

Title	Potential negative effects and heterogeneous distribution of a parasitic copepod Salmincola edwardsii (Copepoda: Lernaeopodidae) on Southern Asian Dolly Varden Salvelinus curilus in Hokkaido, Japan
Author(s)	Hasegawa, Ryota; Ayer, Christopher G.; Umatani, Yoshiyuki; Miura, Kazuki; Ukumura, Miyo; Katahira, Hirotaka; Koizumi, Itsuro
Citation	Parasitology International, 87, 102529 https://doi.org/10.1016/j.parint.2021.102529
Issue Date	2022-04
Doc URL	http://hdl.handle.net/2115/88947
Rights	© 2022. This manuscript version is made available under the CC-BY-NC-ND 4.0 license https://creativecommons.org/licenses/by-nc-nd/4.0/
Rights(URL)	https://creativecommons.org/licenses/by-nc-nd/4.0/
Туре	article (author version)
File Information	Hasegawa et al_HUSCUP_revised.pdf



1	Parasitology International
2	
3	Potential negative effects and heterogeneous distribution of a parasitic copepod
4	Salmincola edwardsii (Copepoda: Lernaeopodidae) on Southern Asian Dolly
5	Varden <i>Salvelinus curilus</i> in Hokkaido, Japan
6	
7	Ryota Hasegawa ^a , Christopher G. Ayer ^a , Yoshiyuki Umatani ^{b,c} , Kazuki Miura ^d , Miyo
8	Ukumura ^e , Hirotaka Katahira ^f & Itsuro Koizumi ^{a, g}
9	
10	^a Graduate School of Environmental Science, Hokkaido University, Sapporo, Hokkaido,
11	060-0810, Japan
12	^b Field Science Center for Northern Biosphere, Hokkaido University, Sapporo,
13	Hokkaido, 060-0809, Japan
14	^c Present address: Nakagawa Experimental Forest, Forest Research Station, Field
15	Science Center for Northern Biosphere, Hokkaido University, Otoineppu,
16	Hokkaido, 098-2501, Japan
17	^d Shiretoko Museum, Honmachi 49, Shari, Shari-gun, Hokkaido, 099-4113, Japan
18	^e Sapporo Wild Salmon Project, 2-1 Makomanai Park, Minami-ku, Sapporo, Hokkaido,
19	005-0017, Japan
20	^f Department of Environmental Science, School of Life and Environmental Science,
21	Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara, Kanagawa, 252-5201,
22	Japan
23	^g Faculty of Environmental Earth Science, Hokkaido University, Sapporo, Hokkaido,
24	060-0810, Japan
25	
26	Corresponding author: Ryota Hasegawa, Graduate School of Environmental Science,
27	Hokkaido University, Sapporo, Hokkaido, 060-0810, Japan, Tel: +81 11 706 2620, Fax:
28	+81 11 706 4864, Email: ryotahase344922@gmail.com
29	
30	Short title: Effects of Salmincola edwardsii on Southern Asian Dolly Varden
31	
32	Declarations of Interest: None

34 Abstract

35 The genus *Salmincola* is an ectoparasitic copepod group commonly infesting the branchial and buccal cavities of salmonids. While negative impacts on hatchery fishes 36 37 have been reported, their impacts on wild fish populations and distribution patterns are 38 critically understudied. In the Shiretoko Peninsula, Hokkaido, Japan, we found parasites 39 belonging to this genus on the branchial cavity of a stream salmonid, Southern Asian 40 Dolly Varden Salvelinus curilus. All parasites recovered were identified as Salmincola 41 edwardsii based on morphological characteristics and partial 28S rDNA sequences. 42 Prevalence was highly heterogeneous even among neighboring streams (0-54.8%, <10)43 km) with the mean intensity among streams being generally low (2.19 parasites/infeted 44 fish). Despite the low intensity, quantile regression analysis showed negative trends 45 between parasite intensity and host condition, suggesting that the infestation of S. 46 edwardsii has a potential negative impact on the host salmonid. In addition, a single 47 copepod was found from an anadromous fish, which could indicate some salinity 48 tolerance of the copepods. It is important to evaluate the effects of Salmincola spp. on 49 host species and determine the limiting factors on the parasite's distribution for proper 50 management. 51 52 Keywords: Salvelinus malma krascheninnikova, Parasitic copepod, Ectoparasite,

