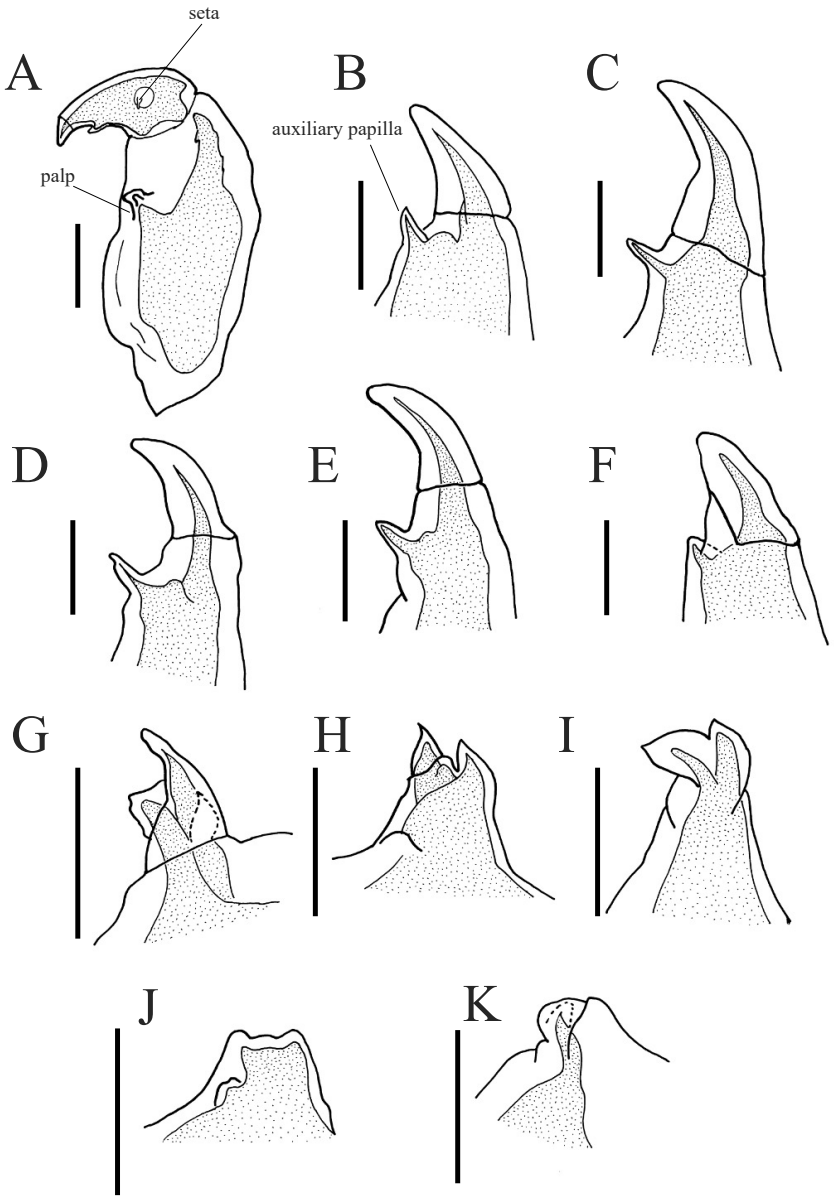
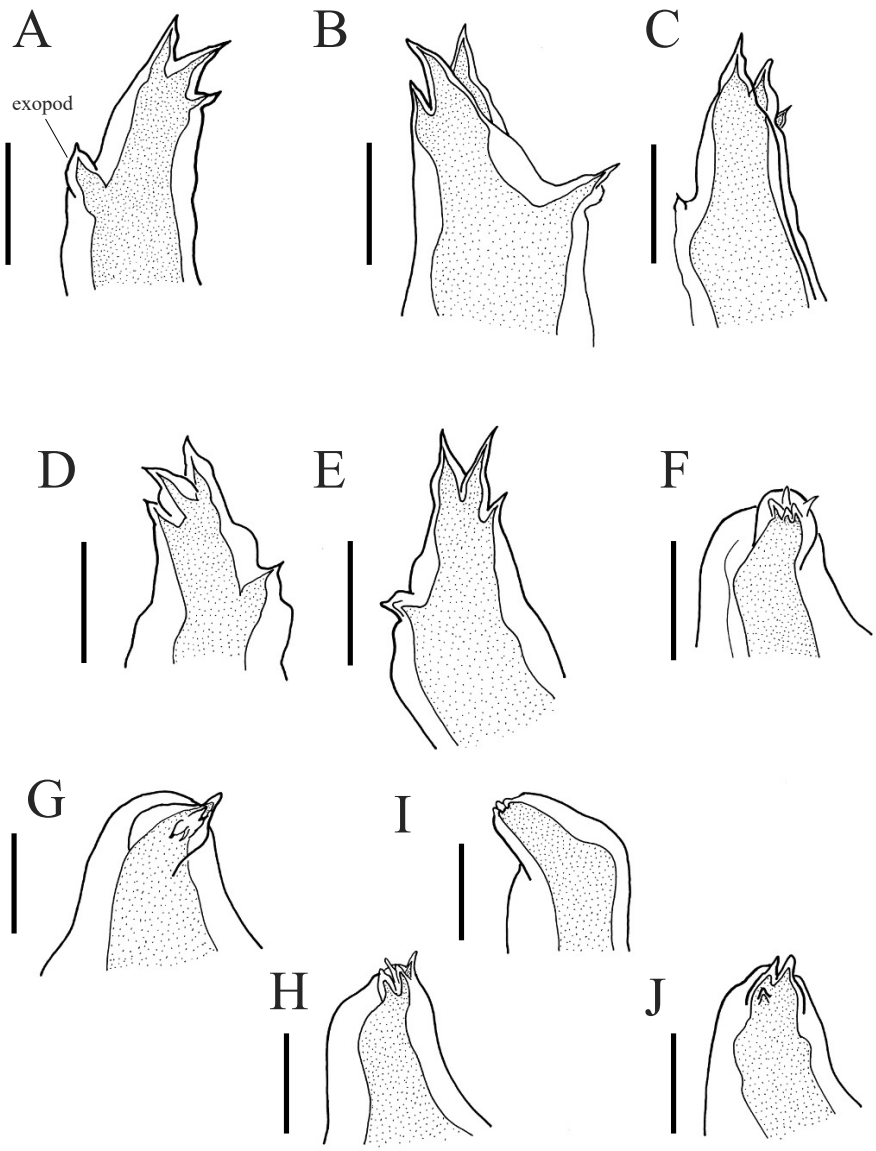


Figure 6. A. Maxillule, lateral (Shiodomari, ID1); B. Same, lateral (SSM, ID15); C. Same, lateral (Bekanbe-ushi, ID11); D. Same, lateral (Fukushima, ID14); E. Same, lateral (Toyama, ID9); F. Antennule, ventral (Shiodomari, ID6); G. Same, lateral (SSM, ID15); H. Same, ventral (Bekanbe-ushi, ID11); I. Same, ventral (Fukushima, ID14); J. Same, ventral (Toyama, ID10). Scale bars: A–J. 3μm.



Appendix paper 2

Basin-wide distribution of *Salmincola* sp. infecting to the mouth cavity of white-spotted charr

Abstract

Understanding parasite distributional patterns is fundamental for elucidating host-parasite relationships. The genus *Salmincola* is an ectoparasitic copepod group specifically infecting freshwater salmonids. Considering their strong association with their hosts, we can predict that the distribution and prevalence (analogues to abundance) of *Salmincola* reflect host salmonids. An alternative hypothesis is that their distribution will be strongly affected by environmental factors like stream drift because they have a free-living stage with low swimming ability. If this is the case, we predict a longitudinal gradient with higher occurrence or infection levels in downstream areas. To estimate the relative strength among factors affecting infection levels, we investigated the distribution pattern of *Salmincola* sp. on wild white-spotted charr *Salvelinus leucomaenis* in a southern Hokkaido river system. Based on data from 19 sites across three seasons, we found that host density and flow velocity affected the prevalence of *Salmincola*. On the other hand, no longitudinal gradient was observed and the prevalence was extremely low in some fragmented habitats (i.e., above dams and waterfalls). This indicates some compensation mechanisms against unidirectional downstream dispersal. We found that parasite prevalence and intensity were much higher in large migratory (anadromous) fish and, therefore, hypothesize that long-distance upstream migration helps the redistribution and population persistence of parasites in upstream areas.

1. Introduction

Parasites account for a large proportion of biomass of living organisms (Dobson et al. 2008; Kuris et al. 2008) and play many important roles in natural systems, such as ecosystem functioning and host population dynamics (Anaya-Rojas et al. 2019; Hudson et al.; 1998; Lafferty et al. 2006; Lafferty et al. 2008; Morton & Silliman 2020; Sato et al. 2012). Despite their potential impacts, however, most parasites have been neglected in ecological studies (Gordy et al. 2020; Poulin 2011), which is partly due to difficulty in finding and identifying them. Accordingly, we have lacked even general patterns for the distribution and abundance of parasites until recently (e.g. Berkhout et al. 2020; Blasco-Costa et al. 2013).

Compared to free-living organisms, parasite distribution can be either simple or complex. For example, some parasites merely follow their host distribution (Arneberg 2002; Hansen & Poulin 2006), resulting in simple distribution patterns. In addition, since parasites *per se* have low mobilities (Poulin 2011), their dispersal and genetic structure mirrors their hosts (Blasco-Costa & Poulin 2013; Criscione & Blouin 2004). However, complex distribution patterns are also expected, because many parasites are affected not only by their hosts but also the external physical environment, that also mediates host abundance and distribution (Berkhout et al. 2020; Grunberg 2021). Physical factors such as temperature, flow velocity and pH level generally affect life-history and transmission of parasites, especially during infectious stages (Baker & Cone 2000; Johnston & Dykeman 1987; Mouritsen 2002; Pietrock & Marcogolies 2003; Thieltges et al. 2008). Although disentangling the factors affecting their distribution is a critical issue in ecology, only a few studies have evaluated the relative importance of ecological factors including host characteristics and external environments affecting parasites (Berkhout et al. 2020; Poulin 1995).

Lotic ecosystems provide a good opportunity to elucidate the relative importance of host characteristics and other external environmental factors on parasite distribution and abundance. This is because unidirectional water flow should be the major physical determinant, especially for parasites that have free-swimming infectious stages (Blasco-Costa et al. 2012, 2013). Passive dispersal by constant stream drift (Müller 1982) generates an infection gradient from upstream to downstream along the river, which may be a common pattern in aquatic parasites (Blasco-Costa et al. 2013). Alternatively, when the influence of host dependency is stronger than stream drift, we will not detect a distributional gradient. This could occur either when host transmission occurs via direct host physical contact, when the transmission window is short, or when host upstream movement compensates for stream drift (e.g. Blasco-Costa et al. 2012).

The genus *Salmincola* (Family Lernaeopodidae), ectoparasitic copepods, and their host salmonids are ideal to evaluate the relative importance of factors affecting parasite distributions in lotic systems. *Salmincola* spp. complete a direct life cycle without intermediate hosts (Kabata & Cousens 1973) and have a short lived (only a few days) free-living stage. Because the infectious copepodids are tiny (i.e. 0.6-0.7 mm; Kabata & Cousens 1973) and seem to have a low swimming ability (Friend 1941; McGladdery & Johnston 1988), they should be strongly affected by stream drift. On the other hand, considering their strong association with their hosts (Kabata 1969), we can also predict that their distribution should be strongly affected by host distribution and dispersal. Salmonids often prefer upstream areas, resulting in high densities (Imanishi 1996; Morita et al. 2016) and they also exhibit long-distanced upstream migration for spawning (Solomon & Templeton 1976). Therefore, we can evaluate the relative strength of host characteristics and the physical environment. That is, if stream drift dominates the parasite distribution, an increase of abundance or occurrence is expected in lower altitude (Blasco-Costa et al. 2012, 2013). Alternatively, if host characteristics

mainly govern parasites distribution, we will observe the opposite pattern because of high host density at higher altitude, as well as upstream host migration.

We tested these predictions by examining the basin-wide distribution pattern of *Salmincola* sp. on wild white-spotted charr (*Salvelinus leucomaenis*) in the Shiodomari River system, southern Hokkaido, Japan. We particularly focused on how host characteristics (density and dispersal) and stream drift (water velocity and altitudinal distribution) affect the infection level of these parasite (i.e. prevalence and intensity). The role of host dispersal can be inferred from the infection level of the migratory (anadromous) form because such individuals are known to undertake long-distance upstream migration before spawning (Quinn 2018). Because *Salmincola* spp. tend to infect larger host individuals (Kabata & Cousens 1977; Bowen & Stedman 1990; Nagasawa et al. 1995), large anadromous fish can carry the parasites in upstream areas effectively. We also evaluated the effects of stream drift and host migration compensation by examining the populations above physical barriers, such as dams and waterfalls. If stream drift and host upstream migration are crucial for the parasite's distribution, we can expect low infection levels in such isolated populations. This is plausible given that even host white-spotted charr often go extinct after dam constructions or show reduced density above dams (Morita & Yamamoto 2002; Morita et al. 2019). Finally, we also examined the effects of other environmental factors, such as water temperature and stream size, which might have affected parasite distributions.

2. Materials and methods

2.1. Study area and species

We conducted our field survey in the Shiodomari River in 2019 (Figure 1). The Shiodomari River system has been designated as a protected freshwater area and is

closed to recreational fishing year-round for all species (Tsuboi & Morita 2004). The upstream areas of this river system are dominated by white-spotted charr, whereas downstream and mainstem are dominated by masu salmon (*Oncorhynchus masou*), freshwater sculpin (*Cottus nozawae*), Siberian stone loach (*Noemacheilus barbatulus*), Japanese dace (*Pseudaspius hakonensis*) and a small number of invasive rainbow trout (*Oncorhynchus mykiss*).

In the Shiodomari River system, white-spotted charr are frequently infected with *Salmincola* sp. (but not other salmonids) and their infections commonly occur in the mouth cavity but not in the gill tissue or on the body surface. To date, five species of the genus *Salmincola* have been recorded from Japan; *S. californiensis* (reported as *S. yamame* in Hoshina & Suenaga 1954; Hoshina & Nishimura 1976; Nagasawa & Urawa 2002), *S. carpionis* (reported as *S. falculata* in Yamaguti 1939; Nagasawa et al. 1995; Nagasawa & Urawa 2002; Nagasawa & Sakaki 2020), *S. stellata* (Nagasawa & Urawa 1991; Nagasawa et al. 1994; Hiramatsu et al. 2001), *S. edwardsii* (Nagasawa 2020a, b; Nagasawa & Kawai 2020) and *S. markewitschi* (Nagasawa 2020c; Nagasawa & Ishiyama, 2021). *S. carpionis* and *S. markewitschi* mainly infect the mouth cavity of the genus *Salvelinus* (Kabata 1969; Nagasawa et al. 1995; Shedko & Shedko 2002). Based on the taxonomic studies proceeded by the first author (R. Hasegawa), we confirmed by genetic analysis that only a single species was present in the river system. However, since morphological variation was quite large, possessing the characteristics of both *S. carpionis* and *S. markewitschi* (Hasegawa unpublished data), we could not conclude which scientific names should be applied without analyzing additional specimens from other areas. Thus, we treated the tentative species as *Salmincola* sp. Heavy infections of *Salmincola* spp. can cause various impacts on host fish in hatchery environments (Herron et al. 2018; Kabata & Cousens 1977; Nagasawa et al. 1998; Sutherland &

Wittrock 1985), whereas the impacts on wild fishes have rarely been reported or may be negligible, possibly due to the low prevalence and intensity in natural conditions (e.g. Amundsen et al. 1997; Ayer et al. 2022; but see Mitro 2016).

White-spotted charr in the Shiodomari River have two types of life-history: some individuals remain in rivers and reproduce as residents, whereas other individuals migrate to the sea or lakes and return to their natal rivers to spawn as migrants (Yamamoto et al. 1992; Morita 2001; Morita et al. 2019). Sea-run or anadromous forms can be infected by *Salmincola* sp. because salinity tolerance is often reported in other members of the genus (Black et al. 1983; Friend 1941; Nagasawa 1998). Above natural waterfalls and man-made dams (i.e., closed-populations), white-spotted charr have a non-anadromous life history (residents; Morita et al. 2000).

