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Potential negative effects and heterogeneous distribution of a parasitic copepod *Salmincola edwardsii* (Copepoda: Lernaeopodidae) on Southern Asian Dolly Varden *Salvelinus curilus* in Hokkaido, Japan

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Short title: Effects of *Salmincola edwardsii* on Southern Asian Dolly Varden

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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, we 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.

Keywords: *Salvelinus malma krascheninnikova*, Parasitic copepod, Ectoparasite, Host-parasite relationship, Condition factor, *Salmincola*

1. Introduction

The genus *Salmincola* (Family Lernaeopodidae), an ectoparasitic copepod group, mainly parasitizes freshwater salmonids [1]. Most species in this genus have a circumpolar distribution like their salmonid hosts [1]. They generally attach to the branchial cavity, buccal cavity, and fins [1, 2], with each species possessing preferred attachment sites and demonstrating strong host specificity, especially at the host genus level [1]. For instance, adult female *S. californiensis* generally attach to the branchial cavity of *Oncorhynchus* spp. [3], while *S. carpionis* commonly attach to the buccal cavity of *Salvelinus* spp. [4]. *Salmincola* spp. have been regarded as serious pests in hatcheries [5–7]. Heavy infestations can cause mechanical damage to gill tissue, which may affect the host's oxygen uptake, swimming performance, and resistance to environmental stressors [3, 8–11]. Some studies suggested that their infestations can also cause a decrease in fecundity [5] and is lethal for fries, or even adult fishes [3, 11].

While some negative impacts have been reported on hatchery or experimental fishes [5–7], 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 [12] or condition [13], whereas many others found no apparent effects [14–19]. 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 [2, 16, 19]. However, there are some cases where *Salmincola* spp. might have significantly affected or even eliminated local populations of stream salmonids [12, 20].

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 [21], host density [22], water temperature [23–25], and water flow [22, 26]. 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 [20, 22, 27]. Such distribution studies are needed, especially in East Asia, the southernmost edge of the distribution for both these copepods and their salmonid hosts [28].

During a survey of Southern Asian Dolly Varden *Salvelinus curilus* (previous studies refered to the same species as *Salvelinus malma* [29], *Salvelinus malma kraschenninnikovi* [30] or *Salvelinus malma kraschenninnikova* [28], but we used this name following Sahashi & Morita [31]) in the Shiretoko Peninsula in eastern Hokkaido, Japan [29], we found ectoparasites identical to *Salmincola edwardsii* on the branchial cavities of Southern Asian Dolly Varden. We recovered these parasite specimens from the hosts and examined their morphology and partial sequences of the 28S ribosomal RNA gene. In this study, we focused particularly on the host use and the regional distribution pattern of this parasite. We 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 [29]. 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 [29, 32, 33]. 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, Fig. 1). For the purpose of the original study [29], 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 [29]. We had frozen 215 Southern Asian Dolly Varden samples after the initial study [29], 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. We 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 mimimal.

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, we dissected the head area of all fish for a more comprehensive examination. When we found parasitic copepods, we 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, we 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), we could not confidently identify them. Thus, we 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. We 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, we 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 [34]. Morphological descriptions were made with the aid of a drawing scope equipped to the light microscope. The morphological terminology followed Kabata [1]. As males of the genus *Salmincola* are a dwarf form [35], 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.

154

155 *2.3. Molecular analysis*

156 Twenty-three specimens, i.e. five from Horobetsu Stream (No. 1 in Fig. 1), seven
157 from Funbe Stream (No. 2), one from Oshobaomabu (No. 6), two from Kamoiunbe
158 Stream (No. 7), three from Chienbetsu (No. 11), two from Okkabake (No. 13) and one
159 from Mosekarubetsu Stream (No. 12) in 2017, and two specimens from Pereke Stream
160 in 2020 were used in the following molecular analysis for species identification. Total
161 genomic DNA was extracted from whole parasites using a PureGene DNA isolation kit
162 (Applied Biosystems) for the former twenty-one samples. For the latter two specimens,
163 a part of the egg sac was used for DNA extraction, lysed in 20 µL of 0.02 N NaOH at
164 98 ℃ for 30 min [36]. We amplified a partial sequence of 28S rDNA region, which is
165 known to be useful for identifying *Salmincola* spp. [37]. The region was amplified with
166 PCR using primers D1a (5'-CCC(C/G)CGTAA(T/C)TTAAGCATAT-3') and D3b
167 (5'-TCCGGAAGGAACCAGCTACTA-3') [38]. The PCR reactions were performed in
168 10 µL and 25 µL volumes for the former and latter specimens, respectively, with
169 thermocycling protocol for gene amplification as follows: initial denaturation at 95 ºC
170 for 2 min, 35 cycles of 95 ºC for 30 s, annealing at 55 ºC for 40 s and extension at 72 ºC
171 for 90 s, followed by a further extension at 72 °C for 8 min. Purified products were
172 cycle sequenced with both the forward and reverse primers (i.e. D1a and D3b). The
173 obtained sequences were analyzed with the software MEGA ver. 10.0.4 [39], and
174 compared with known sequences of *S. edwardsii* from Norway (DQ180346) and North
175 America (KY113080, KY113081) and *S. californiensis* from North America
176 (KY113082, KY113083) [37] from the GenBank database.

