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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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1 **Abstract**

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

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

1 **Introduction**

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

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

1 1998). Furthermore, floodplain ecosystems are generally characterized by dynamic and
2 diverse hydrological connectivity between waterbodies, which sustain freshwater
3 biodiversity through the exchange of nutrients and organisms (Górski et al. 2013;
4 Tockner et al. 1999). However, hydrological connectivity within floodplain ecosystems
5 might foster the spread of invasive species, as active dispersal via watercourses and
6 passive flood transport represent potential advantages for rapid invasion (Höfle et al.
7 2014; Helms et al. 2013; Stoffels et al. 2016). Therefore, environmental factors
8 affecting the invasion success in floodplain ecosystems should be clarified to conserve
9 freshwater biodiversity.

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

1 extremely susceptible to abiotic factors (Bubb et al. 2004; Light 2003; Olsson and
2 Nyström 2009). Furthermore, introduced signal crayfish can adjust their traits
3 depending on the new environment (Hudina et al. 2012; Rebrina et al. 2015). For
4 example, Hudina et al. (2012) reported that signal crayfish at the invasion front have
5 larger claw in Europe. Other recent studies reported morphological adaptations of crayfish
6 in response to water velocity and quality (Haddaway et al. 2012; Perry et al. 2013),
7 which might be a strategy to improve competitive and/or survival ability (Hudina et al.
8 2012; Usio et al. 2016). However, little is known about the vulnerable environments to
9 their invasion and the morphological responses of crayfish in floodplain ecosystems.

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

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

8

9 **Methods**

10 *Study species*

11 In the native range, signal crayfish are found across a diversity of freshwater
12 habitats, from lentic to lotic (e.g., Abrahamsson and Goldman 1970; Bondar et al.
13 2005). This species is generally omnivorous, feeding on a variety of allochthonous and
14 autochthonous resources, such as woody debris, macrophytes, and aquatic insects
15 (Bondar et al. 2005). Signal crayfish have very high mobility. For example, Anastácio et
16 al (2015) reported that this species moved up to 461 m in half a day. The broad
17 ecological niche and high mobility of this species might contribute to its ability to
18 disperse and establish in new habitats.

1

2 *Study site*

3 Field surveys were conducted at 33 floodplain tributary sites and 18 floodplain lake
4 sites along the Shibetsu River in eastern Hokkaido, Japan (Fig. 1). The Shibetsu River
5 originates in Mt. Shibetsu (1,061 m) and runs to the Okhotsk Sea (approximately 78 km
6 in length), and has broad floodplains. The mean annual average discharge from 2004 to
7 2008 was 22.2 m³/s. The climate of this region is subarctic. The floodplains have been
8 developed for agricultural lands. The main river channel was modified and straightened
9 between the 1950s and 1970s (Nakano and Nakamura 2006). The presence of signal
10 crayfish in the Shibetsu River was first detected in 2002 (Ohtaka et al. 2005). The study
11 tributaries were small lowland streams with an average wetted width of 3.39 ± 1.52 m
12 (mean ± SD). The average area of the study lakes was 1.26 ± 1.64 ha (mean ± SD). The
13 average temperature and precipitation in the study year in Shibetsu District were
14 approximately 5.6 °C and 1600 mm, respectively.

15

16 *Tributary surveys*

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

1 2016, crayfish were collected using electrofishing (Model 12-B Battery Powered
2 Backpack Electrofisher; Smith-Root Inc.). Collections were repeated three times at each
3 reach from downstream to upstream. At each reach, we examined the presence or
4 absence of crayfish and calculated crayfish density by dividing the number of
5 individuals by the total water surface area.

6 We measured the environmental factors at both tributary and reach scale. Tributary-
7 scale environmental factors were measured at one central point of the section including
8 three sampling reaches. We measured water temperature, pH and ion concentration
9 (Table 1). Water temperature was automatically measured every 30 minutes using a data
10 logger (HOBO[®] Pendant Temperature/Light 8 K Data Logger; Onset Computer Corp.).
11 The mean water temperature from July to October was used for the analysis, as signal
12 crayfish activity is highest during this warm period of the year (Usio et al. 2007). In the
13 early morning, from 06.00 to 08.00 in August, pH was measured using a pH meter
14 (Portable pH Meter WM-22EP; DKK-TOA Corp.). For ion concentration, we collected
15 200 ml water using a plastic bottle, and quickly preserved the samples in a cooler box
16 with ice packs. We subsequently analyzed the samples using ion chromatography
17 (Dionex ICS-1100 Ion Chromatography; Thermo Fisher Scientific Inc.) in a laboratory.
18 We selected Ca_2^+ , SO_4^{2-} , and NO_3^- as ions that were influential to the survival and

1 growth of freshwater crayfish (Edwards et al. 2016; Rallo and García-Arberas 2002).