2.2. Fish collection and measurement

Sampling was carried out at 17, 15 and 14 sites during three separated seasons (May, July, October), respectively (i.e., 19 sites in total) (Figure 1, Table 1, Supporting Information). Two sampling tributaries have natural waterfalls (head water area of Ito River; Figure 1) and three tributaries have impassable dams installed to control erosion (Sasagoya Stream, Nishimata Stream, Sentarosawa Stream; Figure 1). A high-dam (Yabetsu reservoir) was constructed in the upper area of the main stem (Figure 1). While no fish can access upstream areas above impassable dams from downstream areas, a few fish, including anadromous forms (migrants), can pass some small waterfalls (i.e. Site 16, 18, R. Hasegawa, unpublished data).

In May and October, fish were collected within 100-300 m reaches using a backpack electro-fisher (model 12B; Smith-Root, Inc.) in each site. At each site, we tried to collect at least 30 host individuals to estimate reliable parasite abundance data. In addition, at the sites where dams or waterfalls were present, we started sampling within 150 m from above or below the barrier to compare the infection levels between them, although we could not catch fish in these areas at two sites (Site 17, 19) due to the difficulty of the approach. During July sampling, we estimated host density (see 2.4 Host density estimation) and measured physical environmental variables at 11 sites within a 100 m area as described in the next section. If we could not collect more than 10 fish in a reach, we sampled for additional fish outside of the sampling area (but within the same reach as May and October).

In May, we captured age-1 and older individuals, whereas we did not capture newly emerged fries (age-0) because the average fork length of fries was less than 50 mm in May (Yamamoto & Kato 1984) and a previous study showed that fish less than 50 mm were rarely infected with *Salmincola* sp. (Barndt & Stone 2003). In July, to check the infection pattern, captured fish were categorized into two age classes; age-0 (ca., less than 78 mm) and age-1 and older, based on visual observation and bimodal frequency in a histogram. During the breeding season (October), in addition to the classification of age-0 (ca., less than 89 mm), the age-1 and older individuals were categorized into two life history types: resident and migrant, determined according to their body size and coloration as follows (Ishigaki 1984; Yamamoto et al. 1999). (i) *Residents* were usually brownish and had many small white spots on the sides of the body. The abdomen had a characteristic yellow tinge. (ii) *Migrants* showed silver body color with relatively large white spots on the sides of the body. Some migrants were captured from additional reaches because it was difficult to collect enough samples at

each site. In addition, we also captured masu salmon (in July and October) and rainbow trout (in May and October) to confirm whether infection had occurred or not. Captured fish were anesthetized with FA100 (DS Pharma Animal Health Co., Ltd.) and measured for fork length (FL) to the nearest 1 mm. Since the main attachment sites of *Salmincola* sp. parasitic on white-spotted charr are the body surfaces and buccal cavities (Nagasawa et al. 1995; Nagasawa 2020c), we examined fish body surfaces and buccal cavities for the presence of copepods. When found, we counted the number of individuals and recorded their attachment sites. All the copepods detected were considered as females, because the males are dwarf, attaching to females, and difficult to observe by the naked eye (Kabata & Cousens 1973). As no copepods were found on newly emerged white-spotted charr fries (age-0), we excluded these hosts from all calculations and analyses. In addition, migrants were also excluded from calculations and analyses because of their high infection levels and significant body size differences as discussed below.

2.3. Measurements of the physical environment

Physical environmental factors such as water temperature and flow velocity can influence the development, infection and abundance of *Salmincola* spp. (Conley & Cutis 1993; Mitro & Griffin 2018; Monzyk et al. 2015; Vigil et al. 2016). Therefore, we measured multiple physical environmental factors (water temperature, stream width, stream depth, substrate score, flow velocity) at 11 sites in July (Figure 1; Supporting Information). We established seven measurement points on 11 transects per 100 m reach (i.e., 77 measurement points in total), which were equally spaced longitudinally along each of the sites. Only the flow velocity was measured in the middle of each measurement point (6 points per 1 transect), resulting in a total of 66 measurement points. Water temperatures were recorded with HOBO data loggers (Onset Computer

Corporation, Bourne, MA) from July to October. We set each logger near the riverbed (about 0.3-1.0 m depth) and measured temperature at 1h intervals beginning on the 24th to 31st of July and until the 19th to 24th of October. Stream widths were measured on each transect (i.e., 11 measurement transects). Stream depths were measured on each measurement point. The dominant substrate was visually classified into seven categories and scored as follows: 1, silt and sand (< 2 mm); 2, gravel (2-16 mm); 3, pebble (16-64 mm); 4, cobble (64-256 mm); 5, boulder (> 256 mm); 6, bedrock, a system modified from Bain et al. (1985). Flow velocity was measured with a propeller-type meter (CR-11; Cosmo-Riken, Osaka, Japan) at about 60 % of the depth from the surface to the bed. All variables were calculated in averages for each site and used for the principal components analysis (PCA) and a generalized linear mixed models (GLMMs) as described below. Elevation (m) for each site was determined using 1:25000 scale topographic maps (http://maps.gsi.go.jp) and also included PCA analysis.

2.4 Host density estimation

In general, host density can be a strong predictor of parasite abundance (Anderson & May 1978; Hansen & Poulin 2006). Thus, we estimated charr density in the same reaches as used for environmental factor measurements in July as described above. Charr abundance was estimated by a two-pass removal method (e.g., Riley & Fausch 1992). We set block nets at both ends of the reach to prevent fish from entering or leaving during the sampling. White-spotted charr abundance was calculated by using the model M (bh) in program CAPTURE (White et al. 1978). Reach wide density of charr (number / m^2) was calculated by dividing the estimated number of charr by the reach area (m^2) (Supplementary material 1).

2.5. Statistical analysis

We calculated prevalence (percentage of individuals infected), intensity (the number of individual parasites in a single infected fish) and mean intensity (the average number of parasites among the infected fish) following Bush et al. (1997).

To summarize physical environmental factors (elevation, water temperature, stream width, stream depth, substrate score, flow velocity), we used a principal component analysis (PCA). Only principal components (PC) showing eigenvalue greater than one (Kaiser–Guttman criterion) were selected for further analysis. This resulted in two principal components describing all factors (Table 2).

During the population level analysis, we examined if the prevalence was affected by each principal component, host density (calculated from age-1 and older individuals) and habitat types (i.e., closed or open) by using GLMM with a binomial error distribution and logit link function. The response variable, prevalence, was the binary variable defined as (n, N-n), where n and N indicate number of infected individuals and number of all individuals at each population (i.e., N-n indicates numbers of uninfected individuals), respectively. Explanatory variables were PC1 (continuous variable), PC2 (continuous variable), host density (continuous variable) and habitat type (categorical variable; closed, open). Sampling sites and season (May, July, October) were treated as random effects. Statistical significance ($p \leq 0.05$) was evaluated by likelihood ratio test between the full model and reduced model. We did not include the interaction terms between habitat type and other variables, because preliminary analysis showed any significant effects. Thus, the full model as follows:

(n, N-n) ~ PC1 + PC2 + host density + habitat type (closed, open) + (1 | sites) + (1 | seasons).

Of the 19 sites we captured fish from, for only 11 sites physical environment measurements and charr abundance estimations were conducted. In addition, because we lost water temperature loggers at 3 sites, we conducted PCA analysis using the data of 8 sites. To minimize the effect of this smaller sample size, we firstly conducted GLMM only with elevation and habitat type (closed, open), but without PCs and host density (i.e., 19 sites) and subsequently included all the variables into the analysis (i.e., 8 sites).

To consider the effects of host body size on infection, we also performed an individual level analysis. In this analysis, we constructed GLMM with a binomial error distribution to examine if the probability of infection was affected by fish size and population type (closed or open). The response variable was the binary variable that defined infected or uninfected (infected = 1, uninfected = 0) and explanatory variables were FL and habitat type (closed, open). Sampling sites and season were treated as random effects. Statistical significance ($p \leq 0.05$) was evaluated by likelihood ratio test between the full model and reduced model. Finally, we compared the infection prevalence and mean intensity between residents and migrants in October by Fisher's exact test and Wilcoxon rank-sum test, respectively. We used the package lme4 (Bates et al. 2011) for the mixed model procedures. All the statistical analyses were conducted using R version 3.5.2 (R Core Team 2018).

3. Results

3.1. Basin-wide distribution of *Salmincola* sp.

Salmincola sp. infections on white-spotted charr were present in 15 sites and absent in 4 sites (Table 1). Average prevalence was 26.4 % (0.00–53.6 %) in May, 19.4 % (0.00–38.9 %) in July and 14.3 % (0.00–46.7 %) in October. Average mean intensity was 1.95 (1.00–4.46) in May, 1.54 (1.00–2.50) in July and 1.70 (1.00–2.17) in October. All individual copepods were found from the buccal cavities of age-1 and older white-spotted charr, whereas no copepods were found from newly emerged fries (mean FL: 63.3 mm [39–89 mm]; n = 384), nor other salmonid fishes such as rainbow trout (mean FL: 152.2 mm [90–327 mm]; n = 40) and masu salmon (mean FL: 98.0 mm [49–223 mm]; n = 353).

Contrary to the initial prediction, elevation positively affected the prevalence in the population level analysis (i.e. 19 sites; Table 3a), whereas the significant effect disappeared in the additional analysis (i.e. 8 sites, Table 3b). Differences of prevalence between above and below dam sites were evident even in the same stream (Figure 2, Table 1): GLMM showed that prevalence in closed populations were significantly lower than those of open populations (Table 3a, b), consistent with the second prediction. In two out of three above dam areas (Site 4, 10), no copepods were found across all seasons (Table 1). Although we found two individual copepods at the other closed population above a dam in May (Site 1; Table 1a), the prevalence was evidently low and no copepods were found in July and October (Table 1b, c). The individual level analysis also showed that hosts caught in closed populations showed significantly lower probability of infection (Likelihood-ratio test; G^2 = 5.20, p = 0.02; Figure 3), as well as

a positive effect of fork length on the probability of infection (G^2 = 186.44, p < 0.01; Figure 3).

3.2. Environmental factors affecting the abundance of *Salmincola* sp.

PCA compressed the environmental data into two principal components (PCs) (Table 2). PC1 and PC2 covered 84 % of the total variance (Table 2). Water temperature, stream depth and stream width loaded positively on PC1, whereas elevation and substrate score loaded negatively (Table 2). Flow velocity loaded negatively on PC2 (Table 2).

While PC1 had no significant effect on prevalence, PC2 had a significant negative effect on prevalence, meaning that higher prevalence was detected at sites with higher flow velocity (Table 3b). Also, host density had a significant positive effect on prevalence (Table 3b).

3.3 Comparison of infection level between resident and migratory host fish

Migrant white-spotted charr (n = 21; mean ± SD FL: 358.33 ± 93.07 mm) showed higher prevalence and mean intensity compared to residents (n = 439; mean ± SD FL: 161.34 ± 43.87 mm) (Figure 4). While 15.0 % of residents (n = 66) were infected with *Salmincola* sp., 76.2 % of migrants (n = 16) were infected (Fisher's exact test; p < 0.01). Mean intensity of migrants (3.56 parasites per infected fish) were more than two times higher than that of residents (1.59 parasites per infected fish, Wilcoxon rank-sum test; W = 3232.5, p < 0.01; Figure 4).

4. Discussion

This is one of few studies demonstrating the relative importance of host characteristics and other external factors affecting parasite distribution and/or abundance. We found that host density positively affects parasite prevalence and large migratory fish had much higher prevalence and intensity. No altitudinal distribution was detected, but the prevalence was extremely low in stream reaches above physical barriers. Together, our results suggest that while stream drift is acting in the study system as inferred from low prevalence above barriers, that effect is compensated by high host density in upstream areas and also by upstream migration of large anadromous individuals. This contradicts the presumed “general" pattern of stream parasites (Blasco-Costa et al. 2013) and the ecological mechanisms against the pattern are proposed as below.

4.1. Basin-wide distribution pattern of *Salmincola* sp.

According to previous studies on other aquatic parasites, an infection gradient from upstream to downstream along a river might be a common distribution pattern (Blasco-Costa et al. 2013). Unexpectedly, prevalence for *Salmincola* sp. exhibited positive or no trend with elevation in the present study, suggesting that populations of *Salmincola* sp. can persist in upstream areas even though the swimming ability of the free-living infective stage is low (Friend 1941; Monzyk et al. 2015). A similar phenomenon is known as the “stream drift paradox” where populations of drift-affected aquatic species remain in upstream areas despite the tendency for larvae to drift downstream (e.g., Müller 1982). Some studies have reported that upstream movements by adult aquatic insects may compensate downstream drift of the larvae (see Brittain & Eikeland 1988). The fact that no negative trend was observed in the present study,

implies that other factors compensating for their downstream dispersal may exist in this system.

One of the possible explanations is the spawning migration of host fish. Salmonids, including white-spotted charr, generally move upstream to spawn (Nakamura 1999; Solomon & Templeton 1976). Through this process, *Salmincola* sp. can be transferred by host fishes to upstream areas and among open populations, resulting in population persistence in upstream reaches. Similarly, other studies have already shown that some parasites had no gradient in their abundance along rivers, suggesting that the dispersal abilities of their definitive hosts altered the gradient (Blasco-Costa et al. 2013; Paterson et al. 2019). In particular, migrants may play an important role in the recruitment of copepods during spawning. We found that migrants showed a much higher infection level than residents, which was probably due to their larger body size (Kabata & Cousen 1977; Bowen & Stedman 1990). Migrants return to rivers from the sea during the summer and move upstream to spawn during the autumn (Morita 2001). This long-distance movement from downstream to upstream by highly infected migrants may markedly compensate the copepod's drift from upstream to downstream. However, although some species of the genus *Salmincola* have salinity tolerance (Black et al. 1983; Nagasawa 1998), it remains unclear if this species can survive in saltwater while migrant charrs live in the ocean. It is also unclear during what period migrants are most likely to be infected. More studies are needed to prove this hypothesis.

Skewed distribution and abundance of host fish may also contribute to the population persistence of the copepods in upstream areas, because host density may be the strongest predictor for parasite abundance (Anderson & May 1978; Hansen & Poulin 2006). The density of white-spotted charr is generally higher in upstream reaches because they prefer cold water (Imanishi 1996) and shelter such as rock interstices are

abundant in upstream reaches (Morita et al. 2016). In addition, white-spotted charr tend to prefer upstream habitats when masu salmon co-occur because of interspecific competition (Miyasaka et al. 2003; Nakano 1995), which may be the case in the present study. In fact, host density had a significant positive effect on prevalence and there was a significant positive relationship between host density and elevation in the present study (Pearson's correlation: $r = 0.84$, $p < 0.01$, $n = 11$). Therefore, unidirectional drift may be compensated by high host density in upstream reaches.

Strikingly, we detected a significantly lower infection level in closed populations, especially above dams, suggesting that some populations of *Salmincola* sp. (site 4, 10) had already gone extinct as we predicted. While host fish and their parasites can access open populations freely, they cannot access closed populations from downstream (Morita et al. 2000; Yamamoto et al. 2004). In addition, the copepods would be washed away from the above dam areas by stream drift. This process may cause the extinction of the copepods in some closed populations. This prediction does not contradict with the fact that extremely low prevalence was observed above dams, where migrant forms cannot access.

Extinction of closed populations of copepods could also be accompanied by extinction of the host. Since dams or waterfalls prevent fish from reaching upstream habitats, once they emigrate to areas downstream from these barriers, they are unable to return for reproduction, leading to gradual isolation or extirpation of the upstream population (Morita et al. 2000; Morita & Morita 2007; Yamamoto et al. 2004). Therefore, habitat fragmentation by damming decreases the population size and genetic diversity, and hence increases the extinction rate of freshwater fish (Morita & Yamamoto 2002; Yamamoto et al. 2004). In fact, Morita & Yamamoto (2002) predicted that habitat fragmentation by damming decreases the population size of white-spotted charr, and therefore cause local extinctions. Moreover, Morita et al. (2019)

re-investigated the same populations as the previous study and confirmed that extinction had already occurred in some of these populations. By these mechanisms, *Salmincola* sp. can easily go extinct when white-spotted charr populations are fragmented by dams, because local extinction of host-specific parasites is likely to occur faster than its hosts (Rózsa 1992).

4.2 Environmental factors affecting the infection level

Although there were no significant effects with PC1 (loaded with elevation, temperature, stream depth and width, substrate score), we found significant effects of PC2 (negatively correlated with flow velocity) on prevalence. This result is not consistent with previous studies that found fishes in streams having lower infection levels than lakes, where lower flow may contribute to their infection (Monzyk et al. 2015). As we discussed above, distribution and density of white-spotted charr were highly skewed toward upstream areas, where high water flow and low water temperature is generally observed. This skewed distribution of host fish may interact with other variables, and hence cause these unexpected results. Another possibility is the matter of scale. We measured the average water velocity at the reach level, but the velocity strongly varied at smaller scales, such as pools and riffles. For example, Morita et al. (2016) showed that the average water velocity was lower in upstream reaches compared to lower reaches in a high-gradient river and this was because there were more turbulent and slow flowing microhabitats in the upper reaches created by step-pool geomorphological structures. Therefore, to determine the limiting factors of their distribution, we need to investigate the factors affecting their distribution across a variety of scales (e.g., Fausch et al. 1994).

