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Environmental factors affecting the invasion success and morphological responses of a globally introduced crayfish in floodplain waterbodies

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Key Words

aquatic connectivity, ecosystem management, invasion ecology, invisibility, river-floodplain complex

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Abstract

Floodplain ecosystems that are characterized by high habitat heterogeneity and hydrological connectivity are considered hotspots for freshwater biodiversity. However, these biodiversity-rich areas have been seriously threatened by biological invasions. The signal crayfish (*Pacifastacus leniusculus*) is listed as 100 of the world's worst invasive species, and is a major threat to freshwater biodiversity and ecosystem functioning. Here, we examined environmental factors relating to the invasion success of signal crayfish and their morphological responses in floodplain waterbodies. Classification and regression tree analyses showed that most of the influential factors differed between tributary and lake populations. In floodplain tributaries, the occurrence of crayfish was positively related with water temperature and abundance of leaf cover, while crayfish abundance was highest where large-wood was abundant. In floodplain lakes, crayfish were absent at oxygen-poor sites, and abundant at sites with high connectivity to a main channel. These results indicate that conservation practitioners should consider different environmental factors in accordance with strategies for invasive species management (i.e., offensive or defensive management). Furthermore, we demonstrated morphological differences between tributary and lake populations, with tributary crayfish having wider chelae. These morphological differences might have resulted

from the physical differences between the two types of waterbodies, facilitating the rapid invasion of signal crayfish to floodplain waterbodies. Our study showed that invasion-risk assessments should consider both environmental factors and morphological responses to new environments to understand invasion ecology and to form effective conservation plans and to prioritize management actions.

Introduction

Environmental and biological factors are major determinants of invasion success (Chapple et al. 2012; Drenovsky et al. 2012; Molina-Montenegro et al. 2012). Environmental factors directly and indirectly affect invasion success by regulating species mortality and migration (Hellmann et al. 2008; Pyšek and Richardson 2010). In addition, invasive species can change their behavior, life history, and morphology in response to new environments (Colautti and Barrett 2013; Prentis et al. 2008). Such responses, such as phenotypic plasticity, are considered as one of the main factors facilitating biological invasions (Davidson et al. 2011; Liu et al. 2015). Hence, information on environmental factors affecting invasion success and the responses of invaders to new environments could be used to inform the decisions of managers.

Floodplains are areas of land adjacent to rivers that extend from the banks of river channel to the base of the surrounding valleys, and are maintained by the fluvial actions of flooding and channel migration (Nanson and Croke 1992). Floodplain ecosystems are composed of various types of waterbodies (e.g., lotic, lentic, ephemeral, and semi-aquatic) that are adjacent to the main river channel (Tockner and Stanford 2002; Ward et al. 1999). High physio-chemical heterogeneity may exist within the same waterbody, providing complex and diverse habitats for freshwater fauna (Thomaz et al. 2007; Ward

1998). Furthermore, floodplain ecosystems are generally characterized by dynamic and diverse hydrological connectivity between waterbodies, which sustain freshwater biodiversity through the exchange of nutrients and organisms (Górski et al. 2013; Tockner et al. 1999). However, hydrological connectivity within floodplain ecosystems might foster the spread of invasive species, as active dispersal via watercourses and passive flood transport represent potential advantages for rapid invasion (Höfle et al. 2014; Helms et al. 2013; Stoffels et al. 2016). Therefore, environmental factors affecting the invasion success in floodplain ecosystems should be clarified to conserve freshwater biodiversity.

The distribution of invasive crayfish has rapidly expanded in freshwater ecosystems, profoundly influencing food webs and ecosystem functioning through predation, the spread of pathogens, and the decomposition of organic matter (Ficetola et al. 2012; Jackson et al. 2014; Nilsson et al. 2012; Twardochleb et al. 2013). The signal crayfish, *Pacifastacus leniusculus* (Astacidae), is listed as 100 of the world's worst invasive species (Lowe et al. 2000). Signal crayfish are native to western North America, and were originally introduced into 27 European countries and Japan for aquaculture (Kouba et al. 2014; Usio et al. 2016). The pattern of spread depends on the environmental conditions since the mortality and movement of signal crayfish are

extremely susceptible to abiotic factors (Bubb et al. 2004; Light 2003; Olsson and Nyström 2009). Furthermore, introduced signal crayfish can adjust their traits depending on the new environment (Hudina et al. 2012; Rebrina et al. 2015). For example, Hudina et al. (2012) reported that signal crayfish at the invasion front have larger claw in Europe. Other recent studies reported morphological adaptions of crayfish in response to water velocity and quality (Haddaway et al. 2012; Perry et al. 2013), which might be a strategy to improve competitive and/or survival ability (Hudina et al. 2012; Usio et al. 2016). However, little is known about the vulnerable environments to their invasion and the morphological responses of crayfish in floodplain ecosystems.

The conservation strategies that prevent invasive species from spreading may be either defensive or offensive. Defensive strategies aim to block the establishment of species at currently uninvaded locations, whereas offensive strategies aim to prevent the expansion of species from source locations (Drury and Rothlisberger 2008; Stewart‐Koster et al. 2015). Understanding the relationship between the presence/absence and environmental factors could facilitate project design and/or help the selection of locations that require defensive strategies. Identifying locations with high invader abundance might also allow managers to implement effective offensive control of invasive species.

Here, we examined the environmental factors affecting the invasion success of signal crayfish and their morphological responses in floodplain ecosystems. We focused on two types of floodplain waterbodies, lotic (tributaries) and lentic (lakes), and examined potential environmental factors affecting distribution (presence/absence and abundance) of signal crayfish for each water-body type. In addition, we surveyed habitat-dependent morphological variation by comparing the morphological characteristics between tributary and lake populations.

Methods

Study species

In the native range, signal crayfish are found across a diversity of freshwater habitats, from lentic to lotic (e.g., Abrahamsson and Goldman 1970; Bondar et al. 2005). This species is generally omnivorous, feeding on a variety of allochthonous and autochthonous resources, such as woody debris, macrophytes, and aquatic insects (Bonder et al.2005). Signal crayfish have very high mobility. For example, Anastácio et al (2015) reported that this species moved up to 461 m in half a day. The broad ecological niche and high mobility of this species might contribute to its ability to disperse and establish in new habitats.