2 Reach-scale environmental factors were measured at each sampling reach to calculate

3 environmental heterogeneity within each tributary. We measured the wetted channel

4 width, depth, current velocity, substrate coarseness, habitat cover, and undercut bank

5 ratio of each reach (Table 1). Ten equally spaced transects with three equally spaced

6 measuring points were established at each reach (Fig. 2). The wetted channel width was

7 measured at each transect, and the depth and current velocity were determined at each

8 point. Current velocity was measured at the bed surface using an electromagnetic

9 current meter (FLO-MATE TM Model 2000 Portable Flowmeter; Marsh-McBirney

10 Inc.). The mean of each variable at each reach was used for the analysis. At each

11 measuring point, following Wentworth (1922), the substrate was visually identified and

12 classified as one of the following types: bedrock (fixed rock, 1 point), sand (<2 mm, 2

13 points), pebbles (2–64 mm, 3 points), cobbles (65–256 mm, 4 points), or boulders (>256

14 mm, 5 points). Substrate coarseness was represented by the mean of substrate points at

15 each reach. Habitat cover was divided into leaf cover and large wood. Length and width

16 of study reach were measured to calculate the cover area. We calculated the cover ratio

17 by dividing the total cover area by the total water surface area. The length of undercut

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

3

4 *Lake surveys*

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

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

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

9

10 *Morphological examination of crayfish*

11 How individuals with breeding capacity respond to new environments can directly
12 influence invasion success. Thus, we used reproductive crayfish with $CL \geq 30$ mm
13 (Lewis and Horton 1997) for the morphological examination. We excluded individuals
14 that were in the process of molting or that had unbalanced left and right chelae sizes. In
15 total, we collected 50 individuals (male: $n = 25$, female: $n = 25$) from six tributaries and
16 50 individuals (male: $n = 25$, female: $n = 25$) from eight lakes. The average CL of each
17 group was: 41.2 ± 4.3 mm (tributary males), 40.0 ± 4.2 mm (tributary females), $41.4 \pm$
18 3.8 mm (lake males), and 39.7 ± 2.6 mm (lake females). Seven morphological variables

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

5

6 *Data analysis*

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

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

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

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

3

4 **Results**

5 *Environmental factors affecting invasion*

6 We collected a total of 533 individuals, of which 218 individuals were from
7 tributaries and 315 individuals were from lakes (Supplementary Materials). Mean
8 abundance and occurrence probability was 6.6 inds/m² and 48% in tributaries, and 4.4
9 inds/net and 35% in lakes, respectively (Table 3).

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

1 was 0.33/m² at reaches where large wood covered $\geq 4.94\%$ of the water surface.
2 Crayfish density was 0.12/m² where large wood covered $< 4.94\%$ of the water surface
3 and temperature ≥ 10.93 °C, and 0.003/m² when water temperature < 10.93 °C. Pearson's
4 correlation coefficient between the observed and predicted values was 0.99.

5 The splits of the classification tree for lakes were primarily determined by the
6 level of DO, followed by the presence of the water channel (Fig. 5a). The classification
7 tree predicted that crayfish would be absent from lakes with DO < 4.48 mg/L. For lakes
8 with DO ≥ 4.48 mg/L, sites hydrologically connected to a main channel showed higher
9 crayfish occurrence compared to isolated lakes. The correct classification rate was 83%,
10 and k was 0.65, showing "good" agreement. The regression tree for lakes showed that
11 only water channel influenced crayfish abundance (Fig. 5b). The CPUE of crayfish was
12 0.67 when the water channel was present, and 0.059 when absent. The Pearson's
13 correlation coefficient between the observed and predicted values was 0.99.

14

15 *Morphological measurement of crayfish*

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

1 0.87 for males and females, respectively (Table 4, Fig. 6a, b). GLMs with a likelihood
2 ratio test showed that the morphological difference between habitats was significantly
3 explained by a gradient of PC2 (males: $p < 0.001$, females: $p < 0.001$). Tributary
4 populations showed significantly higher PC2 scores than lake populations (Fig. 6c, d).
5 Out of the seven morphological factors, only ChW had a high positive eigenvector to
6 PC2 (Table 3). The ChW of tributary crayfish was significantly greater than that of lake
7 crayfish, regardless of sex.

8

9 **Discussion**

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

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

7 The classification tree for tributaries showed that the occurrence probability for
8 crayfish was highest where average water temperature was ≥ 10.93 °C. The activity of
9 signal crayfish dramatically increases between 8 °C and 15 °C, and peaks at around
10 20 °C (Nyström and Strand 1996; Rutledge and Pritchard 1981; Usio et al. 2006).
11 Therefore, decreased activity due to low temperature could limit the spread of signal
12 crayfish by depressing their tolerance to water flow (Johnson et al. 2014). In addition,
13 high water temperature enhances crayfish foraging activity (Nyström and Strand 1996),
14 which might be advantageous for their survival and reproduction. Meanwhile, the
15 occurrence probability of crayfish increased in sites with more leaf cover, even under
16 low temperature conditions. Leaves can provide food resources and refuges for signal
17 crayfish (Bondar et al. 2005; Jackson et al. 2014), which might enhance their survival
18 and/or growth rates, mitigating the negative effects of low temperature. The regression

