Spatial network structure and scales differently affect the population size and genetic diversity of the ninespine stickleback in a remnant wetland system

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Running head:

Genetic and demographic connectivity in wetland fish populations

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Summary

1. The management of population size and genetic diversity in fragmented landscapes is the central issue in conservation biology. Functional connectivity between remnant habitat patches affects these parameters. However, the functional connectivity for genetic diversity would be characterized by a greater spatial scale than population size even within the same habitat network. The reason for this difference is that while dispersal frequency generally decreases with increasing distance, only a few immigrants may effectively contribute to gene flow, whereas a certain number of dispersers may be required to influence population abundance.

2. Here, we investigated the effects of habitat network structures on population abundance and genetic diversity of the ninespine stickleback, *Pungitius pungitius*, in remnant wetland ponds in northern Japan. We tested (i) whether both population abundance and genetic diversity are positively related not only to habitat size but also to connectivity and (ii) whether the dispersal effect extends to greater spatial scales in genetic diversity than in population size.

3. We employed a graph theoretic index to measure the degree of pond connectivity. This index can evaluate the connectivity threshold distance above which individuals cannot disperse and clarify the difference in the spatial scale of effective dispersal between population abundance and genetic diversity.

4. Pond connectivity significantly affected the spatial variation of both population abundance and genetic diversity. In contrast, pond size was related only to population abundance. As we predicted, the connectivity threshold distance for genetic diversity was more than two times greater than that for population abundance (12.5 km vs. 5 km, respectively).

5. Our findings indicate that the landscape managers should consider various spatial scales as a conservation unit for the management of a habitat network in accordance with the conservation targets that they establish. We also found that small artificial agricultural ditches and streams may play important roles in sustaining the population networks of wetland organisms.

Introduction

The management of population size and genetic diversity is a major target in conservation biology (Sanderson, 2006). A certain number of individuals is required to ensure the population's persistence over a given period of time (minimum viable population size: Shaffer, 1981; Nunney *et al*., 1993). In contrast, when considering longer-term population persistence, the conservation of genetic diversity becomes more important because genetic diversity is closely related to the evolutionary capacity for adaptation to environmental changes, i.e., shifts in climate and land-use and disease outbreaks (Lande & Shannon, 1996). Consequently, conservation planners need to take effective measures depending on established conservation targets (Groves *et al*., 2002). However, demographic and genetic studies are usually conducted separately with their own aims: unified studies are still lacking (e.g., Kirchner, Robert & Colas, 2006; Koizumi, 2011).

Habitat connectivity, along with habitat size, is the key determinant of population size and genetic diversity in a fragmented landscape. Habitats with higher connectivity generally exhibit a larger population size, primarily because habitat isolation decreases the immigration rate from surrounding habitats (Fahrig & Merriam, 1985; Debinski & Holt, 2000). In dynamic landscapes, habitat connectivity is particularly important to allow a disturbed population to sustain its resilience by promoting emigration (Turner, 1989; Hanski, 1999). Additionally, habitat connectivity enables gene flow, which is essential for maintaining genetic diversity. In smaller populations, gene flow is necessary to prevent genetic loss caused by genetic drift (Frankham, Briscoe & Ballou, 2002). However, human activities in recent years have seriously altered habitat connectivity, causing declines in population abundance and genetic diversity (Young *et al*., 1996; Lindenmayer & Fischer, 2006). Due to anthropogenic impacts,

habitat connectivity conservation has been recognized as a key concern in conservation biology.

Habitat connectivity for a given species can be defined by the distance between scattered habitat patches (i.e., inter-patch distance). In general, the functional connectivity between distant patches drastically decreases when inter-patch distances are beyond the range of species mobility (Metzger & Décamps, 1997; Langevelde, 2000). Therefore, information about this connectivity threshold distance (hereafter, connectivity threshold) is very useful for managing various conservation targets in a fragmented landscape. The connectivity threshold is often estimated using indirect methods that analyze the relationship between the spatial distribution of a target species and habitat connectivity calculated from the habitat configuration (i.e., structural connectivity) (Jacobson & Peres-Neto, 2010). For most organisms, the frequency of dispersal is generally high at a short distance and decreases with increasing distance (e.g., Kot, Lewis & van den Driessche, 1996; Paradis *et al*., 1998). Rare dispersal of individuals does not substantially contribute to promoting the immigration rate; therefore, it may influence population abundance only slightly. In contrast, a few migrants per generation may be sufficient to sustain the genetic diversity of local populations (Mills & Allendorf, 1996). Therefore, genetic diversity may be affected by sporadic long-distance dispersal (Trakhtenbrot *et al*., 2005). This principle indicates that distant connectivity that does not affect population abundance could be important for sustaining genetic diversity. In fact, Jackson & Fahrig (2014) have recently shown via a simulation model that the effect of individual movement is greater on genetic diversity than on population size. However, very few studies have quantitatively assessed the difference between the connectivity thresholds of population abundance and genetic diversity in natural populations. Such information should

112 be useful for determining appropriate/effective management actions depending on
113 conservation targets in real fragmented landscapes.
114 When organisms disperse to a distant habitat, they often utilize multiple habitat patches for
115 passage, resting, and foraging (Fahrig & Merriam, 1994). Hence, the management of a habitat
116 network should consider connectivity at the landscape level, including both direct and indirect
117 habitat connections. In landscape ecology, graph theory has been widely used as a tool to
118 examine the influence of landscape connectivity on species distribution in a habitat network
119 (Galpen, Manseau & Fall, 2011; Rayfield, Fortin & Fall, 2011). The relative importance of
120 each habitat patch in maintaining the overall landscape connectivity can be calculated based
121 on a graph theoretical approach. Consequently, using this approach, conservation planners can
122 quantify connectivity thresholds based on considerations of landscape connectivity (Pascual-
123 Hortal & Saura, 2006; Galpen *et al*., 2011). These practical benefits of the graph theoretical
124 approach have produced an increasing demand for the application of this approach to
125 landscape genetics (Manel & Holderegger, 2013). However, such applications have only
126 recently been developed, and actual implementations in landscape genetics are still limited
127 (but see Neel, 2008; Aavik, Holderegger & Bolliger, 2013).
128 Wetlands represent a unique ecosystem where hydrologic connectivity is sustained by
129 streams and occasional floods. Many freshwater organisms, such as fishes and amphibians,
130 have adapted to such dynamic landscapes, often exhibiting high levels of species diversity and
131 regional endemism (Tockner & Stanford, 2002; Zedler & Kercher, 2005). However, a number
132 of wetlands have become isolated or have disappeared due to human alterations, such as levee
133 construction associated with agricultural development and sedimentation from surrounding
134 land-use practices (Galat *et al*., 1998; Ahn *et al*., 2009). Fragmented lotic patches are currently