Study site

Field surveys were conducted at 33 floodplain tributary sites and 18 floodplain lake sites along the Shibetsu River in eastern Hokkaido, Japan (Fig. 1). The Shibetsu River originates in Mt. Shibetsu (1,061 m) and runs to the Okhotsk Sea (approximately 78 km in length), and has broad floodplains. The mean annual average discharge from 2004 to 2008 was 22.2 m^3/s. The climate of this region is subarctic. The floodplains have been developed for agricultural lands. The main river channel was modified and straightened between the 1950s and 1970s (Nakano and Nakamura 2006). The presence of signal crayfish in the Shibetsu River was first detected in 2002 (Ohtaka et al. 2005). The study tributaries were small lowland streams with an average wetted width of 3.39 ± 1.52 m (mean ± SD). The average area of the study lakes was 1.26 ± 1.64 ha (mean ± SD). The average temperature and precipitation in the study year in Shibetsu District were approximately 5.6 °C and 1600 mm, respectively.

Tributary surveys

In 11 of the floodplain tributaries, we established three 20-m sampling reaches within 500 m of the confluence with the main channel (Fig. 2). During July and August,

2016, crayfish were collected using electrofishing (Model 12-B Battery Powered Backpack Electrofisher; Smith-Root Inc.). Collections were repeated three times at each reach from downstream to upstream. At each reach, we examined the presence or absence of crayfish and calculated crayfish density by dividing the number of individuals by the total water surface area.

We measured the environmental factors at both tributary and reach scale. Tributary-scale environmental factors were measured at one central point of the section including three sampling reaches. We measured water temperature, pH and ion concentration (Table 1). Water temperature was automatically measured every 30 minutes using a data logger (HOBO® Pendant Temperature/Light 8 K Data Logger; Onset Computer Corp.). The mean water temperature from July to October was used for the analysis, as signal crayfish activity is highest during this warm period of the year (Usio et al. 2007). In the early morning, from 06.00 to 08.00 in August, pH was measured using a pH meter (Portable pH Meter WM-22EP; DKK-TOA Corp.). For ion concentration, we collected 200 ml water using a plastic bottle, and quickly preserved the samples in a cooler box with ice packs. We subsequently analyzed the samples using ion chromatography (Dionex ICS-1100 Ion Chromatography; Thermo Fisher Scientific Inc.) in a laboratory. We selected Ca_2^+, SO_4^{2-}, and NO_3^- as ions that were influential to the survival and

growth of freshwater crayfish (Edwards et al. 2016; Rallo and García-Arberas 2002). Reach-scale environmental factors were measured at each sampling reach to calculate environmental heterogeneity within each tributary. We measured the wetted channel width, depth, current velocity, substrate coarseness, habitat cover, and undercut bank ratio of each reach (Table 1). Ten equally spaced transects with three equally spaced measuring points were established at each reach (Fig. 2). The wetted channel width was measured at each transect, and the depth and current velocity were determined at each point. Current velocity was measured at the bed surface using an electromagnetic current meter (FLO-MATE TM Model 2000 Portable Flowmeter; Marsh-McBirney Inc.). The mean of each variable at each reach was used for the analysis. At each measuring point, following Wentworth (1922), the substrate was visually identified and classified as one of the following types: bedrock (fixed rock, 1 point), sand (<2 mm, 2 points), pebbles (2–64 mm, 3 points), cobbles (65–256 mm, 4 points), or boulders (>256 mm, 5 points). Substrate coarseness was represented by the mean of substrate points at each reach. Habitat cover was divided into leaf cover and large wood. Length and width of study reach were measured to calculate the cover area. We calculated the cover ratio by dividing the total cover area by the total water surface area. The length of undercut

bank was measured on the right and left banks, and the undercut bank ratio was calculated by dividing the total undercut bank length by the total bank length.

Lake surveys

During July and August 2016, crayfish were collected from each lake on one occasion using a cylindrical minnow trap (diameter 30 cm, length 65 cm, mesh size 8 mm), with Pacific saury, *Cololabis saira,* cut into quarters as bait. In each lake, four traps were set within 2 m of shore and more than 10 m from each other. The traps were set overnight and retrieved the next morning. We recorded the presence/absence of crayfish and calculated catch per unit effort (CPUE) by dividing the number of individuals per trap by the set time.

We measured 11 variables in lakes: water temperature, pH, ion concentration (Ca_2^+, SO_4^{2-}, NO_3^-), dissolved oxygen, depth, aquatic vegetation ratio, presence/absence of water channel connected to the main channel, shortest Euclidean distance to the main channel, and water surface area of each lake (Table 2). Water temperature and pH were measured once near the shore using the same methods as in the tributary surveys. Water collection and analysis for ion concentration was also conducted using the same methods as the tributary surveys. We measured dissolved oxygen (DO) using a DO

meter (HQd Portable Meter; Hach Company) at the same points. Ten transects with three measuring points were established at each lake. Depth was measured at each point, and the aquatic vegetation ratio along each transect was recorded using visual observations at 10% intervals. The mean of each variable at each lake was used for the analysis. The presence/absence of the water channel was confirmed using field observations. The shortest Euclidian distances to the main channel and water surface area were measured using the Quantum Geographical Information System (QGIS, version 2.14.0).

Morphological examination of crayfish

How individuals with breeding capacity respond to new environments can directly influence invasion success. Thus, we used reproductive crayfish with CL ≥30 mm (Lewis and Horton 1997) for the morphological examination. We excluded individuals that were in the process of molting or that had unbalanced left and right chelae sizes. In total, we collected 50 individuals (male: n = 25, female: n = 25) from six tributaries and 50 individuals (male: n = 25, female: n = 25) from eight lakes. The average CL of each group was: 41.2 ± 4.3 mm (tributary males), 40.0 ± 4.2 mm (tributary females), 41.4 ± 3.8 mm (lake males), and 39.7 ± 2.6 mm (lake females). Seven morphological variables

were measured using a metal caliper (Absolute COOLANT PROOF Caliper IP66; Mitutoyo America Corp.): Carapace Length (CL), Chelae Length (ChL), Chelae Width (ChW), Carapace Length to cervical groove (CLcg), Carapace Width (CW), Abdomen Length (AL), and Abdomen Width (AW) (Fig. 3).

