

Intralacustrine site fidelity and nonrandom mating in the littoral-spawning northern redbelly dace (*Phoxinus eos*)

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Abstract: Natal site fidelity of the northern redbelly dace (*Phoxinus eos*), a common minnow in North America, was confirmed by combining ecological and genetic approaches. A 2-year mark–recapture experiment conducted at four sites separated by 50–450 m strongly supported the propensity of the dace to practice site fidelity during the reproductive period. Individuals recaptured at their marking sites were characterized with five microsatellite loci. Hardy–Weinberg equilibrium and allelic differentiation tests revealed that the fish from different sites significantly differed from a single panmictic and genetically uniform population, thus confirming the homing behaviour of the dace. The detection of a pattern of isolation by distance revealed that migration mostly occurred between nearby sites and decreased as distance from birth site increased. When considering the high population density of dace, their high swimming capability, the distribution of the spawning sites along the littoral zone, and the small size of the lake studied (<5 ha), these results strongly suggest natal site fidelity in this species. The detection of this phenomenon for this species is extremely useful for empirical investigations of factors affecting patterns of isolation by distance and of evolutionary perspectives of natal site fidelity in fishes.

Résumé : Un comportement de fidélité au site de naissance du ventre rouge du nord (*Phoxinus eos*), un cyprin commun d'Amérique du Nord, a été confirmé par la combinaison d'approches écologique et génétique. Une expérience de marquage et recaptures répétées pendant 2 années sur quatre sites distants de 50 à 450 m supporte fortement la propension du cyprin au comportement de fidélité au site en période de reproduction. Les individus recapturés à leur site de marquage ont ensuite été caractérisés avec cinq microsatellites. Les tests d'équilibre de Hardy–Weinberg et de différenciation allélique révèlent que les poissons des différents sites diffèrent significativement d'une population panmictique et génétiquement uniforme, ce qui confirme le comportement de fidélité au site de naissance du cyprin. La détection d'un patron d'isolement par la distance révèle que les échanges de migrants sont plus importants entre des sites rapprochés et diminuent avec une augmentation de la distance entre les sites de naissance et de ponte. En considérant la forte densité de cette population de cyprins, leur importante capacité natale, la distribution des sites de ponte le long de la zone littorale et la petite taille du lac étudié (<5 ha), ces résultats indiquent fortement l'existence d'un comportement de fidélité au site de naissance chez cette espèce. La détection de ce phénomène chez cette espèce la rend extrêmement utile pour l'étude empirique des facteurs qui affectent le patron d'isolement par la distance et des perspectives évolutives de la fidélité au site de naissance chez les poissons.

Introduction

Natural populations are generally genetically structured, with a higher frequency of mating between individuals that are geographically close and (or) that reproduce during the same period. Spatially or temporally structured populations will display different evolutionary properties and trajectories than will a randomly mating population (Hendry and Day 2005). Determining how individuals of a species are organized within a given habitat is a key feature in ecology (e.g., Excoffier 2001) and population management (e.g., Nielsen

1998). The analysis of genetic structure can also reveal evolutionary processes acting on populations.

While physical barriers and habitat discontinuities limit displacements, population structuring may also occur in a continuous habitat; each group of reproducing individuals may be panmictic within the group but isolated to some extent from other groups. In such a case, genetic differentiation can be modeled with Wright's (1943) isolation-by-distance (IBD) model, which predicts a positive correlation between genetic differentiation and geographical distance. The correlation between the geographic locations

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of parents and their offspring is generally related to the species' dispersal properties. Species with limited dispersal capabilities generally display a high level of population structure (e.g., Barluenga and Meyer 2005). Though a species with a high dispersal capability often evolves as a panmictic population (e.g., Teterina et al. 2005; Barluenga et al. 2006), it may display low variance in distance between birth and breeding sites owing to behaviours such as natal site fidelity. Natal site fidelity is characterized by the return and the reproduction of an individual to the site of its birth (Blair and Quinn 1989). For example, anadromous fish usually show high site fidelity for reproduction (Nielsen et al. 1999; Waters et al. 2000; King et al. 2001), which is referred to as philopatry or homing behaviour. When repeated over several generations, natal site fidelity restricts gene flow and allows progressive genetic differentiation and population structuring among spawning populations, even in the absence of geographic barriers (Leider et al. 1984; Varnavskaya et al. 1994; Hendry et al. 1995).

The northern redbelly dace (*Phoxinus eos*) is a small cyprinid fish widely distributed in lakes and streams of North America (Scott and Crossman 1973). Despite its small size (average 51 mm), this species is known to have a good swimming capacity (Trudel and Boisclair 1996) and performs daily migrations between the littoral and pelagic zones of lakes (Naud and Magnan 1988; Gauthier and Boisclair 1997). While the species has the capacity to disperse throughout a lake, individuals do not display a random distribution in the littoral zone and are frequently recaptured at the same locations (Magnin et al. 1976), suggesting that this species exhibits site fidelity behaviour to some extent. However, site fidelity alone is not expected to affect genetic structure unless fish reproduce at their birth site. In lakes, spawning takes place in masses of filamentous algae along the littoral zone (Cooper 1935). Such spawning habitats are likely more abundant and continuous than spawning habitats in tributary streams, which tend to be more discrete and fragmented in distribution (Dynes et al. 1999; Strange and Stepien 2007). Genetic structure in such a species with high dispersal capability and neighbouring spawning sites would then be interpreted as a strong correlation between birth and breeding sites.