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

6 The classification tree for lakes showed that crayfish were absent where DO was
7 <4.48 mg/L, which is consistent with the threshold value previously reported. Harlioğlu
8 (1996) reported that the survivorship of signal crayfish decreases in hypoxic
9 environments with <4.5 mg/L DO. In shallow lentic systems, severe hypoxia frequently
10 occurs due to stagnant water and the respiration of aquatic vegetation (Brönmark and
11 Hansson 2005). Therefore, DO would be a strong factor limiting the spread of signal
12 crayfish in floodplain lakes. We did not include DO in the tributary analyses to keep the
13 model simple. However, we actually found that DO in study lakes was noticeably lower
14 than that in tributaries (Lake: min 0.8 mg/L, max 12.2 mg/L, mean 5.6 mg/L; Tributary:
15 min 9.5 mg/L, max 11.2 mg/L, mean 10.4 mg/L). At oxygen-rich sites (≥ 4.48 mg/L),
16 the occurrence probability of crayfish was highest at sites with connections to other
17 waterbodies. Many previous field studies have demonstrated that signal crayfish have
18 high mobility, up to 400 m/day, in aquatic environments (Anastácio et al. 2015; Wutz

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

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

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

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

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

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

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

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

1 **References**

- 2 Abrahamsson SA, Goldman CR (1970) Distribution, density and production of the
3 crayfish *Pacifastacus leniusculus* Dana in Lake Tahoe, California-Nevada. *Oikos*:83-
4 91.
- 5 Anastácio PM, Banha F, Capinha C et al. (2015) Indicators of movement and space use
6 for two co-occurring invasive crayfish species. *Ecol Indicators* 53:171-181.
- 7 Anderson WE, Simon TP (2015) Length-weight relationship, body morphometrics, and
8 condition based on sexual stage in the rusty crayfish, *Orconectes rusticus* Girard,
9 1852 (Decapoda, Cambaridae) with emphasis on management implications. *Fisheries*
10 and *Aquaculture Journal* 6:129-135.
- 11 Blake M, Nyström P, Hart P (1994) The effect of weed cover on juvenile signal crayfish
12 (*Pacifastacus leniusculus* Dana) exposed to adult crayfish and non-predatory fish.
13 *Ann Zool Fenn* 31:297-306.
- 14 Bondar CA, Bottriell K, Zeron K et al. (2005) Does trophic position of the omnivorous
15 signal crayfish (*Pacifastacus leniusculus*) in a stream food web vary with life history
16 stage or density? *Can J Fish Aquat Sci* 62:2632-2639.
- 17 Brönmark C, Hansson L-A (2005) *The biology of lakes and ponds*. Oxford University
18 Press.

- 1 Brummer T, Byrom A, Sullivan J et al. (2016) Alien and native plant richness and
2 abundance respond to different environmental drivers across multiple gravel
3 floodplain ecosystems. *Divers Distrib* 22:823-835.
- 4 Bubb DH, Lucas MC, Thom TJ (2002) Winter movements and activity of signal
5 crayfish *Pacifastacus leniusculus* in an upland river, determined by radio telemetry.
6 *Aquatic telemetry*. Springer, pp. 111-119.
- 7 Bubb DH, Thom TJ, Lucas MC (2004) Movement and dispersal of the invasive signal
8 crayfish *Pacifastacus leniusculus* in upland rivers. *Freshwat Biol* 49:357-368.
- 9 Chapple DG, Simmonds SM, Wong BB (2012) Can behavioral and personality traits
10 influence the success of unintentional species introductions? *Trends Ecol Evol* 27:57-
11 64.
- 12 Cohen J (1960) A coefficient of agreement for nominal scales. *Educ Psychol Meas*
13 20:37-46.
- 14 Colautti RI, Barrett SC (2013) Rapid adaptation to climate facilitates range expansion of
15 an invasive plant. *Science* 342:364-366.
- 16 Davidson AM, Jennions M, Nicotra AB (2011) Do invasive species show higher
17 phenotypic plasticity than native species and, if so, is it adaptive? A meta - analysis.
18 *Ecol Lett* 14:419-431.

- 1 Drenovsky RE, Grewell BJ, D'antonio CM et al. (2012) A functional trait perspective on
2 plant invasion. *Ann Bot* 110:141-153.
- 3 Drury KL, Rothlisberger JD (2008) Offense and defense in landscape - level invasion
4 control. *Oikos* 117:182-190.
- 5 Edwards BA, Jackson DA, Somers KM (2016) Effects of declining calcium availability
6 on the survival, growth and calcium content of a freshwater crayfish, *Orconectes*
7 *virilis*. *Freshwat Biol* 61:914-922.
- 8 Ficetola GF, Siesa ME, De Bernardi F et al. (2012) Complex impact of an invasive
9 crayfish on freshwater food webs. *Biodivers Conserv* 21:2641-2651.
- 10 Geng Y, van Klinken RD, Sosa A et al. (2016) The relative importance of genetic
11 diversity and phenotypic plasticity in determining invasion success of a clonal weed
12 in the USA and China. *Front Plant Sci* 7:213.
- 13 Gladman Z, Yeomans W, Adams C et al. (2010) Detecting North American signal
14 crayfish (*Pacifastacus leniusculus*) in riffles. *Aquat Conserv: Mar Freshwat Ecosyst*
15 20:588-594.
- 16 Górski K, Buijse A, Winter H et al. (2013) Geomorphology and flooding shape fish
17 distribution in a large - scale temperate floodplain. *River Res Appl* 29:1226-1236.
- 18 Haddaway NR, Mortimer RJ, Christmas M et al. (2012) Morphological diversity and

1 phenotypic plasticity in the threatened British white - clawed crayfish
2 (*Austropotamobius pallipes*). *Aquat Conserv: Mar Freshwat Ecosyst* 22:220-231.