177

178 *2.4. Statistical analysis*

179 We used the infestation parameters described in Bush et al. [40]; those were
180 prevalence (percentage of individuals infested), intensity (the number of individual
181 parasites in a single infested fish), and mean intensity (the average intensity among the
182 infested fish).

183 To assess the effect of the parasite on Southern Asian Dolly Varden, we evaluated if
184 the condition factor (CF) of the fish negatively correlated with the parasite number. CF
185 was calculated as $CF = 10^5 \times SW/FL^3$, where SW is somatic weight (g) and FL is fork
186 length (mm). CF was highly heterogenous among individuals specially within

uninfested fish, and the variance decreased with increasing the parasite number (see Results). Therefore, we 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 [41, 42]. We 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. We first analyzed all host individuals except for one fish with 13 parasites, which was considered to be an outlier (total $n = 211$). We 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). We used the package quantreg [43] for quantile regression analysis. All the statistical analyses were conducted using R.3.5.2 [44]. 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 (Fig. 2A). From the dorsal view, the cephalothorax was tapered from posterior to anterior, and had weak constriction around the middle (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 2M, N). Some were well developed and slender (Fig. 2M), but others were short and thick (Fig. 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 (Fig. 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 (Fig. 2D); tubercle was not observed in some specimens. The exopod was highly inflated, and their distal surface was covered by many large spines (Fig. 2E). Two palps were projected laterally, with one-two small spines around each (Fig. 2E).

The mandible usually had six teeth, but some specimens had seven. The distal four teeth were noticeably larger than the proximal two (Fig. 2F). One pair of maxillipeds was located on the anterior part of the cephalothorax (Fig. 2A). A short and curved claw was present on the distal end of the subchela with a small protrusion near its base (Fig. 2G). One auxiliary papilla (shown as "auxiliary palp" in Ruiz et al. [37]) projected from near the posterior part of the claw (Fig. 2H, I). There were some variations in the number of small spines distributed around the auxiliary papilla (Fig. 2H, I). Some specimens had only 3–4 spines (Fig. 2H), whereas others had many (generally more than 15) (Fig. 2I). Prominent palp, also with some variations, positioned at the medial margin of the corpus (Fig. 2G). Some were biramous (Fig. 2J), while others had three-branched outgrowths, but the middle one was moderate (Fig. 2K). The first maxilla with three subequal papillae at the distal end, had a small exopod near its base (Fig. 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 [37]). 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 [37]).

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 (Fig. 3) as reported in previous studies [9, 37].

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, Fig. 1). The highest value was 54.8% (Funbe, No. 2 in Fig. 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, Fig. 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; Fig. 4a). After excluding the uninfested fish from the analysis, however, a significant negative effect was detected for most of the focal quantiles (Table 2b; Fig. 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; Fig. 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 [45], [18, 46]), *S. carpionis* (reported as *S. falculata* in [47], [4, 18]), *S. stellata* [11, 48, 49], *S. edwardsii* [28, 50, 51] and *S. markewitschi* [52–54]. 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 [1]. Almost all morphology of the copepod specimens in the present study were consistent with *S. edwardsii* specimens in other studies [1, 28, 37, 52]. Thus, we morphologically identified these specimens as *S. edwardsii*. Additionally, *S. edwardsii* was also recently found from rivers in eastern Hokkaido [28, 50, 51] and the Kuril Islands [52].

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 [1]. In the present study, some specimens had numerous spines on the ventral side of the maxilliped tip (Fig. 2I), whereas others had few (Fig. 2H) even in the same population. Kabata [1] 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 [1]. However, Ruiz et al. [37] also found similar spines from North American specimens. Russian and Japanese specimens also had greater or fewer numbers of spines [28, 52]. 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) [1]. 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 [28, 52].

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 [55, 56]. 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 [1, 28].

While we 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 [2, 27]. Their attachment sites are also affected by host body size

and environmental factors like flow velocity [26, 57]. Although copepods were likely to infest the fins and fin bases on small hosts [3, 57], we could not confirm if the smaller fish could be infested on other body parts, because of the lack of small fish samples [29]. However, the main attachment site for *S. edwardsii* seemed to be branchial cavities in our study area, as previous studies reported [28, 52].

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 [15–19] or found significant negative effects only in the cases where the infestation intensity was very high (>100 copepods per host, [13]). 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 [2, 16]. 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 [3, 10, 50] and body surfaces, even at a low-infestation level [27]. We also observed whitened attachment sites, suggesting that the copepods' attachment caused gill lesions. Such damage can severely drain host energy [27], 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.

Our 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. 58, 59]. 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 exclusing 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 our sample size was not small (> 200 host individudals) and this system naturally had relatively low prelevance 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 [41, 42], 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 [60, 61]. To reveal the causal relationship of our results, a mark-recapture study and/or lab experiments are required in a future study [19].

