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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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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

be useful for determining appropriate/effective management actions depending on conservation targets in real fragmented landscapes.

When organisms disperse to a distant habitat, they often utilize multiple habitat patches for passage, resting, and foraging (Fahrig & Merriam, 1994). Hence, the management of a habitat network should consider connectivity at the landscape level, including both direct and indirect habitat connections. In landscape ecology, graph theory has been widely used as a tool to examine the influence of landscape connectivity on species distribution in a habitat network (Galpen, Manseau & Fall, 2011; Rayfield, Fortin & Fall, 2011). The relative importance of each habitat patch in maintaining the overall landscape connectivity can be calculated based on a graph theoretical approach. Consequently, using this approach, conservation planners can quantify connectivity thresholds based on considerations of landscape connectivity (Pascual-Hortal & Saura, 2006; Galpen *et al.*, 2011). These practical benefits of the graph theoretical approach have produced an increasing demand for the application of this approach to landscape genetics (Manel & Holderegger, 2013). However, such applications have only recently been developed, and actual implementations in landscape genetics are still limited (but see Neel, 2008; Aavik, Holderegger & Bolliger, 2013).

Wetlands represent a unique ecosystem where hydrologic connectivity is sustained by streams and occasional floods. Many freshwater organisms, such as fishes and amphibians, have adapted to such dynamic landscapes, often exhibiting high levels of species diversity and regional endemism (Tockner & Stanford, 2002; Zedler & Kercher, 2005). However, a number of wetlands have become isolated or have disappeared due to human alterations, such as levee construction associated with agricultural development and sedimentation from surrounding 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 (A_r) and heterozygosity (H_e) 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

km, 15 km, 17.5 km, and 20 km) for the construction of a hydrologic landscape graph. Here, we set 20 km as the maximum threshold distance for hydrologic connectivity in our study area because the network structures did not change significantly if connectivity distances greater than this maximum were used (Fig. 2).

- Network analysis

The importance of each pond in the network was assessed using the relative decrease in the Integral Index of Connectivity (dIIC; Pascual-Hortal & Saura, 2006) based on the hydrologic landscape graphs. The dIIC was calculated based on the Integral Index of Connectivity (IIC), given by the following:

Eq. (1)

$$IIC = \frac{\sum_{i=1}^n \sum_{j=1}^n a_i \times a_j / (1 + nl_{ij})}{A_L^2}$$

and

Eq. (2)

$$dIIC_k(\%) = \frac{IIC - IIC_{remove,k}}{IIC} \times 100$$

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 region, and nl_{ij} is either the number of links in the shortest watercourse path or the Euclidean distance between ponds i and j . When ponds i and j are not connected to each other, $nl_{ij} = \infty$, 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 habitat network. The value of $dIIC_k$ represents the percent reduction in IIC that occurs when pond k is lost (i.e., the importance of pond k in the network).

The dIIC is based on the concept of habitat availability, which quantifies both inter-patch 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 (W_i) of each model in the cases where multiple equivalent models were detected ($\Delta AICc < 2$). In each averaged model, the explanatory parameters for which the 95% confidence intervals did not include zero were

considered influential parameters. To improve normality, the vegetation cover ratio was arcsine-transformed, and *CPUE* and other explanatory variables were log-transformed.

The relationships between F_{st} and watercourse distance between populations were examined using a Mantel test to assess the presence of IBD. All statistical analyses were conducted using R (R Development Core Team, 2008, ver. 2.15.0), and the MuMin package (dredge function) was used for model averaging (Bartoń, 2012).

Results

We collected a total of 6804 sticklebacks from 24 ponds. *CPUE* ranged from 0 to 669 (Table 1). We collected enough samples for genetic analyses (> 20) at 17 ponds, and a total of 524 individuals were genotyped (Table 1). No significant linkage between pairs of loci was found ($\alpha = 0.05$, $k = 45$). Among nine microsatellite loci, deviation from Hardy–Weinberg equilibrium was significant for STN 163 ($\alpha = 0.05$, initial $k = 153$) in 23.5% of the populations (4 / 17); therefore, eight loci other than STN163 were used for the genetic diversity and statistical analyses. *Ar* and *He* of the sticklebacks in each pond ranged from 5.02 to 8.32 and 0.63 to 0.75, respectively (Table 1).