Figure 1. Map of the sampling tributaries in the Shiodomari River system, southern Hokkaido, Japan. Detailed information on each tributary is shown in Supplementary material 1. Underlined site represents the sites where measurements of environmental factors and fish abundance estimation were conducted. Gray and black circles indicate closed and open populations, respectively.

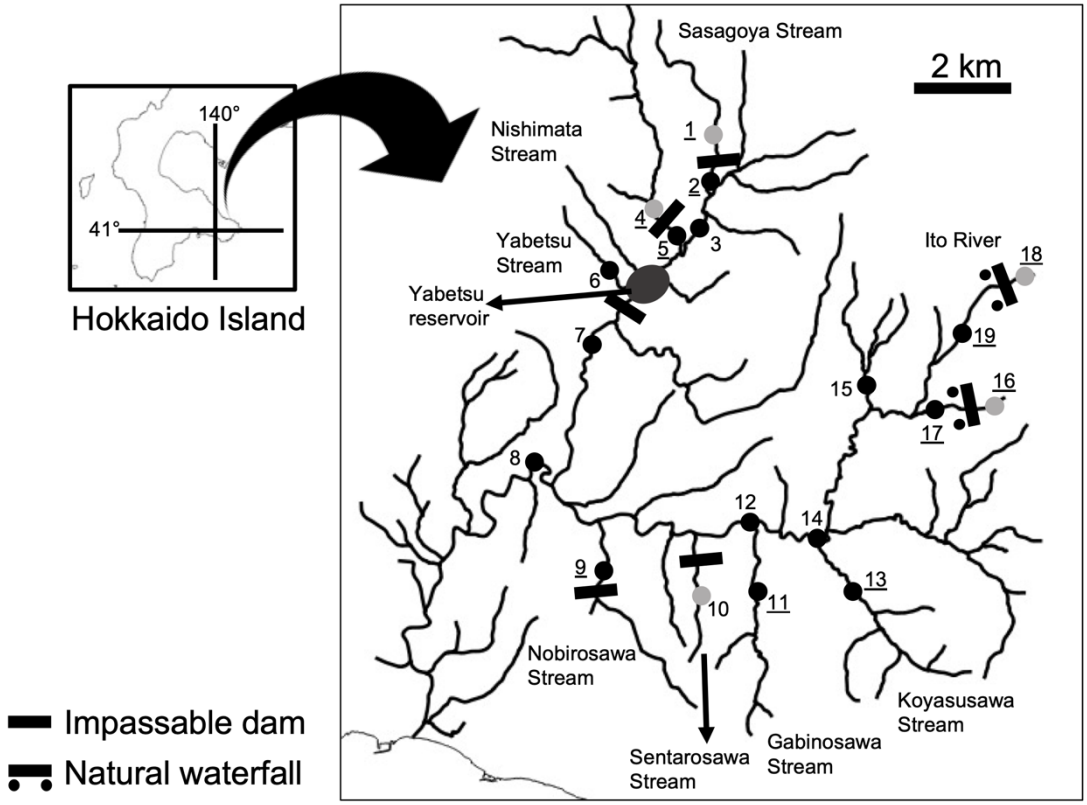


Figure 2. Relationship between infection prevalence and elevation in each season. Open circles and crosses indicate open and closed populations, respectively. Different plots scattered at the same elevation indicate different season (May, July, October).

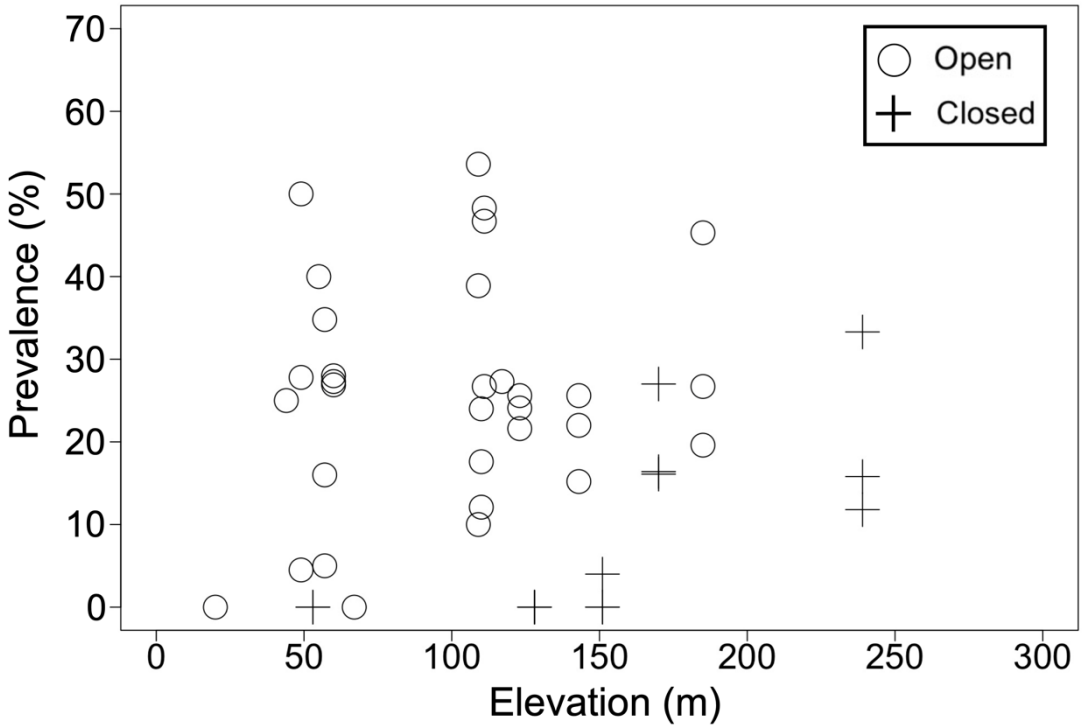


Figure 3. The relationships between parasite infections and host fork length. (a) Logistic relationships between infection probability and fork length. Open circles and crosses indicate open and closed populations, respectively. The curves were estimated by a generalized linear mixed model (GLMM) with binomial error distribution. Barplots indicate the ratio of infected fish for each fork length class. (b) open and (c) closed populations.

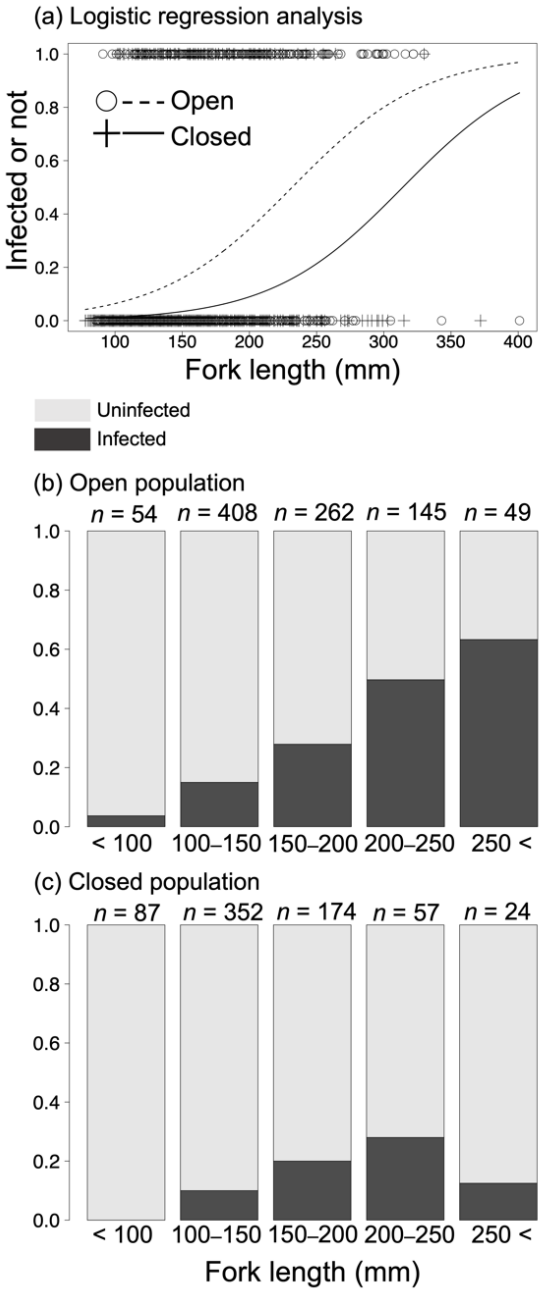
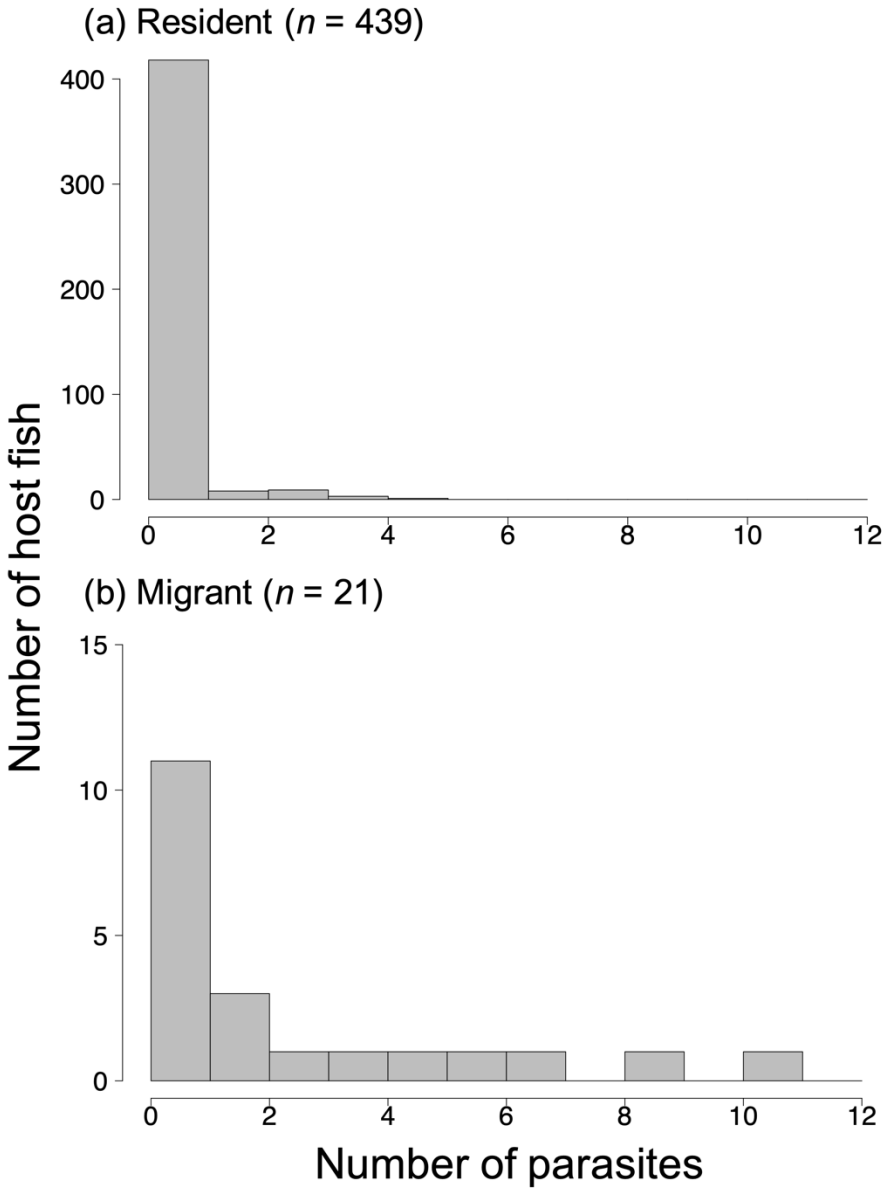


Figure 4. Comparison of parasite number between (a) residents and (b) migrants of white-spotted charr during the spawning season (October).



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Supplementary material 1. Summary of physical characteristics and fish density at each study site in the Shiodomari River system.

Sampling site	1	2	3	4	5	6	7	8
Population type	Closed	Open	Open	Closed	Open	Open	Open	Open
Elevation (m)	151	143	110	128	109	117	67	20
Physical characteristics								
Stream Width (cm)	359	361	-	516	593	-	-	-
Stream depth (cm)	8.4	10.8	-	19.5	18.1	-	-	-
Flow velocity (m/s)	13.8	25.6	-	20.6	28.5	-	-	-
Water temperature (℃)	15.15	-	-	14.98	15.14	-	-	-
Substrate score	3.36	4.26	-	2.49	3.52	-	-	-
Host population structure			-			-	-	-
Age-0 density	0.16	0.15	-	0.00	0.03	-	-	-
Age-1< density	0.07	0.13	-	0.07	0.05	-	-	-
All density	0.22	0.28	-	0.07	0.07	-	-	-

9	10	11	12	13	14	15	16	17	18	19
Open	Closed	Open	Open	Open	Open	Open	Closed	Open	Closed	Open
57	53	49	44	60	55	111	170	123	239	185
330	-	425	-	453	-	-	288	337	245	312
8.5	-	12.5	-	15.2	-	-	9.2	9.1	8.5	10.4
20.8	-	29.2	-	42.8	-	-	43.2	24.1	21.1	31.6
-	-	14.83	-	-	-	-	13.72	14.26	13.86	14.12
3.26	-	3.24	-	3.91	-	-	4.53	2.98	4.08	4.08
	-		-		-	-				
0.02	-	0.01	-	0.01	-	-	0.09	0.22	0.21	0.15
0.02	-	0.01	-	0.01	-	-	0.36	0.18	0.58	0.18
0.04	-	0.03	-	0.02	-	-	0.47	0.40	0.79	0.33

References

Abaunza P, Arroyo NL, Preciado I (2001) A contribution to the knowledge on the morphometry and the anatomical characters of *Pennella balaenopterae* (Copepoda, Siphonostomatoida, Pennellidae), with special reference to the buccal complex. Crustaceana 74: 193–210.

Abrahms B, Hazen EL, Aikens EO, Savoca MS, Goldbogen JA, Bograd SJ et al. (2019) Memory and resource tracking drive blue whale migrations. Proc Natl Acad Sci 116: 5582–5587.

Akaike H (1983) Information measures and model selection. Bull Int Stat Inst 44: 277–291.

Allen JE, Sutherland TE (2014) Host protective roles of type 2 immunity: Parasite killing and tissue repair, flip sides of the same coin. Seminar Immunol 26: 329–340.

Almaraz P (2005) Ecological time-series analysis through structural modelling with latent constructs: concepts, methods and applications. Comptes Rendus Biologies 328: 301–316.

Altizer S, Ostfeld RS, Johnson PT, Kutz S, Harvell CD (2013) Climate change and infectious diseases: from evidence to a predictive framework. Science 341: 514–519.

Amundsen PA, Kristoffersen R, Knudsen R, Klemetsen A (1997) Infection of *Salmincola edwardsii* (Copepoda: Lernaeopodidae) in an age-structured population of Arctic charr—a long-term study. J Fish Biol 51: 1033–1046.

Amundsen PA, Sánchez-Hernández J (2019) Feeding studies take guts–critical review and recommendations of methods for stomach contents analysis in fish. J Fish Biol 95: 1364–1373.

Anderson RM, May RM (1978) Regulation and stability of host-parasite population interactions: I. regulatory processes. J Anim Ecol 219–247.

Anonymous (1989) The present status of salmonid rearing and exhibition. Bull Sapporo Salmon Museum 1: 28–34 (In Japanese).

Anonymous (2006) The status of salmonid rearing, exhibition, and egg stripping for fiscal year 2004. Bull Sapporo Salmon Museum 18: 6 (In Japanese).

Arizono N, Yamada M, Tegoshi T, Yoshikawa M (2012) *Anisakis simplex* sensu stricto and *Anisakis pegreffii*: biological characteristics and pathogenetic potential in human anisakiasis. Foodborne Pathog Dis 9: 517–521.

Arlinghaus R, Laskowski KL, Alós J, Klefoth T, Monk CT, Nakayama S et al. (2017) Passive gear-induced timidity syndrome in wild fish populations and its potential ecological and managerial implications. Fish Fish 18: 360–373.

Arlinghaus R, Abbott JK, Fenichel EP, Carpenter SR, Hunt LM, Alós J et al. (2019) Governing the recreational dimension of global fisheries. Proc Natl Acad Sci 116: 5209–5213.