widespread, although artificial ditches and streams may still connect some habitat patches (Ishiyama, Akasaka & Nakamura, 2014). Therefore, we need to evaluate the effects of such a modified network structure on populations, considering the artificial corridors.

In the present study, we examined how habitat structure affects both the population abundance and the genetic diversity of the ninespine stickleback (*Pungitius pungitius*) in a fragmented wetland network. We used a graph theoretic approach to specifically test two predictions: (i) both population abundance and genetic diversity are positively correlated with habitat size and connectivity and (ii) the connectivity threshold distance is greater for genetic diversity compared with population abundance. Additionally, we examined the influence of local habitat qualities because habitat quality may also be related to population abundance and genetic diversity (e.g., Virgós, 2001; de Vere *et al*., 2009).

Methods

- Study species

The ninespine stickleback is a cold water-adapted fish with a circumpolar distribution in the Northern Hemisphere (Wootton, 1976). Japan is located at the southern limit of its distribution. In Japan, the ninespine stickleback primarily inhabits lentic habitats such as downstream rivers, wetlands, and spring-fed ponds. This species feeds primarily on small crustaceans and chironomid larvae. The spawning season is from May to July in northern Japan. Individuals mature in one year and survive for up to two years (Kawanabe & Mizuno, 1998). This species is currently on the Red Data List of species in many of Japan's districts, primarily due to habitat destruction (Association of Wildlife Research and EnVision, 2007).

- Study area

The field study was conducted in remnant wetland ponds located in the floodplain landscape of the Tokachi River basin, central Hokkaido, northern Japan (42°45’ N, 143°30’ E). The wetland ponds include a permanent floodplain pond, a spring-fed pond, and a cut-off channel. In 2011, the study period, the annual precipitation in the region was 882 mm, and the average temperature was 6.3°C. Since 1900, this region has been rapidly converted to farmland as modern irrigation technology, river channelization, and drainage systems have been introduced (Obihiro Development and Construction Department). Consequently, a large portion of the wetlands disappeared in the early 1900s. Approximately 50 wetland ponds currently remain in this area. Several are fully isolated, whereas others are connected to each other via drainage ditches and streams. The dominant wetland plants are the common cattail (*Typha latifolia*), common reed (*Phragmites australis*), hishi (*Trapa japonica*), Japanese spatterdock (*Nuphar japonicum*) and bladderwort (*Utricularia* spp.).

- Sample collection

From the remnant wetland ponds of the region, we selected 24 ponds with a wide range of connectivity levels as study sites. We collected sticklebacks once from each pond from June to August 2011 (Fig. 1). We set 1 to 6 fyke nets (0.4-m diameter, 2-m bag length, and 6-m wing length), depending on the pond area, in each pond. Each fyke net was stationed near aquatic vegetation (floating leaf macrophytes or emergent macrophytes) for approximately 24 hours. Catch per unit effort (*CPUE*) is commonly used as a relative measure of littoral fish abundance (e.g., Hinch *et al*., 1991; Winemiller *et al.*, 2000). For each of the study ponds, we calculated *CPUE* (number captured per trap per day) to assess the relative population

abundance of the stickleback. In the ponds where fewer than 20 individuals were caught, we performed additional sampling using a D-frame net (0.3-m width, 1.8-m length, and 1-mm mesh size) for 30 minutes near the sampling points for the fyke net. Part of the tail fin was collected from each individual and preserved in 95% ethanol for subsequent genetic analyses. Because juveniles may group with kin, we only sampled mature-sized sticklebacks (> 40 mm in length) to avoid genetic bias.

- Laboratory protocols

A Gentra Puregene Tissue Kit (Qiagen, Valencia, CA) was used for DNA extraction. For the polymerase chain reaction (PCR), we used the following nine microsatellite loci: Ppu1, Ppu6, Ppu7, Ppu10 (Meguro *et al*., 2009), STN96, STN 163, STN173, STN196 (Makinen, Valimaki & Merila, 2007), and Gac1125PBBE (Largider *et al*., 1999). The PCR was run in two multiplex reactions on a Thermal cycler 2720 (Applied Biosystems, Foster City, CA, USA): multiplex 1: Ppu1, Ppu6, Gac1125PBBE, STN96, STN163; multiplex 2: Ppu7, Ppu10, STN173, STN196; 95°C for 15 min, 30 cycles of 94°C for 30 sec, 53°C for 90 sec, 72°C for 60 sec, plus a final extension of 10 min at 72°C. The PCR products were diluted 1:5 and run on a 3130xl Automated Sequencer (Applied Biosystems, Foster City, CA, USA) using a GeneScan-500 LIZ size standard to estimate allele sizes. Alleles were scored with a PEAKSCANNER (Applied Biosystems, Foster City, CA, USA).