The objective of this study was to confirm site fidelity and indirectly assess reproduction at the birth site in a littoral-spawning *P. eos* population. We used ecological observations (mark-recapture (MR)) made on four sites of a lake during the reproductive period combined with genetic analyses on individuals recaptured at their site (DNA polymorphism) to address this objective. The rationale of the experiment is that a correlation between reproduction and birth sites is expected if individuals exhibit natal site fidelity. In such a case, individuals recaptured at a given site are expected to originate and reproduce at that site. If so, population genetics predict that pooling individuals from different mating sites will result in a deviation from Hardy-Weinberg equilibrium (HWE) while individuals from a given site are expected to reproduce panmictically. Secondly, in absence of barriers, individuals may reproduce at a site different from their birth place, but the number of migrants is then expected to decrease as the geographic distance between sites increases, resulting in an IBD pattern.

In the absence of natal site fidelity, individuals are expected to be randomly distributed with respect to their birth site. In such a situation, recaptures are not expected to originate at that site and will represent a single panmictic population. As a result, no deviation from HWE, no genetic differentiation, and no IBD will be observed.

Materials and methods

MR experiment

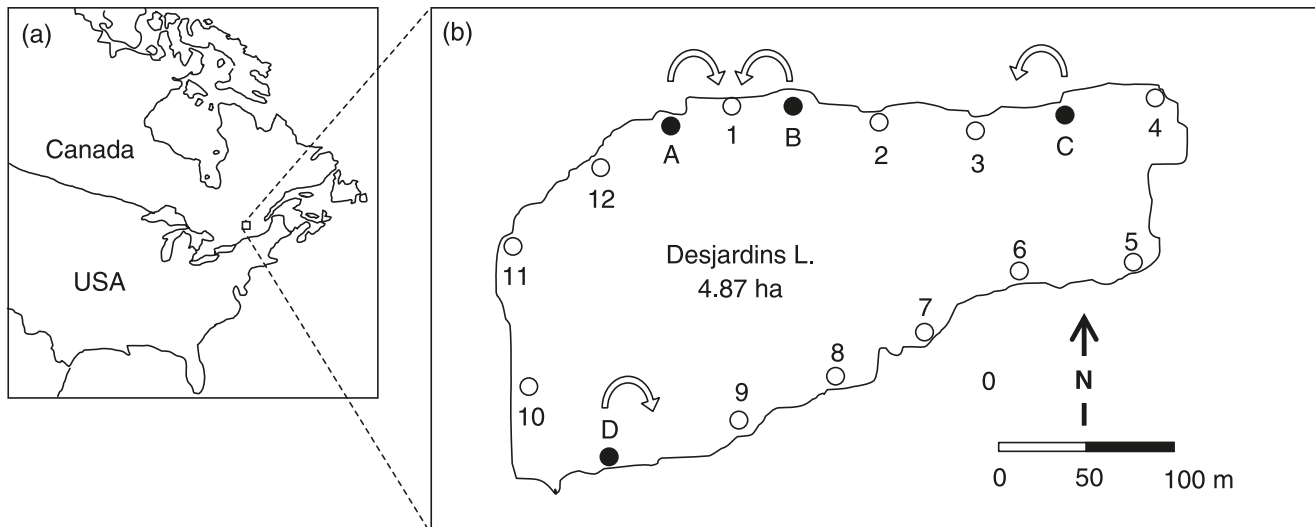
The experiment was conducted in Lake Desjardins, southern Quebec, Canada (45°54'56.1"N, 74°04'26.3"W). This lake is 4.87 ha in surface area and has a perimeter of approximately 1791 m, a mean depth of 2 m, and a maximum depth of 8 m.

To investigate site fidelity during the reproductive period, an MR experiment was performed at four distinct sites (A, B, C, D) separated by 60–400 m to examine the influence of distance on migration (Fig. 1). Four unbaited commercial minnow traps per site, separated from each other by less than 4 m, were set between 0.5 and 1.5 m of depth in the littoral zone for 1 h. The experiment was performed at the end of the spring, which corresponds to the breeding period of *Phoxinus* (Scott and Crossman 1973). According to external characteristics (Scott and Crossman 1973), most individuals observed during the experiment were sexually mature. Females had to be handled with care, since the eggs of many were easily extruded, indicating that they were ready to spawn. Similarly, most of the males had thick pectoral fins; this trait is usually an indication of mating activity, though it remains for some time after mating.

Five marking sessions were performed at each of the four sites from 27 May to 10 June 2004 (Table 1). Approximately the same number of individuals was marked at the different sites, for a total of 7115 (Table 1). Fish from each of the four sites and five marking sessions were marked in a distinctive manner with a fluorescent elastomer using an elastomer tag air-driven injection system (Northwest Marine Technology). Prior to the experiment, we assessed how this tagging method affected *P. eos* survival by keeping 50 tagged and 50 untagged individuals under observation for 3 weeks in aquaria; no mortalities occurred in either group. To confirm that site fidelity is the result of the active return of individuals to their site of capture rather than restricted displacement (e.g., Gerking 1959), fish were released 20–30 m away from their capture site after a brief period of recovery (approximately 1 h). Six recapture sessions were performed in 2004 (28–29 May; 31 May – 1 June; 2–3, 9–10, 11, 15 June). An additional sampling session was performed 1 year later (9 June 2005) to assess site fidelity over a longer period. Each fish caught was examined for the presence of the fluorescent tag. Recaptured individuals were remarked according to the site and day of sampling (during the marking sessions only), allowing us to reconstruct the complete capture history for each individual.

