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Patterns of genetic structure of Eurasian perch (*Perca fluviatilis* L.) in Lake Geneva at the end of the spawning season

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ABSTRACT

The study provides the first analysis of the genetic structure of an economically and ecologically important fish species, Eurasian perch, in Lake Geneva. Twelve microsatellite markers were analyzed for 394 fish sampled at the end of the spawning season from seven sites located all around the lake (between 42 and 61 fish per site). The measure of genetic differentiation, F_{ST}, was low overall (0.0035), but significant for twelve pairwise site comparisons. In contrast, Bayesian cluster analyses indicated no support for the presence of more than one distinct genetic cluster. We conclude that there is only one population in the lake. Therefore, the Eurasian perch should be managed as a single stock.

Introduction

The Eurasian perch, *Perca fluviatilis*, is a temperate freshwater fish species found throughout central and northern Europe, except in southern regions, such as the Iberian Peninsula, central Italy, and the Adriatic and Aegean basins (Kottelat and Freyhof, 2007). Eurasian perch is commercially important in Western Europe, especially in Alpine regions as well as in Benelux and Scandinavian countries (Fontaine, 2004; Fontaine et al., 2009). However, harvest is highly variable (both in quality and quantity) because it depends on environmental conditions (Fontaine, 2004). In addition to commercial fisheries, perch farming started about two decades ago (Fontaine et al., 2009). By 2012, perch aquaculture production rose to 435 tons (FAO, 2014, www.fao.org). In addition to its socio-economic importance, perch is also used as a bioindicator in environmental monitoring studies (Neuman and Sandström, 1996; Olsson et al., 2011). Despite its commercial importance and use as a bioindicator, few studies have examined the genetic variability of *P. fluviatilis*.

Bergek and Björklund (2009) and Olsson et al. (2011) studied the genetic structure of perch populations from the Baltic Sea. Along the Swedish coast and in the archipelago of the Baltic Sea, perch populations were genetically differentiated following a model of isolation by distance. Along the west coast of the Baltic Sea, deep water might represent a barrier to gene flow, leading to differentiation between perch from the North (Gulf of Bothnia) and those from the central area of the sea. However, this differentiation was not strong enough to separate the perch into two different populations (Olsson et al., 2011). In studies of perch populations in lakes, Gerlach et al. (2001) and Bergek and Björklund (2007) reported different patterns, two populations were observed within Lake Constance (Germany) and nine within Lake Erken (Sweden).

Lake Geneva, located between France and Switzerland (46°26′N, 6°33′E), is the largest Alpine and Subalpine lake in Central Europe. It is also the largest natural lake in Western Europe; its volume is approximately 89 km^3 with a surface area of 580 km^2 and a maximum depth of 309 m. The main inflow of Lake Geneva is the Rhône River, which rises in the Alps and flows into the Mediterranean Sea. It is fed by rivers and snow melt from the Alps and the Jura mountain chains (Assouline et al., 2008). This monomictic and mesotrophic lake (Assouline et al., 2008; Gerdeaux, 2011) is generally divided into two sub-lakes: the eastern Upper Lake (86% of the total area) and the western Lower Lake (14% of the total area) (see Fig. 1; Commission Internationale pour la Protection des Eaux du Léman, 2012, www.cipel.org). Eurasian perch have been found in Lake Geneva since the last glacial period; and no records of perch stocking could be found. Catch rates for all species in Lake Geneva are variable but have not exceeded 1000 tons per year from 1976 to 2010. Wild perch catches represented 66.2% of the total volume, and declined to 40.8% between 2006 and 2010,

