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Imperiled freshwater mussels in drainage channels associated with rare agricultural landscape and diverse fish communities

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Abstract

Identification of landscape structures that predict the distribution of aquatic organisms has the potential to provide a practical management tool for species conservation in agricultural drainage channels. We tested the hypothesis that sites with imperiled freshwater mussels have distinct rural landscape structures, and are characterized by the presence of diverse fish communities. In central Japan, the proportion of developed land-use in surrounding areas was compared among sites with mussel populations (mussel sites) and randomly chosen sites (random sites) across multiple spatial scales (with a radius ranging from 100 to 3000 m). Mussel sites were characterized by a much lower proportion of developed land (means = 5-18%) compared with random sites (means = 32-35%) at a scale of ≤ 300 m. The areas that met the landscape criteria for mussel sites across multiple scales constituted only 0.23% of the area that presumed to have suitable slope and elevation as mussel habitat. Landscape metrics derived from mussel sites to locate unknown populations had a low predictability (16.7%). Sites with mussels were located close to each other and had fish communities with higher taxonomic diversity than in sites without mussels. In addition, mussel taxonomic richness was a good predictor of fish community diversity. The quantitative measures of landscape structure may serve as a useful tool when prioritizing or identifying areas for

conservation of mussels and fish if spatially auto-correlated distribution of habitat and other critical environmental factors such as habitat connectivity are also considered.

Keywords: development, indicator, paddy fields, Unionoida, urbanization

Introduction

In Japan, rice paddy fields, which constitute approximately 7% of national land as the most widespread cropland type (>50%; Sato 2001), have been a part of landscapes for centuries, providing habitats for unique sets of species (Natuhara 2012). Of particular importance are the environmental characteristics similar to those of natural floodplains (Elphick 2000) and the presence of a mosaic of different landscape components (i.e., paddy-fields, forests, ponds, and networks of small creeks and ditches) that are closely coupled through food webs (Katoh et al. 2009). Modernization of landscape elements (i.e., land consolidation that converts small land parcels into larger ones with the installation of pipeline systems for irrigation water management) and the urbanization of fields (i.e., land use conversion) are both pressing causes of reduced species diversity in rice-paddy landscapes (Takahashi 1994; Natuhara 2012).

There is a growing body of literature on the ecological roles of drainage channel networks in agricultural landscapes (Herzon and Helenius 2008). The relative importance of ditch communities to regional species diversity has been relatively well studied, with unique species being often identified (Armitage et al. 2003; Williams 2004). Degradation of ditch habitat quality has been related to runoff from agricultural crop fields with high levels of agro-chemicals (pesticides and fertilizers) (Janse et al.

1998; Biggs et al. 2007). Physical modifications of channel structures including concrete lining and the placement of migration barriers, such as vertical drops, have been also related to decreased species diversity in drainage channel systems (Katano et al. 2003; Nagayama et al. 2012; Natuhara 2012). Recent ecological studies with the aim of developing species conservation strategies have emphasized the importance of landscape structure in accounting for species distribution across multiple spatial scales (Gergel 2002; Marchand 2004). Such attempts have been made for organisms in riverine channels (e.g., Wang et al. 2001; Cao et al. 2013) but not in drainage channel networks. The latter tends to be smaller in size, located in more upstream areas, and regulated more strongly (i.e., flow rate) (e.g., Negishi and Kayaba 2009), and thus better understanding of ecosystem processes characteristic of drainage channels is needed.

Identification of areas that harbor high species richness at the local scale and/or make high contributions to species diversity at the regional scale would greatly facilitate prioritization of sites for conservation (Moilanen and Wilson 2009). Such a prioritization approach is critically important in paddy-dominated landscapes because drainage channel management for species conservation inevitably assumes secondary importance to that of increased agricultural production (Herzon and Helenius 2008). Among aquatic organisms, freshwater mussels (Order: Unionoida) can be used as an indicator for biodiversity in low-land aquatic ecosystems, especially other macroinvertebrates and fish (Aldridge et al. 2007; Negishi et al. 2013). Besides, freshwater mussels are one of the most imperiled groups of organisms (Lydeard et al. 2004; Negishi et al. 2008). Several empirical relationships between the community structure and distribution of freshwater mussels and landscape metrics, such as catchment geology, have been reported (Arbuckle and Downing 2002; Daniel and Brown 2013).

Landscape structure in the surrounding areas may affect mussel distribution in agricultural drainage channel. First, urbanization could affect mussel habitat quality because effluents from industrial and household sources impair water quality (Onikura et al. 2006). Local hydraulic conditions, which are a strong factor determining mussel distribution (Morales et al. 2006; Allen and Vaughn 2010), may be altered as a result of flashier runoff hydrograph from impervious urban surfaces (Xu and Wu 2006). Second, the encroachment of non-agricultural human land-uses in surrounding areas often coincide with improvements in agricultural infrastructure in the area, such as the modernization of ditch structures and irrigation systems (J.N. Negishi, personal observation). We hypothesized that sites with remaining populations of freshwater mussels have distinct rural landscape structures. This study aimed to develop a practical scheme for the identification of locations of mussel habitat, where diverse fish communities also occur based on landscape metrics over a large area (> 10,000 km^2). Specific objectives were to: 1) identify the landscape structures (i.e., the areal proportion of non-agricultural areas in surrounding areas) characteristic of mussel habitats at various spatial scales; 2) to test the efficacy of the identified landscape structures in predicting potential mussel habitats; and 3) to test the use of mussel community structure as an indicator of fish community structure. We predicted that mussel habitats in drainage channels are associated with rural landscapes having low levels of non-agricultural human land-uses, and are characterized by the presence of fish communities with relatively high species richness.

Methods

<u>Study site</u>

The study was conducted in the period between April and August, 2011, in the lowlands of Tokai region, including Aichi, Gifu, and Mie prefectures (Fig. 1). We collected as much information about the locations of existing unionoid mussel populations in agricultural channel networks *a priori* as we could find in the published literature, unpublished records, and knowledge from local people and researchers (see

Acknowledgements). Between 23rd and 26th April, each location was visited to confirm the presence of mussels. Our criteria for study site selection were: 1) sites were located in agricultural channels for single (drainage) or dual (irrigation and drainage) purpose(s); 2) sites had > 10 individual live mussels with an approximately 2 person hours search; 3) sites were located a minimum of 800-m channel length from each other; and 4) sites were not connected to each other within the same drainage channel networks before connecting to main river channels in downstream areas. The sites located immediately downstream of agricultural ponds (reservoirs for irrigation water supply) were excluded because there was the possibility that local populations were largely maintained by emigrants from the source populations within the ponds. Consequently, we identified 26 sites (hereafter mussel sites; Fig. 1 and 2). These sites had one of the following unionoid taxa: *Anodonta* spp., *Unio douglasiae nipponensis* Martens, *Inversidens brandti* Kobelt, *Obovalis omiensis* Heimburg, *Pronodularia japanensis* Lea, or *Lanceolaria grayana* Lea. It was later found that one of the mussel sites located downstream of the other in the same drainage channel, and excluded when conducting fish and mussel surveys. Landscape analyses were redone by excluding this site, but it did not change the interpretation of statistical results and negligibly affected the criteria used to quantify landscape structures.

