

Spatial aggregation in small spring-fed tributaries leads to a potential metapopulation structure in a parasitic fish leech

HIROTAKA KATAHIRA^{1,2*}, CHITOSE YAMAZAKI¹, SHO FUKUI¹, CHRISTOPHER G. AYER¹ and ITSURO KOIZUMI¹

¹ Graduate School of Environmental Earth Science, Hokkaido University, N10W5 Sapporo, Hokkaido 060-0810, Japan

² Faculty of Bioresources, Mie University, 1577 Kurima machiya-cho, Tsu, Mie 514-8507, Japan

(Received 12 May 2017; revised 30 June 2017; accepted 8 July 2017)

SUMMARY

Large-bodied ectoparasites are often observed only in low numbers in the field. How such rarely found parasites persist and maintain viable populations has been an intriguing question inadequately addressed. The simplest hypothesis is the existence of distribution hot spots, and another, but not mutually exclusive, possibility is a form of metapopulation structure where local populations are effectively connected via dispersal. In this study, we conducted an intensive epidemiological survey of a piscicolid leech *Taimenobdella amurensis* to elucidate spatial population structure and potential dispersal of this rarely found parasite. Four years of potential-host screening ($n = 20\,664$) from 28 tributaries and 10 main stem reaches in a mountain river system of Hokkaido, northern Japan, revealed that occurrences of *T. amurensis* ($n = 1348$) were confined to spring-fed tributaries. Since most spring-fed tributaries were small (<1 km in length), it would seem to be unlikely for the ectoparasite to form a persistent local population in each tributary. The main host fish was Dolly Varden charr, which is known to disperse among neighbouring tributaries. These findings suggest that, along with the host, the ectoparasite displays a potential metapopulation structure, in which host-dependent dispersion may overcome local extinction by keeping the local populations connected.

Key words: fish leech, population synchrony, rescue effect, host–parasite structure, host preference.

INTRODUCTION

When doing parasitological research in the field, species with infrequent occurrence and/or low intensity are routinely observed (Bush and Holmes, 1986; Norton *et al.* 2003; Poulin, 2007a; Kennedy, 2012). While such rarely found species are noticed only when large outbreaks occur (e.g. *Pennella* sp. from the Pacific saury *Cololabis saira*, Nagasawa *et al.* 1988), patterns of spatial distribution and population dynamics have been poorly understood, as the trait of rarity itself makes quantitative sampling difficult. Moreover, the rare occurrences are often mixed up with accidental infections and/or sampling noise without much consideration (Bush *et al.* 2001). In this context, Bush *et al.* (2001) provided an empathetic comment: ‘There are many parasites that are rare (using any connotation of the word; just why they are rare, and how they circumvent extinction, would be an interesting avenue to explore!’)

Species with a low abundance and/or narrow habitat-use range (Gaston, 1997), are at a disadvantage in avoiding extinction (see Gaston, 1994; Mace and Kershaw, 1997). If a parasite has a narrow range of geographic distribution and/or host use, it risks

being trapped by climate change and/or anthropogenic/catastrophic disturbances causing loss of prime habitat (Bush and Kennedy, 1994; Poulin and Morand, 2004). Small populations risk disappearing simply through demographic and environmental stochastic fluctuations (Menges, 1992) and genetic deterioration (Karron, 1997). Furthermore, if a parasite prefers a specific environment, its local populations are likely to be isolated from each other with increasing the risk of extinction (Price, 1980; Rózsa, 1992; Poulin and Morand, 2004). Nevertheless, rarely found parasites are occupying a particular niche in the given ecosystem to survive, reproduce and pass their genes onto future generations.

To compensate for the risk of extinction, specific population features are expected for rarely found parasites insuring the exploitation of targeted hosts. While asexual species have a chance to persist even with small local populations by self-reproduction (Ogawa *et al.* 2014), sexual species require to find mating partners (Kennedy, 1976). Therefore, sexually reproducing parasites might form spatiotemporal hot spots of infection, thereby finding mating partners and acting as sources of parasite populations to colonize to other patches (Hartvigsen and Halvorsen, 1993, 1994; see also Bush and Kennedy, 1994). Extinction risk will be also reduced by dispersal among local populations, via rescue effects or metapopulation processes (Levins, 1969; Hanski, 1999). Therefore, spatial

* Corresponding author: National Research Center for Protozoan Diseases, Obihiro University of Agriculture & Veterinary Medicine, Nishi-2-13 Inada-cho, Obihiro, Hokkaido 080-8555, Japan. E-mail: paraparparasites@gmail.com

population structure and dispersal ability are fundamental to understanding survival strategies in rarely found parasites.

In this study, we conducted an intensive epidemiological survey of a piscicolid leech *Taimenobdella amurensis*, narrowly distributed in the Far East (Epshtein, 1964; Lukin, 1976; Furness *et al.* 2007), to elucidate spatial population structure and potential dispersal of this rare parasite. We also examined temporal changes of prevalence among local habitats by using 4-year data. When local dynamics are highly synchronous, considerable amount of dispersal among habitat patches is suggested, which results in a large panmictic population structure (Hanski, 1999; Koizumi, 2011). Alternatively, when dynamics are completely independent, a set of isolated populations is suggested. Finally, when local dynamics are moderately synchronous, metapopulation structure with some dispersal among local habitats is suggested (Hanski, 1999; Koizumi, 2011). We have some advantages in the study system to understand population structure and dynamics in rare parasites. First, without sacrificing hosts, each parasite can be easily distinguished in the field due to size and colour (Furness *et al.* 2007). Second, biology of host fishes, including population dynamics and environmental characteristics, have been intensively studied more than 15 years (Koizumi, 2011; Koizumi and Shimatani, 2016), providing useful basic information.

MATERIALS AND METHODS

Study species and area

Taimenobdella amurensis is a fish leech found in the Amur basin and Hokkaido (northern Japan) in the Far East (Epshtein, 1964; Lukin, 1976; Furness *et al.* 2007). This sexually reproducing leech has been reported on various fishes (mainly salmonid fishes) (Furness *et al.* 2007; Nagasawa *et al.* 2009), but is known as a rare species possessing an extremely low prevalence and intensity (Nagasawa *et al.* 2009). Only 16 cases of infection by this parasite were observed from 2001 to 2008 at the Chitose Salmon Aquarium (42°49'58"N, 141°39'33"E) neighbouring the middle-reach of the Chitose River, Hokkaido, Japan (Nagasawa *et al.* 2009). Aquarium staffs keep daily records of leech infections on wild fishes via observation in a room equipped with acrylic underwater-viewing windows (ca. 7 windows with 1 m high × 2 m wide); however, only 30 individuals were recorded during the 7-year survey (Nagasawa *et al.* 2009). As of yet, the population biology, including habitat and detailed host use, of this leech is still largely unknown.