A multistate Cormack-Jolly-Seber (CJS) analysis was performed to estimate the movements among sites, the survival rate (the probability of the animal surviving and remaining in the sample area), and the recapture probabilities for each site. The multistate CJS model for open populations is a generalization of the CJS model (Cormack 1964; Jolly

Fig. 1. Location of the study site: (a) map of northeastern North America; (b) map of Lake Desjardins. Solid circles (A, B, C, D) correspond to marking sites; open circles (1 to 12) represent the additional capture sites; arrows indicate release sites of marked individuals.



(45° 54' 56.1" N, 74° 04' 26.3" W)

Table 1. Number of individuals marked at the four study sites for each of the five marking sessions.

Date	Marking site			
	A	B	C	D
27 May	350	169	115	162
28–29 May	300	300	577	271
31 May – 1 June	497	429	492	212
2–3 June	315	272	157	655
9–10 June	295	547	500	500
Total	1757	1717	1841	1800

1965; Seber 1965) that allows transitions among states (e.g., that individuals of a population can be distributed across multiple sites; Williams et al. 2002). We used the Markovian model to estimate the movement and the survival probabilities independently. Three models that differ in the number of parameters estimated were tested: model D computes estimates under the assumption that survival–transition probabilities and capture probabilities are constant over time, model B assumes that survival–transition probabilities are constant over time but that capture probabilities are time-dependent, and model A assumes both survival–transition and capture probabilities are time dependent. The model with the lowest AIC_c value (Akaike's information criterion corrected for small sample sizes; Burnham and Anderson 2002) was considered the most appropriate model. The analysis was performed using the MSSURVIV program (Hines 1994).

To test the hypothesis of random distribution of marked individuals, χ^2 tests were performed. Because the number of marked individuals varied among sites (especially at the beginning of the MR experiment), the expected numbers of recaptures per site for a given session were adjusted according to the proportion of marked individuals (Table 2).

Because individuals that were not recaptured might

have left their initial marking site but remained close by (restricted-movement paradigm: Rodriguez 2002), extensive sampling was performed to detect fish movements outside of the marking sites and to assess the distribution of migrants along the littoral zone. Twelve additional sites located between the marking sites (Fig. 1) were sampled following the same procedure outlined above on two different dates, 2–3 and 17–18 June 2004. Each fish caught was examined for the presence of marks and released at its site of capture. These direct observations of the number of migrants were used to assess IBD (see below).

Genetic analyses

We characterized the population of a given site by assuming that individuals recaptured at their marking site during the reproductive period reproduce at that site. A small piece of the caudal fin of recaptured individuals was taken and preserved in 95% ethanol. Total DNA was extracted from that piece of tissue by proteinase K digestion followed by phenol–chloroform purification and ethanol precipitation (Sambrook et al. 1989).

Because this lake is known to contain both *P. eos* and hybrids (*P. eos-neogaeus*) (Angers and Schlosser 2007), the proportion of each form was determined from a subsample of 266 individuals randomly selected across recapture dates. Individuals were identified using genetic markers according to Binet and Angers (2005). Briefly, hybrids are perpetuated clonally (Goddard et al. 1998) and are thus characterized by the presence of one set of chromosomes from *P. eos* and one set from *P. neogaeus*. To determine the nuclear genomic composition of an individual, an intron of the growth hormone gene and one of the mesoderm specific transcript gene were amplified by polymerase chain reaction (PCR). Primers of each locus are designed to provide PCR products of different sizes for *P. eos* and *P. neogaeus*, allowing chromosome identification. Individuals that displayed alleles of both sizes were classified as hybrids.

To assess the genetic signature of natal site fidelity, 24 to

Table 2. Number of recaptures at the four marking sites.

Recapture site	Marking site				Total
	A	B	C	D	
2004					
A	151 (20)	2	3	6	162 (2 439)
B	8	150 (27)	8	5	171 (2 724)
C	5	16	127 (21)	10	158 (3 883)
D	8	8	3	104 (7)	123 (3 282)
Total	172	176	141	125	614 (12 328)
2005					
A	15	0	0	2	17 (736)
B	4	19	2	0	25 (848)
C	1	1	11	3	16 (737)
D	2	2	3	11	18 (752)
Total	22	22	16	16	76 (3 073)

Note: The diagonal represents the number of individuals that were recaptured at their marking site. For marking site, multiple recaptures are given in parentheses; for total, numbers in parentheses refer to the total number of captures at the marking site.

44 recaptured individuals identified as *P. eos* were analyzed per site for a total of 139 individuals. These individuals were genotyped with five polymorphic microsatellite DNA loci markers developed for *P. eos*: *Pho-1* and *Pho-2* (Binet and Angers 2005) and *Pho-4*, *Pho-60*, and *Pho-61* (Angers and Schlosser 2007). PCR conditions and polymorphism detection were similar to those published in Binet and Angers (2005).