4.3. Regional distribution pattern and infestation of anadromous fish

Our 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 [20, 22]. In particular, since the genus *Salmincola* is host-specific [1], 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 our 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* [62]. 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,

389 such as construction of dams and logging, which increases water temperature [63].
390 Because the development and life-history of *Salmincola* spp. are strongly affected by
391 water temperature [23–25], such habitat modifications mediate parasite life cycles and,
392 hence, affect parasite load. In addition, construction of dams can change the water
393 current pattern. In general, large pools or glides were often created below or above the
394 dams, which would reduce water current velocity. It is suggested that the copepodids of
395 the genus *Salmincola* can attach to hosts more easily under lower current conditions [22,
396 26]. Furthermore, not only physical but also biological characteristics could affect the
397 distribution and abundance of *S. edwardsii*. For example, the density of hosts generally
398 plays an important role in the sustainability of parasite populations [64, 65], including
399 *Salmincola* [22]. In the future, we should consider multiple variables in identifying the
400 limiting factors of distribution.
401 Since we recovered a copepod from an anadromous individual, it is possible for
402 dispersal of the copepod between streams via anadromous host fish. Indeed, though *S.*
403 *edwardsii* are a freshwater species, living individuals were recovered from hosts
404 captured in the sea or brackish water, suggesting that this species has salinity tolerance
405 [2, 52]. Nagasawa [28] investigated the distribution of *S. edwardsii* from 9 rivers on
406 Hokkaido Island, and found them only from the eastern side of the island, where some
407 fish show anadromy [29, 32, 33, 66]. The author concluded that the anadromy of the
408 hosts may play an important role in its distribution expansion [28]. However, this
409 possibility is limited to the regional scale because the degree of anadromy was low in
410 the Shiretoko Peninsula [32]. If the introduction of this parasite by migrants frequently
411 occurs, the infestation level should be similar among neighboring streams because
412 dispersal of migrants would occur in neighboring streams. However, in the present
413 study, no such pattern was observed. Therefore, dispersal should be insufficient to
414 homogenize the abundance or distribution of *S. edwardsii*. In fact, the probability of
415 dispersal on anadromous fish is possibly very low, because we could find only one
416 copepod from an anadromous form. However, the sample size was very small
417 (anadromous host, $n = 15$), and we cannot rule out the possibility that the infestion of
418 the copepod on the migrant occurred after returning to the stream from the sea. It is
419 necessary to confirm if this population of copepods can survive in saline conditions.
420
421
422 *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, we 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 [67]. 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 [12]. 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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Declarations of interest

None.

References

[1] Z. Kabata, Revision of the genus *Salmincola* Wilson, 1915 (Copepoda: Lernaeopodidae), J. Fish. Res. Board. Can. 26 (1969) 2987–3041. https://doi.org/10.1139/f69-285.

[2] G. A. Black, W. L. Montgomery, F. G. Whoriskey, Abundance and distribution of *Salmincola edwardsii* (Copepoda) on anadromous brook trout, *Salvelinus fontinalis*, (Mitchill) in the Moisie River system, Quebec, J. Fish Biol. 22 (1983) 567–575. https://doi.org/10.1111/j.1095-8649.1983.tb04216.x.

[3] Z. Kabata, B. Cousens, Host–parasite relationships between sockeye salmon, *Oncorhynchus nerka*, and *Salmincola californiensis* (Copepoda: Lernaeopodidae), J. Fish. Res. Board. Can. 34 (1977) 191–202. https://doi.org/10.1139/f77-029.

[4] K. Nagasawa, M. Yamamoto, Y. Sakurai, A. Kumagai, 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 (1995) 178–185. 10.1139/F95-525.

[5] G. A. E. Gall, E. L. Mcclendon, W. E. Schafer, 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 (1972) 345–346. https://doi.org/10.1577/1548-8659(1972)101<345:EOTIOT>2.0.CO;2

[6] W. Piasecki, A. E. Goodwin, J. C. Eiras, B. F. Nowak, Importance of Copepoda in freshwater aquaculture, Zool. Stud. 43 (2004) 193–205.

[7] R. J. Roberts, K. A. Johnson, M. T. Casten, Control of *Salmincola californiensis* (Copepoda: Lernaeapodidae) in rainbow trout, *Oncorhynchus mykiss* (Walbaum): a clinical and histopathological study, J. Fish Dis. 27 (2004) 73–79. https://doi.org/10.1046/j.1365-2761.2003.00508.x.

476 [8] K. Pawaputanon, Effects of parasitic copepod, *Salmincola californiensis* (Dana,
477 1852) on juvenile sockeye salmon, *Oncorhynchus nerka* (Walbaum), Doctoral
478 dissertation, University of British Columbia (1980).
479 https://dx.doi.org/10.14288/1.0095204.
480 [9] D. R. Sutherland, D. D. Wittrock, The effects of *Salmincola californiensis*
481 (Copepoda: Lernaeopodidae) on the gills of farm-raised rainbow trout, *Salmo*
482 *gairdneri*, Can. J. Zool. 63 (1985) 2893–2901. https://doi.org/10.1139/z85-433.
483 [10] C. L. Herron, M. L. Kent, C. B. Schreck, Swimming Endurance in Juvenile
484 Chinook Salmon Infected with *Salmincola californiensis*, J. Aquat. Anim. Health.
485 30 (2018) 81–89. https://doi.org/10.1002/aah.10010.
486 [11] N. Hiramatsu, H. Fukada, M. Kitamura, M. Shimizu, H. Fuda, K. Kobayashi, A.
487 Hara, Serum Immunoglobulin M (IgM) in Sakhalin Taimen (*Hucho perryi*),
488 Suisanzoshoku 49 (2001) 347–355.
489 https://doi.org/10.11233/aquaculturesci1953.49.347.
490 [12] M. G. Mitro, Brook Trout, Brown Trout, and ectoparasitic copepods *Salmincola*
491 *edwardsii*: species interactions as a proximate cause of Brook Trout loss under
492 changing environmental conditions, Trans. Am. Fish. Soc. 145 (2016) 1223–1233.
493 https://doi.org/10.1080/00028487.2016.1219676.
494 [13] S. Kusterle, R. Kristoffersen, A. H. Rikardsen, Population dynamics of *Salmincola*
495 *salmoneus* on Atlantic salmon in a northern Norwegian river, Dis. Aquat. Org. 100
496 (2012) 59–70. https://doi.org/10.3354/dao02489.
497 [14] G. E. Vaughan, D. W. Coble, Sublethal effects of three ectoparasites on fish, J. Fish
498 Biol. 7 (1975) 283–294. https://doi.org/10.1111/j.1095-8649.1975.tb04601.x.
499 [15] II. C. A. Bowen, R. M. Stedman, Host–parasite relationships and geographic
500 distribution of *Salmincola corpulentus* (Copepoda: Lernaeopodidae) on bloater