Sampling was conducted in the upper Sorachi River system in central Hokkaido, Japan (Fig. 1),

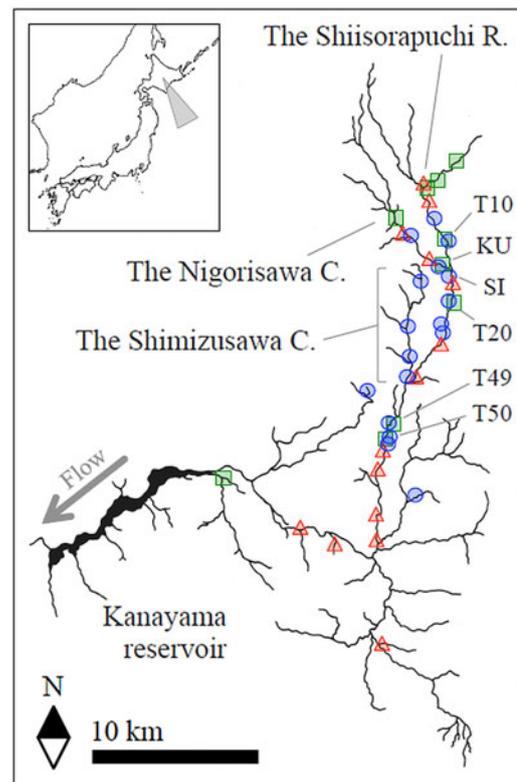


Fig. 1. Map of the river system. Diagrams in the map indicate sampling localities of a spring-fed tributary (circle), non-spring-fed tributary (triangle) and main stem (rectangle). Abbreviations represent site name for additional small-scale surveys.

located upstream from a large reservoir (i.e. Kanayama reservoir; 43°10'12"N, 142°31'48"E). The riverine structure is characterized by more than 100 small tributaries (length, <500 m; base flow, c. 0.01–0.5 m³ s⁻¹; width, c. 0.5–3.0 m; depth, c. 5–20 cm) directly connecting to the main stem channel (base flow, c. 1.0–10 m³ s⁻¹; width, c. 5–30 m; depth, c. 50–100 cm) (see Koizumi and Maekawa, 2003, 2004; Koizumi *et al.* 2006a, b; Koizumi and Shimatani, 2016). In addition to these small tributaries, two branches (i.e. the Nigorisawa Creek and the Shimizusawa Creek) diverge in the upper reaches of the main stem, named as the Shiisorapuchi River (Fig. 1). The substratum of most tributaries consists mainly of pebble and cobble gravels, and other compositions (i.e. sand and silt) can be found depending on the place and stream structure. The main stem of the river generally has a boulder bottom.

These tributaries and creeks of the Shiisorapuchi River can be divided into spring-fed or non-spring-fed types, based on the presence or absence of spring flow that affects water temperature and water discharge variations. Spring-fed tributaries have stable temperatures (5–8 °C) and water discharge throughout the year (Koizumi and Maekawa, 2003; I. Koizumi, unpublished data).

The other type of streams, i.e. non-spring-fed tributaries, are fed by rain and snowmelt runoff, precipitation and shallow ground water; thus, their water temperatures and discharge easily fluctuate on a daily and seasonal basis, ranging from 0 to 16 °C (Koizumi and Maekawa, 2003; I. Koizumi, unpublished data).

Eleven fish species have been reported in the upper Sorachi River system (Koizumi and Maekawa, 2003; Koizumi *et al.* 2012) including far eastern brook lamprey *Lethenteron* sp. (northern form), Rosyface dace *Tribolodon sachalinensis*, Japanese dace *Tribolodon hakonensis*, Siberian stone loach *Nemachelis barbatulus toni*, Hokkaido eight-barbel loach *Lefua costata nikkonis*, Sakhalin taimen *Parahucho perryi*, rainbow trout *Onchorhynchus mykiss*, white-spotted char *Salvelinus leucomaenis leucomaenis*, Dolly Varden, *Salvelinus malma krascheninnikovi*, nine-spined stickleback *Pungitius* sp. (freshwater type) and freshwater sculpin *Cottus nozawae*. Only rainbow trout are a non-native species.

Each species of salmonid, believed to be main hosts (Furness *et al.* 2007; Nagasawa *et al.* 2009), has different distributional patterns and migratory tendencies, which should significantly influence the dynamics of the parasite leech. Dolly Varden occupy upper areas, especially spring-fed tributaries, whereas Sakhalin taimen occupies lower areas, including Kanayama reservoir (Edo *et al.* 2000; Koizumi *et al.* 2012). White-spotted charr are in between but distribute most widely, from upper areas to the reservoir. Most Sakhalin taimen and some white-spotted charr migrate between the river and reservoir, probably moving several dozen kilometres (I. Koizumi, personal observation). They spawn in the main stem and large tributaries but not in small tributaries. Dolly Varden, on the other hand, migrate between small tributaries and the main stem, but not migrating to the reservoir (Koizumi *et al.* 2006a; Ayer *et al.* 2017). They also spawn mostly in small tributaries but not in the main stem. Microsatellite DNA analysis indicates that dispersal of Dolly Varden may be several kilometres (Koizumi *et al.* 2006b). This species forms a metapopulation structure; each tributary-based population is connected to other local populations with a moderate level of dispersal (Koizumi and Maekawa, 2004; Koizumi *et al.* 2006b, 2008; Koizumi, 2011). Non-native rainbow trout are much less abundant and life history is poorly known.

Field samplings

We conducted intensive sampling in spring after snowmelt (16–19 June 2013, 23 May and 15–19 June 2014, 20–27 June 2015, and 23–30 June 2016), which was a part of an annual population census of Dolly Varden (Koizumi *et al.* 2008;

Koizumi, 2011). To investigate seasonal changes of the parasite occurrence and annual life cycle, we conducted additional autumn sampling before snowfall (24–25 October 2013 and 4–5 September and 24–25 October 2014), although the sampling efforts (e.g. number of sites, sampling time) were much lower in autumn sampling. In total, 10 sites of the main stem and 28 tributaries were investigated to cover the entire area of the river system (Fig. 1); the tributaries consisted of 14 spring-fed and 14 non-spring-fed types. At these sites, we caught host fishes using a backpack electrofisher (Model 12B, Smith-Root, Vancouver, Canada). Sampling effort ranged from one to three screening passes with approximately 30–60 min per pass at each site. In addition to this basin-scale investigation, additional small-scale surveys were carried out (1) in a branch (i.e. Shimizusawa Creek) and tributaries (named as KU and SI) to examine the longitudinal distribution and (2) in a side channel of the main stem close to tributaries (i.e. KU, T10, T20, T49 and T50) to discriminate microscale differences in leech occurrence inside and outside the tributary (Fig. 1).

The fishes captured were anaesthetized by FA100 (DS Pharma Animal Health Co., Ltd., Osaka, Japan) in the field, and then measured for their total length or fork length (FL) to the nearest 1 mm and immediately checked for leech infection. All examined fishes were released into recovery sites after the procedure. For species identification, a portion of the leeches found were haphazardly collected and brought back to the laboratory of Hokkaido University, where they were relaxed by dripping 70% ethanol and subsequently refixed with 70–80% ethanol. The fixed specimens were measured for whole body length to the nearest 1 mm. Identification was based on the descriptions provided by Furness *et al.* (2007).

Statistical analyses

Infection indices were calculated using the definition provided by Bush *et al.* (1997).

Host use of *T. amurensis* in the present river system was evaluated with applying dietary-preference indices of Krebs (1999) to the occurrence data: $W_i = O_i/P_i$ and $B_i = W_i/\Sigma W$, where W_i is the infection ratio for host species i , O_i is the proportion of infected host i individuals in the infected individuals of all host fishes, P_i is the proportion of host i in all the examined host fishes and B_i is the standardized value of W_i . Fish with a W_i value above 1.0 can be regarded as preferred hosts for the leech, while values of B_i below or above '1/number of host species' correspond to relative avoidance or preference (see Krebs, 1999).

To elucidate spatial occurrence patterns of leeches in the river system, generalized linear mixed models

(GLMMs) with logit link function were fitted for the prevalence data (i.e. presence or absence of the leech on host individuals) obtained from each site. In this analysis, habitat type (main stem, spring-fed and non-spring-fed tributary) and geographical distance from the downstream end of the river were tested as explanatory variables, with the random effects of sampling site and sampling term taken into account. Geographical distances in each tributary were measured from the inlet of the Kanayama reservoir along with the river line with an open source map (Geospatial Information Authority of Japan; <http://maps.gsi.go.jp>) using Image J 1.46r. The candidate models were selected based on Akaike's information criterion (AIC; Burnham and Anderson, 2002); the difference in AIC value (Δ AIC) between a constructed model and the best model with the lowest AIC value was checked for the model selections.