- Genetic analyses

We tested for linkage disequilibrium between all pairs of loci. We also tested all loci in all populations for deviations from Hardy–Weinberg equilibrium using an exact test. These tests

were implemented in GENEPOP 4.0 (Raymond & Rousset, 1995), and each parameter for the tests was set as follows: Dememorization; 1000, Batch size; 100, Iterations per batch; 1000. Significance levels were adjusted using the Sequential Bonferroni correction (Rice, 1989). Expected allelic richness (*Ar*) and heterozygosity (*He*) were calculated as genetic diversity indices. Allelic richness per population was calculated based on 23 individuals (the lowest sample size; Table 1). F_{st}-values were calculated to assess whether gene flow occurs between populations based on the pattern of isolation-by-distance (IBD). All analyses for genetic diversity and genetic distance were conducted using R (R Development Core Team, 2008, ver. 2.15.0) and the diveRsity package (Keenan *et al*., 2013).

- Hydrologic landscape graph construction

We applied graph theory to assess the relative importance of each wetland pond in the wetland network. To understand and visualize the habitat network using graph theory, we can construct a landscape graph with two components: habitat patches as nodes and habitat connections as links (Minor & Urban, 2008). In the present study, we constructed a hydrologic landscape graph that defined the wetland ponds as nodes and the watercourses (agricultural drainage ditches and streams) as links. A vector map of ponds and watercourses was created using aerial photographs taken in 2005 and 2010 (provided by the Obihiro Development and Construction Department). We included all remnant ponds to construct the graph. Because of the possibility of non-detection of watercourses using the aerial photographs, we conducted a complementary field survey to check for the presence of watercourses. The network structure varies according to the definition of the connectivity threshold. We tested nine connectivity thresholds (0 km, 2.5 km, 5 km, 7.5 km, 10 km, 12.5

227 km, 15 km, 17.5 km, and 20 km) for the construction of a hydrologic landscape graph. Here,
228 we set 20 km as the maximum threshold distance for hydrologic connectivity in our study area
229 because the network structures did not change significantly if connectivity distances greater
230 than this maximum were used (Fig. 2).
231
232 **- Network analysis**
233 The importance of each pond in the network was assessed using the relative decrease in the
234 Integral Index of Connectivity (dIIC; Pascual-Hortal & Saura, 2006) based on the hydrologic
235 landscape graphs. The dIIC was calculated based on the Integral Index of Connectivity (IIC),
236 given by the following:
237 Eq. (1)
238 $$IIC = \frac{\sum_{i=1}^{n}\sum_{j=1}^{n} a_i \times a_j/(1+nl_{ij})}{A_L{}^2}$$
239 and
240 Eq. (2) $$dIIC_k(\%) = \frac{IIC - IIC_{remove,k}}{IIC} \times 100$$
241
242 where a_i and a_j are the areas of ponds *i* and *j*, A_L is the total size of the ponds in the study
243 region, and nl_{ij} is either the number of links in the shortest watercourse path or the Euclidean
244 distance between ponds *i* and *j*. When ponds *i* and *j* are not connected to each other, $nl_{ij} = \infty$,
245 and if $i = j$, $nl_{ij} = 0$. In Eq. (2), $IIC_{remove,k}$ is the IIC value obtained when pond *k* is lost from the
246 habitat network. The value of $dIIC_k$ represents the percent reduction in IIC that occurs when
247 pond *k* is lost (i.e., the importance of pond *k* in the network).
248 The dIIC is based on the concept of habitat availability, which quantifies both inter-patch
249 connectivity (i.e., connectivity) and intra-patch connectivity (i.e., patch size) in measures of

total landscape connectivity. Both habitat connectivity and habitat size are generally related to demographic or genetic properties, which suggests that this integral index can explain the variations in population abundance or genetic diversity simply and sufficiently. A dIIC calculated with a 0-km threshold (dIIC_0 km) is closely related to the size of the studied pond ($r > 0.9$; $n = 24$) because the index under this connectivity threshold treats all ponds as fully isolated from each other (i.e., the index is calculated using only habitat size). Therefore, we used the value of dIIC_0 km as the index of pond size. The dIIC values were calculated using Conefor Sensinode 2.2 software (Saura & Torne, 2009).

- Habitat quality

We measured the water depth, vegetation cover ratio, electrical conductivity (EC), pH, and dissolved oxygen (DO) in the ponds once during the study period. The water depth and vegetation cover ratio were indices of habitat structure, and EC, pH, and DO were indices of water quality. Because these measurements may vary even within a pond, we measured at several sites (water depth: 6-27 sites per pond; water quality indices: 1-6 sites per pond) in each of the ponds depending on the pond area. The average values for these habitat quality indices in each pond were used as explanatory variables. DO generally exhibits diurnal variations and decreases from midnight to early morning because oxygen consumption by aquatic plants increases at this period of time (Brönmark & Hansson, 2005). Accordingly, we measured the water quality indices in the early morning from 6 to 9 AM. Vegetation cover as a percentage of total pond area was recorded in 20% intervals by visual observation and recorded as the vegetation cover ratio.

- Statistical analyses

To examine the relationships of population abundance and genetic diversity to pond importance in a wetland network, we first constructed full models using generalized linear models (GLMs; Burnham & Anderson, 2002) with a Gaussian error distribution and an identity link function. The sample sizes for the analyses of population abundance and genetic diversity were 24 populations and 17 populations, respectively (See "Sample collection"). The response variables were *CPUE*, *Ar* or *He*, and the explanatory variables were the dIIC and the five habitat quality indices (water depth, EC, DO, pH, and vegetation cover ratio).

The dIICs calculated using the different connectivity thresholds were highly correlated with each other ($r > 0.7$). To find the critical connectivity thresholds for population abundance and genetic diversity while avoiding the risk of multicollinearity, we used the dIIC that had the highest correlation with each response variable as an explanatory variable for the full models. For each response variable, we constructed single regression models using each dIIC calculated with a different connectivity threshold and selected the dIIC with the highest correlation (i.e., lowest AICc value; Burnham & Anderson, 2002) among all dIICs. A high correlation was also found between DO and the vegetation cover ratio in 17 populations for the genetic diversity analyses ($r = -0.76$). Therefore, the vegetation cover ratio showing higher correlations with genetic diversity was used as an explanatory variable for the full models.