3 Harlioğlu MM (1996) Comparative biology of the signal crayfish, *Pacifastacus*
4 *leniusculus* (Dana), and the narrow-clawed crayfish, *Astacus leptodactylus*
5 *Eschscholtz.* University of Nottingham.

6 Hellmann JJ, Byers JE, Bierwagen BG et al. (2008) Five potential consequences of
7 climate change for invasive species. *Conserv Biol* 22:534-543.

8 Helms B, Budnick W, Pecora P et al. (2013) The influence of soil type, congeneric cues,
9 and floodplain connectivity on the local distribution of the devil crayfish (*Cambarus*
10 *diogenes* Girard). *Freshwater Science* 32:1333-1344.

11 Höfle R, Dullinger S, Essl F (2014) Different factors affect the local distribution,
12 persistence and spread of alien tree species in floodplain forests. *Basic Appl Ecol*
13 15:426-434.

14 Hudina S, Hock K, Žganec K et al. (2012) Changes in population characteristics and
15 structure of the signal crayfish at the edge of its invasive range in a European river.
16 *Annales de Limnologie-International Journal of Limnology.* EDP Sciences, pp. 3-
17 11.

18 Ishiyama N, Koizumi I, Yuta T et al. (2015) Differential effects of spatial network

- 1 structure and scale on population size and genetic diversity of the ninespine
2 stickleback in a remnant wetland system. *Freshwat Biol* 60:733-744.
- 3 Jackson MC, Jones T, Milligan M et al. (2014) Niche differentiation among invasive
4 crayfish and their impacts on ecosystem structure and functioning. *Freshwat Biol*
5 59:1123-1135.
- 6 Johnson MF, Rice SP, Reid I (2014) The activity of signal crayfish (*Pacifastacus*
7 *leniusculus*) in relation to thermal and hydraulic dynamics of an alluvial stream, UK.
8 *Hydrobiologia* 724:41-54.
- 9 Kouba A, Petrusek A, Kozák P (2014) Continental-wide distribution of crayfish species
10 in Europe: update and maps. *Knowledge and Management of Aquatic Ecosystems*:05
- 11 Landis JR, Koch GG (1977) The measurement of observer agreement for categorical
12 data. *Biometrics*:159-174.
- 13 Lewis SD, Horton HF (1997). Life history and population dynamics of the signal
14 crayfish, *Pacifastacus leniusculus*. Lake Billy Chinook, Oregon. *Freshw Crayfish*
15 11:34-53.
- 16 Light T (2003) Success and failure in a lotic crayfish invasion: the roles of hydrologic
17 variability and habitat alteration. *Freshwat Biol* 48:1886-1897.
- 18 Liu C, Chen Y, Olden JD et al. (2015) Phenotypic shifts in life history traits influence

- 1 invasion success of goldfish in the Yarlung Tsangpo River, Tibet. *Trans Am Fish Soc*
2 144:602-609.
- 3 Lowe S, Browne M, Boudjelas S et al. (2000) 100 of the world's worst invasive alien
4 species: a selection from the global invasive species database. *Invasive Species*
5 Specialist Group Auckland.
- 6 Mazlum Y, Fatih Can M, Eversole A (2007) Morphometric relationship of length–
7 weight and chelae length–width of eastern white river crayfish (*Procambarus acutus*
8 *acutus*, Girard, 1852), under culture conditions. *J Appl Ichthyol* 23:616-620.
- 9 Molina-Montenegro MA, Penuelas J, Munné-Bosch S et al. (2012) Higher plasticity in
10 ecophysiological traits enhances the performance and invasion success of *Taraxacum*
11 *officinale* (dandelion) in alpine environments. *Biol Invasions* 14:21-33.
- 12 Nagasaka A, Nakamura F (1999) The influences of land-use changes on hydrology and
13 riparian environment in a northern Japanese landscape. *Landscape Ecol* 14:543-556.
- 14 Nakano D, Nakamura F (2006) Responses of macroinvertebrate communities to river
15 restoration in a channelized segment of the Shibetsu River, Northern Japan. *River Res*
16 *Appl* 22:681-689.
- 17 Nanson G, Croke J (1992) A genetic classification of floodplains. *Geomorphology*
18 4:459-486.