Armstrong JB, Takimoto G, Schindler DE, Hayes MM, Kauffman MJ (2016) Resource waves: phenological diversity enhances foraging opportunities for mobile consumers. Ecology 97: 1099–1112.

Arneberg P, Folstad I, Karter AJ (1996) Gastrointestinal nematodes depress food intake in naturally infected reindeer. Parasitology 112: 213–219.

Arneberg P (2002) Host population density and body mass as determinants of species richness in parasite communities: comparative analyses of directly transmitted nematodes of mammals. Ecography 25: 88–94.

Arnott SA, Barber I, Huntingford FA (2000) Parasite–associated growth enhancement in a fish–cestode system. Proc R Soc Lond B Biol Sci 267: 657–663.

Arriero E, Pérez-Tris J, Ramírez A, Remacha C (2018) Trade-off between tolerance and resistance to infections: an experimental approach with malaria parasites in a passerine bird. Oecologia 188: 1001–1010.

Atkinson EM, Bateman AW, Dill LM, Krkošek M, Reynolds JD, Godwin SC (2018) Oust the louse: leaping behaviour removes sea lice from wild juvenile sockeye salmon *Oncorhynchus nerka*. J Fish Biol 93: 263–271.

Ayer CG, Morita K, Fukui S, Koizumi I (2022) No apparent effects of the buccal cavity attaching parasite, *Salmincola* sp. (Copepoda: Lernaeopodidae), on a stream salmonid: a mark-recapture study. Ichthyol Res 69: 261–266.

Bagamian KH, Heins DC, Baker JA (2004) Body condition and reproductive capacity of three-spined stickleback infected with the cestode *Schistocephalus solidus*. J Fish Biol 64: 1568–1576.

Bain MB, Finn JT, Booke HE (1985) Quantifying stream substrate for habitat analysis studies. N Am J Fish Manag 5: 499–500.

Baines CB, Diab S, McCauley SJ (2020) Parasitism risk and infection alter host dispersal. Am Nat 196: 119–131.

Barber I, Huntingford FA (1995) The effect of *Schistocephalus solidus* (Cestoda: Pseudophyllidea) on the foraging and shoaling behaviour of three-spined sticklebacks, *Gasterosteus aculeatus*. Behaviour 132: 1223–1240.

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

Barker DE, Cone DK (2000) Occurrence of *Ergasilus celestis* (Copepoda) and *Pseudodactylogryrus anguillae* (Monogenea) among wild eels (*Anguilla rostrata*) in relation to stream flow, pH and temperature and recommendations for controlling their transmission among captive eels. Aquaculture 187: 261–274.

Barndt S, Stone J (2003) Infestation of *Salmincola californiensis* (Copepoda: Lernaeopodidae) in wild coho salmon, steelhead, and coastal cutthroat trout juveniles in a small Columbia River tributary. Trans Am Fish Soc 132: 1027–1032.

Bartholomew JL, Kerans BL, Hedrick RP, Macdiarmid SC, Winton JR (2005) A risk assessment based approach for the management of whirling disease. Rev Fish Sci 13: 205–230.

Bates D, Maechler M, Bolker B, Walker S, Christensen RHB, Singmann H, Grothendieck G (2011) Package "*lme4*". Linear mixed-effects models using S4 classes. R package version 1.

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67: 1–48.

Becker DJ, Albery GF, Kessler MK, Lunn TJ, Falvo CA, Czirják GA et al. (2020) Macroimmunology: The drivers and consequences of spatial patterns in wildlife immune defense. J Anim Ecol 89: 972–995.

Begon M, Harper JL, Townsend CR (1986) Ecology. Individuals, populations and communities. Hoboken, New Jersey, USA: Blackwell scientific publications.

Beldomenico PM, Telfer S, Gebert S, Lukomski L, Bennett M, Begon M (2008) Poor condition and infection: a vicious circle in natural populations. Proc R Soc Lond B Biol Sci 275: 1753–1759.

Beldomenico PM, Telfer S, Gebert S, Lukomski L, Bennett M, Begon M (2009a) The vicious circle and infection intensity: the case of *Trypanosoma microti* in field vole populations. Epidemics 1: 162–167.

Beldomenico PM, Telfer S, Lukomski L, Gebert S, Bennett M, Begon M (2009b) Host condition and individual risk of cowpox virus infection in natural animal populations: cause or effect? Epidemiol Infect 137: 1295–1301.

Beldomenico PM, Begon M (2010) Disease spread, susceptibility and infection intensity: vicious circles? Trends Ecol Evol 25: 21–27.

Berkhout BW, Borregaard MK, Brandl R, Brändle M, Dehling DM, Hof C et al. (2020) Host assemblage and environment shape β-diversity of freshwater parasites across diverse taxa at a continental scale. Glob Ecol Biogeo 29: 38–49.

Bernot RJ, Lamberti GA (2008) Indirect effects of a parasite on a benthic community: an experiment with trematodes, snails and periphyton. Freshw Biol 53: 322–329.

Binning SA, Roche DG, Layton C (2013) Ectoparasites increase swimming costs in a coral reef fish. Biol Lett 9: 20120927.

Binning SA, Shaw AK, Roche DG (2017) Parasites and host performance: incorporating infection into our understanding of animal movement. Integ Comp Biol 57: 267–280.

Biro PA, Post JR (2008) Rapid depletion of genotypes with fast growth and bold personality traits from harvested fish populations. Proc Natl Acad Sci 105: 2919–2922.

Black (1982) Gills as an attachment site for *Salmincola edwardsii* (Copepoda: Lernaeopodidae). J Parasitol 68: 1172–1173.

Black GA, Montgomery WL, Whoriskey FG (1983) Abundance and distribution of *Salmincola edwardsii* (Copepoda) on anadromous brook trout, *Salvelinus fontinalis*, (Mitchill) in the Moisie River system, Quebec. J Fish Biol 22: 567–575.

Blanchet S, Méjean L, Bourque JF, Lek S, Thomas F, Marcogliese DJ et al. (2009a) Why do parasitized hosts look different? Resolving the "chicken-egg" dilemma. Oecologia 160: 37–47.

Blanchet S, Thomas F, Loot G (2009b) Reciprocal effects between host phenotype and pathogens: new insights from an old problem. Trends Parasitol 25: 364–369.

Blanchet S, Rey O, Loot G (2010) Evidence for host variation in parasite tolerance in a wild fish population. Evol Ecol 24: 1129–1139.

Blasco-Costa I, Waters JM, Poulin R (2012) Swimming against the current: genetic structure, host mobility and the drift paradox in trematode parasites. Mol Ecol 21: 207–217.

Blasco-Costa I, Poulin R (2013) Host traits explain the genetic structure of parasites: a meta-analysis. Parasitology 140: 1316–1322.

Blasco-Costa I, Koehler AV, Martin A, Poulin R (2013) Upstream-downstream gradient in infection levels by fish parasites: a common river pattern? Parasitology 140: 266–274.

Blasco-Costa I, Rouco C, Poulin R (2015) Biogeography of parasitism in freshwater fish: spatial patterns in hot spots of infection. Ecography 38: 301–310.

Bolger T, Connolly PL (1989) The selection of suitable indices for the measurement and analysis of fish condition. J Fish Biol 34: 171–182.

Bolnick DI, Snowberg LK, Caporaso JG, Lauber C, Knight R, Stutz WE (2014) Major H istocompatibility C omplex class II b polymorphism influences gut microbiota composition and diversity. Mol Ecol 23: 4831–4845.

Bonte D, Van Dyck H, Bullock JM, Coulon A, Delgado M, Gibbs M et al. (2012) Costs of dispersal. Biol Rev 87: 290–312.

Boone EC, Quinlan HR (2019) Occurrence of *Salmincola* sp. on brook charr *Salvelinus fontinalis* in Lake Superior. Hydrobiologia 840: 301–308.

Boots M, Haraguchi Y (1999) The evolution of costly resistance in host-parasite systems. Am Nat 153: 359–370.

Boots M (2008) Fight or learn to live with the consequences? Trends Ecol Evol 23: 248–250.

Born-Torrijos A, Riekenberg P, van der Meer MT, Nachev M, Sures B, Thieltges DW (2023) Parasite effects on host's trophic and isotopic niches. Trends Parasitol 39: 749–759.

Bowen II CA, Stedman RM (1990) Host–parasite relationships and geographic distribution of *Salmincola corpulentus* (Copepoda: Lernaeopodidae) on bloater (*Coregonus hoyi*) stocks in Lake Huron. Can J Zool 68: 1988–1994.

Boyer N, Réale D, Marmet J, Pisanu B, Chapuis JL (2010) Personality, space use and tick load in an introduced population of Siberian chipmunks *Tamias sibiricus*. J Anim Ecol 79: 538–547.

Braga RR, Bornatowski H, Vitule JRS (2012) Feeding ecology of fishes: an overview of worldwide publications. Rev Fish Biol Fish 22: 915–929.

Brassard P, Rau ME, Curtis MA (1982) Parasite-induced susceptibility to predation in diplostomiasis. Parasitology 85: 495–501.

Brittain JE, Eikeland TJ (1988) Invertebrate drift—a review. Hydrobiologia 166: 77–93.

Brown GP, Kelehear C, Pizzatto L, Shine R (2016) The impact of lungworm parasites on rates of dispersal of their anuran host, the invasive cane toad. Biol Inv 18: 103–114.

Brusca RC, Gilligan MR (1983) Tongue replacement in a marine fish (Lutjanus guttatus) by a parasitic isopod (Crustacea: Isopoda). Copeia 1983: 813–816.

Buck JC, Weinstein SB, Young HS (2018) Ecological and evolutionary consequences of parasite avoidance. Trends Ecol Evol 33: 619–632.

Budischak SA, O'Neal D, Jolles AE, Ezenwa VO (2018) Differential host responses to parasitism shape divergent fitness costs of infection. Funct Ecol 32: 324–333.

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: A practical information-theoretic approach, 2nd ed. New York, USA: Springer Verlag.

Burton T, McKelvey S, Stewart DC, Armstrong JD, Metcalfe NB (2013) Offspring investment in wild Atlantic salmon (*Salmo salar*): relationships with smolt age and spawning condition. Ecol Freshw Fish 22: 317–321.

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83: 575–583.

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83: 575–583.

Bustnes JO, Galaktionov KV (2004) Evidence of a state-dependent trade-off between energy intake and parasite avoidance in Steller's eiders. Can J Zool 82: 1566–1571.

Byrnes JE, Reed DC, Cardinale BJ, Cavanaugh KC, Holbrook SJ, Schmitt RJ (2011) Climate-driven increases in storm frequency simplify kelp forest food webs. Glob Chang Biol 17: 2513–2524.

Cade BS, Noon BR (2003) A gentle introduction to quantile regression for ecologists. Front Ecol Environ 1: 412–420.

Cardon M, Loot G, Grenouillet G, Blanchet S (2011) Host characteristics and environmental factors differentially drive the burden and pathogenicity of an ectoparasite: a multilevel causal analysis. J Anim Ecol 80: 657–667.

Carrascal LM, Senar JC, Mozetich I, Fransec U, Jordi D (1998) Interactions among environmental stress, body condition, nutritional status, and dominance in Great Tits. Auk 115: 727–738.

Carrassón M, Cribb TH (2014) Benign effect of the fish parasitic isopod *Ceratothoa* cf. *imbricata* on *Selenotoca multifasciata* (Scatophagidae) from Australia. Dis Aquat Org 110: 173–180.

Chigbu P (2001) Occurrence and distribution of *Salmincola californiensis* (Copepoda: Lernaeopodidae) on juvenile sockeye salmon (*Oncorhynchus nerka*) in Lake Washington. J Freshw Ecol 16: 615–620.

Chretien E, De Bonville J, Guitard J, Binning SA, Melis E, Kack A et al. (2023) Few studies of wild animal performance account for parasite infections: A systematic review. J Anim Ecol 92: 794–806.

Cohen JM, Venesky MD, Sauer EL, Civitello DJ, McMahon TA, Roznik EA, Rohr JR (2017) The thermal mismatch hypothesis explains host susceptibility to an emerging infectious disease. Ecol Lett 20: 184–193.

Colorni A, Trilles JP, Golani D (1997) *Livoneca* sp. (Flabellifera: Cymothoidae), an isopod parasite in the oral and branchial cavities of the Red Sea silverside *Atherinomorus lacunosus* (Perciformes, Atherinidae). Dis Aquat Org 31: 65–71.

Comas M (2020) Body condition, sex and elevation in relation to mite parasitism in a high mountain gecko. J Zool 310: 298–305.

Combes (2001) Parasitism: The Ecology and Evolution of Intimate Interactions. Chicago, USA: University of Chicago Press.

Cone (1989) The need to reconsider the use of condition indices in fishery science. Trans Am Fish Soc 118: 510–514.

Conley DC, Curtis MA (1993) Effects of temperature and photoperiod on the duration of hatching, swimming, and copepodid survival of the parasitic copepod *Salmincola edwardsii*. Can J Zool 71: 972–976.

Conley DC, Curtis MA (1994) Larval development of the parasitic copepod *Salmincola edwardsii* on brook trout (*Salvelinus fontinalis*). Can J Zool 72: 154–159.

Cooke SJ, Cowx IG (2004) The role of recreational fishing in global fish crises. BioScience 54: 857–859.

Cooke SJ, Twardek WM, Lennox RJ, Zolderdo AJ, Bower SD, et al. (2018) The nexus of fun and nutrition: Recreational fishing is also about food. Fish Fish 19: 201–224.

Cornet S, Biard C, Moret Y (2009) Variation in immune defence among populations of *Gammarus pulex* (Crustacea: Amphipoda). Oecologia 159: 257–269.

Costello MJ (2006) Ecology of sea lice parasitic on farmed and wild fish. Trends Parasitol 22: 475–483.

Costello MJ (2009) How sea lice from salmon farms may cause wild salmonid declines in Europe and North America and be a threat to fishes elsewhere. Proc R Soc Lond B Biol Sci 276: 3385–3394.

Crane AL, Fritts AK, Mathis A, Lisek JC. Barnhart MC (2011) Do gill parasites influence the foraging and antipredator behaviour of rainbow darters, *Etheostoma caeruleum*? Anim Behav 82: 817–823.

Criscione CD, Blouin MS (2004) Life cycles shape parasite evolution: comparative population genetics of salmon trematodes. Evolution 58: 198–202.

Cunjak RA, Power G (1986) Winter habitat utilization by stream resident brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*). Can J Fish Aquat Sci 43: 1970–1981.

Cunningham EJ, Tierney JF, Huntingford FA (1994) Effects of the cestode *Schistocephalus solidus* on food intake and foraging decisions in the three-spined stickleback *Gasterosteus aculeatus*. Ethology 97: 65–75.

Curtis VA (2014) Infection-avoidance behaviour in humans and other animals. Trends Imm 35: 457–464.

Das K, Krzywinski M, Altman N (2019) Quantile regression. Nat Methods 16: 451–452.

Dawkins R (2019) Good strategy or evolutionarily stable strategy? In Sociobiology: beyond nature/nurture? (pp. 331-367). Routledge.

Dearden RP, Herrel A, Pradel A (2023) Evidence for high-performance suction feeding in the Pennsylvanian stem-group holocephalan *Iniopera*. Proc Natl Acad Sci 120: e2207854119.

Decaestecker E, Gaba S, Raeymaekers JA, Stoks R, Van Kerckhoven L, Ebert D, De Meester L (2007) Host–parasite 'Red Queen'dynamics archived in pond sediment. Nature 450: 870–873.

Denda I, Ogawa S (2011) Occurrence of *Salmincola californiensis* on wild Japanese char. Bull Nagano Prefect Fish Experiment Stat 12: 12 (In Japanese).

Dobson AP, May RM (1987) The effects of parasites on fish populations – theoretical aspects. Int J Parasitol 17: 363–370.

Dobson A, Lafferty KD, Kuris AM, Hechinger RF, Jetz W (2008) Homage to Linnaeus: how many parasites? How many hosts? Proc Natl Acad Sci 105: 11482–11489.

Ezenwa VO, Etienne RS, Luikart G, Beja-Pereira A, Jolles AE (2010) Hidden consequences of living in a wormy world: nematode-induced immune suppression facilitates tuberculosis invasion in African buffalo. Am Nat 176: 613–624.

Ezenwa VO, Archie EA, Craft ME, Hawley DM, Martin LB, Moore J et al. (2016) Host behaviour–parasite feedback: an essential link between animal behaviour and disease ecology. Proc R Soc Lond B Biol Sci 283: 20153078.