Data analysis

The relationships between environmental factors and the occurrence and abundance of crayfish were analyzed using classification and regression trees, respectively. Data were analyzed using the tree package developed for freeware R (version 3.1.2). Crayfish presence/absence and abundance (tributary: density, lake: CPUE) were the response variables in the classification and regression trees, respectively. For the tributary analysis, the explanatory variables were water temperature, pH, ion concentration (Ca_2^+, SO_4^{2-}, NO_3^-), wetted channel width, depth, current velocity, substrate coarseness, leaf cover ratio, large wood ratio, and undercut bank ratio. For the lake analysis, the explanatory variables were water temperature, pH, ion concentration (Ca_2^+, SO_4^{2-}, NO_3^-), DO, depth, aquatic vegetation ratio, water channel connecting to the main channel (presence = 1, absence = 0), shortest Euclidian distance to the main channel, and water surface area. The classification tree model was

evaluated using the correct classification rate (Venables and Ripley 2013) and the *k* statistic (Cohen 1960). *k* values of 0–0.4 indicate poor agreement, 0.4–0.75 values indicate good agreement, and 0.75–1.0 values indicate excellent agreement (Landis and Koch 1977). For the regression tree, model accuracy was evaluated using correlations between the observed and expected values.

The relationships between crayfish morphology and waterbody type were analyzed using principal component analysis (PCA) and generalized linear models (GLMs). Previous studies have reported that crayfish morphology is sexually dimorphic (Anderson and Simon 2015; Mazlum et al. 2007). Therefore, we compared the morphology of male and female in the tributary and lake populations separately. First, we used PCA to investigate multivariate differentiation in shell morphology between the two populations using the statistics package developed for R (version 3.6.0). We used scaled morphological variables for the analyses. Second, we conducted GLMs with a Gaussian error distribution and identity link function to detect key morphological variables that differentiate tributary and lake populations. The response variables were the scores of PC1 or PC2, and the explanatory variable was the habitat type (tributary or lake). Model significance was tested using the likelihood ratio test against null models, with significance being set at $\alpha = 0.05$. Based on the results of the GLMs, we judged

variables that had high eigenvectors ($r > 0.7$) to the PCA axis as key morphological factors that significantly differentiated tributary and lake populations.

Results

Environmental factors affecting invasion

We collected a total of 533 individuals, of which 218 individuals were from tributaries and 315 individuals were from lakes (Supplementary Materials). Mean abundance and occurrence probability was 6.6 inds/m^2 and 48% in tributaries, and 4.4 inds/net and 35% in lakes, respectively (Table 3).

For both tributary and lake populations, the most influential factors differed between classification and regression tree analyses. Classification trees for tributaries showed that water temperature and leaf cover were major determinants of crayfish occurrence (Fig. 4a). Crayfish were predicted to occur where average water temperature was ≥10.93 °C. For reaches with water temperature <10.93 °C, crayfish were predicted to occur where leaf cover ≥2.40%, while the probability of crayfish occurrence was 0 for leaf cover <2.40%. The correct classification rate was 88% and *k* was 0.76 (excellent agreement). In the regression tree for tributaries, large wood and water temperature determined the first and second splits, respectively (Fig. 4b). Predicted crayfish density

was 0.33/m^2 at reaches where large wood covered ≥4.94% of the water surface. Crayfish density was 0.12/m^2 where large wood covered <4.94% of the water surface and temperature ≥10.93 °C, and 0.003/m^2 when water temperature <10.93 °C. Pearson's correlation coefficient between the observed and predicted values was 0.99.

The splits of the classification tree for lakes were primarily determined by the level of DO, followed by the presence of the water channel (Fig. 5a). The classification tree predicted that crayfish would be absent from lakes with DO <4.48 mg/L. For lakes with DO ≥4.48 mg/L, sites hydrologically connected to a main channel showed higher crayfish occurrence compared to isolated lakes. The correct classification rate was 83%, and *k* was 0.65, showing "good" agreement. The regression tree for lakes showed that only water channel influenced crayfish abundance (Fig. 5b). The CPUE of crayfish was 0.67 when the water channel was present, and 0.059 when absent. The Pearson's correlation coefficient between the observed and predicted values was 0.99.

Morphological measurement of crayfish

We found morphological differences between the tributary and lake populations. PCA analyses showed that morphological variations of both males and females were well explained by PC1 and PC2; the cumulative proportion of variance was 0.94 and

0.87 for males and females, respectively (Table 4, Fig. 6a, b). GLMs with a likelihood ratio test showed that the morphological difference between habitats was significantly explained by a gradient of PC2 (males: $p < 0.001$, females: $p < 0.001$). Tributary populations showed significantly higher PC2 scores than lake populations (Fig. 6c, d). Out of the seven morphological factors, only ChW had a high positive eigenvector to PC2 (Table 3). The ChW of tributary crayfish was significantly greater than that of lake crayfish, regardless of sex.

Discussion

Habitat and environmental factors that determine invasion capacity provide essential information for implementing suitable management actions. In the present study, we used three-run electrofishing (e.g., Gladman et al. 2010; Light 2003) and trap-fishing (e.g., Abrahamsson and Goldman 1970; Usio et al. 2006), which are effective and widely used approaches for crayfish census in lotic and lentic environments to minimize the risk of false zero records. We found that multiple environmental factors of floodplain waterbodies hierarchically determined the invasion potential of signal crayfish. For both tributary and lake populations, the most influential factors differed between classification and regression tree analyses, indicating that conservation

practitioners should consider different environmental factors depending on the strategies for invasive species management (i.e., offensive or defensive). In addition, the hierarchy of influential variables highlights the need to prioritize management actions. Furthermore, our study confirmed the morphological differences between tributary and lake populations. Such morphological responses might represent a key factor promoting the rapid invasion of signal crayfish in floodplain ecosystems.