Genetic diversity was estimated with the total number of alleles (A), the allelic richness (A_R) using the rarefaction index proposed by El Mousadik and Petit (1996), and with Nei's (1987) measure of gene diversity (H_E) using the software FSTAT version 2.9.3 (Goudet 2001). The significance of differences in genetic diversity among the sites was tested using a paired t test on A , A_R , and H_E values at individual loci (Nei 1987). If natal site fidelity is occurring, individuals do not have the same probability of reproducing with each other, thus such a sample should deviate from Hardy–Weinberg expectations because of an excess of homozygotes (Raymond and Rousset 1995). On the other hand, individuals from a given site are expected to be drawn from a panmictic group that would fit with HWE predictions. This was tested by comparing the HWE of all sites taken together and each site independently using the probability test (HWE) and the global test across loci (homozygote excess) from the GENEPOP program version 3.4 (Raymond and Rousset 1995). The presence of null alleles that may generate homozygote excess and mimic the effect of non-random mating was assessed for each locus and site using the Micro-Checker package, version 2.2.3 (Van Oosterhout et al. 2003), and with the score test (U test) provided with the GENEPOP program (see Girard and Angers 2007).

To assess the consequence of natal site fidelity on genetic structure, allelic differentiation was tested over all sites with Fisher's exact test by means of a Markov chain Monte Carlo variation using the GENEPOP program (Raymond and Rousset 1995). Pairwise comparisons were also computed to identify the most different sites. F_{ST} estimator (Weir and Cockerham 1984) was measured and significance was as-

sessed by permutation of individuals using the GENETIX program, version 4.05.2 (Belkhir et al. 2004).

IBD

A major expectation of the IBD model is that the number of migrants decreases as the distance between the birth and reproductive sites increases. This was tested using the MR results as well as the genetic data. The number of migrants detected during the MR experiment was reported as the sum of all individuals recaptured within each distance interval of 75 m from a marking site. The effective number of migrants exchanged per generation (N_{Em}) between each pair of sites was estimated with the private alleles method (Slatkin 1985; Barton and Slatkin 1986) using the GENEPOP program (Raymond and Rousset 1995). This estimation is recommended when the populations does not fit with an island model of migration or migration–drift equilibrium (Barton and Slatkin 1986). Distance between sites was measured along the littoral zone. The significance of the correlation between the number of migrants and the distance between sites was tested with the Pearson coefficient for MR results and with the Mantel test (Mantel 1967) for N_{Em} .