Fan Y, Chen J, Shirkey G, John R, Wu SR, Park H, Shao C (2016) Applications of structural equation modeling (SEM) in ecological studies: an updated review. Ecol Proc 5: 1–12.

Fast MD (2014) Fish immune responses to parasitic copepod (namely sea lice) infection. Dev Comp Immunol 43: 300–312.

Fausch KD (1984) Profitable stream positions for salmonids: relating specific growth rate to net energy gain. Can J Zool 62: 441–451.

Fausch KD, Nakano S, Ishigaki K (1994) Distribution of two congeneric charrs in streams of Hokkaido Island, Japan: considering multiple factors across scales. Oecologia 100: 1–12.

Fausch KD, Nakano S, Kitano S, Kanno Y, Kim S (2021) Interspecific social dominance networks reveal mechanisms promoting coexistence in sympatric charr in Hokkaido, Japan. J Anim Ecol 90: 515–527.

Ferguson JA, Koketsu W, Ninomiya I, Rossignol PA, Jacobson KC, Kent ML (2011) Mortality of coho salmon (*Oncorhynchus kisutch*) associated with burdens of multiple parasite species. Int J Parasitol 41: 1197–1205.

Filipsson K, Petersson T, Höjesjö J, Piccolo JJ, Näslund J, Wengström N et al. (2018) Heavy loads of parasitic freshwater pearl mussel (*Margaritifera margaritifera* L.) larvae impair foraging, activity and dominance performance in juvenile brown trout (*Salmo trutta* L.). Ecol Freshw Fish 27: 70–77.

Finley RJ, Forrester GE (2003) Impact of ectoparasites on the demography of a small reef fish. Mar Ecol Prog Ser 248: 305–309.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3: 294–299.

Folstad I, Karter AJ (1992) Parasites, bright males, and the immunocompetence handicap. Am Nat 139: 603–622.

Forbes KM, Mappes T, Sironen T, Strandin T, Stuart P, Meri S et al. (2016) Food limitation constrains host immune responses to nematode infections. Biol Lett 12: 20160471.

Fournier DA, Skaug HJ, Ancheta J, Ianelli J, Magnusson A, Maunder MN, Nielsen A, Sibert J (2012) AD model builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. Optim Methods Softw 27: 233–249.

Fraley C, Raftery AE, Scrucca L, Murphy TB, Fop M, Scrucca ML (2012) Package "*mclust*". title gaussian mixture modelling for model based clustering, classification, and density estimation. Available online: https://cran. r-project. org/web/packages/mclust/mclust. pdf (accessed on 27 June 2022).

Friend GF (1941) The life-history and ecology of the gill-maggot *Salmincola salmonea* (L.) (copepod crustacean). Trans Roy Soc Edinburgh 60: 503–541.

Friesen OC, Goellner S, Poulin R, Lagrue C (2020) Parasites shape community structure and dynamics in freshwater crustaceans. Parasitology 147: 182–193.

Fryer G (1961) Variation and systematic problems in a group of lernaeid copepods. Crustaceana 1961: 275–285.

Fryer G (1981) The copepod *Salmincola edwardsii* as a parasite of *Salvelinus alpinus* in Britain, and a consideration of the so-called relict fauna of Ennerdale Water. J Zool 193: 253–268.

Fuess LE, den Haan S, Ling F, Weber JN, Steinel NC, Bolnick DI (2021) Immune gene expression covaries with gut microbiome composition in stickleback. MBio 12: 10–1128.

Fujita M, Yamasaki S, Katagiri C, Oshiro I, Sano K, Kurozumi T et al. (2016) Advanced maritime adaptation in the western Pacific coastal region extends back to 35,000–30,000 years before present. Proc Natl Acad Sci 113: 11184–11189.

Furukawa-Tanaka T (1988) The ecology of salmonid fishes in Japanese mountain streams III. Interactive food segregation between red-spot masu salmon, *Salmo* (*Parasalmo*) *masou*

macrostomus, and Japanese charr, *Salvelinus leucomaenis*, in relation to their social structure. Ph.D. Dissertation, Kyoto University (In Japanese).

Gabelhouse Jr DW (1991) Seasonal changes in body condition of white crappies and relations to length and growth in Melvern Reservoir, Kansas. N Am J Fish Manag 11: 50–56.

Gall GAE, Mcclendon EL, Schafer WE (1972) Evidence on the influence of the copepod (*Salmincola californiensis*) on the reproductive performance of a domesticated strain of rainbow trout (*Salmo gairdneri*). Trans Am Fish Soc 101: 345–346.

Gelman A, Hill J (2007) Data analysis using regression and multilevel/hierarchical models. New York, USA: Cambridge University Press.

Giles N (1980) A stomach sampler for use on live fish. J Fish Biol 16: 441–444.

Giles N (1987) Predation risk and reduced foraging activity in fish: experiments with parasitized and non-parasitized three-spined sticklebacks, *Gasterosteus aculeatus* L. J Fish Biol 31: 37–44.

Gilot-Fromont E, Jégo M, Bonenfant C, Gibert P, Rannou B, Klein F, Gaillard JM (2012) Immune phenotype and body condition in roe deer: individuals with high body condition have different, not stronger immunity. PLoS ONE 9: e45576.

Gleeson DJ, Blows MW, Owens IP (2005) Genetic covariance between indices of body condition and immunocompetence in a passerine bird. BMC Evol Biol 5: 1–9.

Godwin SC, Krkošek M, Reynolds JD, Rogers LA, Dill LM (2018) Heavy sea louse infection is associated with decreased stomach fullness in wild juvenile sockeye salmon. Can J Fish Aquat Sci 75: 1587–1595.

Gonzalez MT, Leiva NV, Sepulveda F, Asencio G, Baeza JA (2021) Genetic homogeneity coupled with morphometric variability suggests high phenotypic plasticity in the sea louse *Caligus rogercresseyi* (Boxshall and Bravo, 2000), infecting farmed salmon (*Salmo salar*) along a wide latitudinal range in southern Chile. J Fish Dis 44: 633–638.

Gordy MA, Koprivnikar J, McPhail B, Hanington PC (2020) Environmental and ecological factors driving trematode parasite community assembly in central Alberta lakes. Int J Parasitol: Parasit Wildl 13: 283–291.

Goto A, Kuroki M, Morita K (2023) Active feeding of anadromous white-spotted char *Salvelinus leucomaenis* at the southern latitudinal rivers. Can J Fish Aquat Sci 80: 1737–1747.

Graham AL, Hayward AD, Watt KA, Pilkington JG, Pemberton JM, Nussey DH (2010) Fitness correlates of heritable variation in antibody responsiveness in a wild mammal. Science 330: 662–665.

Graham AL, Shuker DM, Pollitt LC, Auld SK, Wilson AJ, Little TJ (2011) Fitness consequences of immune responses: strengthening the empirical framework for ecoimmunology. Funct Ecol 25: 5–17.

Grunberg RL (2021) Spatial and seasonal variation in host community structure is weakly concordant with patterns of parasite composition. Freshw Biol 66: 303–316.

Gsell AS, Biere A, de Boer W, de Bruijn I, Eichhorn G, Frenken T et al. (2023) Environmental refuges from disease in host–parasite interactions under global change. Ecology 104: e4001.

Hansen EK, Poulin R (2006) Spatial covariation between infection levels and intermediate host densities in two trematode species. J Helminthol 80: 255–259.

Harper DG (1999) Feather mites, pectoral muscle condition, wing length and plumage coloration of passerines. Anim Behav 58: 553–562.

Hart AG, Carpenter WS, Hlustik-Smith E, Reed M, Goodenough AE (2018) Testing the potential of Twitter mining methods for data acquisition: Evaluating novel opportunities for ecological research in multiple taxa. Methods Ecol Evol 9: 2194–2205.

Härkönen L, Hyvärinen P, Paappanen J, Vainikka A (2014) Explorative behavior increases vulnerability to angling in hatchery-reared brown trout (*Salmo trutta*). Can J Fish Aquat Sci 71: 1900–1909.

Hasegawa R, Koizumi I (2021) Relative importance of host-dependent versus physical environmental characteristics affecting the distribution of an ectoparasitic copepod infecting the mouth cavity of stream salmonid. Ecol Res 36: 1015–1027.

Hasegawa R, Ayer CG, Umatani Y, Miura K, Ukumura M, Katahira H, Koizumi I (2022a) Potential negative effects and heterogeneous distribution of a parasitic copepod *Salmincola edwardsii* (Copepoda: Lernaeopodidae) on southern Asian dolly varden *Salvelinus curilus* in Hokkaido, Japan. Parasitol Int 87: 102529.

Hasegawa R, Katahira H, Koizumi I (2022b) *Salmincola markewitschi* (Copepoda: Lernaeopodidae) or *S. carpionis*? A requirement for taxonomic revision due to their high morphological variations. Folia Parasitologica 69: 025.

Hasegawa R, Koizumi I (2023) Parasites either reduce or increase host vulnerability to fishing: a case study of a parasitic copepod and its salmonid host. Sci Nat 110: 10.

Hasegawa R, Koizumi I (2024) Consistent negative correlations between parasite infection and host body condition across seasons suggest potential harmful impacts of *Salmincola markewitschi* on wild white-spotted charr, *Salvelinus leucomaenis.* Zool Sci. 41

Hendry AP, Beall E (2004) Energy use in spawning Atlantic salmon. Ecol Freshw Fish 13: 185–196.

Hepworth MR, Monticelli LA, Fung TC, Ziegler CG, Grunberg S, Sinha R et al. (2013). Innate lymphoid cells regulate CD4+ T-cell responses to intestinal commensal bacteria. Nature 498: 113–117.

Herron CL, Kent ML, Schreck CB (2018) Swimming endurance in juvenile Chinook Salmon infected with *Salmincola californiensis*. J Aquat Anim Health 30: 81–89.

Hinch SG, Rand PS (1998) Swim speeds and energy use of upriver-migrating sockeye salmon (*Oncorhynchus nerka*): role of local environment and fish characteristics. Can J Fish Aquat Sci 55: 1821–1831.

Hintz WD, Lonzarich DG (2018) Maximizing foraging success: the roles of group size, predation risk, competition, and ontogeny. Ecosphere 9: e02456.

Hiramatsu N, Fukada H, Kitamura M, Shimizu M, Fuda H, Kobayashi K, Hara A (2001) Serum immunoglobulin M (IgM) in Sakhalin taimen (*Hucho perryi*). Aquacult Sci 49: 347–355.

Hodges KE, Boonstra R, Krebs CJ (2006) Overwinter mass loss of snowshoe hares in the Yukon: starvation, stress, adaptation or artefact? J Anim Ecol 75: 1–13.

Hogans WE (1987) Morphological variation in *Pennella balaenoptera* and *P. filosa* (Copepoda: Pennellidae) with a review of the genus *Pennella* Oken, 1816 parasitic on Cetacea. Bull Mar Sci 40: 442–453.

Hoshina H, Suenaga G (1954) On a new species of parasitic copepods from Yamame (salmonid fish) of Japan. J Tokyo Univ Fish 41: 75–79.

Hoshina H, Nishimura T (1976) On a parasitic Copepoda, *Salmincola californiensis* found in a salmonid fish, Yamame *Oncorhynchus masou*. Fish Pathol 11: 153–157 (In Japanese with English abstract).

Hosoya K (2013) Salmonidae. In: Fishes of Japan with pictorial keys to the species, 3rd edition. Nakabo (ed). Hadano, Japan: Tokai University Press. pp 362–367 (In Japanese).

Hothorn T, Bretz F, Westfall P, Heiberger RM, Schuetzenmeister A, Scheibe S, Hothorn MT (2016) Package "*multcomp*". Simultaneous inference in general parametric models. Project for Statistical Computing, Vienna, Austria.

Hua CJ, Zhang D, Zou H, Li M, Jakovlic I, Wu SG, Wang GT, Li WX (2019) Morphology is not a reliable taxonomic tool for the genus *Lernaea*: molecular data and experimental infection reveal that *L. cyprinacea* and *L. cruciata* are conspecific. Parasit Vectors 12: 1–13.

Hudson PJ, Dobson AP, Newborn D (1992) Do parasites make prey vulnerable to predation? Red grouse and parasites. J Anim Ecol 61: 681–692.

Hudson PJ, Dobson AP, Newborn D (1998) Prevention of population cycles by parasite removal. Science 28: 2256–2258.

Hudson PJ, Dobson AP, Lafferty KD (2006) Is a healthy ecosystem one that is rich in parasites? Trends Ecol Evol 21: 381–385.

Hughes NF (1992) Ranking of feeding positions by drift-feeding arctic grayling (*Thymallus arcticus*) in subarctic mountain streams of interior Alaska. Can J Fish Aquat Sci 49: 1999–2008.

Humes AF, Gooding RU (1964) A method for studying the external anatomy of copepods. Crustaceana 6: 238–240.

Huusko ARI, Greenberg L, Stickler M, Linnansaari T, Nykänen M, Vehanen T et al. (2007) Life in the ice lane: the winter ecology of stream salmonids. River Res Appl 23: 469–491.

Huys R, Boxshall GA (1991) Copepod evolution. London: The Ray Society, London. 468pp.

Iaria C, Spanò N, Smeriglio A, Capparucci F, De Benedetto G, Lanteri G et al. (2021) Massive infection of *Cystidicoloides ephemeridarum* in brown trout *Salmo trutta* with skeletal deformities. Dis Aquat Org 143: 159–168.

Ichihara A (1983) Seafood and parasites. New Food Industry 25: 56–67 (In Japanese).

Imanishi K (1996) *Charr and Masu Salmon*. Tokyo, Japan: Heibonsha (In Japanese).

Inaba K, Turley S, Yamaide F, Iyoda T, Mahnke K, Inaba M et al. (1998) Efficient presentation of phagocytosed cellular fragments on the major histocompatibility complex class II products of dendritic cells. J Exp Med 188: 2163–2173.

Ishigaki K (1984) Exploring the Mystery of Charrs. Iwanami-shoten, Tokyo. (In Japanese).

Iwashita Y, Kamijo Y, Nakahashi S, Shindo A, Yokoyama K, Yamamoto A (2013) Food poisoning associated with *Kudoa septempunctata*. J Emerg Med 44: 943–945.

Jackson JA, Hall AJ, Friberg IM, Ralli C, Lowe A, Zawadzka M et al. (2014). An immunological marker of tolerance to infection in wild rodents. PLoS Biol 12: e1001901.

Jakob EM, Marshall SD, Uetz GW (1996) Estimating fitness: a comparison of body condition indices. Oikos 77: 61–67.

Johnson PT, Hoverman JT (2012) Parasite diversity and coinfection determine pathogen infection success and host fitness. Proc Natl Acad Sci 109: 9006–9011.

Johnson PT, Calhoun DM, Riepe T, McDevitt-Galles T, Koprivnikar J (2019) Community disassembly and disease: realistic—but not randomized—biodiversity losses enhance parasite transmission. Proc R Soc Lond B Biol Sci 286: 20190260.

Johnston CE, Dykeman D (1987) Observations on body proportions and egg production on the female parasitic copepod (*Salmincola salmoneus*) from the gills of Atlantic salmon (*Salmo salar*) kelts exposed to different temperatures and photoperiods. Can J Zool 65: 415–419.

Jonsson N, Jonsson B, Hansen LP (1991) Energetic cost of spawning in male and female Atlantic salmon (*Salmo salar* L.). J Fish Biol 39: 739–744.

Kabata Z (1969) Revision of the genus *Salmincola* Wilson, 1915 (Copepoda: Lernaeopodidae). J Fish Board Can 26: 2987–3041.

Kabata Z, Cousens B (1973) Life cycle of *Salmincola californiensis* (Dana 1852) (Copepoda: Lernaeopodidae). J Fish Board Can 30: 881–903.

Kabata Z, Cousens B (1977) Host–parasite relationships between sockeye salmon, *Oncorhynchus nerka*, and *Salmincola californiensis* (Copepoda: Lernaeopodidae). J Fish Board Can 34: 191–202.

Kamiya T, O'dwyer K, Westerdahl H, Senior A, Nakagawa S (2014) A quantitative review of MHC-based mating preference: The role of diversity and dissimilarity. Mol Ecol 23: 5151–5163.

Kanno Y, Locklear ML, Platis NM, Lewis ST (2023) Body condition metrics explain fish movement in experimental streams. J Zool 320: 18–28.

Katahira H, Yamazaki C, Fukui S, Ayer CG, Koizumi I (2017) Spatial aggregation in small spring-fed tributaries leads to a potential metapopulation structure in a parasitic fish leech. Parasitol Open e11.