- 53 Host-parasite relationship, Condition factor, *Salmincola*
- 54

55 1. Introduction

56 The genus *Salmincola* (Family Lernaeopodidae), an ectoparasitic copepod group, 57 mainly parasitizes freshwater salmonids [1]. Most species in this genus have a 58 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 59 60 attachment sites and demonstrating strong host specificity, especially at the host genus 61 level [1]. For instance, adult female S. californiensis generally attach to the branchial 62 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 63 64 hatcheries [5–7]. Heavy infestations can cause mechanical damage to gill tissue, which 65 may affect the host's oxygen uptake, swimming performance, and resistance to 66 environmental stressors [3, 8–11]. Some studies suggested that their infestations can 67 also cause a decrease in fecundity [5] and is lethal for fries, or even adult fishes [3, 11]. 68 While some negative impacts have been reported on hatchery or experimental fishes 69 [5–7], their impacts on wild fish populations have been less understood. Only a few 70 studies have suggested negative impacts on wild host salmonids, such as reduced 71 recruitment [12] or condition [13], whereas many others found no apparent effects 72 [14–19]. Some authors have concluded that the impacts of *Salmincola* spp. on host 73 fishes are negligible in the wild because the prevalence and intensity are generally low 74 compared to hatchery fishes [2, 16, 19]. However, there are some cases where 75 Salmincola spp. might have significantly affected or even eliminated local populations 76 of stream salmonids [12, 20]. 77 Detailed distributional records of Salmincola spp. have also been limited, even 78 though such basic information is important for pest management. The duration of their 79 life cycle and attachment to the host is affected by numerous environmental factors, 80 such as host behavior [21], host density [22], water temperature [23–25], and water flow 81 [22, 26]. Thus, the local environment should affect the parasites' infestation parameters

- 82 (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,
- 84 especially in East Asia, the southernmost edge of the distribution for both these
- 85 copepods and their salmonid hosts [28].
- 86

87 During a survey of Southern Asian Dolly Varden Salvelinus curilus (previous studies 88 refered to the same species as Salvelinus malma [29], Salvelinus malma 89 krascheninnikovi [30] or Salvelinus malma krascheninnikova [28], but we used this 90 name following Sahashi & Morita [31]) in the Shiretoko Peninsula in eastern Hokkaido, 91 Japan [29], we found ectoparasites identical to Salmincola edwardsii on the branchial 92 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 93 94 RNA gene. In this study, we focused particularly on the host use and the regional 95 distribution pattern of this parasite. We also examined if S. edwardsii was found from 96 anadromous (i.e. sea-run) host fish, which could be a possible indication of salinity 97 tolerance in this parasite species.

98

99 2. Material and methods

100 2.1. Host collection and inspection

101 Fish samples used in this study were originally aimed for investigating the anadromy 102 of Southern Asian Dolly Varden in the Shiretoko Peninsula using otolith Sr:Ca ratio 103 [29]. Most Southern Asian Dolly Varden are fluvial (stream resident) in Hokkaido 104 Island, but anadromous (sea-run) fish have been found in some streams in the Shiretoko 105 Peninsula [29, 32, 33]. Southern Asian Dolly Varden were collected by backpack-electrofisher (Smith-Root, Inc., Vancouver, Washington) and cast-net at 14 106 107 streams in the Shiretoko Peninsula, eastern Hokkaido, Japan (Table 1, Fig. 1). For the 108 purpose of the original study [29], Southern Asian Dolly Varden larger than 17 cm were 109 mainly collected. Sampling reaches were around 100-200 m from the mouth of the 110 streams. Field surveys were conducted from October 2006 to November 2006. A total 111 of 218 fish were brought to the laboratory for analyzing otolith Sr:Ca ratios to examine 112 the anadromy of Southern Asian Dolly Varden [29]. We had frozen 215 Southern Asian 113 Dolly Varden samples after the initial study [29], and kept them in storage until the 114 examination of the genus Salmincola in 2017. 115 In 2017, fish body length (fork length: FL) and weight (somatic weight: SW) were 116 measured to the nearest 1 mm and 0.1 g, respectively in the laboratory. We used somatic 117 weight (excluding internal organs) instead of total body weight because some Southern

- 118 Asian Dolly Varden might have released eggs or sperm (samples were collected during
- 119 breeding season), which could cause potential bias when assessing body condition.

4

120 Although some fish exhibited fork length shrinkages due to the long term freezing, fork 121 lengths taken at collection (in 2006) were highly correlated with those measured in 2017 122 (Pearson's r = 0.986). In addition, since all sampled fish had been frozen in the same 123 way, the potential biases due to freezing should be mimimal.

124 The branchial cavity, buccal cavity, body surface, fins and fin bases were examined 125 for the presence of the parasites. Since it was difficult to confirm the presence of 126 copepods on the branchial and buccal cavity, we dissected the head area of all fish for a 127 more comprehensive examination. When we found parasitic copepods, we recorded 128 their attachment sites following two categories: gills (gill filaments and gill arches) and 129 inner opercula. All copepods found were removed and preserved in 90 % ethanol. As 130 one individual had no tail fin, we excluded this-individual from the statistical analysis 131 (though retained it for the calculation of prevalence and mean intensity, see below).

132

133 2.2. Morphological identification of the copepod specimens

134 Since the parasite specimens recovered in 2017 were relatively low quality due to 135 being frozen for a long time (i.e. about 11 years), we could not confidently identify 136 them. Thus, we conducted additional sampling at the Pereke Stream, Shiretoko 137 Peninsula on 26 July 2020. Cast net fishing was performed in four pools of the stream 138 and a total of 30 Southern Asian Dolly Varden were captured. We visually checked the 139 branchial cavity of each collected fish in the field. When infestation of the copepods 140 was confirmed, the infested fish was immediately frozen (i.e. about a week) and sent to 141 the laboratory of Azabu University, Kanagawa prefecture. In the laboratory, we 142 carefully removed the copepods by forceps and preserved them in 70% ethanol for 143 morphological and molecular identification.