- 1 Nilsson E, Solomon C, Wilson K et al. (2012) Effects of an invasive crayfish on trophic
2 relationships in north - temperate lake food webs. *Freshwat Biol* 57:10-23.
- 3 Nyström P, Strand J (1996) Grazing by a native and an exotic crayfish on aquatic
4 macrophytes. *Freshwat Biol* 36:673-682.
- 5 Ohtaka A, Gelder SR, Kawai T et al. (2005) New records and distributions of two North
6 American branchiobdellidan species (Annelida: Clitellata) from introduced signal
7 crayfish, *Pacifastacus leniusculus*, in Japan. *Biol Invasions* 7:149-156.
- 8 Olsson K, Nyström P (2009) Non - interactive effects of habitat complexity and adult
9 crayfish on survival and growth of juvenile crayfish (*Pacifastacus leniusculus*).
10 *Freshwat Biol* 54:35-46.
- 11 Perry WL, Jacks AM, Fiorenza D et al. (2013) Effects of water velocity on the size and
12 shape of rusty crayfish, *Orconectes rusticus*. *Freshw Sci* 32:1398-1409
- 13 Prentis PJ, Wilson JR, Dormontt EE et al. (2008) Adaptive evolution in invasive
14 species. *Trends Plant Sci* 13:288-294.
- 15 Pyšek P, Richardson DM (2010) Invasive species, environmental change and
16 management, and health. *Annu Rev Environ Resour* 35:25-55.
- 17 Rahel FJ (2013) Intentional fragmentation as a management strategy in aquatic systems.
18 *Bioscience* 63:362-372.

- 1 Rahel FJ, Olden JD (2008) Assessing the effects of climate change on aquatic invasive
2 species. *Conserv Biol* 22:521-533.
- 3 Rallo A, García-Arberas L (2002) Differences in abiotic water conditions between
4 fluvial reaches and crayfish fauna in some northern rivers of the Iberian Peninsula.
5 *Aquat Living Resour* 15:119-128.
- 6 Rebrina F, Skejo J, Lucić A et al. (2015) Trait variability of the signal crayfish
7 (*Pacifastacus leniusculus*) in a recently invaded region reflects potential benefits and
8 trade-offs during dispersal. *Aquatic Invasions* 10.
- 9 Riis T, Lambertini C, Olesen B et al. (2010) Invasion strategies in clonal aquatic plants:
10 are phenotypic differences caused by phenotypic plasticity or local adaptation? *Ann*
11 *Bot* 106:813-822.
- 12 Rutledge P, Pritchard A (1981) Scope for activity in the crayfish *Pacifastacus*
13 *leniusculus*. *Am J Physiol Regul Integr Comp Physiol* 240:R87-R92.
- 14 Stewart - Koster B, Olden JD, Johnson PT (2015) Integrating landscape connectivity
15 and habitat suitability to guide offensive and defensive invasive species management.
16 *J Appl Ecol* 52:366-378.
- 17 Stoffels RJ, Rehwinkel RA, Price AE et al. (2016) Dynamics of fish dispersal during
18 river-floodplain connectivity and its implications for community assembly. *Aquat Sci*

- 1 78:355-365.
- 2 Terwei A, Zerbe S, Zeileis A et al. (2013) Which are the factors controlling tree seedling
3 establishment in North Italian floodplain forests invaded by non-native tree species?
4 For Ecol Manage 304:192-203.
- 5 Thomaz SM, Bini LM, Bozelli RL (2007) Floods increase similarity among aquatic
6 habitats in river-floodplain systems. *Hydrobiologia* 579:1-13.
- 7 Tockner K, Pennetzdorfer D, Reiner N et al. (1999) Hydrological connectivity, and the
8 exchange of organic matter and nutrients in a dynamic river–floodplain system
9 (Danube, Austria). *Freshwat Biol* 41:521-535.
- 10 Tockner K, Stanford JA (2002) Riverine flood plains: present state and future trends.
11 *Environ Conserv* 29:308-330.
- 12 Twardochleb LA, Olden JD, Larson ER (2013) A global meta-analysis of the ecological
13 impacts of nonnative crayfish. *Freshw Sci* 32:1367-1382.
- 14 Usio N, Azuma N, Larson ER et al. (2016) Phylogeographic insights into the invasion
15 history and secondary spread of the signal crayfish in Japan. *Ecol Evol* 6:5366-5382.
- 16 Usio N, Nakajima H, Kamiyama R et al. (2006) Predicting the distribution of invasive
17 crayfish (*Pacifastacus leniusculus*) in a Kusiro Moor marsh (Japan) using
18 classification and regression trees. *Ecol Res* 21:271-277.

