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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

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

39

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

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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 (Kato 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 ($> 10,000 \text{ km}^2$). 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

Study site

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 japonensis* 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).

Locating potential sites containing unknown mussel populations

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 $>$

205 100 cells. When randomly chosen 18 candidate cells were placed on areas dominated by
206 large rivers (> 90%), or the areas without noticeable agricultural drainage channel
207 networks, candidate cells were re-chosen by a random selection procedure (this
208 occurred once).

209

210 Mussel and fish surveys

211 Fish and mussel communities were surveyed in the summer period between 8th and 13th
212 August when flow is stable and no noticeable precipitation was recorded in the
213 preceding 2 weeks. In the water management cycles for rice cultivation in the area, this
214 period typically provides the largest amount of water within the channel (Negishi et al.
215 2013). The exact locations of the potential sites within candidate cells were determined
216 as follows: The geographical coordinates for the central points of chosen grid cells were
217 determined by GIS and located in the field by GPS. The sampling sites were chosen by
218 the following criteria: Upon arrival at the location, the closest drainage channel was
219 located. The site suitability was confirmed when it was for drainage or
220 irrigation/drainage purpose(s), had decent water quality and perennial flow, and was of a
221 comparable size to that of the mussel sites. The presence of perennial flow with decent
222 water quality was confirmed by the occurrence of freshwater Mollusca such as

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

227 At each study site, two sections having a longitudinal length 10 times the
228 average wetted surface width (range = 45–320 cm) were set for mussel surveys with a
229 distance of 20 times the width between each section. In each section, electrical
230 conductivity (EC), pH, and water temperature (°C) were recorded using a
231 multi-parameter water quality probe (WM-22EP; DKK-TOA Co., Japan). Mussels were
232 quantified by collecting all mussels within the belt transects (a dimension of 25 × the
233 wetted channel width) equally spaced along a longitudinal profile. The total number of
234 transects was proportional to the section length ($n = 4\text{--}32$) and total areas searched
235 ranged from 0.9 to 36.8 m². Mussels were collected by thoroughly sieving the sediment
236 using baskets (1-cm mesh), identified, enumerated, and measured along their longest
237 shell axes. Fish communities were surveyed by enclosing a section (20 times the length
238 of the wetted width) in the area upstream of the mussel survey sections. To avoid
239 disturbing the fish communities, the survey was conducted prior to the mussel survey.
240 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 m² or 100 m²). 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. japonensis*, 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 ($\pm$ SD) mussel density (individuals/m²), irrespective of taxon, at sites with mussel populations was 29.6 ($\pm$ 57.3) individuals/m². 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

331 did not constrain our analyses to the areas where only the landscape structures varied,
332 with other important factors (see the next paragraph) being more or less controlled when
333 selecting random sites.

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

347 We showed that areas with landscape structures characteristic of mussel sites
348 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. omissa*, and *P. japonensis* 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 m² 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 ($> 10,000 \text{ km}^2$) 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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References

- Allen DC, Vaughn CC (2010) Complex hydraulic and substrate variables limit freshwater mussel species richness and abundance. *J N Am Benthol Soc* 29: 383-394
- Aldridge DC, Fayle TM, Jackson N (2007) Freshwater mussel abundance predicts biodiversity in UK lowland rivers. *Aquat Conserv* 17:554-564
- Arbuckle KE, Downing JA (2002) Freshwater mussel abundance and species richness: GIS relationships with watershed land use and geology. *Can J Fish Aquat Sci* 59:310-316

439 Armitage PD, Szoszkiewicz K, Blackburn JH, Nesbitt I (2003) Ditch communities: a
 440 major contributor to floodplain biodiversity. *Aquat Conserv* 13:165-185
 441 Benda L, Poff NL, Miller D, Dunne T, Reeves G, Pess G, Pollock M (2004). The
 442 network dynamics hypothesis: how channel networks structure riverine habitats.
 443 *BioScience* 54:413-427
 444 Biggs J, Williams P, Whitfield M, Nicolet P, Brown C, Hollis J, Arnold D, Pepper T
 445 (2007) The freshwater biota of British agricultural landscapes and their
 446 sensitivity to pesticides. *Agr Ecosyst Environ* 122:137-148
 447 Cao Y, Huang J, Cummings KS, Holtrop A (2013). Modeling changes in freshwater
 448 mussel diversity in an agriculturally dominated landscape. *Freshwater Science*
 449 32:1205-1218
 450 Daniel WM, Brown KM (2013) Multifactorial model of habitat, host fish, and landscape
 451 effects on Louisiana freshwater mussels. *Freshw Sci* 32:193-203
 452 Elphick CS (2000) Functional equivalency between rice fields and seminatural wetland
 453 habitats. *Conserv Biol* 14:181-191
 454 Fujihara M, Hara K, Short KM (2005) Changes in landscape structure of “yatsu”
 455 valleys: a typical Japanese urban fringe landscape. *Landscape Urban Plan*
 456 70:261-270