Delineation of habitable area

We conducted our study in three regions that contained mussel sites, each of which comprised several neighboring watersheds (Fig. 1). This approach was employed to reduce the possibility that our analyses contained the area in which distribution of mussels is biogeographically limited by the legacy of marine transgressions. The entire area of the three regions is hereafter referred to as the total area (Fig. 2). We recorded the geographical locations of the mussel sites using GPS (CS60, Garmin Co., USA) and registered on GIS (Arcmap, ver. 9.3, ESRI Co., USA). Using the surface elevation distribution (50-m resolution, Geospatial Information Authority of Japan), a digital elevation model (DEM) was generated. A ground surface slope distribution model (50-m resolution; DSM) was also created based on DEM. We calculated the average elevation and slope of each site by calculating average DEM and DSM values within the 100-m radius buffer polygon surrounding each site. We carefully examined the data and excluded the slope values where the 100-m buffer polygon enclosed unusual topographic variations such as adjacent hill slopes because the DSM was particularly sensitive to such errors (this occurred at five sites). Channel slope can limit mussel distribution by potentially mediating hydraulic forces on the benthic habitat conditions

(Arbuckle and Downing 2002). Temperature can also limit mussel distribution because of the physiological thermal tolerance of mussels and/or that of their host fish species whose distribution can determine the distribution of mussels (Galbraith et al. 2010; Schwalb et al. 2011; Negishi et al. 2013; Schwalb et al. 2013). Thus, we have delineated the area that fell within the range of slopes (0.001–3.45%) and elevations (13.0–86.71 m) as a proxy of temperature variation of the mussel sites as potentially habitable for mussels (hereafter habitable area) within the total area (Fig.1 and 2).

Landscape structures of mussel sites

To examine how rare the landscape structures of the mussel sites were in the study area, we randomly chose sites (random sites) within the habitable area (total of 26 sites) (Fig. 2). Random sites were used to represent the average landscape structure present within the habitable area and we did not confirm the occurrence of mussels in random sites. First, continuous square grid cells of 500 × 500 m were generated to cover the habitable area so that all cells were completely contained within (grid cells). A total of 26 grid cells (random cells) was selected so that the number of selections in each region equaled the numbers of mussel sites within each region. Central points of each random cell were considered as random sites (Fig.1). The use of 500 × 500 m grid cells was to assure that randomly selected sites were apart from each other and from mussel sites at least by 800-m channel length (i.e., minimum distances among mussel sites).

We defined the developed areas whose surface was covered by landscape components that are not agricultural fields or natural features such as forests and water bodies (i.e., rivers and lakes). We used nation-wide land use census data (land use database in 1997, Ministry of Land, Infrastructure, Transport and Tourism, 50-m grid resolution), and assigned golf courses, residential areas, transportation fields (e.g., roads and airports), barren land, and commercial districts as the developed landscape components. We created 50-m raster data, with each grid cell being assigned 1 (developed) or 0 (other land-uses). We calculated the proportion of developed areas for mussels as well as random sites at multiple spatial scales (circles with a buffer radius of 100, 200, 300, 500, 1,000, or 3,000 m) using the GIS buffering and zonal and focal statistics functions (Fig. 1).

<u>Locating potential sites containing unknown mussel populations</u>

Based on the landscape structures of known mussel sites defined by land-use patterns at multiple spatial scales, we attempted to identify the locations of unknown sites with mussel populations (i.e., potential sites). We delineated and calculated the area that met the landscape structure criteria for the mussel sites at respective spatial scales; the criteria were set at 95% confidence intervals of the means of the proportion of the developed area for mussel sites. We determined the area that met the criteria of each spatial scale, as well as all spatial scales (i.e., potential areas) from the habitable area using the GIS focal statistics function. For example, the potential area constrained by criteria at all spatial scales refer to the area, any given locations within which had the proportion of developed land in surrounding areas within the criteria for mussel sites at all spatial scales examined (Fig. 2).

We excluded habitable areas in Mie prefecture (see Fig. 1) from the further analyses because distant locations rendered frequent visiting for observations and sampling impractical. We initially attempted to randomly choose candidates for potential sites within the potential area as was done for the selection of random sites using the 500 m grid cells; the selected cells were referred to as candidate cells. We chose 18 candidate cells, the number same as that of initially designated mussel sites. However, preliminary analyses revealed that the number of cells meeting such criteria was too small (< 10 cells) to conduct a random selection (see results for more detail). As an alternative, we selected cells based on the criteria set based on the maximum value of the proportion of developed area at a spatial scale of 500 m (53.8%), which provided >

100 cells. When randomly chosen 18 candidate cells were placed on areas dominated by large rivers (> 90%), or the areas without noticeable agricultural drainage channel networks, candidate cells were re-chosen by a random selection procedure (this occurred once).

Mussel and fish surveys

Fish and mussel communities were surveyed in the summer period between 8th and 13th August when flow is stable and no noticeable precipitation was recorded in the preceding 2 weeks. In the water management cycles for rice cultivation in the area, this period typically provides the largest amount of water within the channel (Negishi et al. 2013). The exact locations of the potential sites within candidate cells were determined as follows: The geographical coordinates for the central points of chosen grid cells were determined by GIS and located in the field by GPS. The sampling sites were chosen by the following criteria: Upon arrival at the location, the closest drainage channel was located. The site suitability was confirmed when it was for drainage or irrigation/drainage purpose(s), had decent water quality and perennial flow, and was of a comparable size to that of the mussel sites. The presence of perennial flow with decent water quality was confirmed by the occurrence of freshwater Mollusca such as

Pleuroceridae sp. and *Viviparidae* sp. Only those whose wetted channel width was within the range of that in the mussel sites were selected. When the closest one did not meet these criteria, the next closest ones were progressively examined until the criteria were met.