- 1 Usio N, Nakata K, Kawai T et al. (2007) Distribution and control status of the invasive
2 signal crayfish (*Pacifastacus leniusculus*) in Japan. *Jap J Limnol* 68:471-482.
- 3 Venables WN, Ripley BD (2013) *Modern applied statistics with S-PLUS*. Springer
4 Science & Business Media.
- 5 Ward J (1998) Riverine landscapes: biodiversity patterns, disturbance regimes, and
6 aquatic conservation. *Biol Conserv* 83:269-278.
- 7 Ward J, Tockner K, Schiemer F (1999) Biodiversity of floodplain river ecosystems:
8 ecotones and connectivity. *Regul Rivers: Res Manage* 15:125-139.
- 9 Wentworth CK (1922) A scale of grade and class terms for clastic sediments. *J Geol*
10 30:377-392.
- 11 Woodward G, Perkins DM, Brown LE (2010) Climate change and freshwater
12 ecosystems: impacts across multiple levels of organization. *Philos Trans R Soc Lond*
13 *B Biol Sci* 365:2093-2106.
- 14 Wutz S, Geist J (2013) Sex-and size-specific migration patterns and habitat preferences
15 of invasive signal crayfish (*Pacifastacus leniusculus* Dana). *Limnologica* 43:59-66.
- 16

Table 1 Environmental characteristics of the study reaches in tributaries. Water quality (water temperature, pH, Ca²⁺, SO₄⁻, NO₃⁻) 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, Ca²⁺, SO₄⁻, NO₃⁻), 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/m²).

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/m²).