144 Morphological examination was carried out using a light microscope (BX53,

145 Olympus Inc., Japan) and a stereo microscope (SZX16, Olympus Inc., Japan). Five

146 copepods were soaked in lactophenol, then dissected under the stereo microscope using

147 the wooden slide method described by Humes & Gooding [34]. Morphological

148 descriptions were made with the aid of a drawing scope equipped to the light

149 microscope. The morphological terminology followed Kabata [1]. As males of the

150 genus *Salmincola* are a dwarf form [35], only females were subject to the morphological

151 examinations. The specimens examined were deposited in the Invertebrates collection

152 of the Hokkaido University Museum (ICHUM 6259, 6260, 6261, 6262, 6263), Sapporo,

153 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 °C 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 °C 170 for 2 min, 35 cycles of 95 °C for 30 s, annealing at 55 °C for 40 s and extension at 72 °C for 90 s, followed by a further extension at 72 °C for 8 min. Purified products were 171 172 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 compared with known sequences of S. edwardsii from Norway (DQ180346) and North 174 175 America (KY113080, KY113081) and S. californiensis from North America 176 (KY113082, KY113083) [37] from the GenBank database.

177

178 2.4. Statistical analysis

We used the infestation parameters described in Bush et al. [40]; 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, we 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

- 187 uninfested fish, and the variance decreased with increasing the parasite number (see
- 188 Results). Therefore, we used quantile regression analysis instead of normal regression
- 189 analysis (e.g. least squares regression analysis). Quantile regression analysis estimates
- 190 any conditional quantiles of a response variable independently (instead of conditional
- 191 mean) and is robust for the data with unequal variance [41, 42]. We calculated focal
- 192 quantiles in steps of 0.1 from tau = 0.1 to tau = 0.9. The response variable was the CF
- 193 and explanatory variables were the number of parasites. We first analyzed all host
- 194 individuals except for one fish with 13 parasites, which was considered to be an outlier
- 195 (total n = 211). We then performed the same analysis focused only on infested fish (n = 211).
- 196 52) because the CF of uninfested fish had large variance and skewed the distribution of
- 197 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].
- 199 Differences were considered significant at p < 0.05.
- 200

3. Results

202 *3.1. Morphological details of the parasite*

203 Each individual body consisted of three major components: cephalothorax, second 204 maxilla, and trunk (Fig. 2A). From the dorsal view, the cephalothorax was tapered from 205 posterior to anterior, and had weak constriction around the middle (Fig. 2B). It was 206 slightly shorter than its trunk (2.19–2.58 mm, mean = 2.40 mm, n = 5) and was 207 separated by slight constriction from the trunk. Second maxilla was extended from each 208 side of the cephalothorax (Fig. 2A), and the distal end was fused forming the base of the 209 bulla. The distal surface of the bulla was convex. Trunk was almost ovoid (2.15–2.98 210 mm, mean = 2.50 mm, n = 5). Two egg sacs were attached at its posterior end (Fig. 2A), 211 though one specimen had only one egg sac. Total body length (excluding egg sacs) was 212 3.54-4.70 mm (mean = 4.15 mm, n = 5).

First antenna, devoid of segmentation, with generally three short setae at its apex

- 214 (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.
- 216 The tips of the biramous sympod had a large spiny pad on the basal surface and were
- 217 composed of an endopod with two segments and an unsegmented exopod (Fig. 2C).
- 218 Large, protruding spiny pads were also present on the lateral side of the basal segment
- 219 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).

225 The mandible usually had six teeth, but some specimens had seven. The distal four 226 teeth were noticeably larger than the proximal two (Fig. 2F). One pair of maxillipeds 227 was located on the anterior part of the cephalothorax (Fig. 2A). A short and curved claw 228 was present on the distal end of the subchela with a small protrusion near its base (Fig. 229 2G). One auxiliary papilla (shown as "auxiliary palp" in Ruiz et al. [37]) projected from 230 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 231 232 specimens had only 3–4 spines (Fig. 2H), whereas others had many (generally more 233 than 15) (Fig. 2I). Prominent palp, also with some variations, positioned at the medial 234 margin of the corpus (Fig. 2G). Some were biramous (Fig. 2J), while others had 235 three-branched outgrowths, but the middle one was moderate (Fig. 2K). The first 236 maxilla with three subequal papillae at the distal end, had a small exopod near its base 237 (Fig. 2L). Each papilla had short seta at its tip.

238

239 *3.2. Molecular analysis*

240 The 708 bp partial 28S rDNA region sequences including gaps were obtained from 241 all twenty-three specimens from fish caught in the Shiretoko Peninsula. Only a single 242 haplotype was detected (under the process for the deposition of GenBank), which 243 showed a 99.72% identity with S. edwardsii collected in Norway (2 bp difference with 244 no gap; GenBank accession numbers is DQ180346) and 99.57 and 99.43% identity with 245 the same species caught in North America (3 bp difference with 0-1 gap; GenBank 246 accession numbers are KY113080 and KY113081 [37]). On the other hand, identities 247 with S. californiensis from North America were 98.72 and 98.58% (8 bp difference with 248 1-3 gap; GenBank accession numbers are KY113082 and KY113083 [37]).