Occurrence surveys of rarely found parasites often produce unreliable data with many absent (i.e. zero-inflated situation), owing to miss examinations of host individuals distributed apart from infectious hot spots or improper timing of sampling when parasite infection rate is low (see Martin *et al.* 2005; Zuur *et al.* 2009). Such sampling biases should violate the general aggression pattern of macroparasites, which is represented by a negative binomial (NB) distribution model (Shaw and Dobson, 1995; Poulin, 2013). Therefore, to investigate whether the probability distribution of the parasite infection follows a NB when considering only the infectious hot spots, model selections and comparisons were conducted based on the abundance data from the most preferred host in the prevalent sites. We compared three error distributions, i.e. NB, zero-inflated Poisson (ZIP) and zero-inflated negative binomial (ZINB) (Zuur *et al.* 2009). Body size of the preferred host, sampling season and year were tested as candidates for explanatory variables in the fittings, with sampling site difference as a random effect. The best models of these probability distributions were subsequently compared with each other, according to the values of AIC.

Due to the seasonal gap in samplings (i.e. spring and autumn), it was also expected that body sizes of the leeches would differ between samplings. This difference was confirmed by the construction of a general linear model (LM) involving categorical explanatory variables of season and year. The leech specimens examined were haphazardly collected through the above-mentioned surveys. AIC was used for the selective criterion for the constructed models, as mentioned above.

We also assessed the level of synchrony in local dynamics to examine a possible population structure and connectivity. We calculated pairwise synchrony of the leech prevalence between the preferred patches (i.e. tributaries and the branch) using

cross-correlation coefficient with no time lag (Bjørnstad *et al.* 1999; see also Koizumi *et al.* 2008). A 95% bootstrap confidence interval for the overall average synchrony was estimated by 10 000 permutations of sampling with replacements of the coefficients (Bjørnstad *et al.* 1999).

All statistical tests were performed using R 3.1.2 (R Development Core Team, 2014). Model fittings in the cases of binomial, NB, ZIP and ZINB error distributions employed the glmmADMB package (Fournier *et al.* 2012).

RESULTS

Infection rate and host preference

In total, 1102 (5.3%) of 20 664 fish examined were infected with the leech during 2013–2016 (Table 1). The number of the leeches recovered was 304, 418, 420 and 206 individuals in the order of the year. Most of the individuals were collected from Dolly Varden (96.07%), while the other fishes, i.e. white-spotted charr (2.60%) and freshwater sculpins (1.26%), were also infected with the leech depending on the site.

Both host-preference indices, i.e. W and B , were always highest in Dolly Varden (Table 1). However, in some sites (uppermost reaches of the main stem and spring-fed tributaries) on the sampling of June 2013, for example, these indices for freshwater sculpin also showed relatively high value as a preferred host.

Leech distribution

The leeches occurred heterogeneously in the river system, confined to spring-fed tributaries and the uppermost reaches of the main stem (Fig. 2). Only three cases of infections were detected from non-spring-fed tributaries (Table 1). The optimal GLMM for the prevalence of leeches was the full model consisting of the all candidate variables (intercept coefficient = -4.17 ± 0.56 , z -value = -7.48 , $P < 0.001$), with a difference of AIC from the second one (Δ AIC = 4.9) consisted of only one variable (i.e. habitat type). Leech prevalence was more frequent in tributaries than in the main stem (partial coefficient of main stem site for spring-fed tributary = -2.13 ± 0.47 , z -value = -4.53 , $P < 0.001$; partial coefficient of non-spring-fed tributary for spring-fed one = -5.33 ± 0.62 , z -value = -8.56 , $P < 0.001$). The possibility of the parasite found in the sites also reflected a tendency to slightly increase with the geographic distance from the inlet (partial coefficient = 0.04 ± 0.017 , z -value = 2.61 , $P = 0.009$).

Additional small-scale surveys in the Shimizusawa Creek observed a clear decrease in prevalence from upstream to downstream (Fig. 3A); with a higher prevalence found in the upper reaches of the creek.

Table 1. Prevalence of the fish leech *Taimenobdella amurensis* in host species and habitats. Bold indicates the habitats where the leeches were found and the preferred hosts provided as *W* and *B* (see details in the text)

Host	Type of locality	2013						2014						2015			2016			
		Spring			Autumn			Spring			Autumn			Spring			Spring			
		Prevalence (%)	Size range mean \pm s.d. (min.–max.)	Index <i>W, B</i>	Prevalence (%)	Size range mean \pm s.d. (min.–max.)	Index <i>W, B</i>	Prevalence (%)	Size range mean \pm s.d. (min.–max.)	Index <i>W, B</i>	Prevalence (%)	Size range mean \pm s.d. (min.–max.)	Index <i>W, B</i>	Prevalence (%)	Size range mean \pm s.d. (min.–max.)	Index <i>W, B</i>	Prevalence (%)	Size range mean \pm s.d. (min.–max.)	Index <i>W, B</i>	
<i>Lethenteron</i> sp.				0, 0															0, 0	
	Non-spring	0/1 (0)	102.0															0/1 (0)	No data	0, 0
<i>Noemachelis barbatulus toni</i>				0, 0														0, 0	0, 0	
	Main stem	0/4 (0)	150.3 \pm 8.2 (140–160)							0/8 (0)	108.5 \pm 35.9 (71–160)									
	Spring-fed																	0/12 (0)	114.1 \pm 37.8 (73–168)	
	Non-spring	0/1 (0)	127.0											0/1 (0)	158.0			0/3 (0)	62.0 \pm 35.0 (13–98)	
<i>Hucho perryi</i>				0, 0			0, 0			0, 0			0, 0							
	Main stem	0/1 (0)	102.0		0/1 (0)	38.0				0/1 (0)	35.0									
	Non-spring						0/2 (0)	57.0 \pm 1.4 (56–58)												
<i>Salvelinus leucomaenis leucomaenis</i>				0.13, 0.06			0, 0			0.03, 0.02			0, 0					0.17, 0.10	0.26, 0.17	
	Main stem	2/40 (5.0)	115.6 \pm 36.8 (31–196)		0/6 (0)	88.0 \pm 18.6 (72–124)			0/19 (0)	39.3 \pm 36.0 (10–162)				0/161 (0)	126.9 \pm 55.5 (52–382)			0/10 (0)	99.0 \pm 54.1 (45–228)	
	Spring-fed	1/6 (16.7)	77.7 \pm 13.4 (60–96)		0/12 (0)	81.5 \pm 26.3 (64–157)			0/221 (0)	64.8 ^a \pm 31.1 (25–190)			0/5 (0)	101.6 \pm 48.2 (48–158)		8/293 (2.7)	64.2 \pm 27.2 (32–194)	11/336 (3.3)	50.6 \pm 14.6 (30–132)	
	Non-spring	0/161 (0)	54.3 \pm 24.6 (27–187)					1/361 (0.3)	54.9 \pm 30.1 (28–283)			0/85 (0)	75.3 \pm 18.1 (54–194)		0/564 (0)	54.7 \pm 23.7 (27–180)		0/802 (0)	48.6 \pm 18.2 (28–218)	
<i>S. malma krascheninnikovi</i>				1.11, 0.48			1.09, 1.00			1.15, 0.64			1.42, 1.00					1.15, 0.71	1.25, 0.83	
	Main stem	7/111 (6.3)	98.6 \pm 34.0 (31–183)		0/4 (0)	94.5 \pm 39.5 (69–153)			0/51 (0)	62.8 \pm 25.6 (12–138)			2/144 (1.4)	108.0 \pm 36.0 (51–209)				0/11 (0)	107 \pm 36.0 (42–165)	
	Spring-fed	205/1291 (15.9)	62.1 \pm 25.4 (22–150)		9/206 (4.4)	94.2 \pm 35.0 (42–200)			301/3345 (9.0)	65.1 ^a \pm 28.8 (21–192)			17/638 (2.7)	94.4 \pm 33.7 (37–240)		355/4111 (8.6)	71.0 \pm 28.8 (21–221)	176/2809 (6.3)	68.4 \pm 27.8 (22–180)	
	Non-spring	0/261 (0)	67.4 \pm 30.9 (23–181)		0/122	90.5 \pm 31.7 (45–200)			1/978 (0.1)	75.3 \pm 36.8 (21–202)			0/289 (0)	99.0 \pm 32.7 (40–213)		0/1384 (0)	60.5 \pm 27.6 (22–189)	1/996 (0.1)	76.1 \pm 31.6 (21–208)	
<i>Cottus nozawae</i>				1.06, 0.46			0, 0			0.61, 0.34			0, 0					0.31, 0.19		
	Main stem	9/64 (14.1)	88.5 \pm 22.7 (35–132)		0/6 (0)	89.5 \pm 23.9 (62–130)			0/17 (0)	80.1 \pm 22.0 (31–110)			0/154 (0)	90.2 \pm 33.4 (14–151)				0/48 (0)	No data	
	Spring-fed	4/26 (15.4)	88.8 \pm 21.0 (30–127)		0/2 (0)	108.0 \pm 2.8 (106–110)			8/192 (4.2)	78.7 ^a \pm 25.7 (27–141)			0/22 (0)	91.7 \pm 25.4 (29–122)		3/174 (1.7)	77.6 \pm 26.0 (27–155)	0/24 (0)	85.5 ^a \pm 15.2 (59–113)	
	Non-spring	0/17 (0)	112.3 ^a \pm 10.0 (103–124)		0/2 (0)	132.5 \pm 6.4 (128–137)			0/9 (0)	107.0 ^a \pm 15.6 (96–118)			0/16 (0)	120.9 \pm 18.8 (95–156)				0/22 (0)	89.9 ^a \pm 33.2 (42–145)	