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

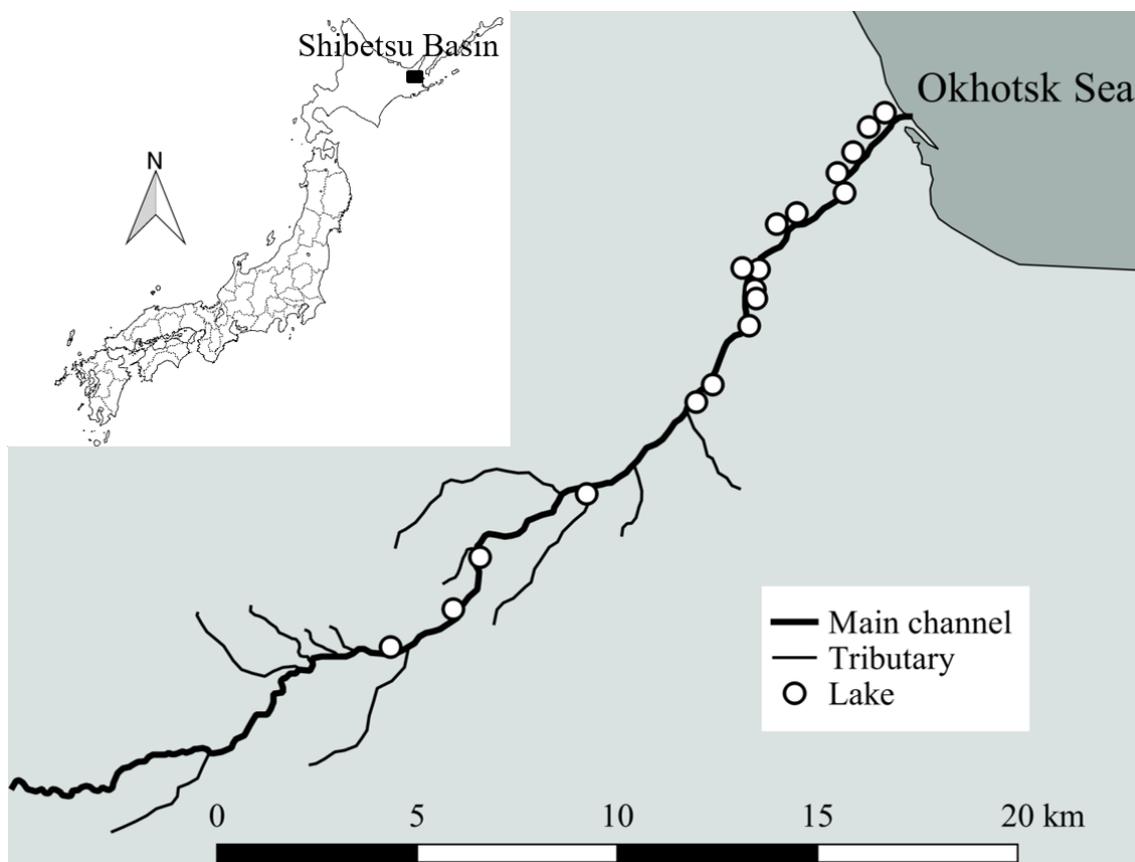


Fig. 2

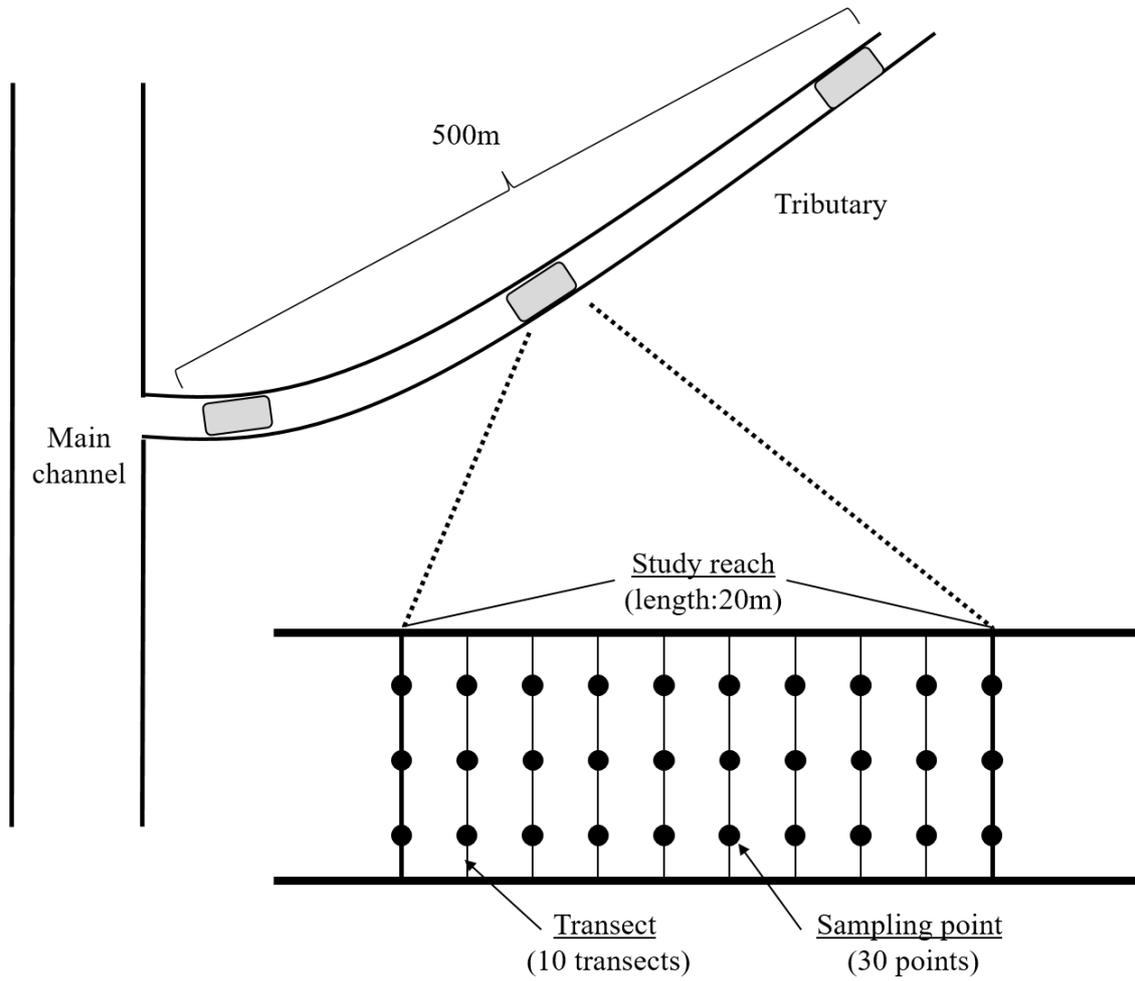


Fig. 3

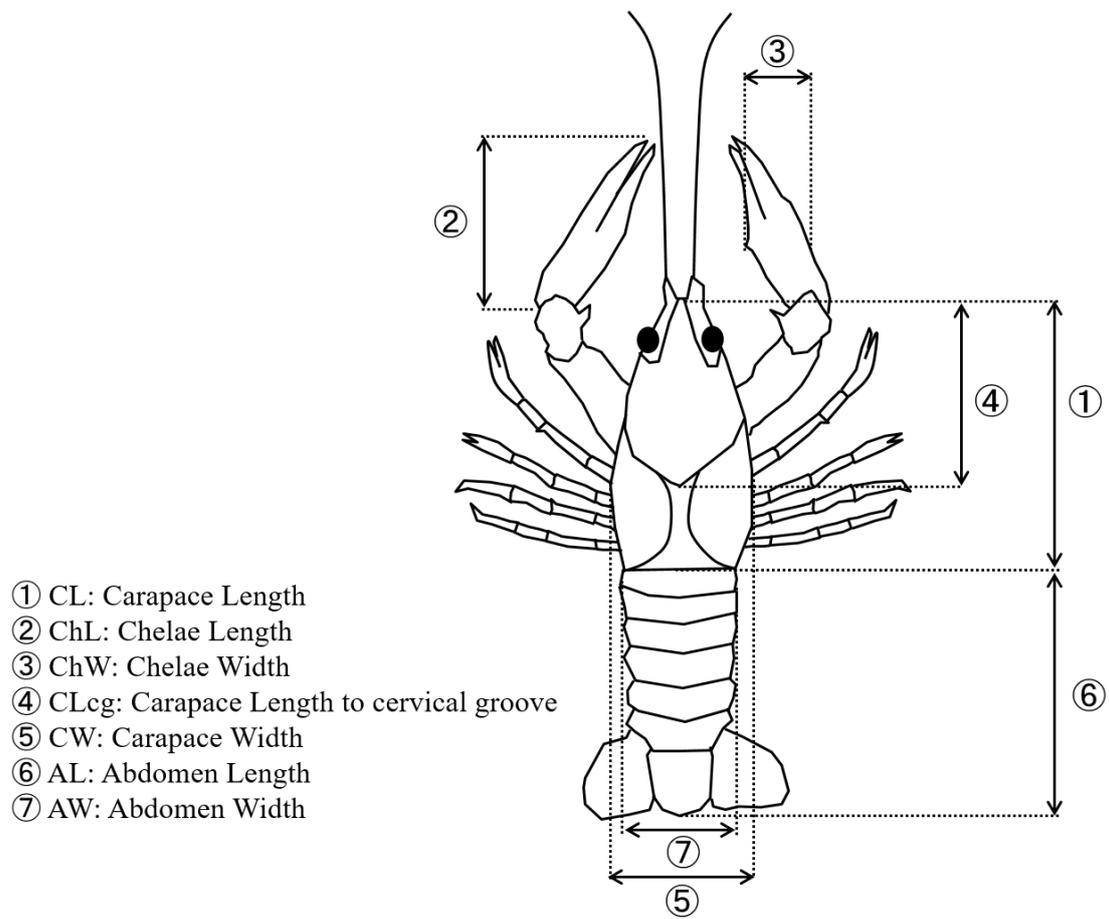
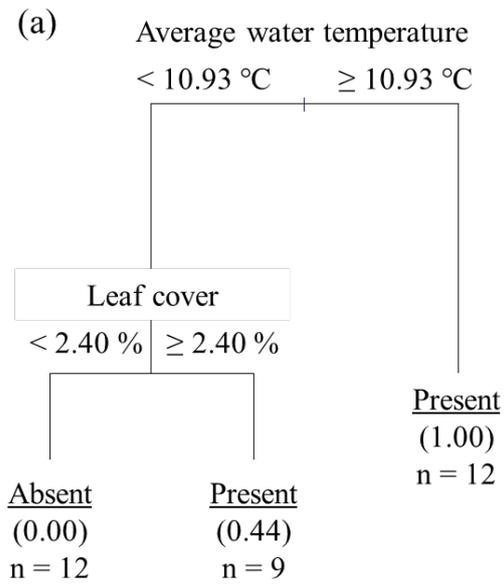
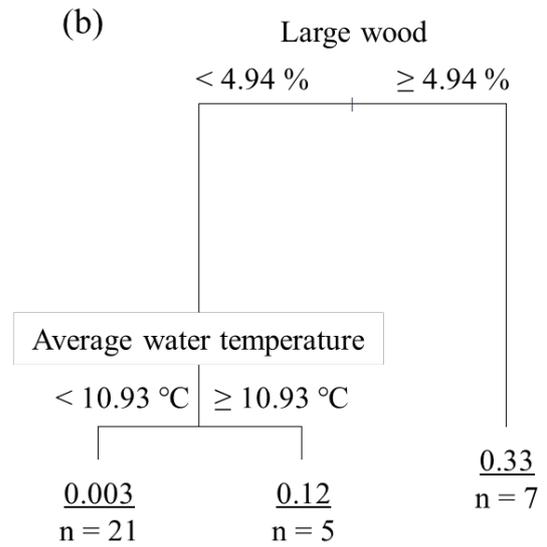


Fig. 4