249

250 *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

253 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
- 256 were anadromous. Only a single copepod had infested an anadromous fish (sampled at
- 257 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
- 259 (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 filamentsturned 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
- 263 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
- 266 54.8% (Funbe, No. 2 in Fig. 1 and Table 1) and the lowest value was 0% (No. 3,
- 267 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.
- 269 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).
- 279

280 **Discussion**

- 281 4.1 The parasite identification and attachment sites
- 282 So far, five species of the genus *Salmincola* have been recorded from Japan; *S.*
- 283 californiensis (reported as S. yamame in [45], [18, 46]), S. carpionis (reported as S.
- 284 *falculata* in [47], [4, 18]), *S. stellata* [11, 48, 49], *S. edwardsii* [28, 50, 51] and *S.*
- 285 markewitschi [52–54]. Of these five species, S. edwardsii is distinguished from the
- 286 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
- 290 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
- 292 S. edwardsii. Additionally, S. edwardsii was also recently found from rivers in eastern
- **293** Hokkaido [28, 50, 51] and the Kuril Islands [52].
- 294 It is well noted that the members of the genus Salmincola showed morphological 295 variations in some body parts among regions, particularly between the Palearctic and 296 the Nearctic regions [1]. In the present study, some specimens had numerous spines on 297 the ventral side of the maxilliped tip (Fig. 2I), whereas others had few (Fig. 2H) even in 298 the same population. Kabata [1] reported that numerous spines on these parts were one 299 of the characteristics of specimens from Eurasia, and spines were few or absent in those 300 of North America [1]. However, Ruiz et al. [37] also found similar spines from North 301 American specimens. Russian and Japanese specimens also had greater or fewer 302 numbers of spines [28, 52]. Trunk length in the present study was 2.15–2.98 mm (mean 303 = 2.50 mm), which was consistent with the previous reports that specimens from the 304 Palearctic region had a longer trunk length (2.96–3.00 mm) than those from the Nearctic 305 (1.60–2.00 mm) [1]. Although it was not simply concluded that there are differences in 306 trunk length between the two regions (Palearctic vs. Nearctic), because other reports 307 showed shorter trunk length even though such specimens were recovered from the same 308 or an adjecent area to the present study [28, 52].
- 309 Overall, considering the high morphological variations despite the small geographic 310 scales, these differences were possibly derived from phenotypic plasticity. Parasitic 311 copepods often change their morphology depending on the ambient environment, such 312 as attachment sites [55, 56]. However, in many cases, the sample size in each population 313 or area was so small that the authors could not refer to the mechanisms producing the 314 variation. Future studies with larger geographic scales and sample sizes will reveal these 315 mechanisms. Another cause of morphological variation was artifacts, as some previous 316 reports-have shown that the method of storage or handling of specimens may cause 317 shrinking or loss of specimen body parts [1, 28]. 318 While we detected all the copepods from the branchial cavities of host fish, some
- 319 previous studies found that infestation of *S. edwardsii* occured on body surfaces such as
- 320 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].

326

327 4.2. Effects of S. edwardsii on host fish

328 To date, while several studies have examined the effects of *Salmincola* spp. on host 329 body condition in the wild, many of them did not find any effects of the copepod 330 infestation [15–19] or found significant negative effects only in the cases where the 331 infestation intensity was very high (>100 copepods per host, [13]). Some researchers, 332 therefore, concluded that Salmincola spp. have negligible effects on host fishes in the 333 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 334 335 infestation of copepods even at low-intensities (max intensity = 7 for statistical analysis). 336 In addition, because the upper bound of condition factor decreased with increasing the 337 numbers of the copepods, the parasite might be a limiting factor for the host condition. 338 These results suggest that a low-intensity of copepods can also reduce the host's body 339 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 340 341 surfaces, even at a low-infestation level [27]. We also observed whitened attachment 342 sites, suggesting that the copepods' attachment caused gill lesions. Such damage can 343 severely drain host energy [27], and negatively affect host condition as a consequence. 344 This histopathological effect of infestation might be the reason for the negative 345 relationship between host fish condition and intensity in the present study. Further 346 pathological studies are required to understand the histopathological effects of copepods 347 on host fish condition in this region. 348 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

11

355 the effects of Salmincola spp. may be the inclusion of uninfested host fishes that have 356 high variations in body condition. Thus, it is worth analyzing the data both including 357 and exclusing uninfested individuals when assessing parasite effects accurately. 358 Second, the sample size was skewed to low intensity individuals: about 70% of 359 infested fish had only 1 or 2 parasites. Although our sample size was not small (> 200 360 host individuals) and this system naturally had relatively low prelevance and intensity, 361 data on heavily infested fishes are crucial to further understanding the effects of 362 Salmincola spp. on host fish condition in wild populations. Nevertheless, the quantile 363 regression analysis clearly showed overall trends for the negative relationship and 364 decrease the variability in host condition. Because the quantile regression analysis can 365 handle the data with unequal variance [41, 42], this will be effective to analyze the 366 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].