At each study site, two sections having a longitudinal length 10 times the average wetted surface width (range = 45–320 cm) were set for mussel surveys with a distance of 20 times the width between each section. In each section, electrical conductivity (EC), pH, and water temperature (°C) were recorded using a multi-parameter water quality probe (WM-22EP; DKK-TOA Co., Japan). Mussels were quantified by collecting all mussels within the belt transects (a dimension of 25 × the wetted channel width) equally spaced along a longitudinal profile. The total number of transects was proportional to the section length (n = 4–32) and total areas searched ranged from 0.9 to 36.8 m^2. Mussels were collected by thoroughly sieving the sediment using baskets (1-cm mesh), identified, enumerated, and measured along their longest shell axes. Fish communities were surveyed by enclosing a section (20 times the length of the wetted width) in the area upstream of the mussel survey sections. To avoid disturbing the fish communities, the survey was conducted prior to the mussel survey. Fish were caught by conducting two passes of catches using triangular scoop nets

(34-cm wide and 33-cm high mouth opening; 44-cm long; 2-mm mesh) aligned perpendicularly to the channel. Furthermore, specific areas such as those with a fast current and along the channel edge were thoroughly searched for fishes using scoop nets (up to 5 min by two or three personnel depending on the channel size). Fish were identified, enumerated, and photographed to digitally estimate their body sizes. Collected organisms were released back to the capture area immediately after the measurements had been taken.

Statistical analyses

Repeated measures two-way analysis of variance (ANOVA) was conducted to examine the effects of spatial scale and site characteristics (mussel sites vs. random sites, or mussel sites vs. potential sites), and their interactions on the proportion of developed areas in surrounding areas. Site identity was included as a repeated measures factor. When significant interaction terms were detected, the proportion of developed lands was compared between site types at each scale using Student's t-tests. A correlogram of Moran's *I* was constructed to assess the degree of spatial autocorrelation in the presence/absence data obtained from field surveys on mussels; presence data from present sites (initially designated mussel sites in addition to some potential sites with mussel populations) whereas absence data from absent sites (remaining potential sites without mussels). Environmental variables (GIS-calculated elevation and slope, water temperature, pH, EC, and flow rate) were compared between present and absent sites using Welch's t-tests. Fish community metrics were calculated as abundance, taxonomic richness, and Shannon-Wiener index. These fish metrics, calculated for both communities, included all taxa and those excluding bitterlings; bitterlings require live mussels for spawning (Negishi et al. 2013). Mussel community metrics were calculated with the pooled data from two sections in terms of abundance and mussel taxonomic richness. Fish and mussel abundance were expressed as density (the number of individuals either per 1 m^2 or 100 m^2). Mussel indicator roles were examined in two ways. First, fish community metrics were compared between present and absent sites using Welch's t-tests. Second, relationships between mussel and fish community metrics were examined by regression analysis (12 cases in total) with the former as independent and the latter as dependent variables. All statistical analyses were conducted in R 2.10.1 (R Development Core Team, 2008) with a significance level of 0.05. The Bonferroni correction was applied to the statistical significance level when appropriate.

Results

The change pattern of the proportions of developed lands in the surrounding areas in response to variable spatial scales differed between the sites with known mussel populations and those of randomly chosen sites (interaction effect: $F_{5, 245}$= 6.8, p < 0.001). The differences in landscape structures between two types of sites were particularly high at relatively small spatial scales (Fig. 3A). The proportions of developed lands were significantly lower for the sites with known mussel populations compared with those of randomly chosen sites (site effect: $F_{1, 49}$ = 12.22, p < 0.001; Fig. 3A). The proportions of developed lands were significantly lower at sites with mussels at the spatial scales of 100, 200, and 300 m when compared at respective spatial scales (Fig. 3A).

The areal extent of lands (potential areas) that fell within the landscape conditions of mussel sites at different spatial scales became disproportionately less with decreasing spatial scale (Table 1). Approximately 7% of the total area was selected as within the slope and elevation range of sites with existing mussel populations (habitable area). The areal extent of the potential area that met the landscape-level criteria of mussel sites across all spatial scales only constituted 0.23% of the habitable area. The potential sites, which were selected based on the criteria of maximal areal proportion of developed land at a scale of 500 m (53.8%), had landscape structures similar to those of mussel sites (Fig. 3B). The change pattern of the proportions of developed lands in the surrounding areas in response to variable spatial scales did not differ between two types of sites (interaction effect: $F_{5, 165} = 0.17$, $p = 0.97$). The potential sites had a proportion of developed lands similar to that of mussel sites in surrounding area (site effect: $F_{1, 33} = 1.16$, $p = 0.29$; Fig. 3B) with a significant effect of spatial scale ($F_{5, 165} = 18.84$, $p < 0.001$).

In total, 2, 128 individuals were found: 1, 764 *P. japanensis*, 118 *L. grayana*, 56 *O. omiensis*, 78 *Inversidens brandti*, 107 *Anodonta* spp., and 5 *U. douglasiae nipponensis*. We found mussels in three out of 18 potential sites (at least one live individual in the quadrat survey) (Fig. 4A). A clear spatial structure was revealed in the distribution of present and absent sites with Moran’s correlogram indicating statistically significant positive autocorrelation for small-distance categories (Fig. 4B). It is important to note that three potential sites with mussels were close to each other or close to the sites with known mussel populations, augmenting the patchy distribution. Sites with mussels (including the three potential sites with mussels) and those without mussels were similar to each other in the measured environmental variables (Table 2). Mean (±SD) mussel density (individuals/m^2), irrespective of taxon, at sites with mussel populations was 29.6 (±57.3) individuals/m^2. In total, 2,390 fish, consisting of 24 taxa, were collected (Appendix); their body length was <15 cm. When compared between sites with and without mussels, fish species richness with all taxa included and taxa excluding bitterling species were both higher at the sites with mussels compared with those without (Fig. 5; $p < 0.001$). The Shannon-Wiener index did not differ among site type ($p > 0.20$). Mussel taxonomic richness was positively associated with fish species richness (both with and without bitterlings) and the Shannon-Wiener index calculated from all taxa included (Fig. 6). Mussel abundance did not have any predictive effect on fish community metrics ($p > 0.12$).

Discussion

We demonstrated that the landscape structure surrounding agricultural drainage channels with imperiled unionoid mussels was characterized by rural landscapes having a significantly lower level of land development compared with common landscapes in the area with similar elevation and slope ranges. This difference was more pronounced when landscape structure was examined at relatively small spatial scales. These results agree with our prediction that mussel habitats possess relatively rare rural landscape features in the region. The interpretation of our findings requires caution because we did not compare landscape structures between the sites with and without mussels. Also, we did not constrain our analyses to the areas where only the landscape structures varied, with other important factors (see the next paragraph) being more or less controlled when selecting random sites.

The absence of GIS-ready digital data resources of drainage channel networks across a large area prevented us from taking into the account spatial attributes of drainage channel networks when delineating areas potentially suitable as mussel habitat. For example, drainage density (i.e., total length of channels in a given unit area; see Benda et al. 2004), which is analogous to habitat availability, could have been particularly meaningful. Thus, it is possible that some of the random sites were selected from areas where highly urban landscapes dominated and as a result few drainage channels existed (relatively low habitat availability). Consequently, the differences in landscape structure between the mussel and random sites can be partly a reflection of the differences in land-use patterns (rural vs. urban) and not necessarily those around drainage channels. Despite such limitations, the results implied that a quantitative measure of rare landscape structure is useful when narrowing down the areas where mussel habitat likely to occur.