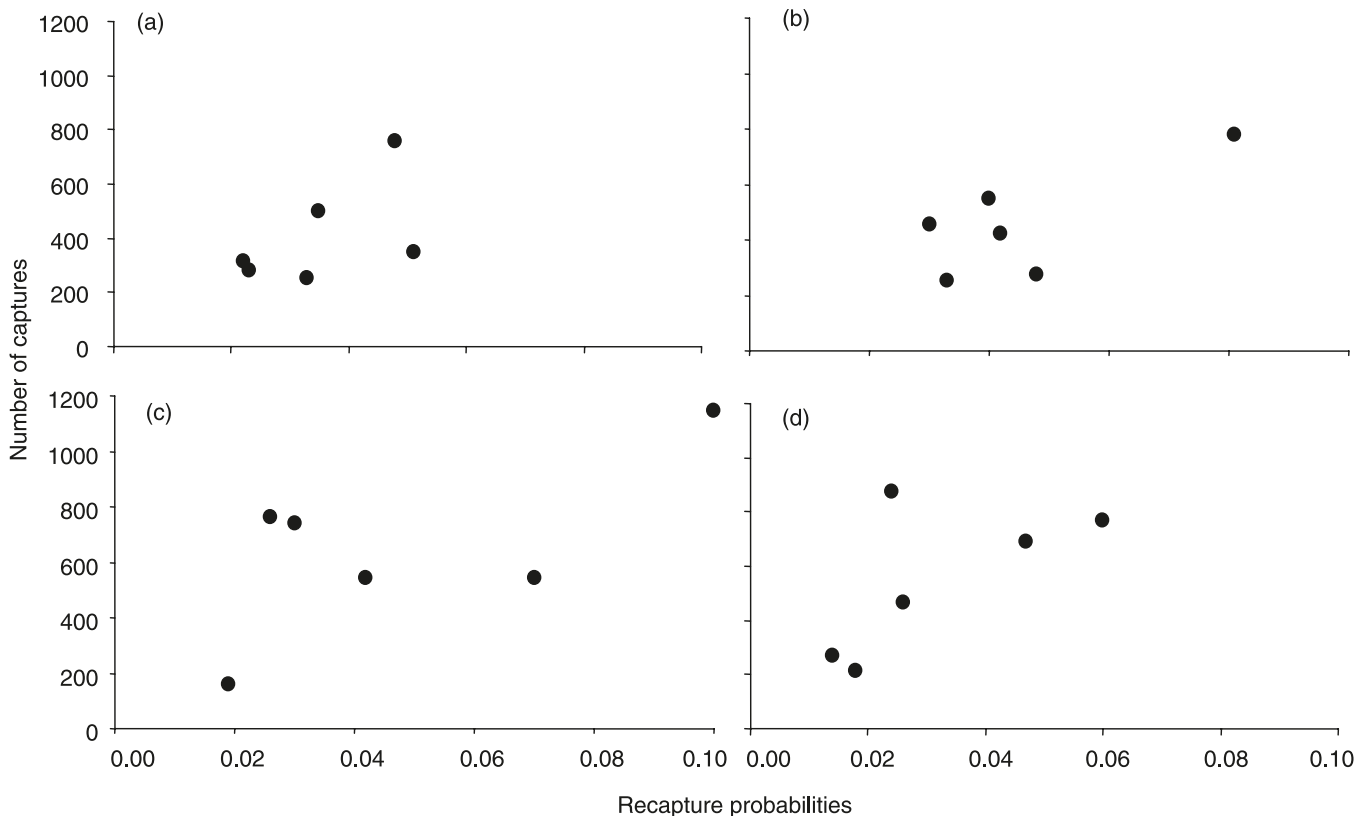
Results

MR experiment

A total of 614 recaptures were made at the four marking sites in 2004 (Table 2). Of these recaptures, 532 (86.6%) were caught at their original marking site in 2004 (Table 2). Furthermore, 63 individuals (11.8%) were captured at their marking site a third time and 12 (2.25%) a fourth time. Similarly, a high proportion of individuals (56 of the 76 recaptures, 74%) were again found on their marking site 1 year after the marking experiment (Table 2).

The model assuming that survival and movement probabilities are constant over time but that recapture probabilities are time dependent (model B) provided the lowest AIC_c value. The result of the multistate CJS analysis indicates that the survival probabilities were high and constant for

Fig. 2. Relationships between the recapture probabilities (multistate Cormack–Jolly–Seber analysis) and the total number of captures for each marking site (A, B, C, D) during the six recapture sessions in 2004.



each site (A: 0.93, B: 0.98, C: 0.76, D: 0.87). These high probabilities indicate that individuals have a high probability of surviving and remaining at their marking site. However, the estimated recapture probabilities are very low (mean \pm standard deviation (SD) = 0.04 ± 0.02), indicating that if an individual was alive and present at a given site, it has a low probability of being recaptured. According to the model selected, recapture probabilities are time dependent, ranging from 0.014 to 0.1, but appear to be related to the success of capture (Fig. 2). In a closed population, recapture probabilities depend on both population size and the number of individuals caught (Davidson and Armstrong 2002).

The result of the multistate CJS analysis indicates that movement probabilities among sites (the probability of an individual to migrate to another site) are very low (Table 3). For example, the probability that an individual marked at site A would move to sites B, C, or D is 0.021, 0.010, and 0.023, respectively. The χ^2 tests used to test for a random distribution of individuals were highly significant for each site and each sampling day in 2004 and 2005 ($P < 0.001$), except for two sites on the first date of recapture (χ^2_3 , site C: $P = 0.216$; site D: $P = 0.04$).

HWE and differentiation

The microsatellite loci were extremely variable and exhibited between 6 and 17 alleles in the 139 individuals recaptured at their marking site (Table 4; Fig. 3). A per site varied from 43 to 48 (Table 5). The difference between the mean A per site (45) and the total A (60) was not observed

on either A_R (41.1 vs. 41.2, Table 5) or H_E (0.7824 vs. 0.7843, Table 5). No significant difference among sites was detected for A ($P > 0.326$), A_R ($P > 0.465$), or H_E ($P > 0.391$). These results indicate heterogeneous distribution of alleles (especially rare alleles that have a minor influence on H_E), but similar levels of diversity among sites.

When all individuals recaptured were considered as a single population, a significant departure from HWE (probability test over all loci: $P = 0.0364$) and a homozygote excess (global test: $P = 0.0163$) were observed (Table 5). This indicates that individuals sampled throughout the lake did not represent a panmictic population. When tested individually, two loci (*Pho-2*, $P = 0.0481$; and *Pho-1*, $P = 0.0650$) displayed significant or nearly significant departure, indicating that significant departure over all loci was not the result of a single marker. On the other hand, individuals recaptured at their marking sites appear to reproduce as a panmictic unit, as no departure from HWE was detected (Table 5). The tests failed to detect null alleles within sites at any of the loci analyzed.

The global test of allelic differentiation is significant ($P = 0.0256$). This suggests that the sites, or at least some of them, represent distinct reproductive units with restricted gene flow between them. Over all loci, significant results of allelic differentiation were observed for two pairwise comparisons (A–C, $P = 0.0158$; A–D, $P = 0.0195$), but these results are no longer significant after sequential Bonferroni correction. These tests also revealed that all pairs of sites differed at least at one locus, and all loci were different for

Table 3. Movement probabilities (\pm standard deviation, SD) between the four marking sites.

Marking site	Recapture site			
	A	B	C	D
A	0.946 \pm 0.01	0.021 \pm 0.007	0.01 \pm 0.005	0.023 \pm 0.009
B	0.0043 \pm 0.003	0.935 \pm 0.02	0.036 \pm 0.01	0.025 \pm 0.009
C	0.009 \pm 0.006	0.035 \pm 0.01	0.927 \pm 0.02	0.029 \pm 0.01
D	0.019 \pm 0.008	0.013 \pm 0.007	0.036 \pm 0.01	0.932 \pm 0.02

Table 4. Characteristics of genetic markers used in this study.