372

373 *4.3. Regional distribution pattern and infestation of anadromous fish*

374 Our results showed that the distribution of S. edwardsii was highly heterogeneous 375 even within a relatively small geographic scale. Previous studies also reported similar 376 results on *Salmincola* spp. and discussed the heterogeneous distribution in terms of 377 habitat connectivity, host extinction and reintroduction [20, 22]. In particular, since the 378 genus Salmincola is host-specific [1], once their host populations go locally extinct, 379 they will also go extinct with their hosts. Thus, local population dynamics and 380 extinction of hosts could be a major factor determining the local abundance of parasites. 381 However, in our system, Southern Asian Dolly Varden populations in the Shiretoko 382 Peninsula are generally healthy, with no recent record of population extirpation or 383 artificial reintroduction (except for a very small population that was significantly 384 influenced by non-native rainbow trout Oncorhynchus mykiss [62]. Therefore, local 385 population dynamics of the host may not be the primary cause for the high variation in 386 parasite abundance. 387 Local environmental differences may explain the heterogeneous distribution. In

388 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 the Shiretoko Peninsula [32]. If the introduction of this parasite by migrants frequently 410 411 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*

423	Infestation of S. edwardsii may affect host health and they have a heterogeneous
424	distribution pattern, even on very small geographic scales like that of the Shiretoko
425	Peninsula in Hokkaido, Japan. However, we know very little about the limiting factors
426	affecting the distribution, prevalence, and intensity of Salmincola spp., which could be
427	critical to proper population management. In particular, Hokkaido Island is the
428	southernmost margin of the Southern Asian Dolly Varden's native range, and
429	populations in the area are thought to be the most vulnerable to climate change [67].
430	Some southern populations of S. edwardsii, in Wisconsin, North America for example,
431	have undergone outbreaks and significantly affected brook trout S. fontinalis, which
432	may be exacerbated by global warming [12]. Additional studies and monitoring are
433	needed to evaluate the effects of S. edwardsii to better understand the epizootics of
434	these ectoparasites.
435	
436	Acknowledgements
437	We acknowledge two anonymous reviewers for their constructive comments to
438	improve our manuscript. We also thank Drs. Munetaka Shimizu and Satoshi Wada,
439	Graduate school of Fisheries Science, Hokkaido University, for providing lab facilities.
440	We would like to express our gratitude to Yuya Eguchi and Erika Yoshida, the
441	laboratory of environmental biology, Azabu University, for helping DNA analysis.
442	
443	Funding
444	This research did not receive any specific grant from funding agencies in the public,
445	commercial, or not-for-profit sectors.
446	
447	Declarations of interest
448	None.
449	

450 References

- 451 [1] Z. Kabata, Revision of the genus Salmincola Wilson, 1915 (Copepoda:
- 452 Lernaeopodidae), J. Fish. Res. Board. Can. 26 (1969) 2987–3041.
- 453 https://doi.org/10.1139/f69-285.
- 454 [2] G. A. Black, W. L. Montgomery, F. G. Whoriskey, Abundance and distribution of
- 455 Salmincola edwardsii (Copepoda) on anadromous brook trout, Salvelinus fontinalis,
- 456 (Mitchill) in the Moisie River system, Quebec, J. Fish Biol. 22 (1983) 567–575.
- 457 https://doi.org/10.1111/j.1095-8649.1983.tb04216.x.
- 458 [3] Z. Kabata, B. Cousens, Host-parasite relationships between sockeye salmon,
- 459 Oncorhynchus nerka, and Salmincola californiensis (Copepoda: Lernaeopodidae), J.
- 460 Fish. Res. Board. Can. 34 (1977) 191–202. https://doi.org/10.1139/f77-029.
- 461 [4] K. Nagasawa, M. Yamamoto, Y. Sakurai, A. Kumagai, Rediscovery in Japan and
- 462 host association of *Salmincola carpionis*. (Copepoda: Lernaeopodidae), a parasite
- 463 of wild and reared freshwater salmonids, Can. J. Fish. Aquat. Sci. 52 (1995)
- **464** 178–185. 10.1139/F95-525.
- 465 [5] G. A. E. Gall, E. L. Mcclendon, W. E. Schafer, Evidence on the influence of the
- 466 copepod (*Salmincola californiensis*) on the reproductive performance of a
- domesticated strain of rainbow trout (*Salmo gairdneri*), Trans. Am. Fish. Soc. 101
- **468** (1972) 345–346.
- 469 https://doi.org/10.1577/1548-8659(1972)101<345:EOTIOT>2.0.CO;2
- 470 [6] W. Piasecki, A. E. Goodwin, J. C. Eiras, B. F. Nowak, Importance of Copepoda in
 471 freshwater aquaculture, Zool. Stud. 43 (2004) 193–205.
- 472 [7] R. J. Roberts, K. A. Johnson, M. T. Casten, Control of Salmincola californiensis
- 473 (Copepoda: Lernaeapodidae) in rainbow trout, Oncorhynchus mykiss (Walbaum): a
- 474 clinical and histopathological study, J. Fish Dis. 27 (2004) 73–79.
- 475 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