(*Coregonus hoyi*) stocks in Lake Huron, Can. J. Zool. 68 (1990) 1988–1994. https://doi.org/10.1139/z90-280.

[16] P. A. Amundsen, R. Kristoffersen, R. Knudsen, A. Klemetsen, Infection of *Salmincola edwardsii* (Copepoda: Lernaeopodidae) in an age-structured population of Arctic charr—a long-term study, J. Fish Biol. 51 (1997) 1033–1046. https://doi.org/10.1111/j.1095-8649.1997.tb01542.x.

[17] P. Chigbu, Occurrence and distribution of *Salmincola californiensis* (Copepoda: Lernaeopodidae) on juvenile sockeye salmon (*Oncorhynchus nerka*) in Lake Washington, J. Freshw. Ecol. 16 (2001) 615–620. https://doi.org/10.1080/02705060.2001.9663853.

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

[19] C. G. Ayer, K. Morita, S. Fukui, I. Koizumi, No apparent effects of the buccal cavity attaching parasite, *Salmincola* sp.(Copepoda: Lernaeopodidae), on a stream salmonid: a mark-recapture study, Ichthyol. Res. online early. https://doi.org/10.1007/s10228-021-00835-0.

[20] M. G. Mitro, J. D. Griffin, Distribution, prevalence, and maximum intensity of the ectoparasitic copepod *Salmincola* cf. *edwardsii* in brook trout in Wisconsin streams, J. Parasitol. 104 (2018) 628–638. https://doi.org/10.1645/17-146.

[21] R. Poulin, M. E. Rau, M. A. Curtis, Infection of brook trout fry, *Salvelinus fontinalis*, by ectoparasitic copepods: the role of host behaviour and initial parasite load, Anim. Behav. 41 (1991) 467–476. https://doi.org/10.1016/S0003-3472(05)80849-8.

[22] R. Hasegawa, I. Koizumi, Relative importance of host dependent vs. physical environmental characteristics affecting the distribution of an ectoparasitic copepod

527 infecting to the mouth cavity of stream salmonid, Ecol. Res. 36 (2021) 1015–1027,
528 doi:10.1111/1440-1703.12262.
529 [23] S. E. McGladdery, C. E. Johnston, Egg development and control of the gill parasite,
530 *Salmincola salmoneus*, on Atlantic salmon kelts (*Salmo salar*) exposed to four
531 different regimes of temperature and photoperiod, Aquaculture 68 (1988) 193–202.
532 https://doi.org/10.1016/0044-8486(88)90352-3.
533 [24] D. C. Conley, M. A. Cutis, Effects of temperature and photoperiod on the duration
534 of hatching, swimming, and copepodid survival of the parasitic copepod *Salmincola*
535 *edwardsii,* Can. J. Zool. 71 (1993) 972–976.
536 [25] E. M. Vigil, K. R. Christianson, J. K. Lepak, P. J. Williams, Temperature effects on
537 hatching and viability of juvenile gill lice, *Salmincola californiensis*, J. Fish Dis. 39
538 (2016) 899–905. https://doi.org/10.1111/jfd.12422.
539 [26] F. R. Monzyk, T. A. Friesen, J. D. Romer, Infection of juvenile salmonids by
540 *Salmincola californiensis* (Copepoda: Lernaeopodidae) in reservoirs and streams of
541 the Willamette River basin, Oregon, Trans. Am. Fish. Soc. 144 (2015) 891–902.
542 https://doi.org/10.1080/00028487.2015.1052558.
543 [27] C. F. White, M. A. Gray, K. A. Kidd, M. S. Duffy, J. Lento, W. A. Monk,
544 Prevalence and intensity of *Salmincola edwardsii* in brook trout in Northwest New
545 Brunswick, Canada, J. Aquat. Anim. Health 32 (2020) 11–20.
546 https://doi.org/10.1002/aah.10091.
547 [28] K. Nagasawa, *Salmincola edwardsii* (Copepoda: Lernaeopodidae) parasitic on
548 southern Asian Dolly Varden, *Salvelinus malma krascheninnikova*, from Hokkaido
549 Island, Japan, with the southernmost distribution record of the copepod in Asia,
550 Spec. Divers. 25 (2020) 197–203. https://doi.org/10.12782/specdiv.25.197.