Based on the full model, we constructed models for all cases using a best-subset procedure (Burnham & Anderson, 2002), and model performance was evaluated using AICc. Then, we averaged all of the models using the Akaike weight (Wi) of each model in the cases where multiple equivalent models were detected (Δ AICc < 2). In each averaged model, the explanatory parameters for which the 95% confidence intervals did not include zero were

296 considered influential parameters. To improve normality, the vegetation cover ratio was
297 arcsine-transformed, and *CPUE* and other explanatory variables were log-transformed.
298 The relationships between F_{st} and watercourse distance between populations were examined
299 using a Mantel test to assess the presence of IBD. All statistical analyses were conducted using
300 R (R Development Core Team, 2008, ver. 2.15.0), and the MuMin package (dredge function)
301 was used for model averaging (Bartoń, 2012).
302
303 **Results**
304 We collected a total of 6804 sticklebacks from 24 ponds. *CPUE* ranged from 0 to 669
305 (Table 1). We collected enough samples for genetic analyses (> 20) at 17 ponds, and a total of
306 524 individuals were genotyped (Table 1). No significant linkage between pairs of loci was
307 found ($\alpha = 0.05$, k = 45). Among nine microsatellite loci, deviation from Hardy–Weinberg
308 equilibrium was significant for STN 163 ($\alpha = 0.05$, initial k = 153) in 23.5% of the
309 populations (4 / 17); therefore, eight loci other than STN163 were used for the genetic
310 diversity and statistical analyses. *Ar* and *He* of the sticklebacks in each pond ranged from 5.02
311 to 8.32 and 0.63 to 0.75, respectively (Table 1).
312 Among the nine connectivity thresholds tested, *CPUE* and genetic diversity were best
313 explained by 5-km (dIIC_5 km) and 12.5-km (dIIC_12.5 km) thresholds, respectively, based
314 on the single regression analyses (Table 2). Therefore, in addition to the local pond-quality
315 indices, we adopted dIIC_5 km and dIIC_12.5 km for the full models of *CPUE* and genetic
316 diversity, respectively.
317 Both population abundance and genetic diversity were related to a wetland pond's
318 importance in maintaining the wetland network (dIIC); the spatial variation of genetic

diversity was influenced by a larger spatial scale than that of abundance. For *CPUE*, only dIIC_5 km was included in the best model; *CPUE* was positively related to the dIIC (Fig. 3). For models constructed for *Ar*, multiple equivalent models were selected (Δ AICc < 2). Accordingly, we averaged all of the models. *Ar* was positively related to dIIC_12.5 km (Fig. 4). For *He*, only pond quality indices were included in the best model; *He* was negatively related to vegetation cover ratio and was positively related to pH (Fig. 5).

The importance of habitat size and connectivity differed between population abundance and genetic diversity. For *CPUE*, the AICc of the model using dIIC_0 km (i.e., considering only pond size) as an explanatory variable was lower than that of the Null model (Δ AICc < 2), and the AICc of the model using dIIC_5 km (i.e., considering both pond size and connectivity) was lower than that of the model using dIIC_0 km (Δ AICc < 2; Table 2). This result indicates that both pond size and connectivity were influential factors for population abundance. For *Ar*, the AICc of the model using dIIC_12.5 km (i.e., considering both pond size and connectivity) was lower than that of the Null model, whereas the AICc of the model using dIIC_0 km (i.e., considering only pond size) as an explanatory variable was higher than that of the Null model (Table 2). This result indicates that genetic diversity was not related to pond size but only to connectivity. Although the genetic differentiation between ponds was relatively low (F_{st} < 0.07), the pattern of IBD was significant (R^2 = 0.37, Fig. 6). One population (p1 in Table 2) that had low connectivity showed high genetic divergence. When we determined IBD without this population, the pattern remained significant but was weaker (F_{st} < 0.03, R^2 = 0.19).

Discussion

342 Our graph theoretical approach demonstrated how the structure of a wetland network affects
343 the spatial variation of both the population abundance and genetic diversity of the ninespine
344 stickleback. The results of this study confirmed that landscape connectivity is important in
345 sustaining a population, as previous studies have reported (e.g., Fahrig & Merriam, 1994). In
346 contrast, habitat size was related only to population abundance and not to genetic diversity.
347 More importantly, we confirmed the prediction that the connectivity threshold of genetic
348 diversity is greater than that of population abundance, as also shown in a recent simulation
349 study (Jackson & Fahrig, 2014). To our knowledge, a difference in influential spatial scale in
350 a habitat network between demographic and genetic connectivity has not previously been
351 demonstrated in the field. Our finding stresses the need for management of a habitat network
352 to consider an appropriate spatial scale for arranged conservation targets.
353
354 **- Influence of habitat connectivity**
355 The network approach based on graph theory showed that the population abundance and *Ar*
356 of the stickleback were positively related to habitat connectivity. In contrast to landscape
357 ecology, the network approach has only recently been applied to landscape genetics; the
358 present study is one of a very few demonstrating the importance of considering the habitat
359 network in conserving genetic diversity. The population abundance and genetic structure of
360 freshwater fishes in lentic systems, like those of other vertebrates, are known to be strongly
361 affected by habitat connectivity (e.g., Uchida & Inoue, 2010; Seymour *et al*., 2013).
362 Particularly in a shallow lentic system such as wetlands, a fish population can be severely
363 damaged by seasonally fluctuating thermal and oxygen conditions (Scheffer & van Nes,
364 2007); hence, connected habitats may function as immigration sources for fishes in such