- 501 (*Coregonus hoyi*) stocks in Lake Huron, Can. J. Zool. 68 (1990) 1988–1994.
- 502 https://doi.org/10.1139/z90-280.
- 503 [16] P. A. Amundsen, R. Kristoffersen, R. Knudsen, A. Klemetsen, Infection of
- 504 *Salmincola edwardsii* (Copepoda: Lernaeopodidae) in an age-structured population
- 505 of Arctic charr—a long-term study, J. Fish Biol. 51 (1997) 1033–1046.
- 506 https://doi.org/10.1111/j.1095-8649.1997.tb01542.x.
- 507 [17] P. Chigbu, Occurrence and distribution of *Salmincola californiensis* (Copepoda:
- 508 Lernaeopodidae) on juvenile sockeye salmon (*Oncorhynchus nerka*) in Lake
- 509 Washington, J. Freshw. Ecol. 16 (2001) 615–620.
- 510 https://doi.org/10.1080/02705060.2001.9663853.
- 511 [18]K. Nagasawa, S. Urawa, Infection of *Salmincola californiensis* (Copepoda:
- 512 Lernaeopodidae) on juvenile masu salmon (*Oncorhynchus masou*) from a stream in
- 513 Hokkaido, Bull. Natl. Salmon Res. Center 5 (2002) 7–12.
- 514 [19]C. G. Ayer, K. Morita, S. Fukui, I. Koizumi, No apparent effects of the buccal
- 515 cavity attaching parasite, *Salmincola* sp.(Copepoda: Lernaeopodidae), on a stream
- salmonid: a mark-recapture study, Ichthyol. Res. online early.
- 517 https://doi.org/10.1007/s10228-021-00835-0.
- 518 [20] M. G. Mitro, J. D. Griffin, Distribution, prevalence, and maximum intensity of the
- 519 ectoparasitic copepod *Salmincola* cf. *edwardsii* in brook trout in Wisconsin streams,
- 520 J. Parasitol. 104 (2018) 628–638. https://doi.org/10.1645/17-146.
- 521 [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
- 523 load, Anim. Behav. 41 (1991) 467–476.
- 524 https://doi.org/10.1016/S0003-3472(05)80849-8.
- 525 [22] R. Hasegawa, I. Koizumi, Relative importance of host dependent vs. physical
- 526 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
- of hatching, swimming, and copepodid survival of the parasitic copepod *Salmincola 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
- hatching and viability of juvenile gill lice, *Salmincola californiensis*, J. Fish Dis. 39
 (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.

- 551 [29] Y. Umatani, T. Arai, K. Maekawa, Flexible seaward migration of Dolly Varden
- 552 *Salvelinus malma* in the Shiretoko Peninsula, Hokkaido, Japan, Ichthyol. Res. 65
- **553** (2018) 202–209. 10.1007/s10228-017-0606-3.
- 554 [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.
- 557 https://doi.org/10.1017/pao.2017.12.
- 558 [31] G. Sahashi, K. Morita, Southern Asian Dolly Varden Charr, *Salvelinus curilus* on
 559 the Higashi-Nodottomari River in Rishiri Island, Rishiri Stud. 40 (2021) 13–20.
- 560 [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.
- 562 https://doi.org/10.1111/j.0022-1112.2005.00672.x.
- 563 [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,
- 565 Environ. Biol. Fish. 83 (2008) 37–44. https://doi.org/10.1007/s10641-007-9249-y.
- 566 [34] A. G. Humes, R. U. Gooding, A method for studying the external anatomy of
- 567 copepods, Crustaceana 6 (1964) 238–240.
- 568 https://doi.org/10.1163/156854064X00650.
- 569 [35]Z. Kabata, B. Cousens, Life cycle of *Salmincola californiensis* (Dana
- 570 1852)(Copepoda: Lernaeopodidae), J. Fish. Res. Board. Can. 30 (1973) 881–903.
- 571 https://doi.org/10.1139/f73-150.
- 572 [36]M. Nakao, M. Sasaki, T. Waki, J. L. Anders, H. Katahira, *Brachylaima asakawai* sp.
- 573 nov. (Trematoda: Brachylaimidae), a rodent intestinal fluke in Hokkaido, Japan,
- with a finding of the first and second intermediate hosts, Parasitol. Int. 67 (2018)
- 575 565–574. https://doi.org/10.1016/j.parint.2018.04.010.