Among the nine connectivity thresholds tested, *CPUE* and genetic diversity were best explained by 5-km (dIIC_5 km) and 12.5-km (dIIC_12.5 km) thresholds, respectively, based on the single regression analyses (Table 2). Therefore, in addition to the local pond-quality indices, we adopted dIIC_5 km and dIIC_12.5 km for the full models of *CPUE* and genetic diversity, respectively.

Both population abundance and genetic diversity were related to a wetland pond's 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 ($\Delta \text{AICc} < 2$). 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 ($\Delta \text{AICc} < 2$), 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 ($\Delta \text{AICc} < 2$; 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

Our graph theoretical approach demonstrated how the structure of a wetland network affects the spatial variation of both the population abundance and genetic diversity of the ninespine stickleback. The results of this study confirmed that landscape connectivity is important in sustaining a population, as previous studies have reported (e.g., Fahrig & Merriam, 1994). In contrast, habitat size was related only to population abundance and not to genetic diversity. More importantly, we confirmed the prediction that the connectivity threshold of genetic diversity is greater than that of population abundance, as also shown in a recent simulation study (Jackson & Fahrig, 2014). To our knowledge, a difference in influential spatial scale in a habitat network between demographic and genetic connectivity has not previously been demonstrated in the field. Our finding stresses the need for management of a habitat network to consider an appropriate spatial scale for arranged conservation targets.

- Influence of habitat connectivity

The network approach based on graph theory showed that the population abundance and *Ar* of the stickleback were positively related to habitat connectivity. In contrast to landscape ecology, the network approach has only recently been applied to landscape genetics; the present study is one of a very few demonstrating the importance of considering the habitat network in conserving genetic diversity. The population abundance and genetic structure of freshwater fishes in lentic systems, like those of other vertebrates, are known to be strongly affected by habitat connectivity (e.g., Uchida & Inoue, 2010; Seymour *et al.*, 2013). Particularly in a shallow lentic system such as wetlands, a fish population can be severely damaged by seasonally fluctuating thermal and oxygen conditions (Scheffer & van Nes, 2007); hence, connected habitats may function as immigration sources for fishes in such

systems (Jackson, Peres-Neto & Olden, 2001). This rescue effect may affect the ninespine stickleback populations in this wetland network both demographically and genetically.

Seymour *et al.* (2013) have reported that the genetic structure of the pond-dwelling threespine stickleback *Gasterosteus aculeatus*, which belongs to the same family (Gasterosteidae) as the ninespine stickleback, was influenced by gene flow among periodically flooded ponds. In the studied floodplain, farmland expansion and stream channelization have drastically fragmented the remaining wetland ponds, and the flooding process has already been lost. However, the watercourse network consisting of agricultural ditches and streams may function as an alternative source of connectivity between populations of ninespine stickleback. Genetic differentiation of the stickleback populations in this study region ($F_{st} < 0.03 - 0.07$) was relatively small compared to that of other lentic populations previously reviewed by Merilä (2013), indicating that gene flow among populations may frequently occur in this highly altered landscape. The observed significant IBD also supported the presence of gene flow between neighboring populations.

- Gap in the connectivity threshold distance between conservation targets

We found that the connectivity threshold of genetic diversity was greater than that of population abundance in a habitat network. This gap between the thresholds may be attributed to the difference in the effective dispersal between population abundance and genetic diversity, as we predicted. The connectivity thresholds for population abundance and A_r were 5 km and 12.5 km, respectively, suggesting that dispersals of less than 5 km may be frequent in this wetland network. For instance, Harvey, Ruggerone & Rogers (1997) reported that ninespine 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 km²; 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 (H_e) 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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Table 1 Population abundance, genetic diversity, and local environments of each studied wetland pond. *CPUE*, population abundance; *N*, sample size for genetic analyses; *Ar*, allelic richness; *He*, expected heterozygosity; *Ho*, observed heterozygosity; Area, pond size; Vegetation, vegetation cover ratio; Depth, water depth; EC, electrical conductivity; DO, dissolved oxygen.

Wetland	Population abundance and genetic diversity					Environments					
	<i>CPUE</i>	<i>N</i>	<i>Ar</i>	<i>He</i>	<i>Ho</i>	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

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

Explanatory variable	<i>CPUE</i>	<i>Ar</i>	<i>He</i>
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

Figure legends

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

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

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

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

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 H_e 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.