Katahira H, Yamamoto A, Masubuchi T, Isshiki T, Watanabe N, Kanaiwa M (2021) A report on potential effects of an ectoparasite *Argulus coregoni* (Crustacea: Branchiura) on ayu under rearing condition. Aquaculture 543: 736980.

Kawaguchi Y, Nakano S (2001) Contribution of terrestrial invertebrates to the annual resource budget for salmonids in forest and grassland reaches of a headwater stream. Freshw Biol 46: 303–316.

Kawanishi R, Kohya N, Sogabe A, Hata H (2019) A comparison of body condition of the yellowstriped butterfish *Labracoglossa argenteiventris* in relation to parasitism by the cymothoid isopod *Ceratothoa arimae*. Parasitol Int 72: 101932.

Kawanobe M (2018) Infection status of *Salmincola carpionis* in the fish hatcheries of Nagano prefecture. Bull Nagano Prefect Fish Experiment Stat 19: 24–28 (In Japanese).

Kawanobe M (2020) Hatching of *Salmincola carpionis* and period of parasitic capacity of the larvae. Bull Nagano Prefect Fish Experiment Stat 19: 24–28 (In Japanese).

Kennedy CR (2012) Can a specialist parasite species of a widespread and common host species be rare? The case of *Spinitectus inermis* (Nematoda: Cystidicolidae) in eels *Anguilla anguilla*. Folia Parasitologica 59: 131–138.

Kéry M, Schaub M (2012) Bayesian population analysis using WinBUGS: A hierarchical perspective. Boston, USA: Academic Press.

Kishi D, Maekawa K (2009) Stream-dwelling Dolly Varden (*Salvelinus malma*) density and habitat characteristics in stream sections installed with low-head dams in the Shiretoko Peninsula, Hokkaido, Japan. Ecol Res 24: 873–880.

Klemme I, Hyvärinen P, Karvonen A (2020) Negative associations between parasite avoidance, resistance and tolerance predict host health in salmonid fish populations. Proc R Soc Lond B Biol Sci 287: 20200388.

Klemme I, Hyvärinen P, Karvonen A (2021) Cold water reduces the severity of parasite-inflicted damage: support for wintertime recuperation in aquatic hosts. Oecologia 195: 155–161.

Knowles SC, Nakagawa S, Sheldon BC (2009) Elevated reproductive effort increases blood parasitaemia and decreases immune function in birds: a meta-regression approach. Funct Ecol 23: 405-415.

Koeck B, Závorka L, Aldvén D, Näslund J, Arlinghaus R, Thörnqvist PO et al. (2019) Angling selects against active and stress-resilient phenotypes in rainbow trout. Can J Fish Aquat Sci 76: 320–333.

Koenker R, Portnoy S, Ng PT, Zeileis A, Grosjean P, Ripley BD (2018) Package "*quantreg*". Cran R-project. org.

Koizumi I, Kanazawa Y, Yamazaki C, Tanaka Y, Takaya K (2017) Extreme winter aggregation of invasive rainbow trout in small tributaries: implications for effective control. Ichthyol Res 64: 197–203.

Komiyama E, Ohtaishi N, Maekawa K (1982) Occurrence of a sea-run type of the Dolly Varden in the Shiretoko Peninsula, Hokkaido, Japan. Ichthyol Res 29: 298–302.

Kotob MH, Menanteau-Ledouble S, Kumar G, Abdelzaher M, El-Matbouli M (2017) The impact of co-infections on fish: a review. Vet Res 47: 1–12.

Krkošek M, Ford JS, Morton A, Lele S, Myers RA, Lewis MA (2007) Declining wild salmon populations in relation to parasites from farm salmon. Science 318: 1772–1775.

Krohn MM, Boisclair D (1994) Use of stereo video system to stimate the energy expenditure of free-swimming fish. Can J Fish Aquat Sci 51: 1119–1127.

Kumagai A (1985) On *Salmincola* parasitic on trout. Annu Rep Iwate Pref Inland Fish Exp Stn Fiscal 1984: 85–93 (In Japanese).

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35: 1547–1549.

Kupfer TR, Le AT (2018) Disgusting clusters: trypophobia as an overgeneralised disease avoidance response. Cogn Emot 32: 729–741.

Kuris AM, Hechinger RF, Shaw JC, Whitney KL, Aguirre-Macedo L, Boch CA et al. (2008) Ecosystem energetic implications of parasite and free-living biomass in three estuaries. Nature 454: 515–518.

Kusterle S, Kristoffersen R, Rikardsen AH (2012) Population dynamics of *Salmincola salmoneus* on Atlantic salmon in a northern Norwegian river. Dis Aquat Org 100: 59–70.

Kutzer MA, Armitage SA (2016) Maximising fitness in the face of parasites: a review of host tolerance. Zoology 119: 281–289.

Kwak ML, Heath AC, Cardoso P (2020) Methods for the assessment and conservation of threatened animal parasites. Biol Conserv 248: 108696.

Lafferty KD, Morris AK (1996) Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. Ecology 77: 1390–1397.

Lafferty KD, Dobson AP, Kuris AM (2006) Parasites dominate food web links. Proc Natl Acad Sci 103: 11211–11216.

Lafferty KD, Allesina S, Arim M, Briggs CJ, De Leo G, Dobson AP et al. (2008) Parasites in food webs: the ultimate missing links. Ecol Lett 11: 533–546.

Lagrue C, Poulin R (2015) Measuring fish body condition with or without parasites: does it matter? J Fish Biol 87: 836–847.

Lanzing WJR, O'Conner PF (1975) Infestation of luderick (*Girella tricuspidata*) populations with parasitic isopods. Mar Freshw Res 26: 355–361.

Larsson S (2005) Thermal preference of Arctic charr, *Salvelinus alpinus*, and brown trout, *Salmo trutta*–implications for their niche segregation. Env Biol Fish 73: 89–96.

Layman CA, Arrington DA, Montana CG, Post DM (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88: 42–48.

Lazzaro BP, Little TJ (2009) Immunity in a variable world. Phil Trans Roy Soc 364: 15–26.

Lebreton JD, Burnham KP, Clobert J, Anderson DR (1992) Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. Ecol Monog 62: 67–118.

Lefcheck JS (2016) piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. Methods Ecol Evol 7: 573–579.

Lemly AD, Esch GW (1984) Effects of the trematode *Uvulifer ambloplitis* on juvenile bluegill sunfish, *Lepomis macrochirus*: ecological implications. J Parasitol 70: 475–492.

Lepak JM, Hansen AG, Hooten MB, Brauch D, Vigil EM (2022) Rapid proliferation of the parasitic copepod, *Salmincola californiensis* (Dana), on kokanee salmon, *Oncorhynchus nerka* (Walbaum), in a large Colorado reservoir. J Fish Dis 45: 89–98.

Liedvogel M, Åkesson S, Bensch S (2011) The genetics of migration on the move. Trends Ecol Evol 26: 561–569.

Lochmiller RL (1996) Immunocompetence and animal population regulation. Oikos 76: 594–602.

Lochmiller RL, Deerenberg C (2000) Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88: 87–98.

Lysne DA, Skorping A (2002) The parasite *Lernaeocera branchialis* on caged cod: infection pattern is caused by differences in host susceptibility. Parasitology 124: 69–76.

Marcogliese DJ (2008) The impact of climate change on the parasites and infectious diseases of aquatic animals. Rev Sci Tech 27: 467–484.

Mattiucci S, Cipriani P, Levsen A, Paoletti M, Nascetti G (2018) Molecular epidemiology of Anisakis and anisakiasis: an ecological and evolutionary road map. Adv Parasitol 99: 93–263.

Mazé-Guilmo E, Loot G, Páez DJ, Lefèvre T, Blanchet S (2014) Heritable variation in host tolerance and resistance inferred from a wild host–parasite system. Proc R Soc Lond B Biol Sci 281: 20132567.

McGladdery SE, Johnston CE (1988) Egg development and control of the gill parasite, *Salmincola salmoneus*, on Atlantic Salmon kelts (*Salmo salar*) exposed to four different regimes of temperature and photoperiod. Aquaculture 68: 193–202.

McNew SM, Knutie SA, Goodman GB, Theodosopoulos A, Saulsberry A, Yépez R et al. (2019) Annual environmental variation influences host tolerance to parasites. Proc R Soc Lond B Biol Sci 286: 20190049.

Merila J (1996) Genetic variation in offspring condition: an experiment. Funct Ecol 10: 465–474.

Minchella DJ, Scott ME (1991) Parasitism: a cryptic determinant of animal community structure. Trends Ecol Evol 6: 250–254.

Mitro MG (2016) Brook trout, brown trout, and ectoparasitic copepods *Salmincola edwardsii*: species interactions as a proximate cause of brook trout loss under changing environmental conditions. Trans Am Fish Soc 145: 1223–1233.

Mitro MG, Griffin JD (2018) Distribution, prevalence, and maximum intensity of the ectoparasitic copepod *Salmincola* cf. *edwardsii* in brook trout in Wisconsin streams. J Parasitol 104: 628–638.

Miura O, Kuris AM, Torchin ME, Hechinger RF, Chiba S (2006) Parasites alter host phenotype and may create a new ecological niche for snail hosts. Proc R Soc Lond B Biol Sci 273: 1323–1328.

Miyasaka H, Nakano S, Furukawa-Tanaka T (2003) Food habit divergence between white-spotted charr and masu salmon in Japanese mountain streams: circumstantial evidence for competition. Limnology 4: 1–10.

Montes MM, Castro-romero R, Martorelli SR (2017) Morphological identification and DNA barcoding of a new species of *Parabrachiella* (Siphonostomatoida: Lernaeopodidae) with aspects of their intraspecific variation. Acta Trop 173: 34–44.

Monzyk FR, Friesen TA, Romer JD (2015) Infection of juvenile salmonids by *Salmincola californiensis* (Copepoda: Lernaeopodidae) in reservoirs and streams of the Willamette River basin, Oregon. Trans Am Fish Soc 144: 891–902.

Morita K, Takashima Y (1998) Effect of female size on fecundity and egg size in white-spotted charr: comparison between sea-run and resident forms. J Fish Biol 53: 1140–1142.

Morita K, Suzuki T (1999) Shifts of food habit and jaw position of white-spotted charr after damming. J Fish Biol 55: 1156–1162.

Morita K, Yamamoto S, Hoshino N (2000) Extreme life history change of white-spotted char (*Salvelinus leucomaenis*) after damming. Can J Fish Aquat Sci 57: 1300–1306.

Morita K (2001) The growth history of anadromous white-spotted charr in northern Japan: a comparison between river and sea life. J Fish Biol 59: 1556–1565.

Morita K, Morita SH (2002) Rule of age and size at maturity: individual variation in the maturation history of resident white-spotted charr. J Fish Biol 61: 1230–1238.

Morita K, Kishi D, Tsuboi JI, Morita S, Arai T (2003) Rainbow trout and brown trout in Shiretoko peninsula, Hokkaido, Japan, Bull Shiretoko Museum 24: 17–26 (In Japanese with English abstract).

Morita K, Arai T, Kishi D, Tsuboi J (2005) Small anadromous *Salvelinus malma* at the southern limits of its distribution. J Fish Biol 66: 1187–1192.

Morita K, Morita S (2007) Alternative life histories and population process of white-spotted charr (salmonid fish). Jap J Ecol 57: 13–24 (In Japanese with English abstract).

Morita K, Morita SH, Yamamoto S (2009) Effects of habitat fragmentation by damming on salmonid fishes: lessons from white-spotted charr in Japan. Ecol Res 24: 711–722.

Morita K, Sahashi G, Tsuboi JI (2016) Altitudinal niche partitioning between white-spotted charr (*Salvelinus leucomaenis*) and masu salmon (*Oncorhynchus masou*) in a Japanese river. Hydrobiologia 783: 93–103.

Morita K, Sahashi G, Miya M, Kamada S, Kanbe T, Araki H (2019) Ongoing localized extinctions of stream-dwelling white-spotted charr populations in small dammed-off habitats of Hokkaido Island, Japan. Hydrobiologia 840: 207–213.

Morita K, Morita SH, Nagasawa T (2011) Seasonal changes in stream salmonid population densities in two tributaries of a boreal river in northern Japan. Ichthyol Res 58: 134–142.

Morton A, Routledge RD (2006) Fulton's condition factor: is it a valid measure of sea lice impact on juvenile salmon? N Am J Fish Manag 26: 56–62.

Morton JP, Silliman BR (2020) Parasites enhance resistance to drought in a coastal ecosystem. Ecology 101: e02897.

Mouritsen KN (2002) The *Hydrobia ulvae-Maritrema subdolum* association: influence of temperature, salinity, light, water-pressure and secondary host exudates on cercarial emergence and longevity. J Helminthol 76: 341–347.

Mrugała A, Wolinska J, Jeschke JM (2023) A meta-analysis of how parasites affect host consumption rates. Oikos e09700.

Müller K (1982) The colonization cycle of freshwater insects. Oecologia 52: 202–207.

Murphy CA, Gerth W, Arismendi I (2020) Hatching and survival of the salmon ‘gill maggot’ *Salmincola californiensis* (Copepoda: Lernaeopodidae) reveals thermal dependence and undocumented naupliar stage. Parasitology 147: 1338–1343.

Nadler SA, Perez-ponce de leon G (2011) Integrating molecular and morphological approaches for characterizing parasite cryptic species: implications for parasitology. Parasitology 138: 1688–1709.

Nagasawa K (1984) The finding of *Pennella* sp.(Copepoda: Pennellidae) on the saury, *Cololabis saira*, in the western and central north pacific ocean and the okhotsk sea parasites of the saury, *Cololabis saira* (Brevoort). Fish Pathol 18: 205–208.

Nagasawa K, Urawa S (1991) New records of the parasitic copepod *Salmincola stellatus* from Sakhalin taimen (*Hucho perryi*) in Hokkaido, with a note on its attachment site. Sci Rep Hokkaido Salmon Hatchery 45: 57–59.

Nagasawa K, Watanabe JR, Kimura S, Hara A (1994) Infection of *Salmincola stellatus* (Copepoda: Lernaeopodidae) on Sakhalin taimen *Hucho perryi* reared in Hokkaido. Bull Fish Sci, Hokkaido Univ 45: 109–112.

Nagasawa K, Yamamoto M, Sakurai Y, Kumagai A (1995) Rediscovery in Japan and host association of *Salmincola carpionis* (Copepoda: Lernaeopodidae), a parasite of wild and reared freshwater salmonids. Can J Fish Aquat Sci 52: 178–185.

Nagasawa K, Ikuta K, Kitamura S (1997) Distribution of *Salmincola carpionis* (Copepoda: Lernaeopodidae) in the buccal cavity of salmonids. Bull Natl Res Inst Aquacult 26: 35–39.

Nagasawa K (1998) Alive freshwater parasitic copepods (*Salmincola californiensis*) found on the gills of ocean-caught Steelhead trout (*Oncorhynchus mykiss*). Salmon Rep Ser 45: 277–279.

Nagasawa K, Ikuta K, Nakamura H, Shikama T, Kitamura S (1998) Occurrence and effects of the parasitic copepod *Salmincola carpionis* on salmonids in the Nikko District, central Japan. J Mar Syst 15: 269–272.

Nagasawa K, Urawa S (2002) Infection of *Salmincola californiensis* (Copepoda: Lernaeopodidae) on juvenile masu salmon (*Oncorhynchus masou*) from a stream in Hokkaido. Bull Nat Salmon Res Center 5: 7–12.

Nagasawa K (2020a) *Salmincola edwardsii* (Copepoda: Lernaeopodidae) parasitic on Southern Asian Dolly Varden, *Salvelinus malma kraschenninnikova*, from Hokkaido Island, Japan, with the southernmost distribution record of the copepod in Asia. Spec Divers 25: 197–203.

Nagasawa K (2020b) Gill lesions caused by the parasitic copepod *Salmincola edwardii* in the southern Asian Dolly Varden, *Salvelinus malma kraschenninnikova,* from Hokkaido, Japan. Nat. Kagoshima 47: 121–124 (In Japanese with English abstract).

Nagasawa K (2020c) *Salmincola markewitschi* (Copepoda: Lernaeopodidae) parasitic on whitespotted char, *Salvelinus leucomaenis*, in a moutain stream of Honshu Island, central Japan. Spec Divers 25: 369–375.

Nagasawa K, Ishikawa T (2017) *Salmincola carpionis* (Copepoda: Lernaeopodidae) parasitic on salmonids cultured at a fish farm in Tochigi Prefecture, Japan. Bull Tochigi Prefect Museum 34: 1–4 (In Japanese with English abstract).