365 systems (Jackson, Peres-Neto & Olden, 2001). This rescue effect may affect the ninespine
366 stickleback populations in this wetland network both demographically and genetically.
367 Seymour *et al*. (2013) have reported that the genetic structure of the pond-dwelling
368 threespine stickleback *Gasterosteus aculeatus*, which belongs to the same family
369 (Gasterosteidae) as the ninespine stickleback, was influenced by gene flow among
370 periodically flooded ponds. In the studied floodplain, farmland expansion and stream
371 channelization have drastically fragmented the remaining wetland ponds, and the flooding
372 process has already been lost. However, the watercourse network consisting of agricultural
373 ditches and streams may function as an alternative source of connectivity between populations
374 of ninespine stickleback. Genetic differentiation of the stickleback populations in this study
375 region (F_{st} < 0.03 - 0.07) was relatively small compared to that of other lentic populations
376 previously reviewed by Merilä (2013), indicating that gene flow among populations may
377 frequently occur in this highly altered landscape. The observed significant IBD also supported
378 the presence of gene flow between neighboring populations.
379
380 **- Gap in the connectivity threshold distance between conservation targets**
381 We found that the connectivity threshold of genetic diversity was greater than that of
382 population abundance in a habitat network. This gap between the thresholds may be attributed
383 to the difference in the effective dispersal between population abundance and genetic diversity,
384 as we predicted. The connectivity thresholds for population abundance and *Ar* were 5 km and
385 12.5 km, respectively, suggesting that dispersals of less than 5 km may be frequent in this
386 wetland network. For instance, Harvey, Ruggerone & Rogers (1997) reported that ninespine
387 stickleback and threespine stickleback in Black River, Alaska seasonally migrate up to several

kilometers to suitable spawning habitat. Millet *et al*. (2013), studying the threespine stickleback in a lake in Iceland (total lake area approximately 37 km^2; maximum geographic distance between sampling points approximately 8.0 km), found no IBD among threespine stickleback populations and found that gene flow might occur extensively across the lake. These previous reports may support 5 km as a reasonable spatial scale for sustaining the frequent dispersal of the sticklebacks in the present study.

- Influence of habitat size

In fragmented landscapes, habitat size as well as habitat connectivity are the influential factors determining population abundance and genetic diversity (Fahrig, 2003). Nevertheless, habitat size was positively related only to population abundance, whereas habitat connectivity was positively related to both population abundance and genetic diversity. Why was genetic diversity affected only by connectivity and not by habitat size? One of the major processes by which habitat reduction affects genetic diversity is the reduction in population size, which in turn increases coincidental changes in allele frequencies (i.e., genetic drift; Frankham *et al*., 2002). It may take a relatively long time for habitat reduction to decrease genetic diversity because genetic drift is generally caused by genetic fluctuations across multiple generations. In contrast, gene flow may influence genetic diversity more rapidly because gene flow is the direct process that supplies new alleles to populations. Therefore, loss of genetic diversity in small populations caused by genetic drift may be substantially mitigated by gene flow (Frankham *et al*., 2002). Such a difference in effective time scales between genetic drift and gene flow might have masked the influence of habitat size on genetic diversity in the present study.

- Influence of wetland qualities on genetic diversity

Expected heterozygosity (*He*) was negatively related to the vegetation cover ratio and positively related to pH, whereas the vegetation cover ratio was negatively correlated with dissolved oxygen. In shallow lentic systems, dense aquatic vegetation can cause serious oxygen depletion and mass mortality of fishes via an increase in the consumption of dissolved oxygen (i.e., summer kill, Brönmark & Hansson, 2005). Water acidification also has negative impacts on freshwater fishes, such as a decrease in mortality at the early life stage (Sayer, Reader & Dalziel, 1993). These unsuitable environments associated with low dissolved oxygen and pH may have caused serious declines in the population size of the stickleback in the past and reduced genetic diversity via a population bottleneck.

In contrast to heterozygosity, allelic richness was not related to local environments but rather to habitat connectivity. This result is contrary to expectation because a bottleneck would reduce allelic richness more rapidly than heterozygosity (Frankham *et al*., 2002). However, in the presence of gene flow, the response of allelic richness to a population bottleneck caused by environmental changes might show patterns differing from complete fragmentation. For example, only a few immigrants would contribute to increased allelic richness if they had alleles that differed from those of the recipient population. Consequently, the influence of past bottlenecks may be decreased by the presence of habitat connectivity. So few immigrants, however, would not immediately increase heterozygosity, especially when the population size was large. This consideration could partly explain the discordance between heterozygosity and allelic richness, although other possibilities may exist.

- Conservation implications

Our most important finding in designing a habitat network is that the critical connectivity threshold for genetic diversity was greater than that for population abundance. This finding emphasizes that landscape managers need to consider the appropriate spatial scale according to the set time scale for population conservation. Specifically, long-distance connections among habitats should be conserved or restored if long-term population persistence (i.e., genetic diversity conservation) is set as a conservation target. In contrast, population abundance should be preferentially restored or maintained if population conservation is urgently needed. In this situation, the efficient conservation of the habitat connections affecting population abundance with a minimal cost is important, and short-distance connections maintaining population abundance should be preferentially conserved.

The genetic diversity of the stickleback was not related to habitat size but was related to habitat connectivity in the studied wetland network, indicating that management priority should be placed on the conservation of connectivity in terms of a genetic context. A large part of the natural connectivity among the studied wetland ponds had been sustained by natural flooding, but that has disappeared as a result of the human landscape modifications in this region. Nevertheless, the human-created watercourse network, e.g., agricultural ditches, appears to function as an alternative connectivity for the ninespine stickleback. In general, these watercourses are widely distributed in agricultural landscapes (Williams *et al.*, 2004). Hence, we believe that a population network of wetland animals can be maintained or restored by conserving the existing semi-natural or artificial watercourses, even in highly altered landscapes. Moreover, population abundance was related not only to the wetland's

connectivity but also to the size. Therefore, wetland management considering both wetland connectivity and size should be required in view of the demographic context.

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References

Aavik T., Holderegger R. & Bolliger J. (2013) The structural and functional connectivity of the grassland plant *Lychnis flos-cuculi*. *Heredity,* **112,** 471-478.

Ahn Y.S., Nakamura F., Kizuka T. & Nakamura Y. (2009) Elevated sedimentation in lake records linked to agricultural activities in the Ishikari River floodplain, northern Japan. *Earth Surface Processes and Landforms,* **34,** 1650-1660.