We showed that areas with landscape structures characteristic of mussel sites across multiple spatial scales constituted only a fraction of habitable area in the region

(0.23%) and was much less when compared with the areas estimated at respective spatial scales (Table 2). This suggests that mussel sites can be more accurately defined by considering landscape metrics quantified at multiple spatial scales. Our findings underscore the importance of considering landscape structures at multiple spatial scales when explaining organism distribution patterns (also see Marchand 2004; Stephens et al. 2004). Furthermore, landscape structure characteristic of mussel sites were more pronounced and became rarer at relatively small spatial scales. Cao et al. (2013) examined the relationships between landscape metrics and riverine mussel habitat conditions and also reported the disproportionately strong influences of landscape conditions in a close proximity (i.e., riparian zone). In the study region, the increase of developed land surface within the area in a relatively short distance from mussel sites likely causes a disproportionately large drop in potential habitat availability and may degrade mussel habitat quality more severely.

Unknown mussel populations were found at only a few potential sites (16.7%), indicating limited usefulness of landscape structure as a single predictor of mussel habitat distribution. A patchy distribution of sites with mussels was apparent, and an understanding and incorporation of its cause can improve the predictive model of mussel distribution. Patchy distribution of mussels (mussel beds) is relatively well reported at within-channel scales as well as at broader regional to continental scales (Vaughn and Taylor, 2000; Strayer 2008). At larger scales, in particular, host fish species distribution may be the one of the most important factors (Vaughn and Taylor 2000; Schwalb et al. 2011). A patchy distribution can be caused by natural barriers (e.g., drainage divide) for the dispersal of mussels (or host fish species), or as a result of human-induced fragmentation and shrinkages of formerly broad distribution range (Strayer 2008). Fish migrate up from rivers to drainage channels or rice paddies via drainage channels seasonally (e.g., Katano et al. 2003). The placements of vertical drops impassable for fish is common in modernized drainage channel networks (Miyamoto 2007). In agricultural channels, therefore, the reduction or loss of connectivity among channels and/or between channels and downstream rivers might have fragmented mussel habitat by limiting movement of host fish with attached glochidia. Other potentially important factors on a local scale, such as flow velocity, hydraulic forces, and physical channel structures, may also play a role in controlling the distribution of mussels (Allen and Vaughn 2010; Matsuzaki et al. 2011; Nagayama et al. 2012; Negishi et al. 2013). These factors independently or in a combination with other factors can affect habitat conditions of mussels directly or indirectly by limiting distribution of host fish species. Future studies should examine the relative importance of multiple factors such as habitat fragmentation and local habitat conditions in relation to natural dispersal ranges of host fish species (Schwalb et al. 2011; Cao et al. 2013; Schwalb et al. 2013).

Fish species richness was higher at sites with mussels compared to sites without mussels despite of similar physico-chemical habitat conditions. This is consistent with the findings that the occurrence of mussels is associated with the presence of relatively species-rich fish communities (e.g., Vaughn and Taylor, 2000; Negishi et al. 2013). Mussel species such as *I. brandti*, *O. omiensis*, and *P. japanensis* co-occur with bitterlings through host-parasite relationships (Kitamura 2007; Terui et al. 2011). Importantly, when the bitterlings were removed, significant differences in fish community species richness remained. This implies that the greater fish species richness observed in the mussel sites was not the sole consequence of the addition of species having a commensal relationship with mussels, but also several other ecological processes (Negishi et al. 2013). Furthermore, the mussel taxonomic diversity gradient predicted fish community taxonomic richness with mussels and the Shannon-Wiener index relatively well. We previously tested fish and mussel communities in a <100 m^2 area including some of the mussel sites used in the present study and reported the relationships between fish and mussel community structure in different seasons (Negishi et al. 2013). A discrepancy with the present study is that Negishi et al. (2013) reported less explanatory power of mussels for fish community structure in the summer period. A cause of such a discrepancy may be scale-dependent relationships between community structure of fish and mussels (e.g., Schwalb et al. 2013). The mechanisms between species richness and fish community structure also merit future research. We argue that unionid mussel can be used as an indicator of fish habitat quality over a large scale (> 10,000 km^2) at least within the area having elevation and slope ranges of mussel sites.

Our overall findings suggest that the quantitative measures of landscape structure may serve as a useful tool when prioritizing or identifying areas for conservation of mussels and fish if spatially auto-correlated distribution of habitat and other critical environmental factors such as local habitat quality and habitat connectivity are also considered. It is important to recognize the landscape structure in rural landscape has changed in recent decades largely because of abandonment of agricultural lands and land development associated with urbanizations (Nakamura and Short 2001; Fujihara et al. 2005). Therefore, the landscape criteria quantified in the present study should not be taken as an ideal habitat condition for mussels. Information on historical distribution of mussels and land-use changes across a large area would provide crucial insights into optimal habitat condition for mussels.

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Table 1: Areal extent of the potential area that met the criteria of mussel site landscape structures at different spatial scales and their relative availability (%) relative to total and habitable areas. For example, 7.41% of total area is considered suitable for mussel habitat based on the range of elevation and slope of sites with mussels; 9.64% of the habitable area was considered suitable for mussel habitat when considering landscape structure obtained for the surrounding area of mussel sites at the scale of 100 m (the area within a circular buffer having a radius of 100 m).

	Area (km^2)	Availability (%)
Total area	6,399.32	
Habitable area	473.89	7.41
3000-m buffer criteria	131.26	27.70*
1000-m buffer criteria	113.55	23.96*
500-m buffer criteria	97.68	20.61*
300-m buffer criteria	64.63	13.64*
200-m buffer criteria	58.24	12.29*
100-m buffer criteria	45.68	9.64*
Multiple-scale criteria	1.07	0.23*

* These values were calculated as a proportion relative to the habitable area

Table 2 General characteristics of study reaches with (present) and without (absent) freshwater mussels. Means ± SD are shown. Statistical significance as the result of Weltch's t-tests are also shown; significance level is Bonferroni-corrected ($p = 0.05/6$).

	Present (n=20†)	Absent (n=15)	Statistical significance
Elevation (m)	43.61±18.16	32.88±16.72	$p = 0.08$
Slope (%)	0.69±0.57	0.57±0.43	$p = 0.67$
Temp. (˚C)	27.45±2.10	26.42±1.35	$p = 0.08$
pH	7.12±0.69	7.80±0.62	$p = 0.01$
EC (μS/cm)	9.81±7.15	9.41±3.52	$p = 0.83$
Flow rate (m^3)	0.19±0.31	0.15±0.11	$p = 0.63$

† Sample size was 15 for slope as the 5 sites were excluded because of unusual land surface slope was estimated due to the presence of steep hillslope near the channel.

Appendix: Taxon list and general characteristics of fish communities in the study reaches. Abundances for the sites with and without mussels are shown; mean ± SD (maximum values; minimum values were all zero).