The classification tree for tributaries showed that the occurrence probability for crayfish was highest where average water temperature was ≥10.93 °C. The activity of signal crayfish dramatically increases between 8 °C and 15 °C, and peaks at around 20 °C (Nyström and Strand 1996; Rutledge and Pritchard 1981; Usio et al. 2006). Therefore, decreased activity due to low temperature could limit the spread of signal crayfish by depressing their tolerance to water flow (Johnson et al. 2014). In addition, high water temperature enhances crayfish foraging activity (Nyström and Strand 1996), which might be advantageous for their survival and reproduction. Meanwhile, the occurrence probability of crayfish increased in sites with more leaf cover, even under low temperature conditions. Leaves can provide food resources and refuges for signal crayfish (Bondar et al. 2005; Jackson et al. 2014), which might enhance their survival and/or growth rates, mitigating the negative effects of low temperature. The regression

tree showed that large wood was the most influential factor determining crayfish density. Large wood is a primary refuge for crayfish against currents and predation, improving the survivorship and growth rate of juveniles (Blake et al. 1994). Such ecological benefits for invasive crayfish might facilitate their invasion and reproductive success.

The classification tree for lakes showed that crayfish were absent where DO was <4.48 mg/L, which is consistent with the threshold value previously reported. Harlioğlu (1996) reported that the survivorship of signal crayfish decreases in hypoxic environments with <4.5 mg/L DO. In shallow lentic systems, severe hypoxia frequently occurs due to stagnant water and the respiration of aquatic vegetation (Brönmark and Hansson 2005). Therefore, DO would be a strong factor limiting the spread of signal crayfish in floodplain lakes. We did not include DO in the tributary analyses to keep the model simple. However, we actually found that DO in study lakes was noticeably lower than that in tributaries (Lake: min 0.8 mg/L, max 12.2 mg/L, mean 5.6 mg/L; Tributary: min 9.5 mg/L, max 11.2 mg/L, mean 10.4 mg/L). At oxygen-rich sites (≥4.48 mg/L), the occurrence probability of crayfish was highest at sites with connections to other waterbodies. Many previous field studies have demonstrated that signal crayfish have high mobility, up to 400 m/day, in aquatic environments (Anastácio et al. 2015; Wutz

and Geist 2013). The results of the present study indicate that the highly mobile signal crayfish could facilitate their spread to uncolonized lakes using water channels in well-connected floodplain landscapes. The regression tree analyses for lakes also demonstrated the impact of hydrological connectivity; crayfish abundance was higher at sites with more water connections. Ishiyama et al. (2015) showed that watercourse networks among floodplain ponds increased native fish abundance. Hydrological connections with other waterbodies might also support constant immigration and rapid recovery from episodic disturbances, contributing to the increased abundance of invasive crayfish. In contrast to tributary sites, we found that water temperature in lakes did not influence their abundance. Water temperature in lakes was higher than that of tributaries by 3.5 °C on average (Tables 1, 2); thus, most lakes had a suitable temperature environment for signal crayfish activity. Consequently, water temperature in lakes would have limited effects on the occurrence and abundance of crayfish.

Our study revealed differences in two morphological traits between tributary and lake populations; tributary crayfish had wider chelae than lake crayfish, regardless of sex. Perry et al. (2013) previously reported this morphological trend between lotic and lentic populations in rusty crayfish (*Orconectes rusticus*). We could not clearly identify the cause of the morphological changes (i.e., local adaption or phenotypic plasticity). In

general, invasive populations that have high genetic diversity tend to show higher phenotypic variation compared to local adaption (Geng et al. 2016; Riis et al. 2010). However, the signal crayfish population in the Shibetsu Basin probably became established in recent years (Ohtaka et al. 2005); thus, this population likely has relatively low genetic diversity compared to other populations formerly introduced to Hokkaido Island (Usio et al. 2016). Moreover, Haddaway et al. (2012) provided evidence for phenotypic plasticity in the carapace width of white-clawed crayfish using relocation experiments. Therefore, we speculate that morphological changes to the chelae of signal crayfish were caused by phenotypic plasticity. The broader chelae of tributary populations might contribute to their establishment in new environments by deflecting water flow pressure.

Although signal crayfish have only recently been introduced into the Shibetsu River Basin, we found that the level of invasion was high in both floodplain tributaries and lakes. This finding highlights the urgent need for the management of this highly invasive species. Our results have several management implications. We found that signal crayfish occurred in warmer tributaries and oxygen-rich lakes. For a defensive strategy, uncolonized waterbodies with such high invasibility should be prioritized for monitoring and management. Recently, human activities have increased global water

temperatures in lotic systems (Rahel and Olden 2008; Woodward et al. 2010). Thus, suppressing an increase in water temperature (e.g., maintenance of riparian forest, Nagasaka and Nakamura 1999) might be one effective preventive action. Crayfish abundance was highest in tributaries with large wood volume and in well-connected lakes. For an offensive strategy, removal of crayfish from waterbodies with such high invasibility might prevent secondary spread. Intentional fragmentation has been recognized as one of the beneficial strategy for preventing the spread of non-native species (Rahel 2013). The findings of the present study might also contribute to selecting potential sources that need isolating (i.e., high-density sites) from the threatened floodplain ecosystems. However, our study only provides a snapshot of the fluctuating environment of floodplain waterbodies. For example, signal crayfish were found in some lakes with no water channels, indicating that episodic flooding of the main channel might transport signal crayfish to floodplain lakes (Bubb et al. 2002; Bubb et al. 2004). In addition, further morphological trends in lotic populations could be tested by other sampling techniques. For instance, electrofishing was used in the tributary census, but this approach might not catch individuals occupying deep burrows, which might have a different morphology to other individuals. Further studies

accounting for the environmental variability of floodplains are essential to obtain an accurate understanding of the invasion success of signal crayfish.