Association of Wildlife Research and EnVision. (2007) *Search System of Japanese Red Data*.

Bartoń K. (2012) MuMIn: multi-model inference. R package version 1.

Brönmark C. & Hansson L.A. (2005) *The biology of lakes and ponds,* Oxford University Press.

Burnham K.P. & Anderson D.R. (2002) *Model selection and multimodel inference: a practical information-theoretic approach,* Springer.

Debinski D.M. & Holt R.D. (2000) A survey and overview of habitat fragmentation experiments. *Conservation Biology,* **14,** 342-355.

De Vere N., Jongejans E., Plowman A. & Williams E. (2009) Population size and habitat quality affect genetic diversity and fitness in the clonal herb Cirsium dissectum. *Oecologia,* **159,** 59-68.

Epps C.W., Palsbøll P.J., Wehausen J.D., Roderick G.K., Ramey R.R. & Mccullough D.R. (2005) Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep. *Ecology Letters,* **8,** 1029-1038.

Fahrig L. (2003) Effects of habitat fragmentation on biodiversity. *Annual review of ecology, evolution, and systematics,* **34,** 487-515.

Fahrig L. & Merriam G. (1985) Habitat patch connectivity and population survival. *Ecology,* **66,** 1762-1768.

494 Fahrig L. & Merriam G. (1994) Conservation of fragmented populations. *Conservation*
495 *Biology,* **8,** 50-59.
496 Frankham R., Briscoe D.A. & Ballou J.D. (2002) *Introduction to conservation genetics*,
497 Cambridge University Press.
498 Galat D.L., Fredrickson L.H., Humburg D.D., Bataille K.J., Bodie J.R., Dohrenwend J.,
499 Gelwicks G.T., Havel J.E., Helmers D.L. & Hooker J.B. (1998) Flooding to restore
500 connectivity of regulated, large-river wetlands natural and controlled flooding as
501 complementary processes along the lower Missouri River. *BioScience,* **48,** 721-733.
502 Galpern P., Manseau M. & Fall A. (2011) Patch-based graphs of landscape connectivity: a
503 guide to construction, analysis and application for conservation. *Biological*
504 *Conservation,* **144,** 44-55.
505 Groves C.R., Jensen D.B., Valutis L.L., Redford K.H., Shaffer M.L., Scott J.M., Baumgartner
506 J.V., Higgins J.V., Beck M.W. & Anderson M.G. (2002) Planning for biodiversity
507 conservation: putting conservation science into practice a seven-step framework for
508 developing regional plans to conserve biological diversity, based upon principles of
509 conservation biology and ecology, is being used extensively by the nature
510 conservancy to identify priority areas for conservation. *BioScience,* **52,** 499-512.
511 Hanski I. (1999) *Metapopulation ecology,* Oxford University Press.
512 Harvey C., Ruggerone G. & Rogers D. (1997) Migrations of three-spined stickleback, nine-
513 spined stickleback, and pond smelt in the Chignik catchment, Alaska. *Journal of fish*
514 *biology,* **50,** 1133-1137.
515 Hinch S.G., Collins N.C. & Harvey H.H. (1991) Relative abundance of littoral zone fishes:
516 biotic interactions, abiotic factors, and postglacial colonization. *Ecology,* **72,** 1314-

517 1324.
518 Ishiyama N., Akasaka T. & Nakamura F. Mobility-dependent response of aquatic animal
519 species richness to a wetland network in an agricultural landscape. *Aquatic sciences*
520 **76,** 437-449.
521 Jackson D.A., Peres-Neto P.R. & Olden J.D. (2001) What controls who is where in freshwater
522 fish communities the roles of biotic, abiotic, and spatial factors. *Canadian Journal of*
523 *Fisheries and Aquatic Sciences,* **58,** 157-170.
524 Jackson N.D. & Fahrig L. (2014) Landscape context affects genetic diversity at a much larger
525 spatial extent than population abundance. *Ecology,* **95,** 871-881.
526 Jacobson B. & Peres-Neto P.R. (2010) Quantifying and disentangling dispersal in
527 metacommunities: how close have we come? How far is there to go? *Landscape*
528 *ecology,* **25,** 495-507.
529 Kawanabe Y. & Mizuno N. (1998) *Freshwater fishes of Japan*. Jama to Keikoku-sha.
530 Keenan K., Mcginnity P., Cross T.F., Crozier W.W. & Prodöhl P.A. (2013) diveRsity: An R
531 package for the estimation and exploration of population genetics parameters and
532 their associated errors. *Methods in Ecology and Evolution,* **4,** 782-788.
533 Kirchner F., Robert A. & Colas B. (2006) Modelling the dynamics of introduced populations
534 in the narrow−endemic Centaurea corymbosa: a demo-genetic integration. *Journal of*
535 *Applied Ecology,* **43,** 1011-1021.
536 Koizumi I. (2011) Integration of ecology, demography and genetics to reveal population
537 structure and persistence: a mini review and case study of stream-dwelling Dolly
538 Varden. *Ecology of freshwater fish,* **20,** 352-363.
539 Kot M., Lewis M.A. & Van Den Driessche P. (1996) Dispersal data and the spread of

invading organisms. *Ecology,* **77,** 2027-2042.

Lande R. & Shannon S. (1996) The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution,* **50,** 434-437.

Langevelde F. (2000) Scale of habitat connectivity and colonization in fragmented nuthatch populations. *Ecography,* **23,** 614-622.

Largiader C., Fries V., Kobler B. & Bakker T. (1999) Isolation and characterization of microsatellite loci from the three-spined stickleback (*Gasterosteus aculeatus L.*). *Molecular Ecology,* **8,** 342-344.

Lindenmayer D.B. & Fischer J. (2006) *Habitat fragmentation and landscape change: an ecological and conservation synthesis,* Island Press.

Makinen H.S., Valimaki K. & Merila J. Cross-species amplification of microsatellite loci for nine-spined stickleback *Pungitius pungitius, Annales Zoologici Fennici,* **44,** 218-224.