[29] Y. Umatani, T. Arai, K. Maekawa, Flexible seaward migration of Dolly Varden *Salvelinus malma* in the Shiretoko Peninsula, Hokkaido, Japan, Ichthyol. Res. 65 (2018) 202–209. 10.1007/s10228-017-0606-3.

[30] H. Katahira, C. Yamazaki, S. Fukui, C. G. Ayer, I. Koizumi, Spatial aggregation in small spring-fed tributaries leads to a potential metapopulation structure in a parasitic fish leech, Parasitol. Open 3 (2017) e11. https://doi.org/10.1017/pao.2017.12.

[31] G. Sahashi, K. Morita, Southern Asian Dolly Varden Charr, *Salvelinus curilus* on the Higashi-Nodottomari River in Rishiri Island, Rishiri Stud. 40 (2021) 13–20.

[32] K. Morita, T. Arai, D. Kishi, J. Tsuboi, Small anadromous *Salvelinus malma* at the southern limits of its distribution, J. Fish Biol. 66 (2005) 1187–1192. https://doi.org/10.1111/j.0022-1112.2005.00672.x.

[33] Y. Umatani, T. Arai, K. Maekawa, Variation in migratory history of Dolly Varden in a stream with an artificial dam in the Shiretoko Peninsula, Hokkaido, Japan, Environ. Biol. Fish. 83 (2008) 37–44. https://doi.org/10.1007/s10641-007-9249-y.

[34] A. G. Humes, R. U. Gooding, A method for studying the external anatomy of copepods, Crustaceana 6 (1964) 238–240. https://doi.org/10.1163/156854064X00650.

[35] Z. Kabata, B. Cousens, Life cycle of *Salmincola californiensis* (Dana 1852)(Copepoda: Lernaeopodidae), J. Fish. Res. Board. Can. 30 (1973) 881–903. https://doi.org/10.1139/f73-150.

[36] M. Nakao, M. Sasaki, T. Waki, J. L. Anders, H. Katahira, *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 (2018) 565–574. https://doi.org/10.1016/j.parint.2018.04.010.

[37] C. F. Ruiz, J. M. Rash, D. A. Besler, J. R. Roberts, M. B. Warren, C. R. Arias, S. A. Bullard, 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 (2017) 377–389. https://doi.org/10.1645/16-165.

[38] B. M. von Reumont, K. Meusemann, N. U. Szucsich, E. Dell'Ampio, V. Gowri-Shankar, D. Bartel, J. W. Wägele, Can comprehensive background knowledge be incorporated into substitution models to improve phylogenetic analyses? A case study on major arthropod relationships, BMC Evol. Biol. 9 (2009) 119. http://www.biomedcentral.com/1471-2148/9/119.

[39] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, MEGA X: molecular evolutionary genetics analysis across computing platforms, Mol. Biol. Evol. 35 (2018) 1547–1549. https://doi.org/10.1093/molbev/msy096.

[40] A. O. Bush, K. D. Lafferty, J. M. Lotz, A. W. Shostak, Parasitology meets ecology on its own terms: Margolis et al. revisited, J. Parasitol. (1997) 575–583. https://doi.org/10.2307/3284227.

[41] B. S. Cade, B. R. Noon, A gentle introduction to quantile regression for ecologists, Front. Ecol. Environ. 1 (2003) 412–420. https://doi.org/10.1890/1540-9295(2003)001[0412:AGITQR]2.0.CO;2.

[42] K. Das, M. Krzywinski, N. Altman, Quantile regression, Nat. Methods 16 (2019) 451–452. https://doi.org/10.1038/s41592-019-0406-y.

[43] R. Koenker, S. Portnoy, P. T. Ng, A. Zeileis, P. Grosjean, B. D. Ripley, Package ‘quantreg’, Cran R-project. org. (2018).

[44] R Core team Team, R: a language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria (2017) https://www.r-project.org/.

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

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

[47] S. Yamaguti, Parasitic copepods from fishes of Japan, Part 6, Lernaeopodidae, I, Volumen Jubliare pro Professore Sadao Yoshida, 2 (1939) 529–578. 25 pls.

[48] K. Nagasawa, S. Urawa, 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 (1991) 57–59.

[49] K. Nagasawa, J. R. Watanabe, S. Kimura, A. Hara, Infection of *Salmincola stellatus* (Copepoda: Lernaeopodidae) on Sakhalin taimen *Hucho perryi* reared in Hokkaido, Bull. Fac. Fish. Hokkaido Uni. 45 (1994) 109–112.

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

[51] K. Nagasawa, K. Kawai, The parasitic copepod *Salmincola edwardsii* from sothern Asian Dolly Varden, *Salvelinus malma krascheninnikova,* 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 (2020c) 129–132 (in Japanese with English abstract).

[52] M. B. Shedko, S. V. Shedko, Parasitic copepods of the genus *Salmincola* (Lernaeopodidae) from the far eastern chars *Salvelinus* (Salmonidae) with description of the new species *S. markewitschi*, Zoologicheskii Zhurnal 81 (2002) 141–153 (in Russian with English abstract).