Floodplain waterbodies are generally characterized by high habitat connectivity and heterogeneity. In such complex ecosystems, many invasive species from many taxonomic groups exist (Brummer et al. 2016; Stoffels et al. 2016; Terwei et al. 2013). These abiotic and biotic complexities of floodplain ecosystems complicate the management of invasive species by ecologists and managers. In this study, we demonstrated that environmental factors and the biological traits of signal crayfish determined their invasion success in floodplain waterbodies. The decision tree models constructed in the present study closely fitted the observed data. However, the key influencing factors identified at our site might differ to those of other sites exposed to different conditions; thus, studies at other regions are required to determine the replicability of our results, particularly when using larger sample sizes. However, we believe that the study concept of linking influential factors on the presence/absence or abundance of invaders to defensive and offensive management measures against invasive species could be applied broadly, can have generality. Besides, phenotypic plasticity can contribute to invasion success of other crustacean as well as our target species. An invasion risk assessment framework that incorporates both environmental

and biological traits could provide a useful approach for prioritizing target invasive species and associated management actions in complex river-floodplain environments with high invasion risk.

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Table 1 Environmental characteristics of the study reaches in tributaries. Water quality (water temperature, pH, Ca^{2+}, SO_4^-, NO_3^-) was observed at one point in each tributary. Wetted width was observed at 30 points in each tributary (10 transects × 3 reaches). Depth, current velocity, and substrate coarseness were observed at 90 points in each tributary (3 points × 10 transects × 3 reaches). Means of these four variables were first calculated per reach, and then calculated for all reaches combined. Leaf cover, large wood, and undercut bank were observed in each reach.

Environment	Number of observations in each tributary	Mean	±	SD
Water temperature (°C)	n = 1	10.89	±	1.06
pH	n = 1	7.31	±	0.20
Ca^{2+} (mg/L)	n = 1	12.85	±	4.88
SO_4^- (mg/L)	n = 1	7.81	±	3.41
NO_3^- (mg/L)	n = 1	5.67	±	3.62
Wetted width (m)	n = 30	3.39	±	1.52
Depth (cm)	n = 90	23.77	±	5.98
Current velocity (m/s)	n = 90	0.33	±	0.15
Substrate coarseness (points)	n = 90	2.63	±	0.61
Leaf cover (%)	n = 3	3.56	±	3.28
Large wood (%)	n = 3	2.68	±	3.22
Undercut bank (%)	n = 3	11.17	±	9.30

Table 2 Environmental characteristics of the study lakes. Water quality (water temperature, DO, pH, Ca^{2+}, SO_4^-, NO_3^-), shortest Euclidian distance, and water surface area were recorded in each lake. Depth was observed in each sampling point (3 points × 10 transects). Aquatic vegetation was observed in each transect (10 transects). Means of these two variables were first calculated per lake, and then calculated for all lakes combined.

Environment	Number of observations in each lake	Mean	±	SD
Water temperature (°C)	n = 1	14.40	±	1.20
DO (mg/L)	n = 1	5.57	±	3.07
pH	n = 1	6.60	±	0.33
Ca^{2+} (mg/L)	n = 1	9.39	±	3.47
SO_4^- (mg/L)	n = 1	5.21	±	5.44
NO_3^- (mg/L)	n = 1	2.40	±	4.42
Depth (cm)	n = 30	105.40	±	52.98
Aquatic vegetation (%)	n = 10	16.67	±	13.72
Shortest Euclidian distance (m)	n = 1	150.33	±	74.01
Water surface area (ha)	n = 1	1.26	±	1.64
Hydrologic connection to a main channel	-	Presence: 8 sites Absence: 10 sites		

Table 3 Crayfish abundance and probability of occurrence in each study tributary and lake. In each tributary and lake, we set three reach and four minnow traps, respectively.

Site ID	Mean abundance	Probability of occurrence
	Tributary Population	
	(inds/m^2)	(%)
T1	0.0	0
T2	0.3	33
T3	0.7	67
T4	0.3	33
T5	19.0	100
T6	5.7	100
T7	23.0	100
T8	0.0	0
T9	23.7	100
T10	0.0	0
T11	0.0	0
	Lake Population	
	(inds/net)	(%)
L1	0.0	0
L2	0.0	0
L3	0.0	0
L4	2.0	50
L5	0.0	0
L6	0.0	0
L7	0.3	25
L8	11.5	100
L9	0.0	0
L10	28.8	100
L11	0.0	0
L12	13.3	100
L13	13.8	100
L14	0.0	0
L15	8.3	75
L16	1.0	75
L17	0.0	0
L18	0.0	0

Table 4 Eigenvectors and percent variance explained by 1st and 2nd principal component (PC). High eigenvectors for each PC (>0.7) are shown in boldface.

	Male		Female	
	PC1	PC2	PC1	PC2
% variance	87.9	6.4	78.0	8.5
Cumulative %	87.9	94.3	78.0	86.5
Eigenvector				
CL	-0.385	0.002	-0.401	-0.167
ChL	-0.383	0.354	-0.4	0.138
ChW	-0.346	**0.733**	-0.314	**0.861**
CLcg	-0.387	-0.274	-0.399	-0.361
AL	-0.37	-0.463	-0.392	-0.211
AW	-0.386	-0.191	-0.378	0.079
CW	-0.386	-0.101	-0.353	-0.175

* CL – carapace length, ChL – chelae length, ChW – chelae width, CLcg – carapace length to cervical groove, AL – abdomen length, AW – abdomen width, CW – carapace width.

Figure Captions

Fig. 1 Map of the study tributaries and lakes in eastern Hokkaido, Japan.

Fig. 2 Schematic showing the reaches, transects, and measuring points for tributary surveys.

Fig. 3 Measured morphological body parts of the signal crayfish.

Fig. 4 (a) Classification tree and (b) regression tree for signal crayfish from tributary reach sites (n = 33). The length of the vertical line below each split corresponds to the importance of the variable. (a) The values in parentheses at the terminal nodes indicate the probability of crayfish occurrence. (b) Each terminal node shows the predicted crayfish density (individuals/m^2).

Fig. 5 (a) Classification tree and (b) regression tree for signal crayfish from lake sites (n = 18). The length of the vertical line below each split corresponds to the importance of the variable. (a) The values in the parentheses at the terminal nodes indicate the probabilities of crayfish occurrence. (b) Each terminal node shows the predicted crayfish density (individuals/m^2).

Fig. 6 Morphological differences between tributary and lake populations. (a) Distribution of male crayfish in the multivariate morphospace described by a principal component analysis (PCA). (b) Distribution of female crayfish in the multivariate morphospace described by PCA. (c) Difference of

PC scores between tributary and lake male populations. (d) Difference in the PC scores between tributary and lake female populations.