Locus	Diversity indices			Null allele tests	
	A	H_O	H_E	M-C	UT
<i>Pho-1</i>	15 (10.5 \pm 1.3)	0.8613 (0.8642 \pm 0.0637)	0.8426 (0.8404 \pm 0.0117)	No	\geq 0.1210
<i>Pho-2</i>	17 (13.8 \pm 2.1)	0.8582 (0.8574 \pm 0.0256)	0.8927 (0.8913 \pm 0.0128)	No	\geq 0.0868
<i>Pho-4</i>	6 (5.5 \pm 0.6)	0.6978 (0.6886 \pm 0.0522)	0.7326 (0.7285 \pm 0.0440)	No	\geq 0.0742
<i>Pho-60</i>	11 (8 \pm 0.8)	0.7742 (0.7873 \pm 0.0904)	0.7738 (0.7665 \pm 0.0384)	No	\geq 0.0819
<i>Pho-61</i>	11 (7.8 \pm 1.3)	0.6379 (0.6376 \pm 0.0332)	0.6800 (0.6853 \pm 0.0586)	No	\geq 0.1564

Note: For each locus, data is given for the number of alleles (A), observed heterozygosity (H_O), Nei's gene diversity (H_E) over all sites (mean \pm standard deviation, SD). Results of the null allele tests performed on each site are also given (M-C, Micro-Checker; UT, U test).

at least one pair of sites. These results indicated a very low divergence among sites, but altogether, the sites did not represent a genetically uniform population. The F_{ST} estimate over all sites is consistent with this interpretation, being very low (0.00530) but significant after permutation of individuals ($P = 0.0458$).

Migration and IBID

The littoral sampling over the 16 sites (including the four marking sites) allowed the recapture of 129 and 238 individuals for the first and the second sampling sessions, respectively. Of these recaptures, 73 (56.6%) and 134 (56.3%) were caught at their marking site during the first and the second sampling sessions, respectively. When considering the number of captures per site, the proportion of faithful individuals (2–3 June: 73/1434 = 5.09%; 17–18 June: 134/1510 = 8.87%) was higher than the total number of migrants captured at the 15 other sites (2–3 June: 56/4853 = 1.15%; 17–18 June: 104/7027 = 1.48%). Chi-squared tests performed between faithful individuals and migrants are highly significant for both periods ($\chi^2 > 80$, $P > 0.00001$).

These results indicate that the migrants are not randomly distributed along the littoral zone. In addition, we observed a decrease in the number of migrants with an increase in the distance between marking and capture sites for both the MR results and the genetic data (Fig. 4). The correlation between the number of migrants (log) estimated by the MR experiment and the distance (log) between the marking site and the recapture site (excluding recaptures at marking sites) is high and significant for both dates (2–3 June 2004: $r = -0.856$, $P = 0.015$; 17–18 June 2004: $r = -0.986$, $P < 0.0001$). The number of migrants per generation (N_{EM}) estimated with the private alleles method varied from 4.5 to 9.5 between sites. The Mantel test between N_{EM} and distance between sites (Fig. 4) is also highly significant ($r = -0.951$, $P < 0.0001$).

Discussion

Site fidelity

The MR experiments confirmed previous observations (Magnin et al. 1976) that *P. eos* does not distribute randomly along the littoral zone but rather exhibits site fidelity. Most individuals were recaptured at their marking site, and several were recaptured three or four times at the same location. Given the low recapture probabilities, recapturing individuals several times at the same site indicates that there is a strong propensity for individuals to return to their site of capture. The very low movement probability among sites that was estimated by the multistate CJS analysis confirmed that there is a higher probability of recapturing an individual at its marking site than at any other sample site in the lake.

The results of this study also confirm that site fidelity is not the result of restricted displacement during the reproductive period (e.g., Gerking 1959; Hert 1992). Recaptures of the marked individuals in all sites along the littoral zone show that this cyprinid has a high displacement capacity (Trudel and Boisclair 1996). Nevertheless, most individuals were recaptured at their marking site even though they had been displaced by 20–30 m. For example, individuals marked at sites A and B (separated by 60 m) were released midway between the two sites. Individuals were mostly recaptured at their marking sites, while very few were recaptured at the release site. This indicates that fish left the release site and actively returned to their site of capture, showing evidence of site fidelity. In addition, the recapture of individuals at their marking sites 1 year after the marking session clearly indicates that *P. eos* shows natal site fidelity and possesses mechanisms allowing site recognition in the littoral zone. Even though these mechanisms have been extensively studied in salmonid species, they are poorly understood in other freshwater fish species (e.g., Werner and Lannoo 1994).

Fig. 3. Allelic frequencies at the five microsatellite loci for the sampled sites. Each circle represents one allele and the size of the circle represents its frequency. The y axis indicates relative allele size. Letters correspond to sites from Lake Desjardins (A, B, C, D).