457 Galbraith HS, Spooner DE, Vaughn CC (2010) Synergistic effects of regional climate
 458 patterns and local water management on freshwater mussel communities. *Biol*
 459 *Conserv* 143: 1175-1183

460 Gergel SE, Turner MG, Miller JR, Melack JM, Stanley EH (2002) Landscape indicators
 461 of human impacts to riverine systems. *Aquat Sci* 64:118-128

462 Herzon I, Helenius J (2008) Agricultural drainage ditches, their biological importance
 463 and functioning. *Biol Conserv* 141:1171-1183

464 Janse JH, Peter JTM, Puijenbroek V (1998) Effects of eutrophication in drainage
 465 ditches. *Environ Pollut* 102:547-552

466 Katano O, Hosoya K, Iguchi KI, Yamaguchi M, Aonuma Y, Kitano S (2003) Species
 467 diversity and abundance of freshwater fishes in irrigation ditches around rice
 468 fields. *Environ Biol Fish* 66:107-121

469 Katoh K, Sakai S, Takahashi T (2009) Factors maintaining species diversity in
 470 satoyama, a traditional agricultural landscape of Japan. *Biol Conserv*
 471 142:1930-1936

472 Kitamura J (2007) Reproductive ecology and host utilization of four sympatric bitterling
 473 (*Acheilognathinae*, *Cyprinidae*) in a lowland reach of the Harai River in Mie,
 474 Japan. *Environ Biol Fish* 78:37-55

475 Lydeard C, Cowie RH, Ponder WF, Bogan AE, Bouchet P, Clark SA, Cummings KS,
 476 Frest TJ, Gargominy O, Herbert DG, Hershler R, Perez KE, Roth B, Seddon M,
 477 Strong EE, Thompson FG (2004) The global decline of nonmarine mollusks.
 478 BioScience 54:321-330

479 Marchand MN (2004) Effects of habitat features and landscape composition on the
 480 population structure of a common aquatic turtle in a region undergoing rapid
 481 development. Conserv Biol 18:758-767

482 Matsuzaki SS, Terui A, Kodama K, Tada M, Yoshida T, Washitani I (2011) Influence
 483 of connectivity, habitat quality and invasive species on egg and larval
 484 distributions and local abundance of crucian carp in Japanese agricultural
 485 landscapes. Biol Conserv 144:2081-2087

486 Miyamoto K (2007) Harmonization of irrigation system development and ecosystem
 487 preservation in paddy fields. Paddy Water Environ 5:1-4

488 Moilanen A, Wilson KA (2009) Spatial conservation prioritization : quantitative
 489 methods and computational tools. Oxford University Press, Oxford [etc.].

490 Morales Y, Weber LJ, Mynett AE, Newton TJ (2006) Effects of substrate and
 491 hydrodynamic conditions on the formation of mussel beds in a large river. J N
 492 Am Benthol Soc 25:664-676

493 Nagayama S, Negishi JN, Kume M, Sagawa S, Tsukahara K, Miwa Y, Kayaba Y
 494 (2012) Habitat use by fish in small perennial agricultural canals during
 495 irrigation and non-irrigation periods. Ecol Civil Eng 15:147-160 (in Japanese
 496 with English abstract)

497 Nakamura T, Short K (2001). Land-use planning and distribution of threatened wildlife
 498 in a city of Japan. Landscape Urban Plan 53:1-15

499 Natuhara Y (2012) Ecosystem services by paddy fields as substitutes of natural
 500 wetlands in Japan. Ecol Eng 56:97-106