^a A few samples excluded due to measurement error.

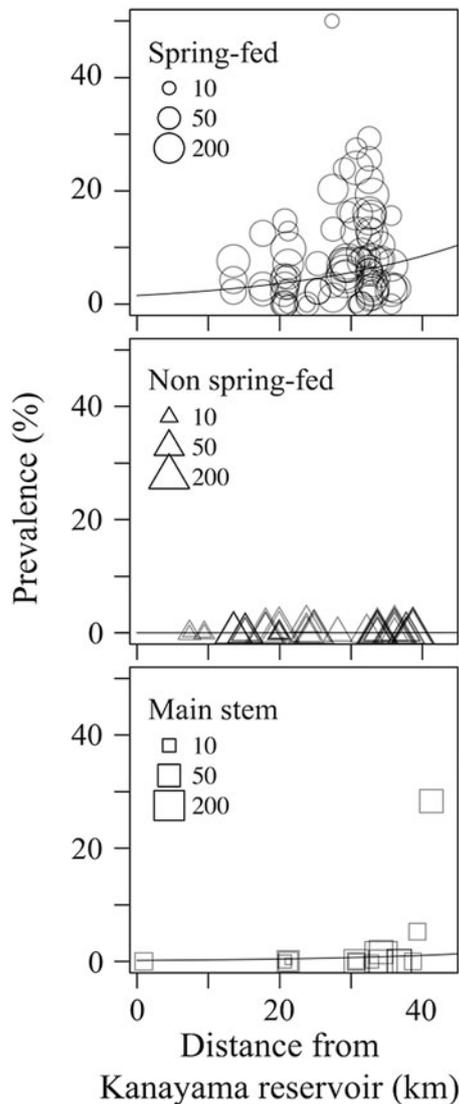


Fig. 2. Spatial distribution of the fish leech *Taimenobdella amurensis* in the river system. Each plot indicates the leech prevalence from one sampling site and term. Size of each shape represents the sample size of the all fish examined.

On the contrary, prevalence in the small tributaries showed similar levels between the upper and lower reaches (Fig. 3B). Furthermore, leech infection was never found outside of the tributaries, compared with inside (Fig. 3C).

Abundance in the preferred habitat

The leech infection ranged from one to five individuals per host fish (Fig. 4A), with a mean 0.110 and deviance 0.146 in the prevalent sites (i.e. spring-fed tributaries). As a result of the model fitting, the NB distribution was selected as the optimal error distribution explaining the abundance data, representing the minimum AIC value among NB, ZIP ($\Delta AIC = 38.7$) and ZINB ($\Delta AIC = 2.0$) distributions. The optimal GLMM model consisted of all candidates of the explanatory variables (i.e. host

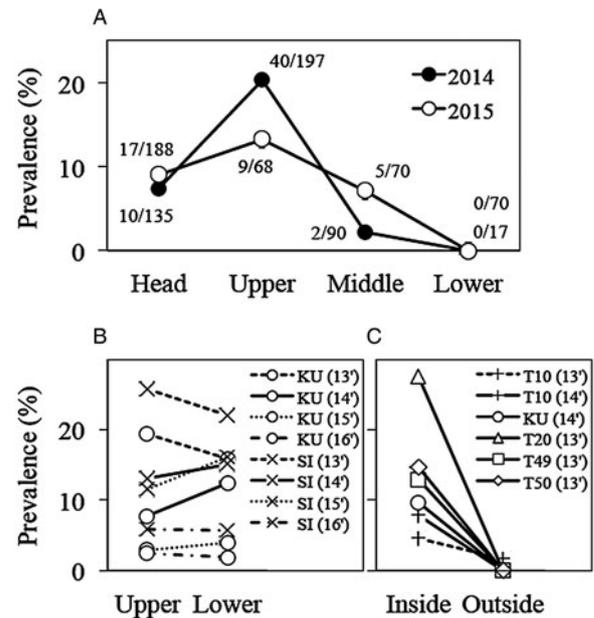


Fig. 3. Habitat use in and around the preferred patches. (A) Longitudinal distributions within the creek and (B) within the small tributaries, and (C) occurrence between inside and outside of the tributary. The leech prevalence in all fishes examined was provided as infected/examined in (A). Abbreviations in (B) and (C) are site names provided in Fig. 1.

body size, season and year) (intercept coefficient = -5.65 ± 0.30 , z -value = -19.04 , $P < 0.001$); leech abundance positively (but only slightly) associated with the body size of the most preferred host, i.e. Dolly Varden (partial coefficient = 0.023 ± 0.0012 , z -value = 18.94 , $P < 0.001$) (Fig. 4B); infection levels in Dolly Varden were higher in spring than in autumn (partial coefficient of spring for autumn = 2.30 ± 0.21 , z -value = 11.15 , $P < 0.001$) and were low in 2014–2016 compared with 2013 (partial coefficient of 2014 for 2013 = -0.80 ± 0.094 , z -value = -8.54 , $P < 0.001$; that of 2015 for 2013 = -0.96 ± 0.095 , z -value = -10.10 , $P < 0.001$; that of 2016 for 2013 = -1.20 ± 0.11 , z -value = -10.95 , $P < 0.001$) (Fig. 4C).