Nagasawa K, Sakaki M (2020) Infection of *Salmincola carpionis* (Copepoda: Lernaeopodidae) on whitespotted charr, *Salvelinus leucomaenis* (Salmonidae), reared in northern Honshu, Japan. Nat Kagoshima 46: 113–115.

Nagasawa K, Kawai T (2020) The parasitic copepod *Salmincola edwardsii* from southern Asian Dolly Varden, *Salvelinus malma kraschenninnikova,* in the Shari River, Hokkaido, Japan, with a note on the hosts and geographical distribution of the copepod in the northern Japan. Nat. Kagoshima 47: 129–132 (In Japanese with English abstract).

Nagasawa K, Ishiyama N (2021) *Salmincola markewitschi* (Copepoda: Lernaeopididae), a parasite of whitespotted charr, *Salvelinus leucomaenis*, from Ishikawa Prefecture, central Japan. Taxa, Proc Japan Soc Syst Zool 50 (2021) 11–19.

Nagasawa K (2021) Two copepods *Salmincola edwardsii* and *Salmincola markewitschi* (Lernaeopodidae) parasitic on chars (*Salvelinus* spp.) reared in a Salmon Museum, northern Japan. Spec Divers 26: 137–143.

Nagasawa K, Yamauchi S, Murano Y, Yamamoto N (2021) The copepod *Salmincola stellata* (Lernaeopodidae) parasitic on aquarium-held Japanese huchen, *Parahucho taimen*, in Hokkaido, northern Japan. Nat Kagoshima 48: 9–14 (In Japanese with English abstract).

Nagasawa K, Urawa S (2022) Occurrence of S*almincola edwardsii* (Olsson, 1869) and *Salmincola markewitschi* Shedko & Shedko, 2002 (Copepoda: Lernaeopodidae) on stream-dwelling salmonids in eastern Hokkaido, Japan, with observations on the morphology of the copepods. Crust Res 51: 91–101.

Nagoshi M, Sakai T (1980) Relation between body size and food of the amago (Oncorhynchus rhodurus) from Hirakura Stream, Mie Prefecture, Japan. Jap J Ichthyol 26: 342–350 (In Japanese with English summary).

Nakamura T (1999) Spawning activities of the fluvial Japanese charr *Salvelinus leucomaenis* and incubation of their eggs at the artificial spawning sites. Nippon Suisan Gakkaishi 65: 434–440 (In Japanese with English Abstract).

Nakamura T, Maruyama T, Watanabe S (2002) Residency and movement of stream-dwelling Japanese charr, *Salvelinus leucomaenis*, in a central Japanese mountain stream. Ecol Freshw Fish 11: 150–157.

Nakano S (1995) Competitive interactions for foraging microhabitats in a size-structured interspecific dominance hierarchy of two sympatric stream salmonids in a natural habitat. Can J Zool. 73: 1845–1854.

Nakano S, Kitano F, Maekawa K (1996) Potential fragmentation and loss of thermal habitats for charrs in the Japanese archipelago due to climatic warming. Freshw Biol 36: 711–722.

Nakano S, Kawaguchi Y, Taniguchi Y, Miyasaka H, Shibata Y, Urabe H et al. (1999) Selective foraging on terrestrial invertebrates by rainbow trout in a forested headwater stream in northern Japan. Ecol Res 14: 351–360.

Nakano S, Fausch KD, Koizumi I, Kanno Y, Taniguchi Y, Kitano S, Miyake Y (2020) Evaluating a pattern of ecological character displacement: charr jaw morphology and diet diverge in sympatry versus allopatry across catchments in Hokkaido, Japan. Biol J Linn Soc 129: 356–378.

Nakao M, Sasaki M, Waki T, Anders JL, Katahira H (2018) *Brachylaima asakawai* sp. nov. (Trematoda: Brachylaimidae), a rodent intestinal fluke in Hokkaido, Japan, with a finding of the first and second intermediate hosts, Parasitol Int 67: 565–574.

Neff BD, Cargnelli LM (2004) Relationships between condition factors, parasite load and paternity in bluegill sunfish, *Lepomis macrochirus*. Environ Biol Fish 71: 297–304.

Neal T, Kent ML, Sanders J, Schreck CB, Peterson JT (2021) Laboratory infection rates and associated mortality of juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) from parasitic copepod (*Salmincola californiensis*). J Fish Dis 44: 1423–1434.

Neilson JD (1992) Sources of error in otolith microstructure examination. Otolith microstructure examination and analysis. Can J Fish Aquat Sci 117: 115–125.

Nicoletto PF (1995) Offspring quality and female choice in the guppy, *Poecilia reticulata*. Anim Behav 49: 377–387.

Nishimura T, Hoshina T (1977) On a parasitic Copepoda, *Salmincola californiensis* found in a salmonid fish, *Salvelinus leucomaenis pluvius*. J Tokyo Univ Fish 63: 67–70 (In Japanese with English abstract).

Norris K (1999) A trade-off between energy intake and exposure to parasites in oystercatchers feeding on a bivalve mollusc. Proc R Soc Lond B Biol Sci 266: 1703–1709.

O'Connell-Milne SA, Poulin R, Savage C, Rayment W (2016) Reduced growth, body condition and foot length of the bivalve Austrovenus stutchburyi in response to parasite infection. J Exp Mar Biol Ecol 474: 23–28.

O'Connor S, Ono R, Clarkson C (2011) Pelagic fishing at 42,000 years before the present and the maritime skills of modern humans. Science 334: 1117–1121.

Ogura Y, Hasegawa K (2023) Infection status of masu salmon *Oncorhynchus masou* by the parasite *Salmonema* cf. *ephemeridarum* (Nematoda, Cystidicolidae). J Fish Biol *in press.*

Ooue K, Terui A, Urabe H, Nakamura F (2017) A delayed effect of the aquatic parasite *Margaritifera laevis* on the growth of the salmonid host fish *Oncorhynchus masou masou*. Limnology 18: 345–351.

Österling EM, Ferm J, Piccolo JJ (2014) Parasitic freshwater pearl mussel larvae (*Margaritifera margaritifera* L.) reduce the drift-feeding rate of juvenile brown trout (*Salmo trutta* L.). Env Biol Fish 97: 543–549.

Ostfeld RS, Glass GE, Keesing F (2005) Spatial epidemiology: an emerging (or re-emerging) discipline. Trends Ecol Evol 20: 328–336.

Östlund-Nilsson S, Curtis L, Nilsson GE, Grutter AS (2005) Parasitic isopod *Anilocra apogonae*, a drag for the cardinal fish *Cheilodipterus quinquelineatus*. Mar Ecol Prog Ser 287: 209–216.

Parker D, Booth AJ (2013) The tongue-replacing isopod *Cymothoa borbonica* reduces the growth of largespot pompano *Trachinotus botla*. Mar Biol 160: 2943–2950.

Paterson S, Vogwill T, Buckling A, Benmayor R, Spiers AJ, Thomson NR et al. (2010) Antagonistic coevolution accelerates molecular evolution. Nature 464: 275–278.

Paterson RA, Knudsen R, Blasco-Costa I, Dunn AM, Hytterød S, Hansen H (2019) Determinants of parasite distribution in Arctic charr populations: catchment structure versus dispersal potential. J Helminthol 93: 559–566.

Paterson JE, Blouin-Demers G (2020) High tolerance of two parasites in ornate tree lizards reduces the fitness costs of parasitism. J Zool 312: 102–110.

Pawaputanon K (1980) Effects of parasitic copepod, *Salmincola californiensis* (Dana, 1852) on juvenile sockeye salmon, *Oncorhynchus nerka* (Walbaum). Doctoral dissertation, University of British Columbia.

Peig J, Green AJ (2009) New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118: 1883–1891.

Perrot-Minnot MJ, Bollache L, Lagrue C (2020) Distribution of *Pomphorhynchus laevis sl* (Acanthocephala) among fish species at a local scale: importance of fish biomass density. J Helminthol 94: 1–10.

Peterson RH, Sutterlin AM, Metcalfe JL (1979) Temperature preference of several species of Salmo and Salvelinus and some of their hybrids. J Fish Board Can 36: 1137–1140.

Piasecki W, Goodwin AE, Eiras JC, Nowak BF (2004) Importance of Copepoda in freshwater aquaculture. Zool Stud 43: 193–205.

Piertney SB, Oliver MK (2006) The evolutionary ecology of the major histocompatibility complex. Heredity 96: 7–21.

Pietrock M, Marcogliese DJ (2003) Free-living endohelminth stages: at the mercy of environmental conditions. Trends Parasitol 19: 293–299.

Plummer M (2017) JAGS version 4.3.0 user manual. http://mcmc-jags.sourceforge.net/.

Poulin R, Curtis MA, Rau ME (1991) Size, behaviour, and acquisition of ectoparasitic copepods by brook trout, *Salvelinus fontinalis*. Oikos 61: 169–174.

Poulin R (1995) Phylogeny, Ecology, and the Richness of Parasite Communities in Vertebrates: Ecological Archives M065-001. Ecol Monog 65: 283–302.

Poulin R (2011) Evolutionary ecology of parasites. 2nd edn. Princeton, USA: Princeton University Press.

Poulin R, Latham ADM (2002) Parasitism and the burrowing depth of the beach hopper *Talorchestia quoyana* (Amphipoda: Talitridae). Anim Behav 63: 269–275.

Poulin R, Maure F (2015) Host manipulation by parasites: a look back before moving forward. Trends Parasitol 31: 563–570.

Poulin R (2019) Best practice guidelines for studies of parasite community ecology. J Helminthol 93: 8–11.

Prokop P, Fedor P (2013) The effects of parasites on human behaviour: An evolutionary perspective. Probl Psychol 21st Century 5: 46–64.

Quinn TP (2018) The behavior and ecology of Pacific salmon and trout. Seattle, USA: University of Washington press.

R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

R Core Team (2023) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rajput ZI, Hu SH, Chen WJ, Arijo AG, Xiao CW (2006) Importance of ticks and their chemical and immunological control in livestock. J Zhejiang Univ Sci B 7: 912–921.

Railsback SF, Harvey BC, Hayse JW, LaGory KE (2005) Tests of theory for diel variation in salmonid feeding activity and habitat use. Ecology 86: 947–959.

Ramsay C, Rohr JR (2021) The application of community ecology theory to co-infections in wildlife hosts. Ecology 102: e03253.

Ramsay C, Rohr JR (2023a) Identity and density of parasite exposures alter the outcome of coinfections: Implications for management. J Appl Ecol 60: 205–214.

Ramsay C, Rohr JR (2023b) Ontogeny of immunity and potential implications for co-infection. Phil Tran Roy Soc B 378: 20220127.

Råberg L, Sim D, Read AF (2007) Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. Science 318: 812–814.

Råberg L, Graham AL, Read AF (2009) Decomposing health: tolerance and resistance to parasites in animals. Phil Trans Roy Soc B 364: 37–49.

Råberg L (2014) How to live with the enemy: understanding tolerance to parasites. PLoS Biol 12: e1001989.

Rau ME, Caron FR (1979) Parasite-induced susceptibility of moose to hunting. Can J Zool 57: 2466–2468.

Reimers E, Kjørrefjord AG, Stavøstrand SM (1993) Compensatory growth and reduced maturation in second sea winter farmed Atlantic salmon following starvation in February and March. J Fish Biol 43: 805–810.

Riera Romo M, Pérez-Martínez D, Castillo Ferrer C (2016) Innate immunity in vertebrates: an overview. Immunology 148: 125–139.

Riley SC, Fausch KD (1992) Underestimation of trout population size by maximum-likelihood removal estimates in small streams. N Am J Fish Manag 12: 768–776.

Ripley B, Venables B, Bates DM, Hornik K, Gebhardt A, Firth D, Ripley MB (2013) Package ”*mass*”. Cran r, 538: 113–120.

Robert RJ, Johnson KA, Casten MT (2004) Control of *Salmincola californiensis* (Copepoda: Lernaeapodidae) in rainbow trout, *Oncorhynchus mykiss* (Walbaum): a clinical and histopathological study. J Fish Dis 27: 73–79.

Robinson ML, Gomez-Raya L, Rauw WM, Peacock MM (2008) Fulton's body condition factor K correlates with survival time in a thermal challenge experiment in juvenile Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*). J Therm Biol 33: 363–368.

Rohr JR, Schotthoefer AM, Raffel TR, Carrick HJ, Halstead N, Hoverman JT et al. (2008) Agrochemicals increase trematode infections in a declining amphibian species. Nature 455: 1235–1239.

Rojas A, Solano-Brenes D, Muniz DG, Machado G (2019) Gone with the rain: negative effects of rainfall on male reproductive success in a nest-building arachnid. Behav Ecol 30: 1145–1156.

Rózsa L (1992) Points in question. Endangered parasite species. Int J Parasitol 22: 265–266.

Ruiz CF, Rash JM, Besler DA, Roberts JR, Warren MB, Arias CR et al. (2017) Exotic "gill lice" species (Copepoda: Lernaeopodidae: *Salmincola* spp.) infect rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) in the southeastern United States. J Parasitol 103: 377–389.

Sahashi G, Morita K (2021) Southern Asian Dolly Varden Charr, *Salvelinus curilus* on the Higashi-Nodottomari river in Rishiri Island. Rishiri Stud 40: 13–20.

Sala-Bozano M, Van Oosterhout C, Mariani S (2012) Impact of a mouth parasite in a marine fish differs between geographical areas. Biol J Linn Soc 105: 842–852.

Sallinen S, Norberg A, Susi H, Laine AL (2020) Intraspecific host variation plays a key role in virus community assembly. Nature comm 11: 5610.

Sanchez CA, Becker DJ, Teitelbaum CS, Barriga P, Brown LM, Majewska AA (2018) On the relationship between body condition and parasite infection in wildlife: a review and meta-analysis. Ecol Lett 21: 1869–1884.

Sasser KT, Weber JN (2023) A call for more ecologically and evolutionarily relevant studies of immune costs. Evol Ecol 37: 203–214.

Sato T, Egusa T, Fukushima K, Oda T, Ohte N, Tokuchi N et al. (2012) Nematomorph parasites indirectly alter the food web and ecosystem function of streams through behavioural manipulation of their cricket hosts. Ecol Lett 15: 786–793.

Sauter CM, & Morris RS (1995) Dominance hierarchies in cattle and red deer (*Cervus elaphus*): their possible relationship to the transmission of bovine tuberculosis. New Zealand Vet J 43: 301–305.

Schloesser RW, Fabrizio MC (2017) Condition indices as surrogates of energy density and lipid content in juveniles of three fish species. Trans Am Fish Soc 146: 1058–1069.

Seegrist DW, Gard R (1972) Effects of floods on trout in Sagehen Creek, California. Trans Am Fish Soc 101: 478–482.

Shanebeck KM, Besson AA, Lagrue C, Green SJ (2022) The energetic costs of sub-lethal helminth parasites in mammals: a meta-analysis. Biol Rev 97: 1886–1907.

Shariff M, Richards RH, Sommerville C (1980) The histopathology of acute and chronic infections of rainbow trout *Salmo gairdneri* Richardson with eye flukes, *Diplostomum* spp. J Fish Dis 3: 455–465.

Shchelinsky VE (2020) Large mammal hunting and use of aquatic food resources in the Early Palaeolithic (finds from Early Acheulean sites in the southern Azov Sea region). Quat Int 541: 182–188.

Shedko MB, Shedko SV (2002) Parasitic copepods of the genus *Salmincola* (Lernaeopodidae) from the far eastern chars *Salvelinus* (Salmonidae) with description of the new species *S. markewitschi*. Zool Zhur 81: 141–153 (In Russian with English abstract).

Shedko MB, Pospekhov VV, Atrashkewitschi GI (2005a) New data on fauna of the freshwater parasitic copepods of the genus *Salmincola* (Copepoda: Lernaeopodidae) of fishes from the northern coast of the Sea of Okhotsk. In: Makarchenko E. (Ed.), Vladimir Ya. Levanidov's Biennial Memorial Meetings 3. Dalnauka, Vladivostok. pp. 421–434. (In Russian with English abstract).

Shedko MB, Shedko SV, Vinogradov SA (2005b) Fauna of the freshwater parasitic copepods of the family Lernaeopodidae (Crustacea: Copepoda) of fishes from Sakhalin Island. In: Bogatov VV, Barkalov V Yu, Lelej AS, Makarchenko EA, Storozhenko S Yu (Eds). Flora and Fauna of Sakhalin Island (Materials of International Sakhalin Island Project). Dalnauka, Vladivostok, pp. 52–63 (In Russian with English abstract).

Shedko SV, Shedko MB, Miroshnichenko IL, Nemkova GA (2023) DNA identification of parasitic copepods *Salmincola* (Copepoda, Siphonostomatoida, Lernaeopodidae): variability and rate of evolution of the mitochondrial cytochrome c oxidase subunit I gene. Genetica 59: 1154–1164 (In Russian with English abstract).