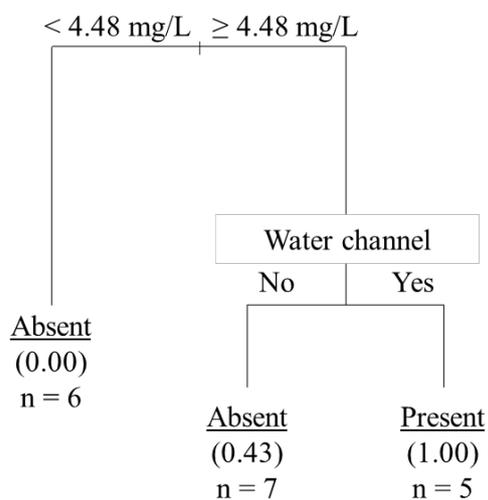
Correct classification rate = 88 %
k statistic = 0.76



Observed vs. Predicted $r = 0.99$

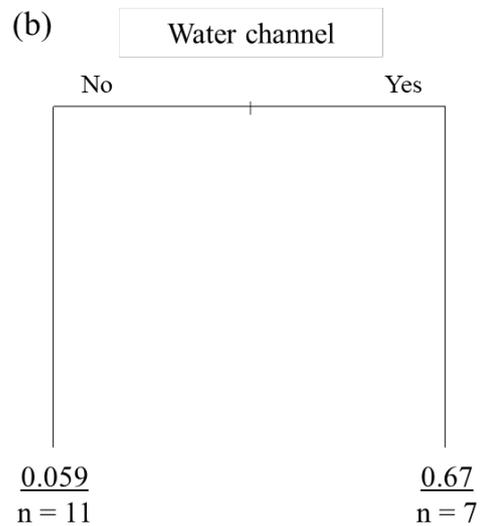
Fig. 5

(a) Dissolved oxygen



Correct classification rate = 83 %
 k statistic = 0.65

(b)



Observed vs. Predicted $r = 0.99$

Fig. 6