Seasonal changes in the leech body size

Small individuals were mainly found in spring (mean \pm s.d. = 6.80 ± 2.62 mm), whereas larger leeches were found in autumn (mean \pm s.d. = 18.52 ± 6.03 mm) (Fig. 4D). This is most remarkable in 2013 with non-overlapping size distribution, whereas the difference was little smaller in 2014. The optimal LM, consisting of one explanatory variable (i.e. season), supported an approximately 2.72 (= $18.52/6.80$ mm) times difference in the leech body size on average (intercept coefficient = 6.88 ± 0.33 , t -value = 21.02 , $P < 0.001$; partial coefficient of autumn for spring = 11.72 ± 0.62 , t -value = 18.90 , $P < 0.001$).

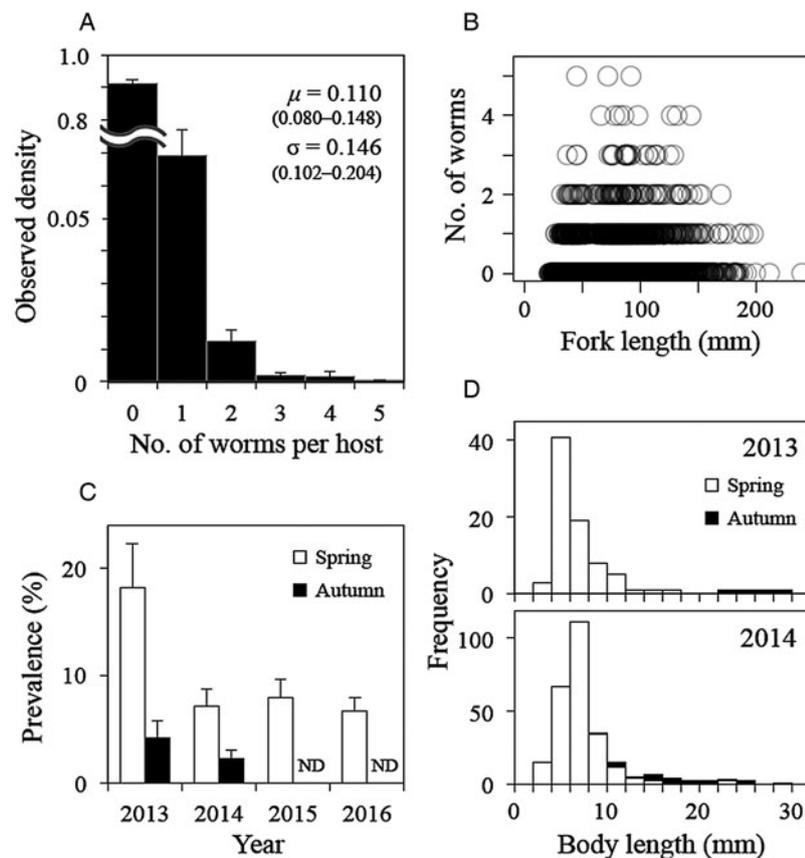


Fig. 4. Infection status of the leech on the preferred host, Dolly Varden, in the prevalent habitats (i.e. spring-fed tributaries). (A) Histogram of the leech infection; estimated parameters and 95% bootstrap confidence intervals (in parentheses, 10 000 permutation) of mean (μ) and variance (σ) in negative binomial distribution are provided. (B) A relationship between the number of worms and the host's FL, (C) seasonal and annual changes in the leech prevalence, (D) seasonal changes in the body size of the leech ($n = 79, 4, 248, 21$ in Spring and Autumn of 2013 and those of 2014, respectively).

Synchrony of the leech prevalence

As a result of pairwise cross-correlation calculated from 11 prevalent sites (consisted of the Shimizusawa Creek and 10 spring-fed tributaries) where the leech infection was continuously investigated in the spring season for 4 years, moderate level of synchrony was detected, with average correlation coefficient 0.315 and a 95% bootstrap confidence interval 0.145–0.479.

DISCUSSION

We demonstrated highly aggregated distribution in an ectoparasite at a small spatial scale (<30 km, within a single watershed), suggesting that local populations can exist at the tributary level and that individuals found in the main stem are due to sporadic passive dispersal depending on host movement. In this case, if we survey only in the main stem, we would consider the leech occurrence being low (2.3% prevalence in the whole, see also Nagasawa *et al.* 2009), but if we survey in small spring-fed tributaries, which may be normally overlooked, they are not very uncommon. This kind of highly heterogeneous distribution may be a characteristic of many parasites with infrequent

occurrence and/or low intensity (Kennedy, 2012). At the same time, however, local population sizes of the fish leech may still be small due to the small sizes of tributaries. We discuss potential population structure and persistence of the ectoparasite, considering the host and parasite life cycles and the factors affecting the narrow suitable habitat.

Life cycle and habitat requirement of the parasitic leech

Taimenobdella amurensis is most likely to have an annual life cycle because small individuals were only found in spring and large individuals in autumn. Decrease in the prevalence from spring to autumn should include both mortality and detachment from the host after ripening and readying to spawn, probably on stream substrates (see Sawyer, 1986). Temporal variations in growth are also suggested from the annual difference in size distributions (Fig. 4D). Timing and duration leeches can attach to the host would significantly affect growth rates, which depend on host density and environmental conditions (Sawyer, 1986).

Although life history of freshwater fish leeches is poorly understood, one of the few good examples

of salmon leeches, *Cystobranchus salmositicus*, shows a remarkable adaptation to host life history (Becker and Katz, 1965). This leech has an annual life cycle adjusted to anadromous salmonids, with hatching from cocoons when host fishes return from sea to rivers in September for spawning, and it subsequently grows up and reproduces prior to the host's death in December (Becker and Katz, 1965). Dolly Varden in this river undergo a similar life cycle as anadromous salmonids: age-1+ juveniles, biasing towards females, migrate from natal tributaries to the main stem to grow, and returned to tributaries for spawning after 0.5–2.5 years of feeding in the main stem (Koizumi *et al.* 2006a; Ayer *et al.* 2017). In addition, the main stem is apparently unsuitable habitat for the leech, at least for reproduction, which acts similarly to the ocean for the salmon leech. In spring, age-0+ Dolly Varden are most abundant in spring-fed tributaries with some age-1+ fish (60–110 mm), whereas large migratory individuals (130–200 mm) move to the tributaries in autumn (Koizumi *et al.* 2006a). Interestingly, leeches emerged in spring and were small enough to attach age-0+ fish, and large leeches were occasionally found on migratory individuals (unpublished data). Therefore, these leeches might partly adjust to the life history of Dolly Varden.

High aggregation of *T. amurensis* in small spring-fed tributaries and the uppermost reaches of the main stem suggest that some environmental factors limit the distribution of this leech. In fact, cold water, high dissolved oxygen and stable water current are required for some freshwater leeches (see Sawyer, 1986), which are all traits related to spring-fed streams. In particular, because *T. amurensis* is a northern species (Epshtein, 1964; Lukin, 1976; Furness *et al.* 2007), one would expect the maximum temperature to be a limiting factor. However, non-spring-fed tributaries and the main stem in the present river system are generally <15 °C and *T. amurensis* can tolerate >20 °C for more than a week (H. Katahira, unpublished data). There might be a critical stage for the development of individuals that is related to water temperature (Amat-Valero *et al.* 2013). For example, eggs might be unable to hatch under near-freezing temperatures, which could explain the concentration in spring-fed tributaries, where the winter stable temperature is relatively high.

The low occurrence of the leech in the main stem might also be due to the anti-parasite behaviours of fishes. In other fish–ectoparasite systems, avoidance responses to parasite presence by move to different microhabitats, such as near the surface and open areas, have been reported (Poulin and Fitzgerald, 1989; Poulin *et al.* 1991; see also Curtis, 2014). In addition to this, scraping against the substrate because of irritation from ectoparasite infection is

well described in cage culture (Shimura, 1983; Woo and Shariff, 1990). We have also observed scraping behaviour of Dolly Varden in an aquarium when leeches were attached (unpublished data). Thus, physical conditions in the main stem with a rocky bottom substrate and high-flow open areas, which is totally differed from that in the tributaries, may work to negatively impact leech existence.