Family	Species name	Present (n = 20)	Absent (n = 15)
Cyprinidae			
	Rhodeus ocellatus ocellatus	17.12±45.86 (194.44)	0
	Tanakia lanceolata	35.81±94.88 (395.83)	0
	Tanakia limbata	190.86±404.25 (1693.12)	0
	Carassius sp.	6.43±18.00 (74.07)	2.22±8.31 (33.33)
	Nipponocypris sieboldii	85.78±173.04 (732.64)	40.30±150.77 (604.44)
	Nipponocypris temminckii	46.37±101.26 (324.79)	2.47±9.24 (37.04)
	Zacco platypus	6.82±23.67 (105.82)	10.15±21.54 (71.11)
	Abbottina rivularis	9.29±39.31 (180.56)	0
	Gnathopogon elongatus	65.12±208.52 (958.33)	3.54±9.16 (30.86)
	Hemibarbus barbus	0	1.19±4.43 (17.78)
	Pseudogobio esocinus	4.83±11.91 (48.61)	0
	Sarcocheilichthys variegatus variegatus	0.17±0.76 (3.47)	0
	Rhynchocypris logowskii steindachneri	1.10±4.07 (18.52)	7.11±21.78 (86.42)
	Rhynchocypris oxycephalus jouyi	0.17±0.76 (3.47)	0
	Pseudorasbora parva	4.08±16.63 (76.39)	0
Adrianichthyidae			
	Oryzias latipes	59.48±183.58 (833.33)	225.91±613.96 (2455.56)
Cobitidae			
	Cobitis biwae	1.66±4.48 (15.87)	29.71±107.92 (433.33)
	Cobitis sp.	1.19±5.19 (23.81)	0
	Misgurnus anguillicaudatus	63.48±103.56 (370.37)	109.06±199.33 (740.74)
	Paramisgurnus dabryanus	0	3.29±12.32 (49.38)
Gobiidae			
	Rhinogobius sp.	42.67±36.65 (128.47)	75.64±134.90 (444.44)
Odontobutidae			
	Odontobutis obscura	0.69±3.03 (13.89)	0
Petromyzontidae			

	Lethenteron reissneri	0	0.82±3.08 (12.35)
Siluridae			
	Silurus asotus	0	0.46±1.73 (6.94)

Figure captions

Figure 1: Location of the study area (A), study sites where unionoid mussel populations were present (mussel sites; n = 26) (B), an example of measurements of landscape structure in relation to multiple buffers surrounding the study sites (C), an example of the area potentially suitable as mussel habitat (habitable area; see the text for details) shown in white within region A (D), and an example of sites with mussels (mussel sites; filled circles) and sites randomly selected (random sites; open circles) (E). The regions A, B, and C in (B) denote watersheds within which random site selections were conducted. The sites enclosed with a broken line (n = 18) in (B) were used when testing mussel/fish relationships. The gray areas in (C) and scratched area in (E) denote the land use categorized as non-agricultural developed use such as urban areas.

Figure 2: A diagram depicting the procedure of delineating total area, habitable area, and potential area in association with sites with mussels (mussel sites), random sites, and potential sites.

Figure 3: Means ± SE of proportion of developed non-agricultural areas surrounding the study sites with mussels (mussel sites; filled circles) and randomly chosen sites (random sites; open triangles) at different spatial scales of buffer radii used to calculate landscape metrics. In (A), random sites ($n = 26$) were selected within the boundary of several watersheds and the range of elevation and ground surface slopes for the mussel sites (Fig. 1). In (B), the selection of potential sites ($n = 18$) was further constrained by the range of landscape characteristics at the spatial scale of 500 m. Asterisks indicate statistically significant pair-wise differences at respective spatial scales using Student's t-tests with Bonferroni corrections ($p = 0.05/6$; $p = 0.005$).

Figure 4: Spatial distribution of sites with mussels (filled circles) and without mussels (open circles) (A) and spatial correlogram of the occurrence (presence or absence) of mussels, where the abscissa is distance classes and the ordinate Moran's *I* coefficient (B). In (A), three sites accompanied by arrow denote sites that were initially chosen as potential sites and had mussel populations whereas the gray areas denote the habitable area. **Significant with Bonferroni-corrected probability ($p = 0.05/10$; $p = 0.005$). *Significant at the probability level $p = 0.05$ (i.e., before applying the Bonferroni correction).

Figure 5: Box plots showing median (central thick lines), 25%, and 75% quartile ranges around the median (box width) of taxonomic richness of fish community of all data (A) and data excluding bitterling species (B) for sites with (present) and without (absent) mussels. The sample size for each group was 21 and 15 for present and absent sites, respectively. Only those with statistically significant differences are shown; the statistical significance of Welch's t-test was corrected with Bonferroni corrections ($p = 0.05/6$; $p = 0.008$).

Figure 6: Relationships between mussel taxonomic richness and fish community metrics; Shannon-Wiener index of fish community (A), taxonomic richness of fish community (B), and taxonomic richness of fish community of data excluding bitterling species (C). Statistically significant regressions are shown as solid lines with coefficients of determination (R^2). Only those with statistically significant differences regressions are shown; statistical significance levels were corrected with Bonferroni corrections ($p = 0.05/6$; $p = 0.008$).