501 Negishi JN, Kayaba Y (2009). Effects of handling and density on the growth of the
 502 unionoid mussel *Pronodularia japonensis*. J N Am Benthol Soc 28:821-831

503 Negishi JN, Kayaba Y, Tsukahara K, Miwa Y (2008) Towards conservation and
 504 restoration of habitats for freshwater mussels (Unionoida). Ecol Civil Eng
 505 11:195-211. (in Japanese with English abstract)

506 Negishi JN, Nagayama S, Kume M, Sagawa S, Kayaba Y, Yamanaka Y (2013)
 507 Unionoid mussels as an indicator of fish communities: A conceptual framework
 508 and empirical evidence. Ecol Indic 24:127-137

509 Onikura N, Nakajima J, Eguchi K, Inui R, Higa E, Miyake T, Kawamura K, Matsui S,
 510 Oikawa S (2006) Changes in distribution of bitterlings, and effects of

511 urbanization on populations of bitterlings and unionid mussels in Tatara River
 512 System, Kyusyu, Japan. J Jpn Soc Water Environ 29:837-842 (in Japanese with
 513 English abstract)
 514 R Development Core Team (2008) R: a language and environment for statistical
 515 computing. R Foundation for Statistical Computing, Vienna, Austria.
 516 Sato H (2001) The current state of paddy agriculture in Japan. Irrig Drain 50:91-99
 517 Schwalb AN, Cottenie KARL, Poos MS, Ackerman JD (2011). Dispersal limitation of
 518 unionid mussels and implications for their conservation. Freshw Biol
 519 56:1509-1518
 520 Schwalb AN, Morris TJ, Mandrak NE, Cottenie K (2013) Distribution of unionid
 521 freshwater mussels depends on the distribution of host fishes on a regional scale.
 522 Divers Distrib 19:446–454
 523 Stephens SE, Koons DN, Rotella JJ, Willey DW (2004) Effects of habitat fragmentation
 524 on avian nesting success: a review of the evidence at multiple spatial scales.
 525 Biol Conserv 115:101-110
 526 Strayer DL (2008) Freshwater mussel ecology—a multi-factor approach to distribution
 527 and abundance. University of California Press, Berkeley and Los Angeles.
 528 Takahashi Y (1994) Characteristics of rice field irrigation: The Japanese experience. Int

529 J Water Resour D 10:425-430

530 Terui A, Shin-ichiro SM, Kodama K, Tada M, Washitani I (2011) Factors affecting the

531 local occurrence of the near-threatened bitterling (*Tanakia lanceolata*) in

532 agricultural canal networks: strong attachment to its potential host mussels.

533 Hydrobiologia 675:19-28

534 Vaughn CC, Taylor CM (2000) Macroecology of a host-parasite relationship.

535 Ecography 23:11-20.

536 Wang L, Lyons J, Kanehl P, Bannerman R (2001). Impacts of urbanization on stream

537 habitat and fish across multiple spatial scales. Environ Manage 28:255-266

538 Williams P (2004) Comparative biodiversity of rivers, streams, ditches and ponds in an

539 agricultural landscape in Southern England. Biol Conserv 115:329-341

540 Xu YJ, Wu K (2006) Seasonality and interannual variability of freshwater inflow to a

541 large oligohaline estuary in the Northern Gulf of Mexico. Estuaries Coast Shelf

542 S 68:619-626

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 $\pm$ SD are shown. Statistical significance as the result of Welch'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 $\pm$ 18.16	32.88 $\pm$ 16.72	$p = 0.08$
Slope (%)	0.69 $\pm$ 0.57	0.57 $\pm$ 0.43	$p = 0.67$
Temp. (°C)	27.45 $\pm$ 2.10	26.42 $\pm$ 1.35	$p = 0.08$
pH	7.12 $\pm$ 0.69	7.80 $\pm$ 0.62	$p = 0.01$
EC (μ S/cm)	9.81 $\pm$ 7.15	9.41 $\pm$ 3.52	$p = 0.83$
Flow rate (m ³)	0.19 $\pm$ 0.31	0.15 $\pm$ 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 $\pm$ SD (maximum values; minimum values were all zero).

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

	<i>Lethenteron reissneri</i>	0	0.82±3.08 (12.35)
Siluridae			
	<i>Silurus asotus</i>	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 $\pm$ 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 ($p = 0.05/6$; $p = 0.008$).

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