Fig. 1

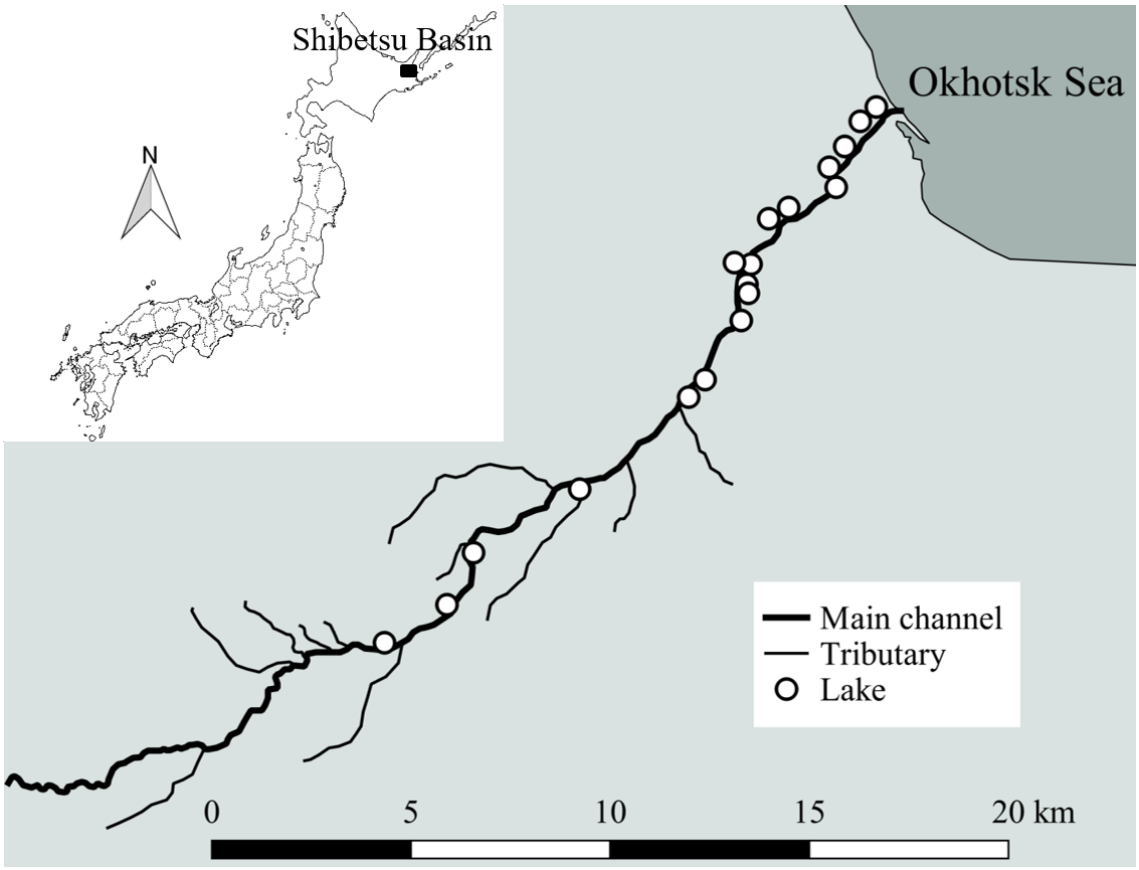

Shibetsu Basin
N
Okhotsk Sea
Main channel
Tributary
Lake
0
5
10
15
20 km

Fig. 2

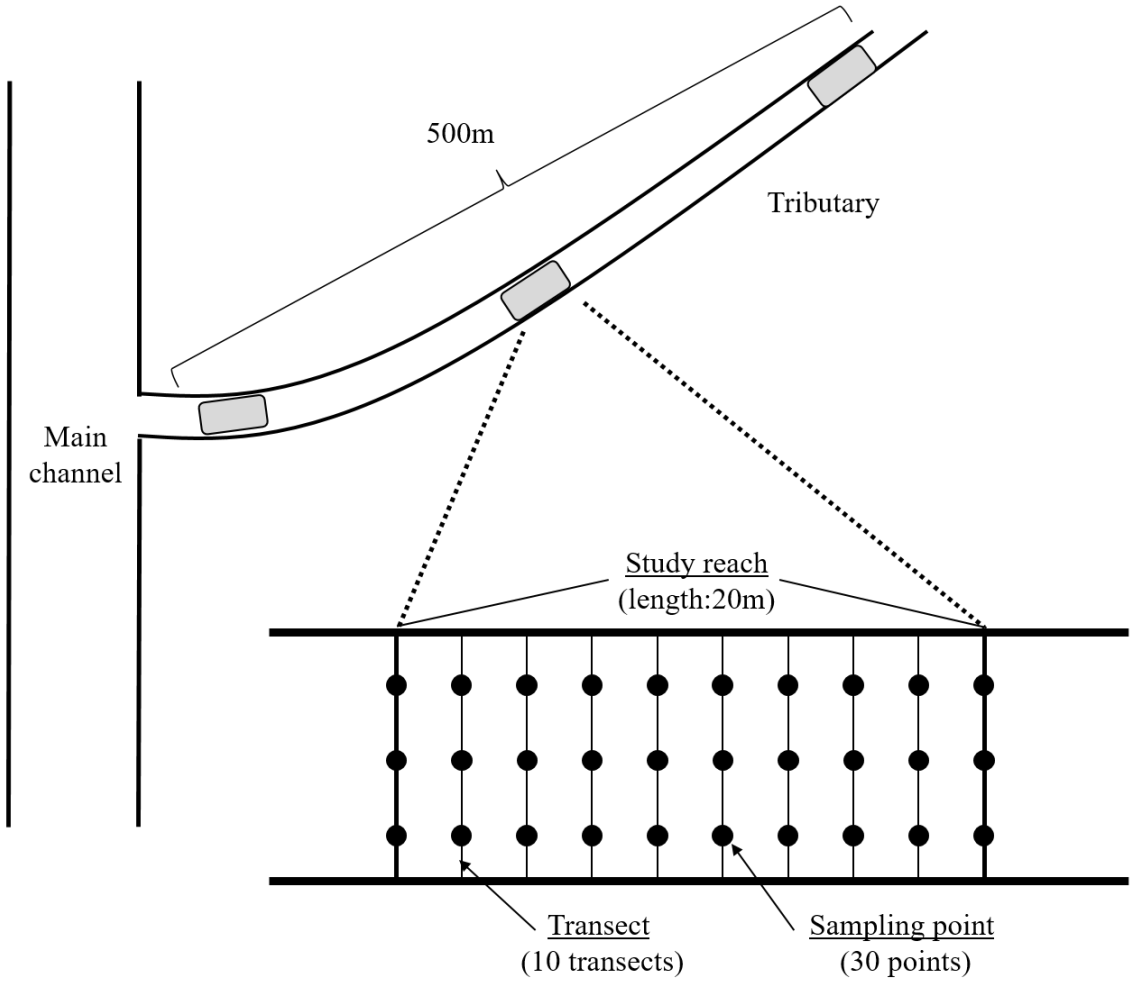
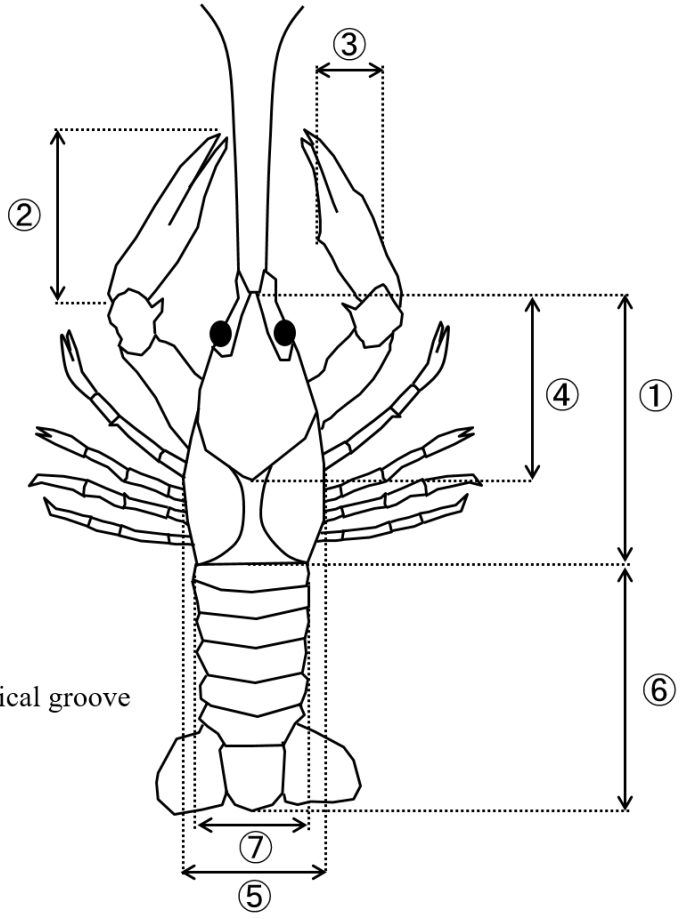
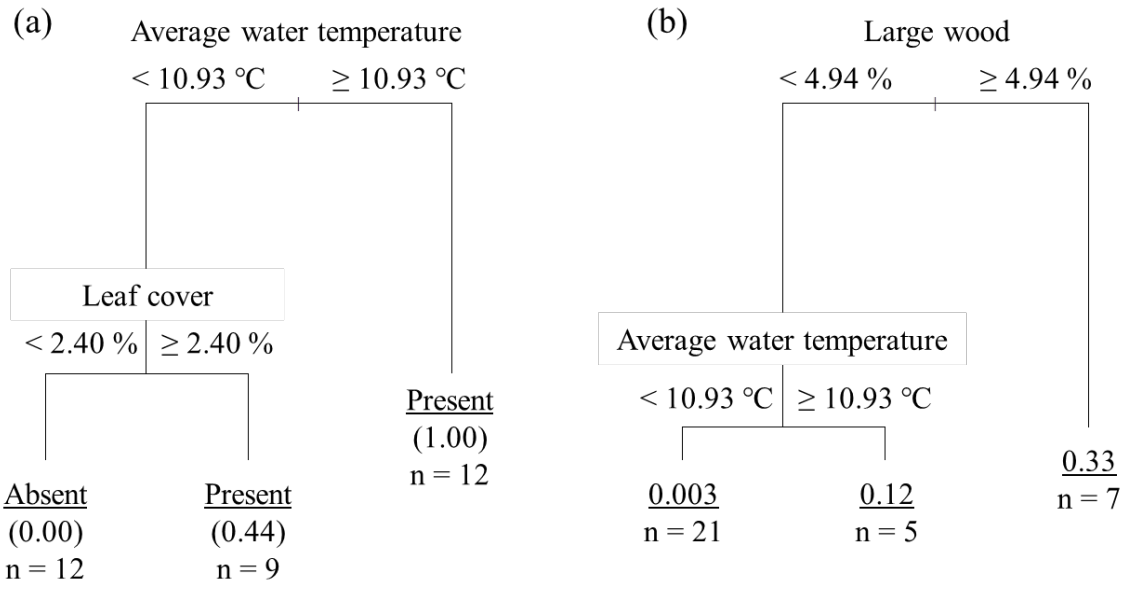

500m
Tributary
Main
channel
Study reach
(length:20m)
Transect
(10 transects)
Sampling point
(30 points)

Fig. 3



① CL: Carapace Length
② ChL: Chelae Length
③ ChW: Chelae Width
④ CLcg: Carapace Length to cervical groove
⑤ CW: Carapace Width
⑥ AL: Abdomen Length
⑦ AW: Abdomen Width