- 576 [37] C. F. Ruiz, J. M. Rash, D. A. Besler, J. R. Roberts, M. B. Warren, C. R. Arias, S. A.
- 577 Bullard, Exotic "gill lice" species (Copepoda: Lernaeopodidae: *Salmincola* spp.)
- 578 infect rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*)
- in the southeastern United States, J. Parasitol. 103 (2017) 377–389.
- 580 https://doi.org/10.1645/16-165.
- 581 [38] B. M. von Reumont, K. Meusemann, N. U. Szucsich, E. Dell'Ampio, V.
- 582 Gowri-Shankar, D. Bartel, J. W. Wägele, Can comprehensive background
- 583 knowledge be incorporated into substitution models to improve phylogenetic
- analyses? A case study on major arthropod relationships, BMC Evol. Biol. 9 (2009)
- 585 119. http://www.biomedcentral.com/1471-2148/9/119.
- 586 [39] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, MEGA X: molecular
- 587evolutionary genetics analysis across computing platforms, Mol. Biol. Evol. 35
- 588 (2018) 1547–1549. https://doi.org/10.1093/molbev/msy096.
- 589 [40] A. O. Bush, K. D. Lafferty, J. M. Lotz, A. W. Shostak, Parasitology meets ecology
- 590 on its own terms: Margolis et al. revisited, J. Parasitol. (1997) 575–583.
- 591 <u>https://doi.org/10.2307/3284227</u>.
- 592 [41]B. S. Cade, B. R. Noon, A gentle introduction to quantile regression for ecologists,
- **593** Front. Ecol. Environ. 1 (2003) 412–420.
- 594 https://doi.org/10.1890/1540-9295(2003)001[0412:AGITQR]2.0.CO;2.
- 595 [42] K. Das, M. Krzywinski, N. Altman, Quantile regression, Nat. Methods 16 (2019)
- 596 451–452. https://doi.org/10.1038/s41592-019-0406-y.
- 597 [43] R. Koenker, S. Portnoy, P. T. Ng, A. Zeileis, P. Grosjean, B. D. Ripley, Package
 598 'quantreg', Cran R-project. org. (2018).
- 599 [44] R Core team Team, R: a language and environment for statistical computing, R
- 600 Foundation for Statistical Computing, Vienna, Austria (2017)
- 601 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.
- 604 [46] T. Hoshina, T. Nishimura, On a parasitic Copepoda, Salmincola californiensis
- found in a salmonid fish, Yamame *Oncorhynchus masou*, Fish Pathol. 11 (1976)
- 606 153–157 (in Japanese with English abstract).
- 607 [47] S. Yamaguti, Parasitic copepods from fishes of Japan, Part 6, Lernaeopodidae, I,
- 608 Volumen Jubliare pro Professore Sadao Yoshida, 2 (1939) 529–578. 25 pls.
- 609 [48] K. Nagasawa, S. Urawa, New records of the parasitic copepod Salmincola stellatus
- 610 from Sakhalin taimen (*Hucho perryi*) in Hokkaido, with a note on its attachment
- 611 site, Sci. Rep. Hokkaido Salmon Hatchery 45 (1991) 57–59.
- 612 [49] K. Nagasawa, J. R. Watanabe, S. Kimura, A. Hara, Infection of Salmincola
- 613 *stellatus* (Copepoda: Lernaeopodidae) on Sakhalin taimen *Hucho perryi* reared in
 614 Hokkaido, Bull. Fac. Fish. Hokkaido Uni. 45 (1994) 109–112.
- 615 [50]K. Nagasawa, Gill lesions caused by the parasitic copepod Salmincola edwardii in
- 616 the southern Asian Dolly Varden, *Salvelinus malma krascheninnikova*, from
- 617 Hokkaido, Japan, Nat. Kagoshima 47 (2020b) 121–124 (in Japanese with English
 618 abstract).
- 619 [51]K. Nagasawa, K. Kawai, The parasitic copepod *Salmincola edwardsii* from sothern

620 Asian Dolly Varden, *Salvelinus malma krascheninnikova*, in the Shari River,

- 621 Hokkaido, Japan, with a note on the hosts and geographical distribution of the
- 622 copepod in the northern Japan, Nat. Kagoshima 47 (2020c) 129–132 (in Japanese
- 623 with English abstract).
- 624 [52] M. B. Shedko, S. V. Shedko, Parasitic copepods of the genus *Salmincola*

625 (Lernaeopodidae) from the far eastern chars *Salvelinus* (Salmonidae) with

- description of the new species *S. markewitschi*, Zoologicheskii Zhurnal 81 (2002)
- 627 141–153 (in Russian with English abstract).