Sheldon BC, Verhulst S (1996) Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends Ecol Evol 11: 317–321.

Shipley B (2009) Confirmatory path analysis in a generalized multilevel context. Ecology 90: 363–368.

Sih A, Mathot KJ, Moiron M, Montiglio PO, Wolf M, Dingemanse NJ (2015) Animal personality and state–behaviour feedbacks: a review and guide for empiricists. Trends Ecol Evol 30: 50–60.

Slavík O, Horký P, Douda K, Velíšek J, Kolářová J, Lepič P (2017) Parasite-induced increases in the energy costs of movement of host freshwater fish. Physiol Behav 171: 127–134.

Smit NJ, Bruce NL, Hadfield KA (2014) Global diversity of fish parasitic isopod crustaceans of the family Cymothoidae. Int J Parasitol: Parasit and Wildl 3: 188–197.

Sokolov SG, Shedko MB, Protasov EN, Frolov EV (2012) Parasites of the inland water fishes of Sakhalin Island. In: Bogatov VV, Barkalov V Yu, Lelej AS, Makarchenko EA, Storozhenko SY. (Eds). Flora and Fauna of North-West Pacific Islands (Materials of International Kuril Island and International Sakhalin Island Projects). Dalnauka, Vladivostok, pp. 179–216 (In Russian with English abstract).

Solomon DJ, Templeton RG (1976) Movements of brown trout *Salmo trutta* L. in a chalk stream. J Fish Biol 9: 411–423.

Souza JS, da Cruz Canellas BG, Sakabe R, Dos Santos LN, Dos Santos AFGN (2019) The parasitic isopod *Mothocya nana* drives dietary shifts and poorer condition of Brazilian silversides *Atherinella brasiliensis*. Dis Aquat Org 132: 229–239.

Spangenberg DK, Fuhrman AE, Larsen DA, Beckman BR (2023) A correlation between seasonally changing photoperiod, whole body lipid, and condition factor in juvenile spring Chinook salmon (*Oncorhynchus tshawytscha*). PLoS ONE 18: e0285380.

Spitzer CA, Anderson TW, Sikkel PC (2022) Habitat associations and impacts on a juvenile fish host by a temperate gnathiid isopod. Int J Parasitol: Parasit Wildl 17: 65–73.

Stankowska-Radziun M, Radziun K (1993) Observations on the development of *Salmincola edwardsii* (Olsson, 1869) (Copepoda: Lernaeopodidae) parasitizing the Arctic charr (*Salvelinus alpinus* (L.)) in the Hornsund region (Vest Spitsbergen). Acta Ichthyologica et Piscatoria 23: 107–114.

Sutherland DR, Wittrock DD (1985) The effects of *Salmincola californiensis* (Copepoda: Lernaeopodidae) on the gills of farm-raised rainbow trout, *Salmo gairdneri*. Can J Zool 63: 2893–2901.

Sutton SG, Bult TP, Haedrich RL (2000) Relationships among fat weight, body weight, water weight, and condition factors in wild Atlantic salmon parr. Trans Am Fish Soc 129: 527–538.

Suyama S, Masuda Y, Yanagimoto T, Chow S (2019) Genetic and morphological variation in *Pennella* sp. (Copepoda: Siphonostomatoida) collected from Pacific saury, *Cololabis saira*. Mar Biodivers 49: 1233–1245.

Tadiri CP, Dargent F, Scott ME (2013) Relative host body condition and food availability influence epidemic dynamics: a *Poecilia reticulata*-*Gyrodactylus turnbulli* host-parasite model. Parasitology 140: 343–351.

Takegawa Y, Kawaguchi Y, Mitsuhashi H, Taniguchi Y (2017) Implementing species distribution models to predict the impact of global warming on current and future potential habitats of white-spotted char (*Salvelinus leucomaenis*) for effective conservation planning in Japan. Japan. J. Conserv Ecol 22: 121–134 (In Japanese).

Takayama H, Ohara S, Okamoto Y, Sato N (1999) The present status of multigenerational rearing of salmonids at the Sapporo Salmon Museum, and a note on their age and size at sexual maturity, number of eggs taken, and egg weight. Bull Sapporo Salmon Museum 11: 18–38 (In Japanese).

Telfer S, Lambin X, Birtles R, Beldomenico P, Burthe S, Paterson S, Begon M (2010) Species interactions in a parasite community drive infection risk in a wildlife population. Science 330: 243−246.

Temple SA (1987) Do predators always capture substandard individuals disproportionately from prey populations? Ecology 68: 669–674.

Terui A, Ooue K, Urabe H, Nakamura F (2017) Parasite infection induces size-dependent host dispersal: consequences for parasite persistence. Proc R Soc Lond B Biol Sci 284: 20171491.

Thieltges DW, Jensen KT, Poulin R (2008) The role of biotic factors in the transmission of free-living endohelminth stages. Parasitology 135: 407–426.

Thompson CD, Meeuwig JJ (2022) Sharks are the preferred scraping surface for large pelagic fishes: Possible implications for parasite removal and fitness in a changing ocean. PLoS ONE 17: e0275458.

Tierney JF (1994) Effects of *Schistocephalus solidus* (Cestoda) on the food intake and diet of the three-spined stickleback, *Gasterosteus aculeatus*. J Fish Biol 44: 731–735.

Tokyo Metropolitan Market Sanitation Inspection Center (1990) Handbook of parasites in seafoods. 2: 45pp. Tokyo, Japan: Tokyo Metropolitan Market Sanitation Inspection Center (In Japanese).

Tompkins DM, White AR, Boots M (2003) Ecological replacement of native red squirrels by invasive greys driven by disease. Ecol Lett 6: 189–196.

Toscano BJ, Newsome B, Griffen BD (2014) Parasite modification of predator functional response. Oecologia 175: 345–352.

Treasurer JW, Hastie LC, Hunter D, Duncan F, Treasurer CM (2006) Effects of (*Margaritifera margaritifera*) glochidial infection on performance of tank-reared Atlantic salmon (*Salmo salar*). Aquaculture 256: 74–79.

Tsuboi JI, Morita K (2004) Selectivity effects on wild white-spotted charr (*Salvelinus leucomaenis*) during a catch and release fishery. Fish Res 69: 229–238.

Tsuboi JI, Morita K, Sahashi G, Kuroki M, Baba S, Arlinghaus R (2021) Species-specific vulnerability to angling and its size-selectivity in sympatric stream salmonids. Can J Fish Aquat Sci 78: 1470–1478.

Umatani Y, Arai T, Maekawa K (2008) Variation in migratory history of Dolly Varden in a stream with an artificial dam in the Shiretoko Peninsula, Hokkaido, Japan. Environ Biol Fish 83: 37–44.

Umatani Y, Arai T, Maekawa K (2018) Flexible seaward migration of Dolly Varden *Salvelinus malma* in the Shiretoko Peninsula, Hokkaido, Japan. Ichthyol. Res. 65: 202–209.

van der Most PJ, de Jong B, Parmentier HK, Verhulst S (2011) Trade-off between growth and immune function: a meta-analysis of selection experiments. Funct Ecol 25: 74–80.

Vaughan GE, Coble DW (1975) Sublethal effects of three ectoparasites on fish. J Fish Biol 7: 283–294.

Vicente J, Fierro Y, Martinez M, Gortazar C (2004) Long-term epidemiology, effect on body condition and interspecific interactions of concomitant infection by nasopharyngeal bot fly larvae (*Cephenemyia auribarbis* and *Pharyngomyia picta*, Oestridae) in a population of Iberian red deer (*Cervus elaphus hispanicus*). Parasitology 129: 349–361.

Vigil EM, Christianson KR, Lepak JK, Williams PJ (2016) Temperature effects on hatching and viability of juvenile gill lice, *Salmincola californiensis*. J Fish Dis 39: 899–905.

Vigneshwaran P, Ravichandran S, Prema M (2019) Parasitic isopod *Cymothoa eremita* (Brünnich 1783)(Isopoda: Cymothoidae) affects the growth of black pomfret *Parastromateus niger* (Bloch 1795) in the southeast coast of India. Thalassas: An Inte J Mar Sci 35: 109–115.

Villa SM, Goodman GB, Ruff JS, Clayton DH (2016) Does allopreening control avian ectoparasites? Biol Lett 12: 20160362.

Vijendravarma RK, Kraaijeveld AR, Godfray HCJ (2009) Experimental evolution shows *Drosophila melanogaster* resistance to a microsporidian pathogen has fitness costs. Evolution 63: 104–114.

Vivas Muñoz JC, Feld CK, Hilt S, Manfrin A, Nachev M, Köster et al. (2021) Eye fluke infection changes diet composition in juvenile European perch (*Perca fluviatilis*). Sci Rep 11: 3440.

von Königslöw V, Fornoff F, Klein AM (2022) Wild bee communities benefit from temporal complementarity of hedges and flower strips in apple orchards. J Appl Ecol 59: 2814–2824.

von Reumont BM, Meusemann K, Szucsich NU, Dell'Ampio E, Gowri-Shankar V, Bartel D (2009) Can comprehensive background knowledge be incorporated into substitution models to improve phylogenetic analyses? A case study on major arthropod relationships. BMC Evol Biol 9: 119.

Voutilainen A, Figueiredo K, Huuskonen H (2008) Effects of the eye fluke *Diplostomum spathaceum* on the energetics and feeding of Arctic charr *Salvelinus alpinus*. J Fish Biol 73: 2228–2237.

Wainwright PC, McGee MD, Longo SJ, Patricia Hernandez L (2015) Origins, innovations, and diversification of suction feeding in vertebrates. Integ Comp Biol 55: 134–145.

Waite TA, Field KL (2007) Foraging with others: Games social foragers play. Pages 331–362 in Stephens DW, Brown JS, Ydenberg RC (eds). Foraging behavior and ecology. Chicago, Illinois, USA: University of Chicago Press.

Wakabayashi S (1997) Infection of whitespotted charr, *Salvelinus leucomaenis*, reared in Toyama Prefecture by *Salmincola carpionis* (Copepoda: Lernaeopodidae). Bull Toyama Prefect Fish Res Inst 9: 35–40 (In Japanese with English abstract).

Walter TC, Boxshall G (2020) World of Copepods database. *Salmincola* Wilson C. B., 1915. Accessed through: World Register of Marine Species at: http://www.marinespecies.org/aphia.php?p=taxdetails&id=135610 (6th December 2023).

Warner DA, Johnson MS, Nagy TR (2016) Validation of body condition indices and quantitative magnetic resonance in estimating body composition in a small lizard. J Experimental Zool Part A: Ecological Genetics and Physiology 325: 588–597.

Watanabe H, Ishii T (2000) On the occurrence of *Salmincola*. Ann. Rep. Fukushima Prefect Inland Fish Exp Stat Fisc 1998: 41 (In Japanese).

Watz J, Otsuki Y, Nagatsuka K, Hasegawa K, Koizumi I (2019) Temperature-dependent competition between juvenile salmonids in small streams. Freshw Biol 64: 1534–1541.

Webb TJ, Hurd H (1999) Direct manipulation of insect reproduction by agents of parasite origin. Proc R Soc Lond B Biol Sci 266: 1537–1541.

Weber JN, Kalbe M, Shim KC, Erin NI, Steinel NC, Ma L et al. (2017) Resist globally, infect locally: a transcontinental test of adaptation by stickleback and their tapeworm parasite. Am Nat 189: 43–57.

Weber JN, Steinel NC, Peng F, Shim KC, Lohman BK, Fuess LE et al. (2022) Evolutionary gain and loss of a pathological immune response to parasitism. Science 377: 1206–1211.

Weese DJ, Schwartz AK, Bentzen P, Hendry AP, Kinnison MT (2011) Eco-evolutionary effects on population recovery following catastrophic disturbance. Evol Appl 4: 354–366.

Weinstein MP, Heck KL (1977) Biology and host-parasite relationships of *Cymothoa excisa* (Isopoda, Cymothoidae) with three species of snappers (Lutjanidae)on the Caribbean coast of Panama. Fishery Bull Fish Wildl Serv Aust 49: 128–185.

Weinstein SB, Buck JC, Young HS (2018) A landscape of disgust. Science 359: 1213–1214.

Welicky RL, Parkyn DC, Sikkel PC (2018) Host-dependent differences in measures of condition associated with *Anilocra* spp. parasitism in two coral reef fishes. Environ Biol Fish 101: 1223–1234.

Werner EE, Hall DJ (1974) Optimal foraging and the size selection of prey by the bluegill sunfish (*Lepomis macrochirus*). Ecology 55: 1042–1052.

White GC, Burnham KP, Otis DL, Anderson DR (1978) User's manual for program CAPTURE. Logan, Utah, USA: Utah State University Press.

White CF, Gray MA, Kidd KA, Duffy MS, Lento J, Monk WA (2020) Prevalence and intensity of *Salmincola edwardsii* in brook trout in northwest New Brunswick, Canada. J Aquat Anim Health 32: 11–20.

Wikel SK (1996) Host immunity to ticks. Annl Rev Entomol 41: 1–22.

Wilber MQ, Weinstein SB, Briggs CJ (2016) Detecting and quantifying parasite-induced host mortality from intensity data: method comparisons and limitations. Int J Parasitol 46: 59–66.

Wilder SM, Raubenheimer D, Simpson SJ (2016) Moving beyond body condition indices as an estimate of fitness in ecological and evolutionary studies. Funct Ecol 30: 108–115.

Wilson DS, Coleman K, Clark AB, Biederman L (1993) Shy-bold continuum in pumpkinseed sunfish (*Lepomis gibbosus*): An ecological study of a psychological trait. J Comp Psychol 107: 250–260.

Wilson AD, Binder TR, McGrath KP, Cooke SJ, Godin JGJ (2011) Capture technique and fish personality: angling targets timid bluegill sunfish, *Lepomis macrochirus*. Can J Fish Aquat Sci 68: 749–757.

Wilson AD, Brownscombe JW, Sullivan B, Jain-Schlaepfer S, Cooke SJ (2015) Does angling technique selectively target fishes based on their behavioural type? PLoS ONE 10: e0135848.

Wood CL, Byers JE, Cottingham KL, Altman I, Donahue MJ, Blakeslee AM (2007) Parasites alter community structure. Proc Natl Acad Sci 104: 9335–9339.

Wood CL, Johnson PT (2015) A world without parasites: exploring the hidden ecology of infection. Front Ecol Environ 13: 425–434.

Yamada H, Wada S (2021) Morphological evolution reduces downstream displacement in juvenile landlocked salmon. Evolution 75: 1850–1861.

Yamada H, Wada S (2023) Interpopulation variation of behavioural and morphological traits that affect downstream displacement of the juvenile white-spotted charr *Salvelinus leucomaenis*. J Fish Biol 102: 1168–1176.

Yamaguti S (1939) Parasitic copepods from fishes of Japan, Part 6, Lernaeopodidae, I, Volumen Jubliare pro Professore Sadao Yoshida, 2: 529–578. 25 pls.

Yamamoto A, Nagasawa K (1999) *Salmincola carpionis*, a parasitic copepod of cultured salmonids. Rep Yamanashi Prefect Fish Tech Center 26: 26–27 (In Japanese).

Yamamoto A, Nagasawa K (2001) *Salmincola carpionis*, a parasitic copepod of cultured salmonids–II. Experimental infection, infection period, number of spawning and speed of egg development. Rep Yamanashi Prefect Fish Tech Center 28: 14–17 (In Japanese).

Yamamoto S, Nakano S, Tokuda Y (1992) Variation and divergence of the life history of Japanese white-spotted charr *Salvelinus leucomaenis* on an artificial lake-inlet stream system. Jap J Ecol 42: 149–157 (In Japanese with English abstract).

Yamamoto S, Morita K, Goto A (1999) Geographic variations in life-history characteristics of white-spotted charr. Can J Zool 77: 871–878.

Yamamoto S, Morita K, Koizumi I, Maekawa K (2004) Genetic differentiation of white-spotted charr (*Salvelinus leucomaenis*) populations after habitat fragmentation: spatial–temporal changes in gene frequencies. Conserv Genet 5: 529–538.

Yang X, Cox-Foster DL (2005) Impact of an ectoparasite on the immunity and pathology of an invertebrate: evidence for host immunosuppression and viral amplification. Proc Natl Acad Sci 102: 7470–7475.

Yazdanbakhsh M, Kremsner PG, Van Ree R (2002) Allergy, parasites, and the hygiene hypothesis. Science 296: 490–494.

Zippin C (1958) The removal method of population estimation. J Wildl Manag 22: 82–90.