[53] K. Nagasawa, *Salmincola markewitschi* (Copepoda: Lernaeopodidae) Parasitic on Whitespotted Char, *Salvelinus leucomaenis*, in a Moutain Stream of Honshu Island, Central Japan, Spec. Divers. 25 (2020c) 369–375. https://doi.org/10.12782/specdiv.25.369.

[54] K. Nagasawa, N. Ishiyama, *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.

[55] W. E. Hogans, 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 (1987) 442–453.

[56] P. Abaunza, N. L. Arroyo, I. Preciado, 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 (2001) 193–210. https://doi.org/10.1163/156854001750096292.

[57] G. A. Black, Gills as an attachment site for *Salmincola edwardsii* (Copepoda: Lernaeopodidae), J. Parasitol. 68 (1982) 1172–1173.

[58] A. D. Lemly, G. W. Esch, Effects of the trematode *Uvulifer ambloplitis* on juvenile bluegill sunfish, *Lepomis macrochirus*: ecological implications, J. Parasitol. 70 (1984) 475–492. https://doi.org/10.2307/3281395.

[59] S. A. O'Connell-Milne, R. Poulin, C. Savage, W. Rayment, Reduced growth, body condition and foot length of the bivalve Austrovenus stutchburyi in response to parasite infection, J. Exp. Mar. Biol. Ecol. 474 (2016) 23–28. https://doi.org/10.1016/j.jembe.2015.09.012.

[60] P. M. Beldomenico, S. Telfer, S. Gebert, L. Lukomski, M. Bennett, M. Begon, Poor condition and infection: a vicious circle in natural populations. Proc. R. Soc. B 275 (2008) 1753–1759. https://doi.org/10.1098/rspb.2008.0147.

[61] P. M. Beldomenico, M. Begon, Disease spread, susceptibility and infection intensity: vicious circles? Trends Ecol. Evol. 25 (2010) 21–27. https://doi.org/10.1016/j.tree.2009.06.015.

[62] K. Morita, D. Kishi, J. Tsuboi, S. Morita, T. Aarai, Rainbow trout and brown trout in Shiretoko Peninsula, Hokkaido, Japan, Bull. Shiretoko Museum 24 (2003) 17–26 (in Japanese with English abstract).

[63] D. Kishi, K. Maekawa, 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 (2009) 873–880. https://doi.org/10.1007/s11284-008-0562-5.

[64] R. M. Anderson, R. M. May, Regulation and stability of host-parasite population interactions: I. regulatory processes, J. Anim. Ecol. (1978) 219–247. https://doi.org/10.2307/3933.

[65] P. Arneberg, Host population density and body mass as determinants of species richness in parasite communities: comparative analyses of directly transmitted nematodes of mammals, Ecography. 25 (2002) 88–94. https://doi.org/10.1034/j.1600-0587.2002.250110.x.

[66] E. Komiyama, N. Ohtaishi, K. Maekawa, Occurrence of a sea-run type of the Dolly Varden in the Shiretoko Peninsula, Hokkaido, Japan, Ichthyol. Res. 29 (1982) 298–302. https://doi.org/10.11369/jji1950.29.298.

[67] S. Nakano, F. Kitano, K. Maekawa, Potential fragmentation and loss of thermal habitats for charrs in the Japanese archipelago due to climatic warming, Freshw. Biol. 36 (1996) 711–722. https://doi.org/10.1046/j.1365-2427.1996.d01-516.x.

Table

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

Fig. 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.