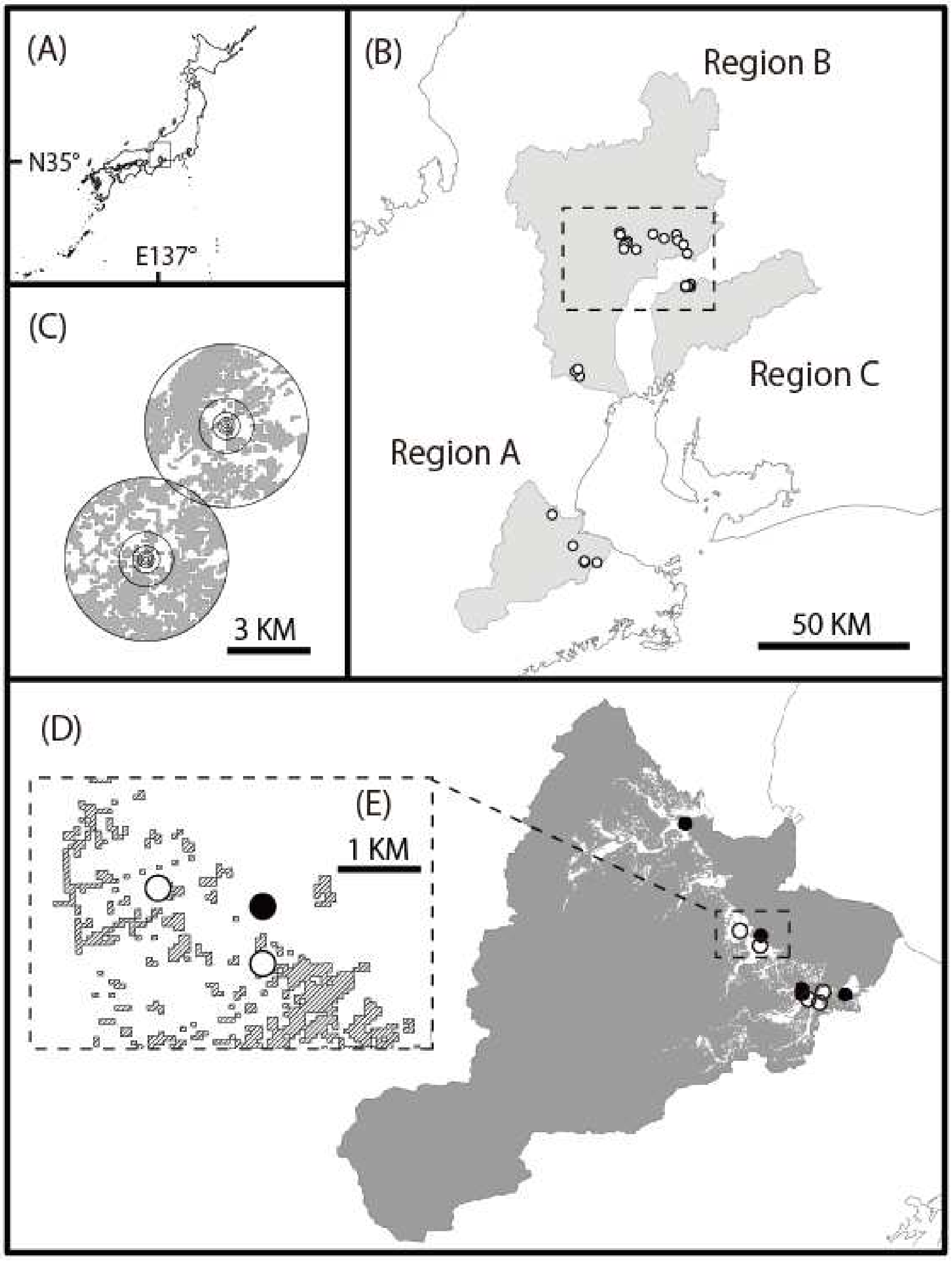


Fig. 1 Negishi et al.

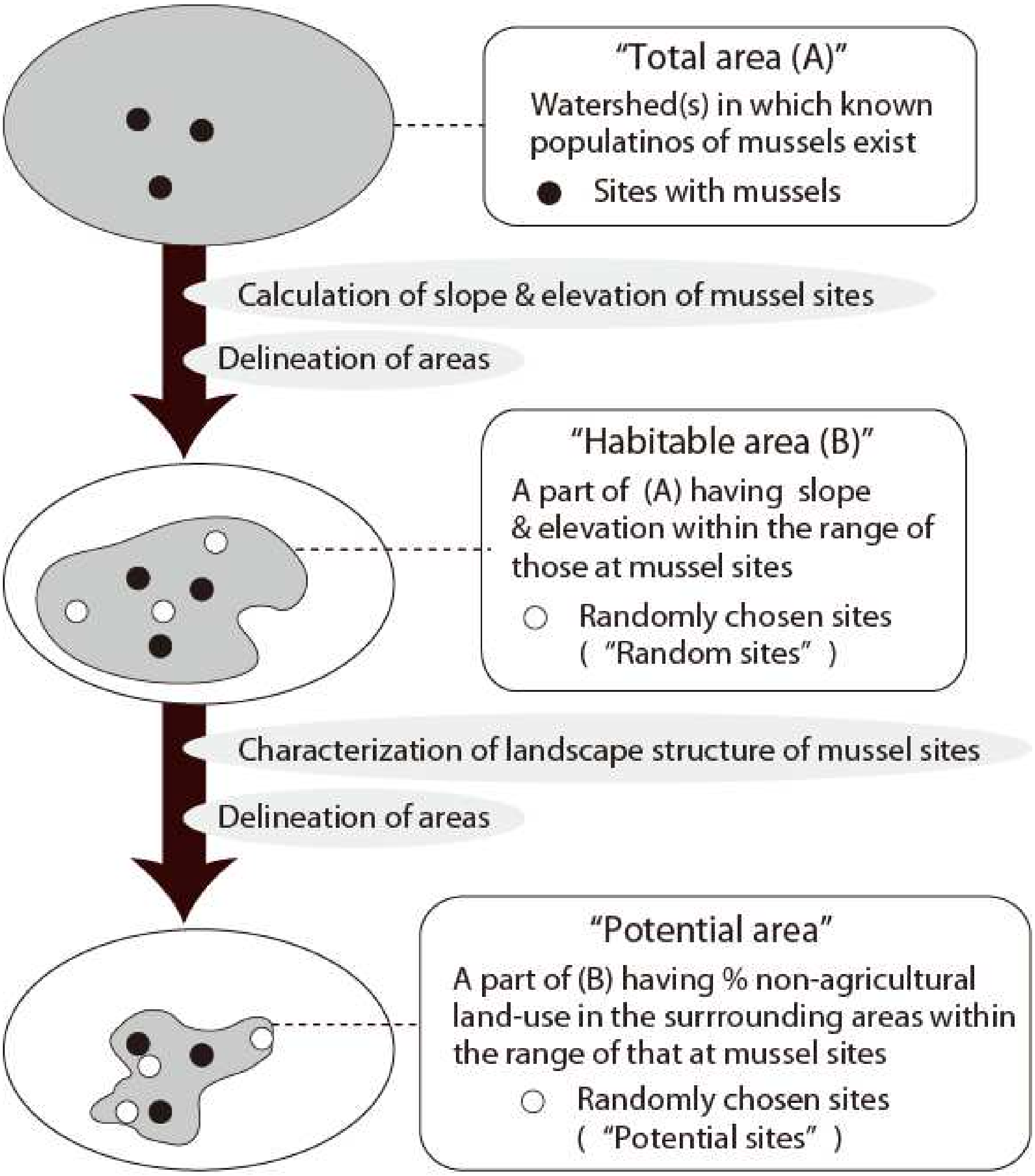


Fig. 2 Negishi et al.