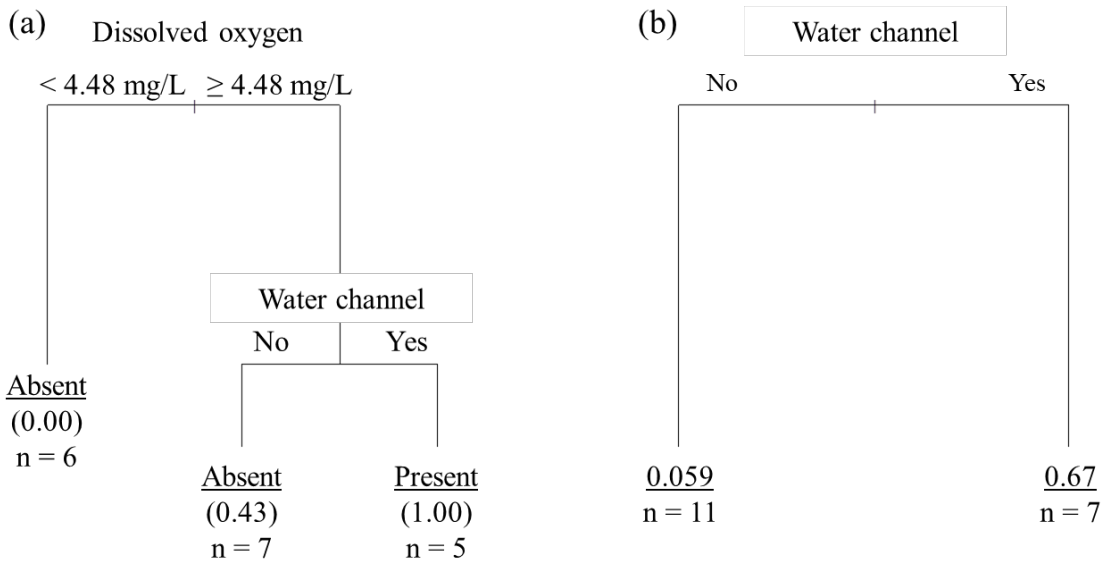
Fig. 4



Correct classification rate = 88 %
k statistic = 0.76

Observed vs. Predicted r = 0.99

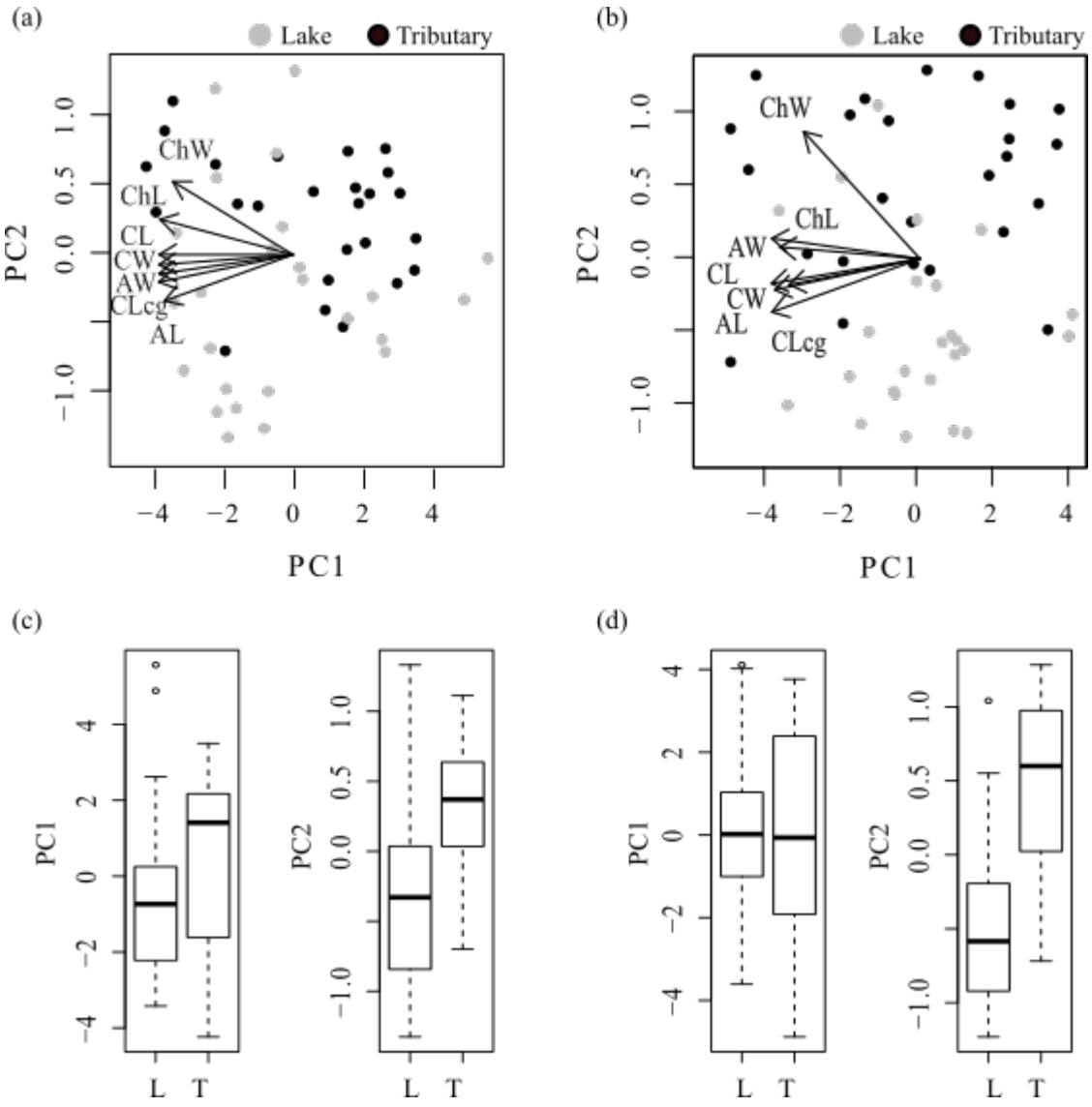
Fig. 5



Correct classification rate = 83 %
k statistic = 0.65

Observed vs. Predicted r = 0.99

Fig. 6


(a)
Lake
Tributary
ChW
ChL
CL
CW
AW
CLcg
AL
PC2
PC1
(b)
Lake
Tributary
ChW
ChL
AW
CL
CW
AL
CLcg
PC2
PC1
(c)
PC1
PC2
L
T
(d)
PC1
PC2
L
T