Manel S. & Holderegger R. (2013) Ten years of landscape genetics. *Trends in Ecology & Evolution,* **28,** 614-621.

Meguro Y.I., Takahashi H., Takeshima H., Nishida M. & Goto A. (2009) Isolation and characterization of 13 microsatellite loci in the nine-spined stickleback (*Pungitius pungitius*) and cross-species amplification in 5 stickleback species (family *Gasterosteidae*). *Conservation Genetics Resources,* **1,** 31-34.

Merilä J. (2013) Nine-spined stickleback (*Pungitius pungitius*): an emerging model for evolutionary biology research. *Annals of the New York Academy of Sciences,* **1289,** 18-35.

Metzger J.P. & Décamps H. (1997) The structural connectivity threshold: an hypothesis in conservation biology at the landscape scale. *Acta Oecologica,* **18,** 1-12.

563 Millet A., Kristjánsson B.K., Einarsson Á. & Räsänen K. (2013) Spatial phenotypic and
564 genetic structure of threespine stickleback (*Gasterosteus aculeatus*) in a
565 heterogeneous natural system, Lake Mývatn, Iceland. *Ecology and evolution,* **3,** 3219-
566 3232.
567 Mills L.S. & Allendorf F.W. (1996) The one-migrant-per-generation rule in conservation and
568 management. *Conservation Biology,* **10,** 1509-1518.
569 Minor E.S. & Urban D.L. (2008) A Graph−Theory Framework for Evaluating Landscape
570 Connectivity and Conservation Planning. *Conservation Biology,* **22,** 297-307.
571 Neel M.C. (2008) Patch connectivity and genetic diversity conservation in the federally
572 endangered and narrowly endemic plant species *Astragalus albens* (Fabaceae).
573 *Biological Conservation,* **141,** 938-955.
574 Nunney L. & Campbell K.A. (1993) Assessing minimum viable population size: demography
575 meets population genetics. *Trends in Ecology & Evolution,* **8,** 234-239.
576 Obihiro Development and Construction Department. http://www.ob.hkd.mlit.go.jp/ (in
577 Japanese, accessed April 2013)
578 Paradis E., Baillie S.R., Sutherland W.J. & Gregory R.D. (1998) Patterns of natal and
579 breeding dispersal in birds. *Journal of Animal Ecology,* **67,** 518-536.
580 Pascual-Hortal L. & Saura S. (2006) Comparison and development of new graph-based
581 landscape connectivity indices: towards the priorization of habitat patches and
582 corridors for conservation. *Landscape ecology,* **21,** 959-967.
583 Rayfield B., Fortin M.J. & Fall A. (2011) Connectivity for conservation: a framework to
584 classify network measures. *Ecology,* **92,** 847-858.
585 Raymond M. & Rousset F. (1995) GENEPOP (version 1.2): population genetics software for

586 exact tests and ecumenicism. *Journal of heredity,* **86,** 248-249.
587 R Development Core Team. (2005) R: A language and environment for statistical computing.
588 ISBN 3-900051-07-0. R Foundation for Statistical Computing. Vienna, Austria, 2013.
589 url: http://www. R-project. org.
590 Rice W.R. (1989) Analyzing tables of statistical tests. *Evolution,* **43,** 223-225.
591 Sanderson E.W. (2006) How many animals do we want to save? The many ways of setting
592 population target levels for conservation. *BioScience,* **56,** 911-922.
593 Saura S. & Torne J. (2009) Conefor Sensinode 2.2: a software package for quantifying the
594 importance of habitat patches for landscape connectivity. *Environmental Modelling &*
595 *Software,* **24,** 135-139.
596 Sayer M., Reader J. & Dalziel T. (1993) Freshwater acidification: effects on the early life
597 stages of fish. *Reviews in Fish Biology and Fisheries,* **3,** 95-132.
598 Scheffer M. & Van Nes E.H. (2007) Shallow lakes theory revisited: various alternative
599 regimes driven by climate, nutrients, depth and lake size. *Hydrobiologia,* **584,** 455-
600 466.
601 Seymour M., Räsänen K., Holderegger R. & Kristjánsson B.K. (2013) Connectivity in a pond
602 system influences migration and genetic structure in threespine stickleback. *Ecology*
603 *and evolution,* **3,** 492-502.
604 Shaffer M.L. (1981) Minimum population sizes for species conservation. *BioScience,* **31,** 131-
605 134.
606 Tockner K. & Stanford J.A. (2002) Riverine flood plains: present state and future trends.
607 *Environmental conservation,* **29,** 308-330.
608 Trakhtenbrot A., Nathan R., Perry G. & Richardson D.M. (2005) The importance of

609 long‐distance dispersal in biodiversity conservation. *Diversity and Distributions,* **11,**
610 173-181.
611 Turner M.G. (1989) Landscape ecology: the effect of pattern on process. *Annual Review of*
612 *Ecology and Systematics,* **20,** 171-197.
613 Uchida Y. & Inoue M. (2010) Fish species richness in spring-fed ponds: effects of habitat size
614 versus isolation in temporally variable environments. *Freshwater Biology,* **55,** 983-
615 994.
616 Virgós E. (2001) Role of isolation and habitat quality in shaping species abundance: a test
617 with badgers (*Meles meles L.*) in a gradient of forest fragmentation. *Journal of*
618 *Biogeography,* **28,** 381-389.
619 Williams P., Whitfield M., Biggs J., Bray S., Fox G., Nicolet P. & Sear D. (2004) Comparative
620 biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in
621 Southern England. *Biological Conservation,* **115,** 329-341.
622 Winemiller K.O., Tarim S., Shormann D. & Cotner J.B. (2000) Fish assemblage structure in
623 relation to environmental variation among Brazos River oxbow lakes. *Transactions of*
624 *the American Fisheries Society,* **129,** 451-468.
625 Wootton R.J. (1976) *Biology of the sticklebacks,* Academic Press., London.
626 Young A., Boyle T. & Brown T. (1996) The population genetic consequences of habitat
627 fragmentation for plants. *Trends in Ecology & Evolution,* **11,** 413-418.
628 Zedler J.B. & Kercher S. (2005) Wetland resources: status, trends, ecosystem services, and
629 restorability. *Annual Review of Environment and Resources,* **30,** 39-74.
630

631 **Table 1** Population abundance, genetic diversity, and local environments of each studied
632 wetland pond. *CPUE*, population abundance; N, sample size for genetic analyses; *Ar*, allelic
633 richness; *He*, expected heterozygosity; *Ho*, observed heterozygosity; Area, pond size;
634 Vegetation, vegetation cover ratio; Depth, water depth; EC, electrical conductivity; DO,
635 dissolved oxygen.