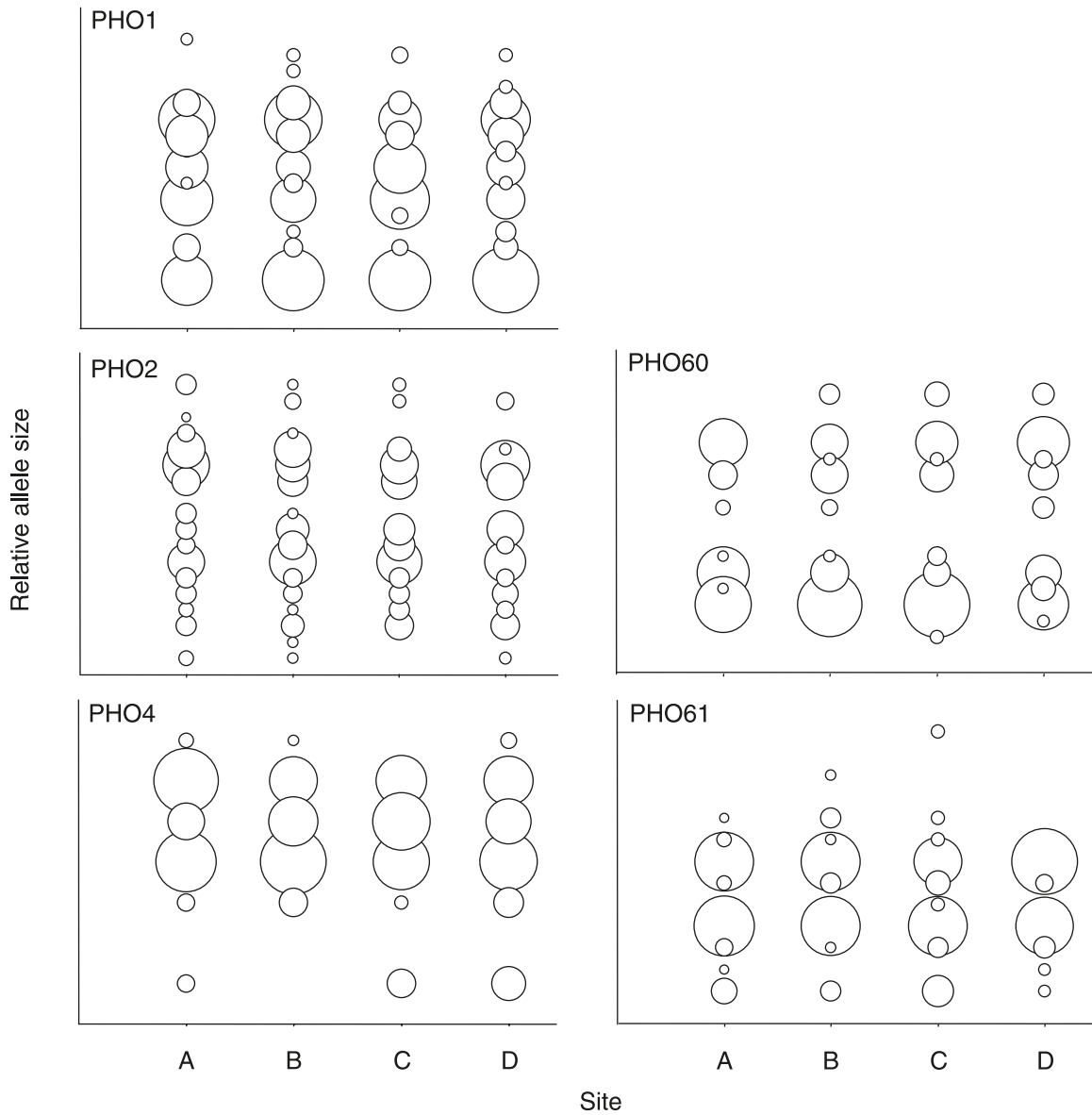
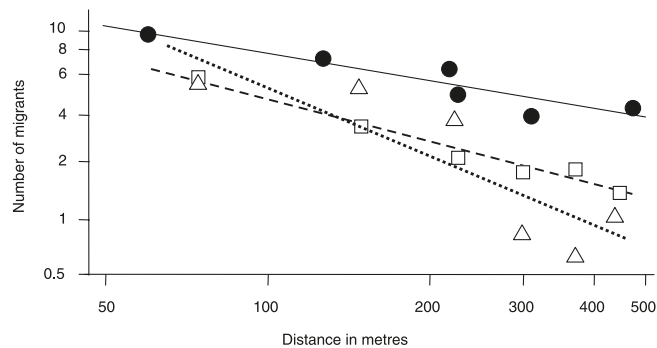


Table 5. Number of individuals analyzed (*n*), number of alleles (*A*) (allelic richness (*A_R*) in parentheses) estimated from 21 individuals, observed heterozygosity (*H_O*) and Nei's gene diversity (*H_E*) over all loci, and *P* value of Hardy-Weinberg equilibrium (HWE) probability test and global test across loci (homozygote excess).

Site	<i>n</i>	<i>A</i> (<i>A_R</i>)	<i>H_O</i>	<i>H_E</i>	HWE probability test	Homozygote excess
A	44	46 (39.6)	0.7689	0.7720	0.7591	0.1124
B	37	48 (41.3)	0.7439	0.7751	0.3854	0.3494
C	24	43 (41.9)	0.7917	0.7926	0.1395	0.4135
D	34	45 (41.5)	0.7636	0.7898	0.9297	0.0851
Total	139	60 (41.2)	0.7659	0.7843	0.0364*	0.0163*
Mean	34.8	45.5 (41.1)	0.7670	0.7824		

**P* < 0.05.

Fig. 4. Relationships between the number of migrants and the distance between marking and capture sites. Triangles and squares refer to recaptures made during the littoral sampling of 2–3 June and 17–18 June 2004, respectively. Solid circles refer to the number of migrants per generation ($N_E m$) estimated from genetic data.