- 628 [53] K. Nagasawa, *Salmincola markewitschi* (Copepoda: Lernaeopodidae) Parasitic on
- 629 Whitespotted Char, *Salvelinus leucomaenis*, in a Moutain Stream of Honshu Island,
- 630 Central Japan, Spec. Divers. 25 (2020c) 369–375.
- 631 https://doi.org/10.12782/specdiv.25.369.
- 632 [54] K. Nagasawa, N. Ishiyama, Salmincola markewitschi (Copepoda: Lernaeopididae),
- 633 a parasite of whitespotted charr, *Salvelinus leucomaenis*, from Ishikawa Prefecture,
- 634 central Japan, Taxa, Proc. Japan. Soc. Syst. Zool. 50 (2021) 11–19.
- 635 [55] W. E. Hogans, Morphological variation in *Pennella balaenoptera* and *P. filosa*
- 636 (Copepoda: Pennellidae) with a review of the genus *Pennella* Oken, 1816 parasitic
- 637 on Cetacea, Bull. Mar. Sci. 40 (1987) 442–453.
- 638 [56] P. Abaunza, N. L. Arroyo, I. Preciado, A contribution to the knowledge on the
- 639 morphometry and the anatomical characters of *Pennella balaenopterae* (Copepoda,
- 640 Siphonostomatoida, Pennellidae), with special reference to the buccal complex,
- 641 Crustaceana 74 (2001) 193–210. https://doi.org/10.1163/156854001750096292.
- 642 [57] G. A. Black, Gills as an attachment site for *Salmincola edwardsii* (Copepoda:
- 643 Lernaeopodidae), J. Parasitol. 68 (1982) 1172–1173.
- 644 [58] A. D. Lemly, G. W. Esch, Effects of the trematode *Uvulifer ambloplitis* on juvenile
- bluegill sunfish, *Lepomis macrochirus*: ecological implications, J. Parasitol. 70
- 646 (1984) 475–492. https://doi.org/10.2307/3281395.
- 647 [59] S. A. O'Connell-Milne, R. Poulin, C. Savage, W. Rayment, Reduced growth, body
- 648 condition and foot length of the bivalve Austrovenus stutchburyi in response to
- 649 parasite infection, J. Exp. Mar. Biol. Ecol. 474 (2016) 23–28.
- 650 https://doi.org/10.1016/j.jembe.2015.09.012.
- 651 [60] P. M. Beldomenico, S. Telfer, S. Gebert, L. Lukomski, M. Bennett, M. Begon, Poor
- 652 condition and infection: a vicious circle in natural populations. Proc. R. Soc. B 275
- 653 (2008) 1753–1759. https://doi.org/10.1098/rspb.2008.0147.

- 654 [61] P. M. Beldomenico, M. Begon, Disease spread, susceptibility and infection
- 655 intensity: vicious circles? Trends Ecol. Evol. 25 (2010) 21–27.
- 656 https://doi.org/10.1016/j.tree.2009.06.015.
- 657 [62]K. Morita, D. Kishi, J. Tsuboi, S. Morita, T. Aarai, Rainbow trout and brown trout
- 658 in Shiretoko Peninsula, Hokkaido, Japan, Bull. Shiretoko Museum 24 (2003) 17–26
- 659 (in Japanese with English abstract).
- 660 [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
- 662 Shiretoko Peninsula, Hokkaido, Japan, Ecol. Res. 24 (2009) 873–880.
- 663 https://doi.org/10.1007/s11284-008-0562-5.
- 664 [64] R. M. Anderson, R. M. May, Regulation and stability of host-parasite population
- interactions: I. regulatory processes, J. Anim. Ecol. (1978) 219–247.
- 666 https://doi.org/10.2307/3933.
- 667 [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.
- 670 https://doi.org/10.1034/j.1600-0587.2002.250110.x.
- 671 [66] E. Komiyama, N. Ohtaishi, K. Maekawa, Occurrence of a sea-run type of the Dolly
- 672 Varden in the Shiretoko Peninsula, Hokkaido, Japan, Ichthyol. Res. 29 (1982)
- 673 298–302. https://doi.org/10.11369/jji1950.29.298.
- 674 [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.
- 676 Biol. 36 (1996) 711–722. https://doi.org/10.1046/j.1365-2427.1996.d01-516.x.
- 677
- 678

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	$173235~(196\pm19)$	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 \pm 16)	30.0	1.56 (1–3)
8	Aidomari	1	0	198	0	-
9	Kikiribetsu	2	2	$194197~(196\pm2)$	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	$161-205 (184 \pm 15)$	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	1.

(a)				
tau = 0.1				
	Estimate	SE	<i>t</i> -value	<i>p</i> -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	<i>t</i> -value	<i>p</i> -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	<i>t</i> -value	<i>p</i> -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	<i>t</i> -value	<i>p</i> -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	<i>t</i> -value	<i>p</i> -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	<i>t</i> -value	<i>p</i> -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	<i>t</i> -value	<i>p</i> -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	<i>t</i> -value	<i>p</i> -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	<i>t</i> -value	<i>p</i> -value
Intercept	1.127	0.015	73.245	< 0.01
Parasite number	-0.030	0.017	-1.744 0.082	
(b)				
tau = 0.1				
	Estimate	SE	<i>t</i> -value	<i>p</i> -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	<i>t</i> -value	<i>p</i> -value
Intercept	0.918	0.045	20.218	< 0.01

0.014

-1.367

0.178

-0.020

Parasite number

tau = 0.3

Parasite number

tau = 0.5				
	Estimate	SE	<i>t</i> -value	<i>p</i> -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	<i>t</i> -value	<i>p</i> -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	<i>t</i> -value	<i>p</i> -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	<i>t</i> -value	<i>p</i> -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	<i>t</i> -value	<i>p</i> -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	<i>t</i> -value	<i>p</i> -value
Intercept	1.115	0.056	20.015	< 0.01

0.018

-1.665

0.102

-0.030

tau	=	0.9
tau	_	0.9

	Estimate	SE	<i>t</i> -value	<i>p</i> -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.





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.