High frequency of infection on Dolly Varden is apparently due to the parasite's habitat requirements. Spring-fed tributaries are mostly dominated by Dolly Varden (Koizumi and Maekawa, 2004) possibly due to temperature-dependent competition (Taniguchi and Nakano, 2000). When other fishes occurred in spring-fed tributaries or the cold upper main stem, the leech attached to other fishes, such as sculpin and white-spotted charr. It is also reported in another stream that *T. amurensis* attached to different host species, such as sticklebacks and freshwater gobies (Nagasawa *et al.* 2009). Therefore, the aggregated distribution is not due to host specificity but habitat requirements.

Possible metapopulation structure in T. amurensis

Self-sustaining populations of *T. amurensis* may be expected in some large tributaries (e.g. the Shimizusawa Creek), but not in others. Because many tributaries are small, the population size of Dolly Varden, the main host fish, are also small with 30–100 adults or <1000 individuals including juveniles (Koizumi, 2011; Koizumi and Shimatani, 2016). Simply calculated from the mean abundance data of the parasite, there should be, at most, a few hundred individuals attached to host fishes in each tributary; the total population size would not be large, given the limited host resources. Piscicolid leeches are also known to deposit multiple clutches (i.e. cocoons containing eggs) on substrates (see Sawyer 1986), but reproductive rate in the present leech may not be high, considering from restricted recruitment in the spring season. Thus, it is unlikely that the leeches could form self-sustaining populations in most of the small tributaries.

For small populations, immigrants from other patches have an important role in population persistence (Hartvigsen and Halvorsen, 1993, 1994; see also Kennedy, 2001, 2012). In this context, dispersal of Dolly Varden is a key transporter of the parasite. Genetic analysis indicated that gene flow occurs among neighbouring tributaries and dispersal distance of Dolly Varden may be 3–5 km (Koizumi *et al.* 2006b). If the leech immigrants are supplied via the host dispersal, stable tributary populations of the leech could conceivably to be sustained for a long time. Although the data are not yet sufficient (i.e. 4 years in 11 sites) and a slightly decreasing trend was observed during the study period, a moderate level of synchrony was observed in leech

population dynamics. This suggests that dispersal is not so high as to connect all tributary populations functioning as a single panmictic population, but not so low for the populations to be completely isolated from each other.

Host dispersion as a vehicle has a powerful role for parasite migration and exchanges among local individuals (Poulin, 2007a). It thus seems obvious if a host species forms a metapopulation structure, its parasite also forms a metapopulation structure. Rather, the putative metapopulation structure of the present parasite may be more prominent than host fish with the possibility of frequent local extinction and re-colonization, because local population sizes are smaller and its vagility should be less than that of the host. Empirical evidence of host–parasite co-structuring is critically lacking and further research deserves attention.

Implications to ecological laws

As shown by one of the few known ecological laws in parasitism (Poulin, 2007b), spatial aggregation in almost all macroparasites, including rarely found species with small mean abundance, is explained by a probability of a NB distribution (Shaw and Dobson, 1995; Poulin, 2013). According to this law, zero-inflated data can be excluded, even in rarely found parasites, when field samplings are properly designed. The present leech infection follows a negative binominal distribution within a preferred habitat (i.e. spring-fed tributary), suggesting that our sampling detected the infectious hot spots of the leech while avoiding false zero data as much as possible. The few occurrences in the density survey in the Chitose River from 2001 to 2008 (Nagasawa *et al.* 2009) appear to be due to the middle-main stem location of the aquarium apart from the main hot spot of the parasite. Since the Chitose River system is composed of spring-fed tributaries (Kawai *et al.* 2013), the patchy feature is also expected as similar to the present river system with an annually reproducing population of tributary.

In some common species, occurrences have been thought to continuously decrease along with river line from downstream to upstream (see Blasco-costa *et al.* 2013), following the River Continuum Concept that explains the longitudinal gradient of biomass in various aquatic organisms (Vannote *et al.* 1980). The inverse trend in our case is clearly due to the specific habits of the leech preferring spring-fed conditions that are also found in the uppermost reaches where the river size is decreasing at branch level, such as the Shimizusawa Creek. The groundwater-rich environment in the headwater area is necessary to account for the ectoparasite infection as a probable factor. Further, the river ecosystem is characterized by not only the

unidirectional factor from top to bottom, but also by discontinuous environmental factors (Poole, 2002), such as riffle and pool structures originating from large woody debris (Fausch and Northcote, 1992; Wonzell and Swanson, 1999), tributary confluences (Benda *et al.* 2004; Fernandes *et al.* 2004) and stream connectivity related to patch adjacency (Lowe *et al.* 2006). Since ectoparasite habits are sensitive to abiotic factors in addition to interactions with their hosts (Chubb, 1977; Sawyer, 1986; Marcogliese and Cone, 1996; Kearns, 2004), hot spots of some species may be explained by such discontinuous fluvial environments within a drainage system, as well as under spring-fed conditions.

The patchy distribution of *T. amurensis* also indicates that the parasite burden differs between the habitat types. This heterogeneous spatial use deserves further attention, specifically in regard to local adaptations in the host–parasite system (Kalts and Shykoff, 1998). There is a possibility that specific selective pressure by the leech infection affects the host fish traits, especially in the spring-fed tributaries. For example, diversity of major histocompatibility complex in the local host populations can be changed with parasite presence (Wegner *et al.* 2003; Rauch *et al.* 2006). Given the immunogenetic differences partly relating to the host demography (e.g. fitness; Kalbe *et al.* 2009), life history traits in the preferred host Dolly Varden should be compared between the leech-abundant hot spots (i.e. spring-fed tributaries), fewer leech hot spots (i.e. the uppermost area of the main stem) and no leech environments (i.e. non spring-fed tributaries).

Conclusion

This large-scale survey demonstrated the heterogeneous spatial use of the rare stream parasite, *T. amurensis*, within a mountain watershed. This hot spot-dependent distribution further suggests that the parasite has a metapopulation structure at a small spatial scale, retained by immigrants transported via host migratory behaviour between the tributaries. Metapopulation structure is often presumed in many parasites due to the nature of patchy habitats (i.e. host individuals or specific areas) (Poulin, 2007a; Criscione, 2008; McCoy, 2009), but empirical data remain limited, except for some common species (Bruyndonckx *et al.* 2009; Dharmarajan *et al.* 2016) or at large spatial scales (on the order of 100–1000 km) (McCoy *et al.* 2003). The heterogeneous occurrence of parasites also provides a good opportunity to evaluate the local adaptation of host species (see Kalts and Shykoff, 1998). Due to the ease of collecting a large amount of data without sacrificing host species, the leech–fish system in this river may be an ideal strategy to study the ecology and evolution of host–parasite interactions.

ACKNOWLEDGEMENTS

We thank H. Omiya, an official staff of the town hall of Minami-Furano, for helping the field samplings. We also express our gratitude for the members of the laboratory of Animal Ecology, Graduate School of Environmental Earth Science, Hokkaido University, for assistance and providing useful comments to our investigation. We thank the editor and two anonymous reviewers for their kind and helpful comments.

FINANCIAL SUPPORT

This study was partially financially supported by Grant-in-Aid for JSPS Fellows to H.K. (grant number 254625) and by a Grant-in-Aid for Young Scientists (B) to I.K. (grant number 23770013).