636

	Population abundance and genetic diversity					Environments					
Wetland	*CPUE*	N	*Ar*	*He*	*Ho*	Area (ha)	Vegetation (%)	Depth (cm)	EC (ms/m)	pH	DO (mg/l)
p1	4.67	28	5.02	0.63	0.54	0.4	100	42.9	15.1	6.5	1.1
p3	9.19	32	6.47	0.67	0.67	1.5	20	107.1	22.2	6.3	2.4
p5	257.61	35	6.68	0.69	0.68	6.0	100	76.0	8.0	6.6	1.5
p7	108.22	35	7.51	0.71	0.72	9.0	60	79.0	15.1	6.3	4.2
p11	3.27	—	—	—	—	6.4	40	109.3	11.4	6.7	6.7
p12	669.04	28	7.17	0.67	0.63	2.4	100	79.4	13.2	6.3	1.2
p13	283.54	34	7.90	0.70	0.69	1.7	60	51.8	14.7	6.3	1.2
p15	87.32	31	8.32	0.75	0.72	3.4	40	78.3	21.9	6.8	2.4
p16	2.00	33	7.52	0.70	0.70	0.4	100	61.7	15.5	7.0	1.1
p18	2.14	—	—	—	—	0.3	40	139.0	6.7	6.7	1.3
p20	118.94	23	8.17	0.74	0.72	1.0	20	36.9	22.7	7.1	5.8
p21	4.94	30	7.67	0.70	0.64	5.1	60	98.4	11.5	6.8	5.6
p22	67.45	32	7.84	0.69	0.71	3.2	80	91.0	13.0	6.6	2.0
p23	26.78	31	8.24	0.72	0.67	1.7	80	57.7	20.4	6.8	1.2
p25	38.04	31	7.57	0.69	0.70	1.5	100	63.3	15.8	7.0	0.8
p27	18.72	30	7.49	0.70	0.70	0.2	100	76.8	16.0	7.0	0.8
p28	5.85	35	7.68	0.66	0.61	0.4	80	49.6	12.3	5.9	1.4
p29	137.11	30	6.42	0.70	0.67	3.5	100	90.0	9.3	6.8	0.4
p30	8.18	—	—	—	—	11.4	100	95.0	12.1	6.6	4.4
p31	0	—	—	—	—	0.1	80	45.8	2.5	6.3	1.0
p33	0	—	—	—	—	0.4	80	65.9	26.3	6.3	1.6
p34	1.81	—	—	—	—	0.0	100	85.4	15.6	6.7	0.6
p35	5.48	26	7.89	0.74	0.74	1.4	80	125.0	23.5	6.4	2.4
p36	0	—	—	—	—	0.1	20	41.3	13.2	5.8	5.2

637 **Table 2** AICc values of models that were constructed to find the critical connectivity
638 thresholds for the response variables. “Null” indicates the null model without the explanatory
639 variable. The lowest AICc value for each response variable is indicated by boldface.

640

Explanatory variable	*CPUE*	*Ar*	*He*
Null	63.30	45.86	-66.80
dIIC_0 km	60.10	48.43	-63.83
dIIC_2.5 km	54.19	48.41	-64.56
dIIC_5 km	**52.46**	47.67	-65.51
dIIC_7.5 km	53.61	46.82	-66.32
dIIC_10 km	54.48	44.88	-66.42
dIIC_12.5 km	55.24	**43.15**	**-67.23**
dIIC_15 km	55.24	43.83	-66.72
dIIC_17.5 km	56.55	48.46	-64.00
dIIC_20 km	55.28	48.84	-64.16

641 **Figure legends**

642 **Fig. 1** A wetland network in the Tokachi Plain. A filled circle indicates that the pond was
643 included in the analyses for population abundance and genetic diversity. An open circle
644 indicates that the pond was included only in the analysis for population abundance. Non-
645 studied wetland ponds are represented by lozenges. Watercourses are represented by solid
646 lines.

647

648 **Fig. 2** Relationship between the area of a maximum component and the connectivity threshold
649 distance. Here, “component” indicates a cluster of ponds directly or indirectly connected to
650 each other.

651

652 **Fig. 3** Relationship between population abundance (*CPUE)* and a wetland pond’s importance
653 in maintaining the wetland network (dIIC). The numbers shown beside dIIC indicate
654 connectivity thresholds used for dIIC calculation. Filled circles indicate wetlands included in
655 the genetic diversity analysis. Both variables were transformed for the statistical analysis.
656 Solid line shows the estimated *CPUE* based on the best model.

657

658 **Fig. 4** Relationship between genetic diversity indices and dIIC. The numbers shown beside
659 dIIC indicate connectivity thresholds used for dIIC calculation. *Ar* and *He* indicate expected
660 allelic richness and heterozygosity, respectively. Both explanatory variables were transformed
661 for the statistical analysis. Solid line shows the estimated *Ar* based on model averaging.

662

663 **Fig. 5** Relationship between the expected heterozygosity (*He*) and pond quality indices.

Vegetation indicates a vegetation cover ratio. Both explanatory variables were transformed for the statistical analysis. Solid line shows the estimated *He* based on model averaging.

Fig. 6 Relationship between the genetic distance (F_{st}) and watercourse distances. Open circles show an outlier corresponding to a specific population (p1) having low connectivity.