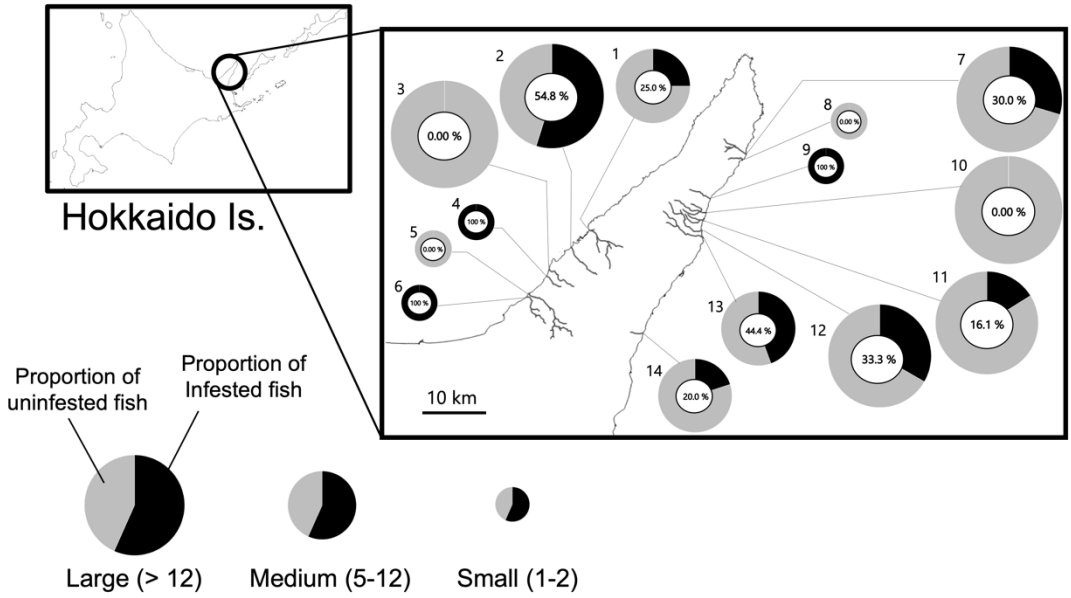
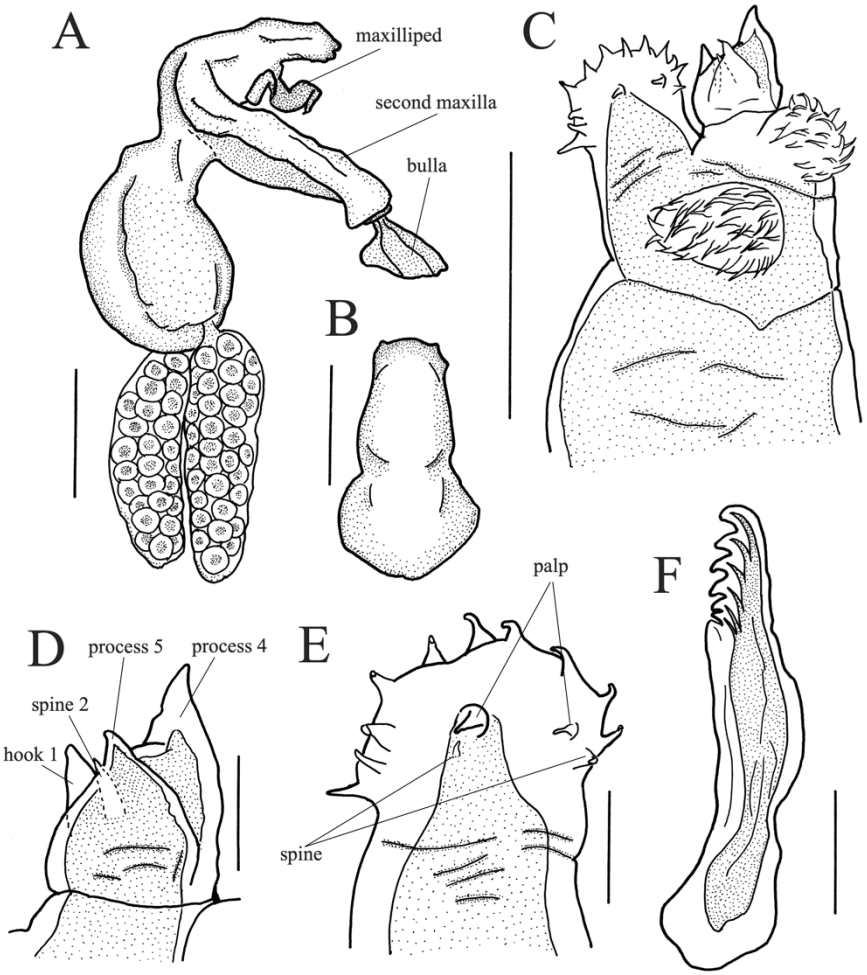


Fig. 2. Adult female *Salmincola edwardsii* (Copepoda: Lernaeopodidae) from Southern Asian Dolly varden *Salvelinus curilus,* from the Shiretoko Peninsula, Hokkaido, Japan. ID indicates the specimen's ID. A. Entire (lateral view, ID4); B. cephalothorax (dorsal view, ID5); C. second antenna, entire (lateral 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.