REFERENCES

- Amat-Valero, M., Calero-Torralbo, M. A. and Valera, F.** (2013). Temperature during the free-living phase of an ectoparasite influences the emergence pattern of the infective phase. *Parasitology* **140**, 1357–1367.
- Ayer, C. G., Katahira, H., Fukui, S. and Koizumi, I.** (2017). Seasonal patterns of downstream movement in partially migratory stream-dwelling Dolly Varden. *Ecology of Freshwater Fish*. Online available only. doi: 10.1111/eff.12342.
- Becker, C. D. and Katz, M.** (1965). Distribution, ecology, and biology of the salmonid leech, *Piscicola salmositica* (Rhynchobdellae: Piscicolidae). *Journal of Fisheries Research Board of Canada* **22**, 1175–1195.
- Benda, L., Poff, N. L., Miller, D., Dunne, T., Reeves, G., Pess, G. and Pollock, M.** (2004). The network dynamics hypothesis: how channel networks structure riverine habitats. *BioScience* **54**, 413–427.
- Bjørnstad, O. N., Ims, R. A. and Lambin, X.** (1999). Spatial population dynamics: analysing patterns and processes of population synchrony. *Trends in Ecology and Evolution* **14**, 427–432.
- Blasco-costa, I., Koehler, A. V., Martin, A. and Poulin, R.** (2013). Upstream-downstream gradient in infection levels by fish parasites: a common river pattern? *Parasitology* **140**, 266–274.
- Bruyndonckx, N., Henry, I., Christe, P. and Kerth, G.** (2009). Spatio-temporal population genetic structure of the parasitic mite *Spinturnix bechsteini* is shaped by its own demography and the social system of its bat host. *Molecular Ecology* **18**, 3581–3592.
- Burnham, K. P. and Anderson, D. R.** (2002). *Model Selection and Multimodel Inference: a Practical Information-theoretic Approach*, 2nd Edn. Springer, New York, USA.
- Bush, A. O. and Holmes, J. C.** (1986). Intestinal helminths of lesser scaup ducks: patterns of association. *Canadian Journal of Zoology* **64**, 132–141.
- Bush, A. O. and Kennedy, C. R.** (1994). Host fragmentation and helminth parasites: hedging your bets against extinction. *International Journal for Parasitology* **24**, 1333–1343.
- Bush, A. O., Lafferty, K. D., Lotz, J. M. and Shostak, A. W.** (1997). Parasitology meets ecology on its own terms: Margolis *et al.* revised. *Journal of Parasitology* **83**, 575–583.
- Bush, A. O., Fernández, J. C., Esch, G. W. and Seed, J. R.** (2001). *Parasitism: the Diversity and Ecology of Animal Parasites*. Cambridge University Press, Cambridge, UK.
- Chubb, J. C.** (1977). Seasonal occurrence of helminths in freshwater fishes. Part I. Monogenea. *Advances in Parasitology* **15**, 133–199.
- Criscione, C. D.** (2008). Parasite co-structure: broad and local scale approaches. *Parasite – Journal De La Societe Francaise De Parasitologie* **15**, 439–443.
- Curtis, V. A.** (2014). Infection-avoidance behaviour in humans and other animals. *Trends in Immunology* **35**, 457–464.
- Dharmarajan, G., Beasley, J. C., Beatty, W. S., Olson, Z. H., Fike, J. A. and Rhodes, O. E., Jr.** (2016). Genetic co-structuring in host-parasite systems: empirical data from raccoons and raccoon ticks. *Ecosphere* **7**, e01269.
- Edo, K., Kawamura, H. and Higashi, S.** (2000). The structure and dimensions of redds and egg pockets of the endangered salmonid, *Sakhalin taimen*. *Journal of Fish Biology* **56**, 890–904.
- Epshtein, V. M.** (1964). Towards a zoogeographical characterization of the fish leeches of the Amur basin. *Doklady Akademii Nauk SSSR* **159**, 1179–1182 (In Russian).
- Fausch, K. D., and Northcote, T. G.** (1992). Large woody debris and salmonid habitat in a small coastal British Columbia stream. *Canadian Journal of Fisheries and Aquatic Sciences* **49**, 682–693.
- Fernandes, C. C., Podos, J. and Lundberg, J. G.** (2004). Amazonian Ecology: tributaries enhance the diversity of electric fishes. *Science* **305**, 1960–1962.
- Fournier, D. A., Skaug, H. J., Ancheta, J., Ianello, J., Magnusson, A., Maunder, M. N., Nielsen, A. and Sibert, J.** (2012). AD model builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optimization Methods and Software* **27**, 233–249.
- Furness, S., Williams, J. I., Nagasawa, K. and Bureson, E. M.** (2007). A collection of fish leeches (Hirudinida: Piscicolidae) from Japan and surrounding waters, including redescription of three species. *Journal of Parasitology* **93**, 875–883.
- Gaston, K. J.** (1994). *Rarity*. Chapman & Hall, London, UK.
- Gaston, K. J.** (1997). What is rarity? In *The Biology of Rarity: Causes and Consequences of Rare-common Differences* (ed. Kunin, W. E. and Gaston, K. J.), pp. 30–47. Chapman & Hall, London, UK.
- Hanski, I.** (1999). *Metapopulation Ecology*. Oxford University Press, Oxford, UK.
- Hartvigsen, R. and Halvorsen, O.** (1993). Common and rare trout parasites in a small landscape system. *Parasitology* **106**, 101–105.
- Hartvigsen, R. and Halvorsen, O.** (1994). Spatial patterns in the abundance and distribution of parasites of freshwater fish. *Parasitology Today* **10**, 28–31.
- Kalbe, M., Eizaguirre, C., Dankert, I., Reusch, T. B. H., Sommerfeld, R. D., Wegner, K. M. and Milinski, M.** (2009). Lifetime reproductive success is maximized with optimal major histocompatibility complex diversity. *Proceedings of the Royal Society, B* **276**, 925–934.
- Kalts, O. and Shykoff, J. A.** (1998). Local adaptation in host-parasite systems. *Heredity* **81**, 361–370.
- Karron, J. D.** (1997). Genetic consequences of different patterns of distribution and abundance. In *The Biology of Rarity: Causes and Consequences of Rare-common Differences* (ed. Kunin, W. E. and Gaston, K. J.), pp. 174–189. Chapman & Hall, London, UK.
- Kawai, H., Ishiyama, N., Hasegawa, K. and Nakamura, F.** (2013). The relationship between the snowmelt flood and the establishment of non-native brown trout (*Salmo trutta*) in streams of the Chitose River, Hokkaido, northern Japan. *Ecology of Freshwater Fish* **22**, 645–653.
- Kearn, G. C.** (2004). *Leeches, lice and Lampreys: a Natural History of Skin and Gill Parasites of Fishes*. Springer, Dordrecht, The Netherlands.
- Kennedy, C. R.** (1976). Reproduction and dispersal. In *Ecological aspects of Parasitology* (ed. Kennedy, C. R.), pp. 143–160. North-Holland Publishing Company, Amsterdam, The Netherlands.
- Kennedy, C. R.** (2001). Metapopulation and community dynamics of helminth parasites of eels *Anguilla anguilla* in the River Exe system. *Parasitology* **122**, 689–698.
- Kennedy, C. R.** (2012). Can a specialist parasite species of a widespread and common host species be rare? The case of *Spinitectus inermis* (Nematoda: Cystidicolidae) in eels *Anguilla anguilla*. *Folia Parasitologica* **59**, 131–138.
- Koizumi, I.** (2011). Integration of ecology, demography and genetics to reveal population structure and persistence: a mini review and case study of stream-dwelling Dolly Varden. *Ecology of Freshwater Fish* **20**, 352–363.
- Koizumi, I. and Maekawa, K.** (2003). Spawning migration of stream-dwelling Dolly Varden in spring-fed tributaries of the Shiisorapuchi River, Japan. *Journal of Freshwater Ecology* **18**, 321–331.
- Koizumi, I. and Maekawa, K.** (2004). Metapopulation structure of stream-dwelling Dolly Varden charr in inferred from patterns of occurrence in the Sorachi River basin, Hokkaido, Japan. *Freshwater Biology* **49**, 973–981.
- Koizumi, I. and Shimatani, I. K.** (2016). Socially induced reproductive synchrony in a salmonid: an approximate Bayesian computation approach. *Behavioral Ecology* **27**, 1386–1396.
- Koizumi, I., Yamamoto, S. and Maekawa, K.** (2006a). Female-biased migration of stream-dwelling Dolly Varden in the Shiisorapuchi River, Hokkaido, Japan. *Journal of Fish Biology* **68**, 1513–1529.
- Koizumi, I., Yamamoto, S. and Maekawa, K.** (2006b). Decomposed pairwise regression analysis of genetic and geographic distances reveals a metapopulation structure of stream-dwelling Dolly Varden charr. *Molecular Ecology* **15**, 3175–3189.
- Koizumi, I., Yamamoto, S., Nomoto, K. and Maekawa, K.** (2008). Synchrony in local population dynamics of stream-dwelling Dolly Varden: do genetically similar groups show similar demography? *Population Ecology* **50**, 367–377.
- Koizumi, I., Hasegawa, K. and Kishi, D.** (2012). Do small tributaries function as refuges from floods? A test in a salmonid-dominated mountainous river. *Ecology of Freshwater Fish* **21**, 165–167.
- Krebs, C. J.** (1999) *Ecological Methodology*, 2nd Edn. Benjamin Cummings, Menlo Park, USA.