The high survival probability (which includes the probability of individuals to be present at their site) indicates that the low recapture success is not the result of high mortality and (or) movement of individuals to other sites. Elastomer tags have been successfully used with many organisms, including fishes (Dewey and Zigler 1996; Frederic 1997), and we observed no mortality in either tagged or untagged fish after 3 weeks in aquaria. The low recapture success thus appears to be due to the high population density. Assuming each site to be a closed population, size estimates (number of captures divided by recapture probability; Davidson and Armstrong 2002) varied from 10 000 to 18 800 individuals per site. While these estimates must be considered with caution, they reveal the high population density in the littoral zone. The passive capture of an average of 87 to 139 individuals per trap per hour and the increase of recapture probability with the number of captures are consistent with the high population density hypothesis.

Natal site fidelity

Genetic analyses confirmed that the site fidelity observed in the littoral zone during the reproductive period of *P. eos* is more specifically natal site fidelity. While a direct assessment of natal site fidelity would have been to tag fish before they left their birth site and show that individuals spawn at the site they were born, genetic analyses provide an indirect assessment of natal site fidelity. The HWE tests clearly revealed that each local site (or at least some of them), and not the whole lake, is organized as a panmictic reproductive unit. Such results could occur only if individuals return to their birth site for reproduction. If individuals chose their spawning sites randomly, the entire population would have evolved as a single panmictic unit, even if these individuals subsequently returned to the same sites. These results are consistent with the numerous studies that report natal site fidelity in freshwater fish species (L'Abée-Lund and Vallestad 1985; Crossman 1990; Ridgway et al. 1991), though the phenomenon has rarely been confirmed with genetic analyses (Gross et al. 1994; Miller et al. 2001; Adams et al. 2006).

Given the scale of the study site, the presence of numerous spawning sites along the littoral area, and the high population density, differentiation among sampling sites, even if very small, reflects the natal site fidelity behaviour in this

species. However, the fact that HWE over all individuals, allelic differentiation, and F_{ST} provide significant results revealed that gene flow among sites is not high enough to homogenize the genetic composition of sites (at least some of them). The very low levels of differentiation is a hallmark of recent intralacustrine populations of fish; F_{ST} values among populations that diverge since the end of Pleistocene ranging from 0.002 to 0.043 were observed for sockeye salmon (*Oncorhynchus nerka*) (Varnavskaya et al. 1994), while a value of 0.03 was reported for stone loach (*Barbatula barbatula*) of Lake Constance (Barluenga and Meyer 2005) and 0.029 was found for walleye (*Sander vitreus vitreus*) in the Great Lakes (Strange and Stepien 2007). Examples of large differentiation generally occur when multiple evolutionary groups coexist, such as the result of colonization from distinct glacial origins (e.g., Barluenga and Meyer 2005; Fraser and Bernatchez 2005). Intralacustrine populations are also generally characterized by scarce and discrete spawning sites (Dynes et al. 1999; Miller et al. 2001; Strange and Stepien 2007). The presence of a few discrete spawning zones may restrict gene flow among spawning sites, as the probability of straying is expected to be reduced in the absence of alternative spawning sites near the birth site.

The pattern of IBD detected from genetic polymorphism represents concrete evidence of the long-term organization resulting from natal site fidelity. Our results indicate that most of the individuals reproduce at their birth site and that migrants reproduce less frequently as the distance between the birth and breeding sites increases. The number of migrants estimated from genetic data are expected to differ from those based on the MR experiment; the former integrate over a long period the effects of migrants that are reproducing, while the latter represent a snapshot of the migration. Nevertheless, the slopes of both estimates are consistent with what would be expected if the number of migrants that reproduced successfully in the new habitat were proportional to the total number of migrants. The extensive recapture sessions conducted to detect fish movements outside of the marking sites confirmed that a substantial number of individuals moved along the littoral zone after being marked, but that their distribution was not random. Most individuals were recaptured close to their marking site, and their abundance decreased as the distance from the marking site increased.

In conclusion, this study reveals that in spite of a continuous distribution in the littoral zone and a high dispersal capability, fish may display a structured genetic organization at a very fine geographical scale. To our knowledge, the northern redbelly dace from Lake Desjardins shows an IBD pattern occurring at a much more restricted geographical scale than what has been previously observed. However, studies of IBD at a fine geographical scale are not common in temperate fish (Carlsson and Nilsson 2000; Barluenga and Meyer 2005). We believe that this species represents an excellent model for the study of the evolutionary perspectives of natal site fidelity and of the empirical factors characterizing IBD, such as population density (Gandon and Rousset 1999), distance between sites and barriers to dispersal (Taylor et al. 2003), and time since colonization (Crispo and Hendry 2005). Because the lakes inhabited by *P. eos* present very different characteristics, this species allows the investigation of many of these aspects.

Additional features characteristic of this species make natal site fidelity of particular interest. First, this species displays a large variability in phenotypic characters (Schlosser et al. 1998). Natal site fidelity may then facilitate morphological differentiation and the formation of local adaptations within a given lake (Taylor 1991). Second, in the *Phoxinus eos-neogaeus* complex, the clonal hybrids are generally found in sympatry with *P. eos* (Angers and Schlosser 2007) and depend on their sperm for perpetuation (Goddard and Dawley 1990). While a sexual species is expected to have a larger ecological niche than asexual hybrids (Vrijenhoek 1994), natal site fidelity may reinforce the spatial isolation of a portion of the sexual species during the reproduction and prevent their extinction.

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