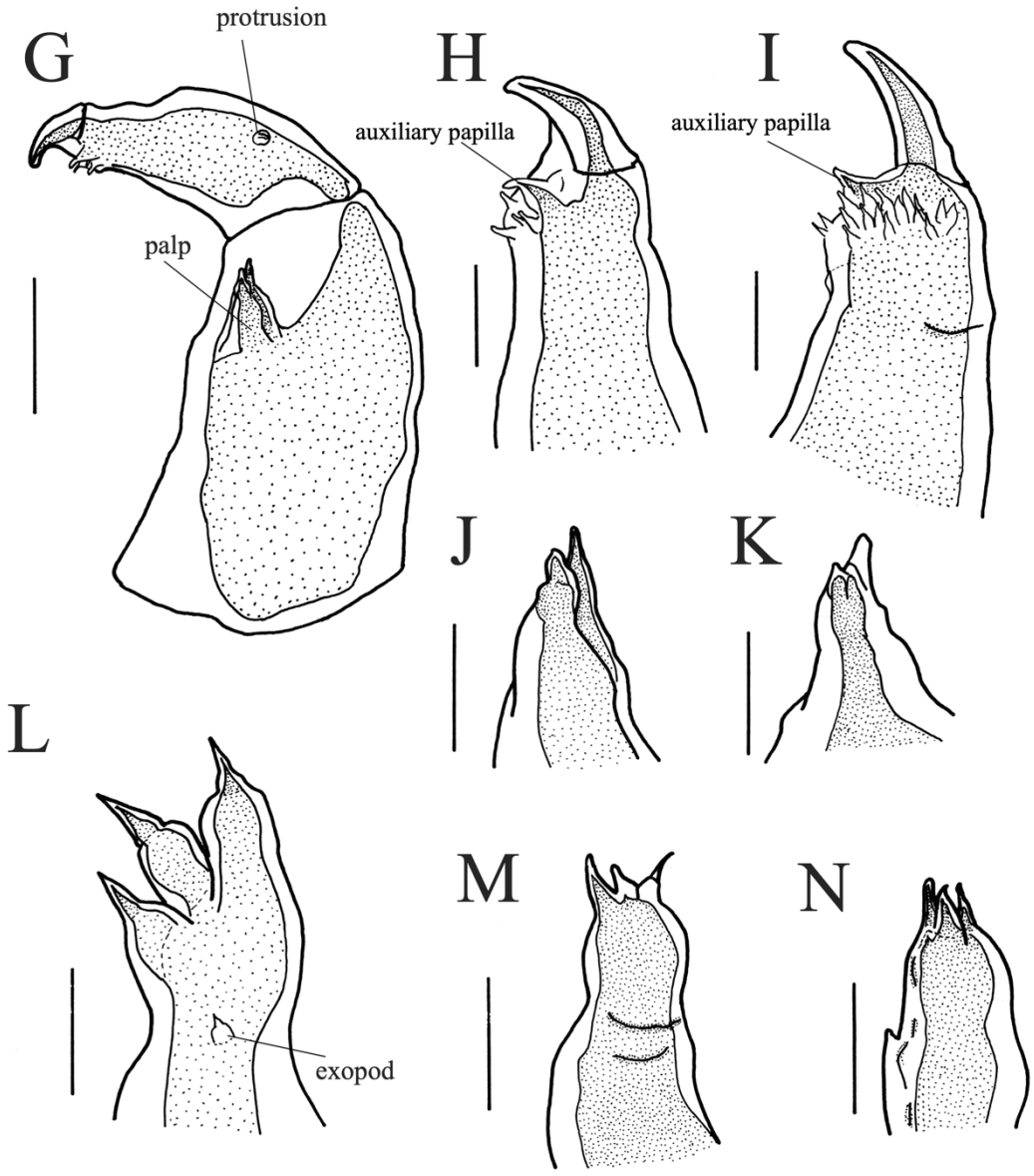

G
protrusion
palp
H
auxiliary papilla
I
auxiliary papilla
J
K
L
exopod
M
N

Fig. 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.

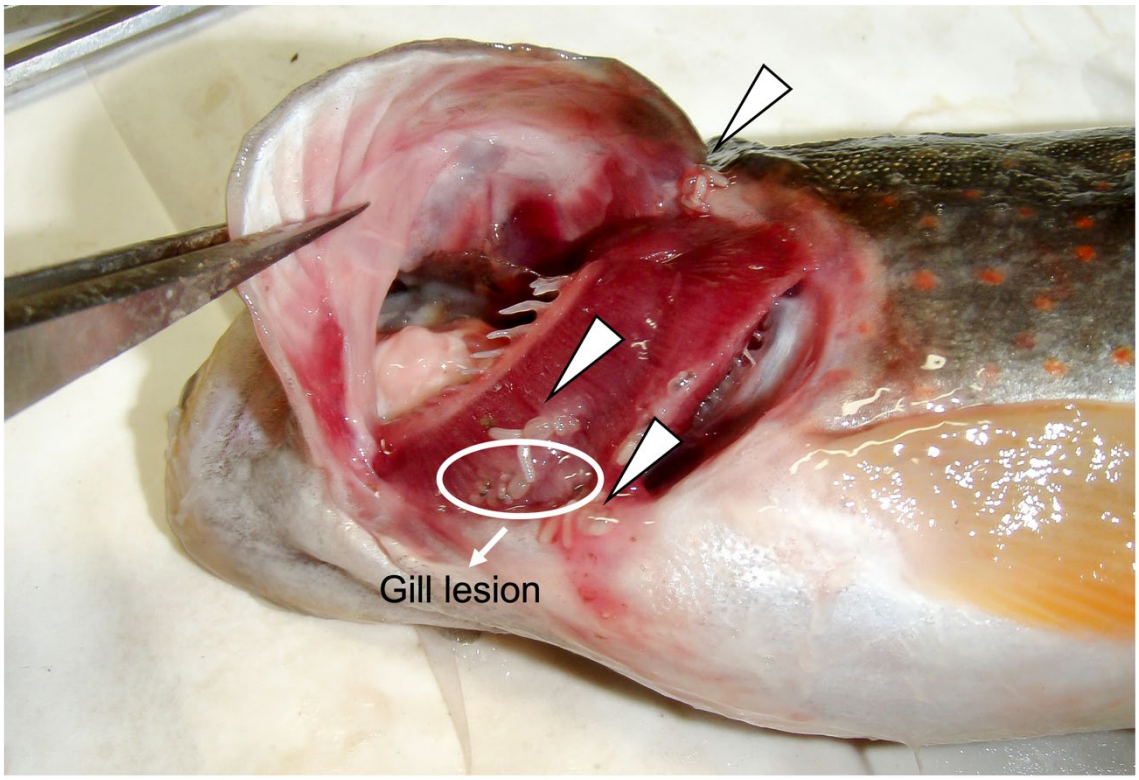


Fig. 4. The relationship between the condition factor of Southern Asian Dolly Varden *Salvelinus curilus* and the number of *Salmincola edwardsii*, analyzed by a quantile regression. (a) the results analyzed with all fish (except for the individual with 13 parasites) and (b) the results analyzed with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