- Levins, R.** (1969). Some demographic and genetic consequences of environmental heterogeneity for biological control. *Bulletin of the Entomological Society of America* **15**, 237–240.
- Lowe, W. H., Likens, G. E. and Power, M. E.** (2006). Linking scales in stream Ecology. *BioScience* **56**, 591–597.
- Lukin, E. I.** (1976). *Fauna of the USSR. Leeches, Vol. 1. Leeches of Fresh and Brackish Water Bodies*. Nauka, Leningrad, USSR. (In Russian).
- Mace, G. M. and Kershaw, M.** (1997). Extinction risk and rarity on an ecological timescale. In *The Biology of Rarity: Causes and Consequences of Rare-common Differences* (ed. Kunin, W. E. and Gaston, K. J.), pp. 130–149. Chapman & Hall, London, UK.
- Marcogliese, D. J. and Cone, D. K.** (1996). On the distribution and abundance of eel parasites in Nova Scotia: influence of pH. *Journal of Parasitology* **82**, 389–399.
- Martin, T. G., Wintle, B. A., Rhodes, J. R., Kuhnert, P. M., Field, S. A., Low-Choy, S. J., Tyre, A. J. and Possingham, H. P.** (2005). Zero tolerance ecology: improving ecological inference by modeling the source of zero observations. *Ecology Letters* **8**, 1235–1246.
- McCoy, K. D.** (2009). Host-parasite determinants of parasite population structure: lessons from bats and mites on the importance of time. *Molecular Ecology* **18**, 3545–3547.
- McCoy, K. D., Boulinier, T., Tirard, C. and Michalakis, Y.** (2003). Host-dependent genetic structure of parasite populations: differential dispersal of seabird tick host races. *Evolution* **57**, 288–296.
- Menges, E. S.** (1992). Stochastic modeling of extinction in plant populations. In *Conservation Biology: the Theory and Practice of Nature Conservation, Preservation and Management* (ed. Fiedler, P. L. and Jain, S. K.), pp. 253–275. Chapman & Hall, New York, USA.
- Nagasawa, K., Imai, Y. and Ishida, K.** (1988). Long-term changes in the population size and geographical distribution of *Pennella* sp. (Copepoda) on the saury, *Cololabis saira*, in the western North Pacific Ocean and adjacent seas. *Hydrobiologia* **167/168**, 571–577.
- Nagasawa, K., Kikuchi, M., Arakane, Y. and Yusa, S.** (2009). New data on the hosts and attachment sites of the fish leech *Taimenobdella amurensis* (Hirudinida, Piscicolidae). *Biogeography* **11**, 3–8.
- Norton, J., Lewis, J. W. and Rollinson, D.** (2003). Parasite intracommunity diversity in eels: a reflection of local component community diversity. *Parasitology* **127**, 475–482.
- Ogawa, K., Shirakashi, S. and Ishitani, H.** (2014). Insemination of the monogenean *Neobenedenia girellae* (Capsalidae, Benedeniinae). *Parasitology International* **63**, 473–478.
- Poole, G. C.** (2002). Fluvial landscape ecology: addressing uniqueness within the river discontinuum. *Freshwater Biology* **47**, 641–660.
- Poulin, R.** (2007a). *Evolutionary Ecology of Parasites*, 2nd Edn. Princeton University Press, Princeton, USA.
- Poulin, R.** (2007b). Are there general laws in parasitic ecology? *Parasitology* **134**, 763–776.
- Poulin, R.** (2013). Explaining variability in parasitic aggregation levels among host samples. *Parasitology* **140**, 541–546.
- Poulin, R. and Fitzgerald, G. J.** (1989). Risk of parasitism and microhabitat selection in juvenile sticklebacks. *Canadian Journal of Zoology* **67**, 14–18.
- Poulin, R. and Morand, S.** (2004). *Parasite biodiversity*. Smithsonian Institution Press, Washington, DC, USA.
- Poulin, R., Rau, M. E. and Curtis, M. A.** (1991). Infection of brook trout fry, *Salvelinus fontinalis*, by ectoparasitic copepods: the role of host behaviour and initial parasite load. *Animal Behaviour* **41**, 467–476.
- Price, P. W.** (1980) *Evolutionary Biology of Parasites*. Princeton University Press, Princeton, USA.
- Rauch, G., Kalbe, M. and Reusch, T. B. H.** (2006). One day is enough: rapid and specific host-parasite interactions in a stickleback-trematode system. *Biology Letters* **2**, 382–384.
- R Development Core Team** (2014). R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- Rózsa, L.** (1992). Points in question endangered parasite species. *International Journal for Parasitology* **22**, 265–266.
- Sawyer, R. T.** (1986). *Leech biology and Behaviour, Vol. 2. Feeding Biology, Ecology, and Systematics*. Clarendon Press, Oxford, UK.
- Shaw, D. J. and Dobson, A. P.** (1995). Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology* **111** (Suppl.), S111–S133.
- Shimura, S.** (1983). Seasonal occurrence, sex ratio and site preference of *Argulus coregoni* Thorell (Crustacea: Brachiura) parasitic on cultured freshwater salmonids in Japan. *Parasitology* **86**, 537–552.
- Taniguchi, Y. and Nakano, S.** (2000). Condition-specific competition: implications for the altitudinal distribution of stream fishes. *Ecology* **81**, 2027–2039.
- Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R. and Cushing, C. E.** (1980). The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 130–137.
- Wegner, K. M., Kalbe, M., Kurtz, J., Reusch, T. B. H. and Milinski, M.** (2003). Parasite selection for immunogenetic optimality. *Science* **301**, 1343.
- Wonzell, S. M. and Swanson, F. J.** (1999). Floods, channel change, and the hyporheic zone. *Water Resources Research* **35**, 555–567.
- Woo, P. T. K. and Shariff, M.** (1990). *Lernaea cyprinacea* L. (Copepoda: Caligidae) in *Helistoma temmincki* Cuvier and Valenciennes: the dynamics of resistance in recovered and naïve fish. *Journal of Fish Diseases* **13**, 485–494.
- Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A. and Smith, G. M.** (2009). *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York, USA.