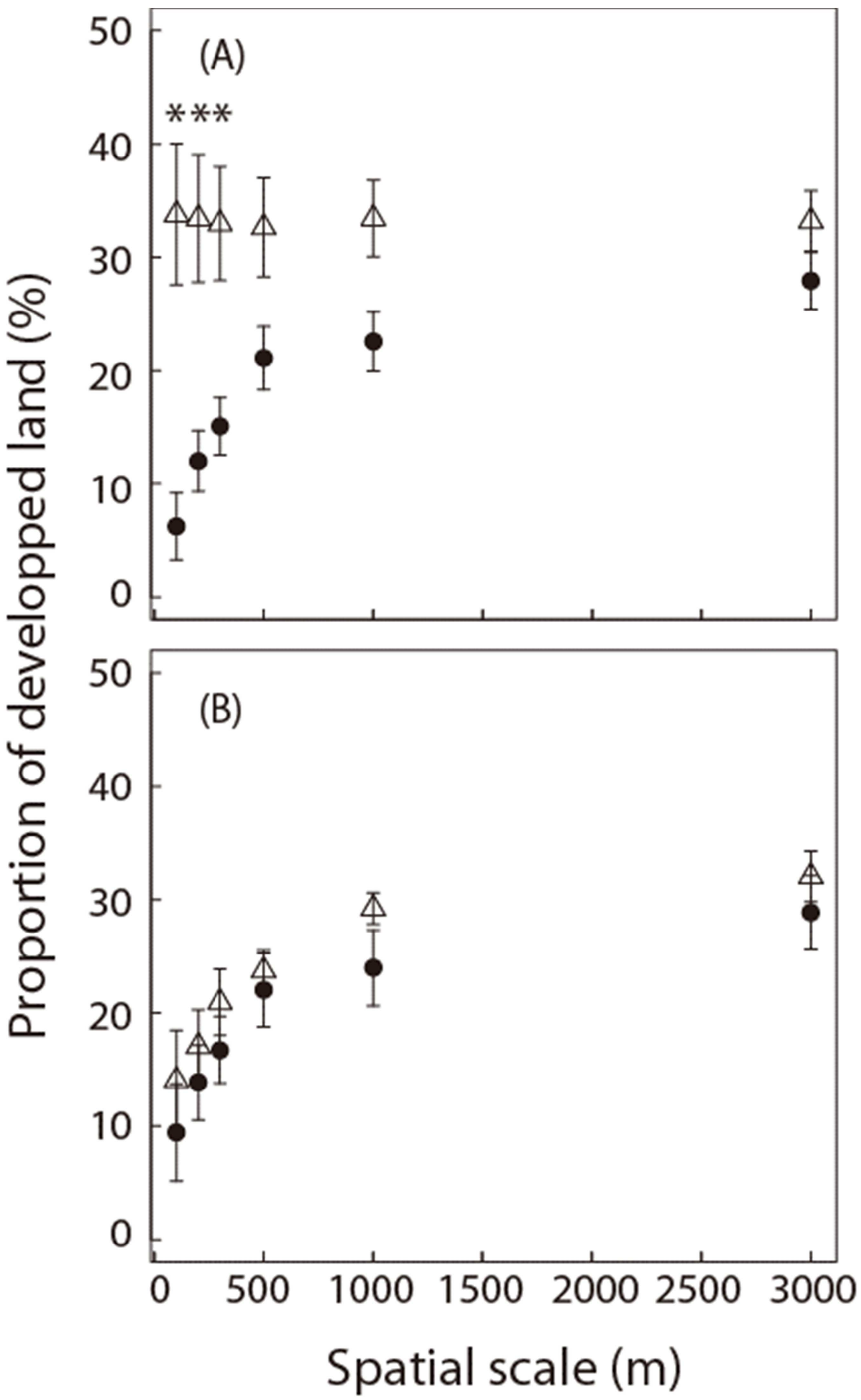
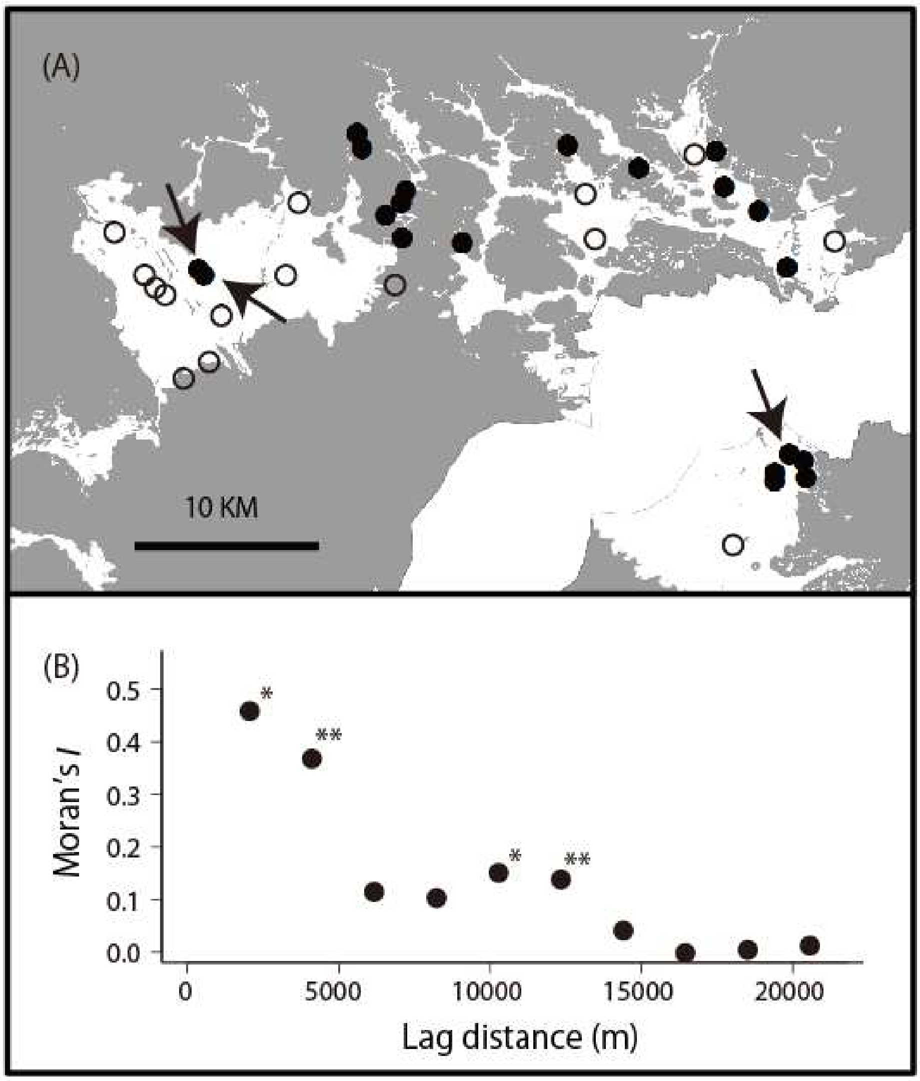


Fig. 3 Negishi et al.



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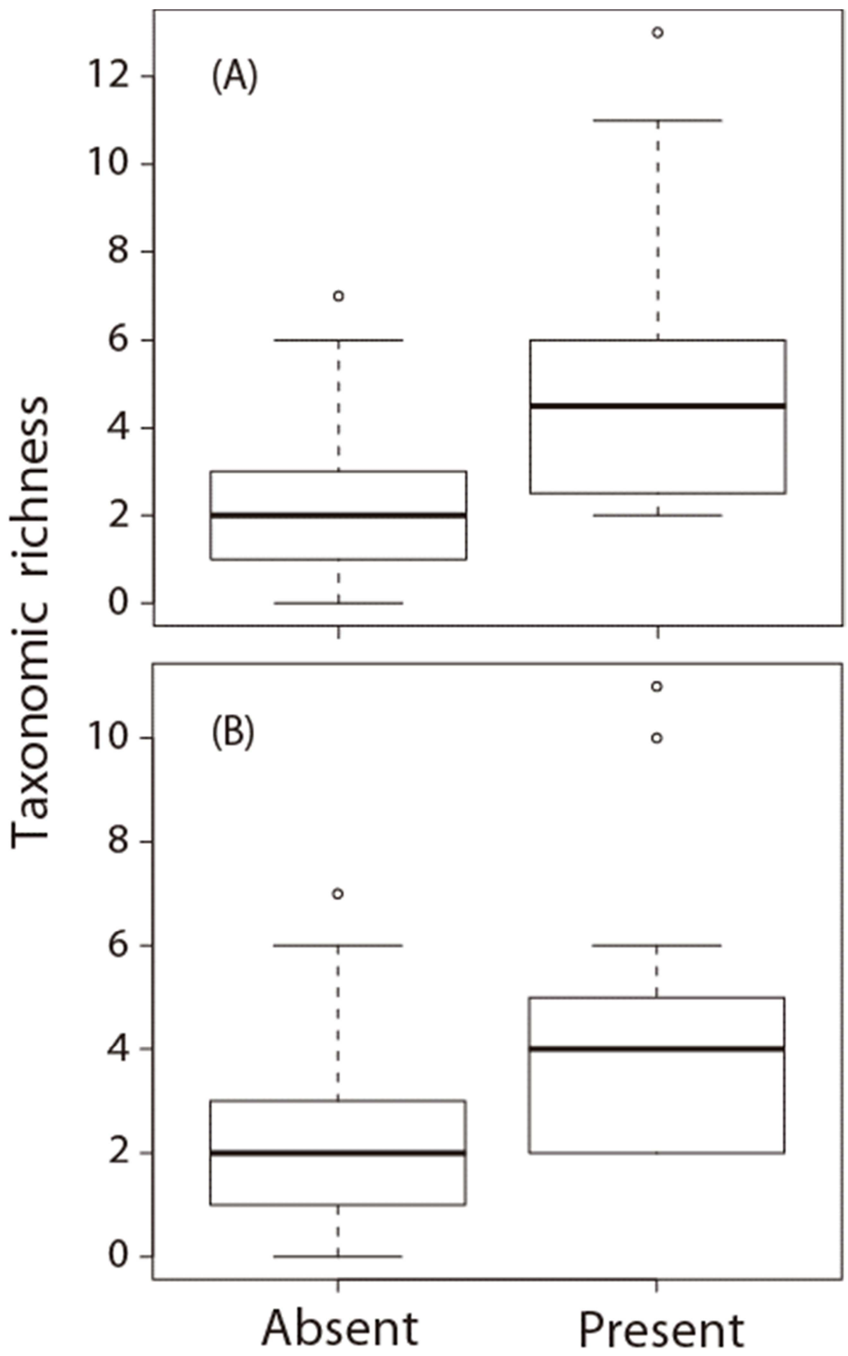

Fig. 5 Negishi et al.

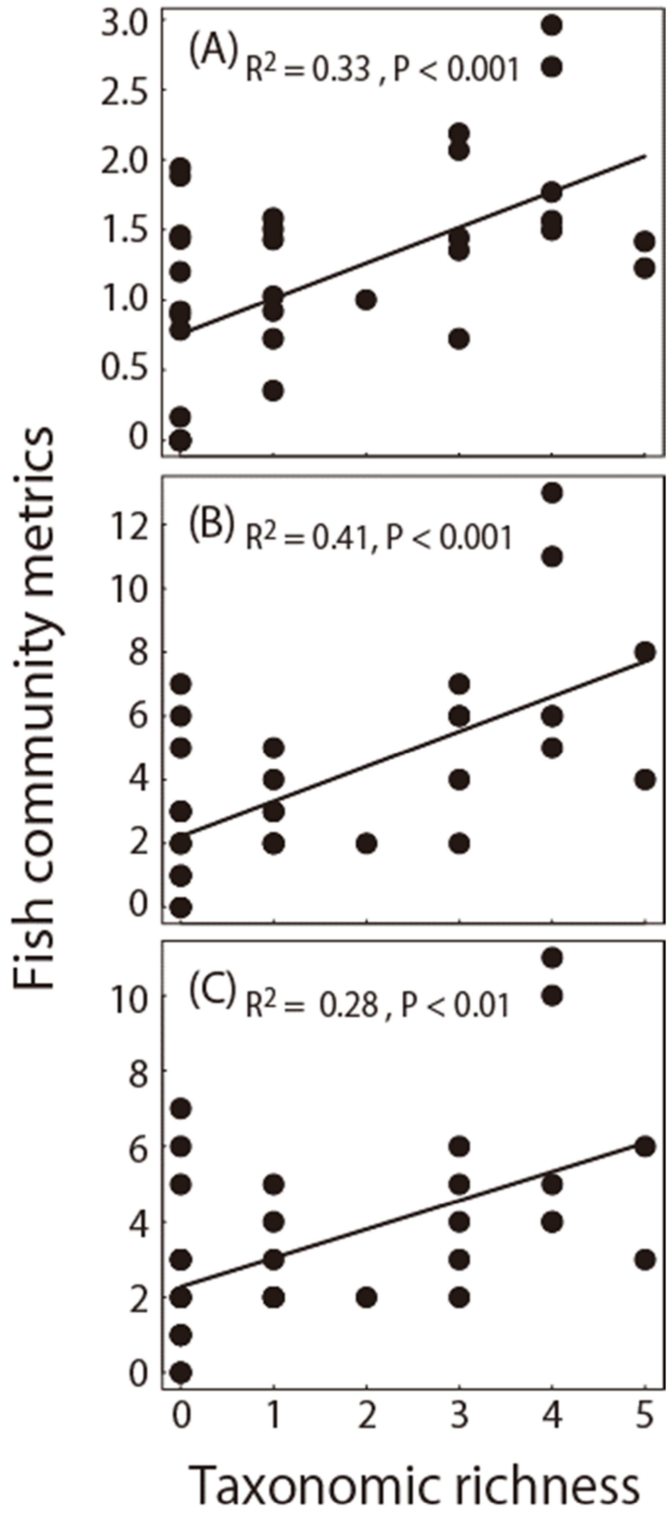

Fig. 6 Negishi et al.