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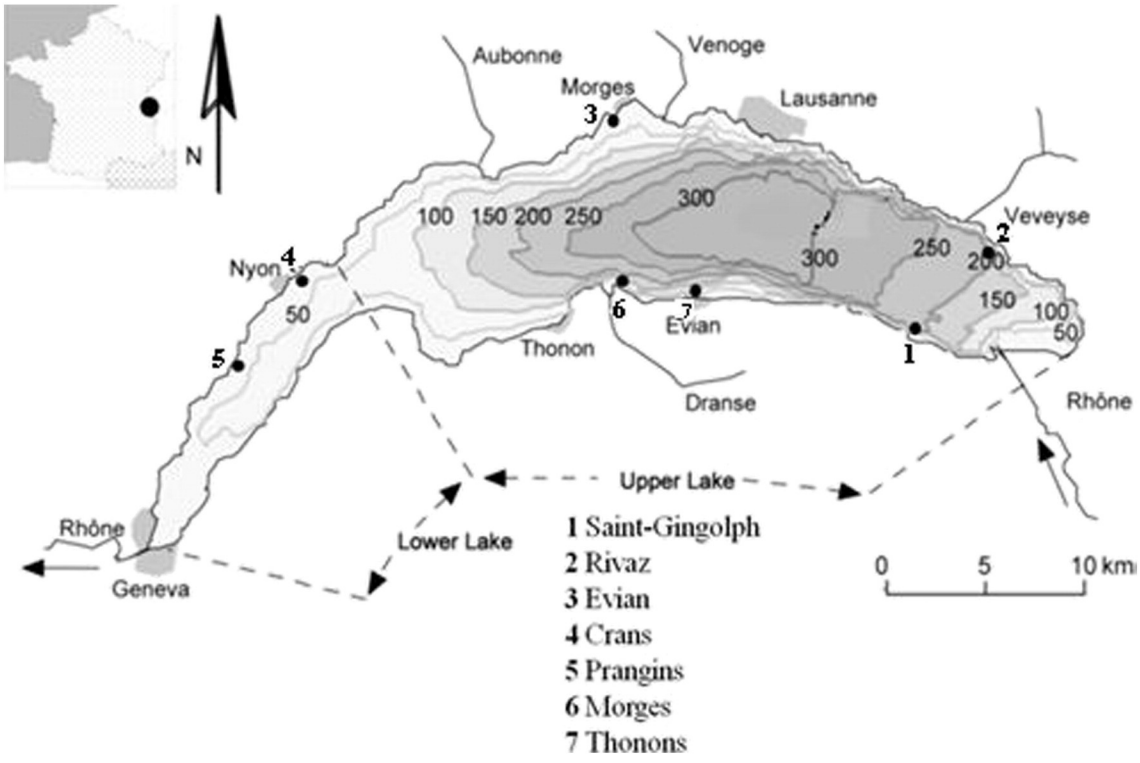

Fig. 1. Map showing the seven sampling sites around Lake Geneva.

amounting to 250 tons per year (Commission Internationale pour la Protection des Eaux du Léman, 2012, www.cipel.org). In addition to fisheries, wild perch inhabiting Lake Geneva are also used to develop aquaculture production both in French and Swiss farms.

A better understanding of the genetic structure of perch populations within Lake Geneva would be valuable for both fisheries and domestication process management. In the latter case, uncontrolled choice of wild breeders may alter genetic variability, thus reducing the potential of selection available for domestication (Vandeputte and Launey, 2004). This study described the genetic structure of Eurasian perch in Lake Geneva using 12 polymorphic microsatellite markers.

Materials and methods

Fish sampling

As perch can exhibit a homing behavior (Järv, 2000; Zomora and Moreno-Amich, 2002) and return to their natal site to spawn, the genetic structure was evaluated at the end of the spawning season. Perch were sampled during the first 4 days of June 2012, i.e., at the end of the spawning season which is closed to fishing (the month of May). Fish were collected at seven sites around the lake (Fig. 1). Sample sizes varied from 42 specimens in Thonon-Les-Bains to 61 in Saint-Gingolph and Clarens. Fish were caught by professional fishermen using seine nets, gill nets or traps, at a depth of 5 to 20 m. Fish size varied from 8.0 to 39.3 cm. Fin clips were stored in 95% ethanol for genetic analysis.

DNA extraction, amplification, and microsatellite genotyping

DNA was extracted with a modified high-salt DNA extraction protocol (Aljanabi and Martinez, 1997). Twelve microsatellite markers were used: *PflaL1, PflaL2, PflaL4, PflaL6, PflaL9, PflaL10* (Leclerc et al., 2000), and *YP60, YP78, YP111* (Li et al., 2007) that were previously developed for yellow perch (*Perca flavescens*); *SviL7* (Wirth et al., 1999), and *Svi17, Svi18* (Borer et al., 1999) that were developed for walleye (*Sander vitreus*). For each sample, PCR and genotyping were conducted in four multiplexes using the QIAGEN Multiplex reactions PCR Plus Kit and fluorescently labeled primers (VIC, NED, 6-FAM and PET). The design of multiplex was based on the size ranges of the different loci to avoid multiplexing loci with overlapping allele size ranges that were labeled with the same fluorescent dye and on the optimized annealing temperatures (Table 1). PCR reactions were carried out in a total volume of 10 μL containing 1 μL of genomic DNA (40 ng/μL), 5 μL of Master mix (Qiagen), and 1 μL of primer mix at a concentration recommended by the kit's manufacturer. Amplifications were performed in a BioRad DNA thermal cycler as follows: 5 min at 95 °C, followed by 30 cycles of 30 s at 95 °C, 90 s at the annealing temperature, and 30 s at 72 °C, with a final extension of 45 min at 60 °C. PCR products for each sample (3 μL) were mixed with 15 μL of Hi-Di™ Formamide and 0.2 μL of the size standard GeneScan™ 600 LIZ® (Applied Biosystems). They were then genotyped and visualized using an ABI 3130XL Prism automated sequencer.

Genetic analysis

Allele sizes were determined with GENEMAPPER 4.0 software. Potential errors (allelic dropouts, stuttering or null alleles) were investigated with the MICROCHECKER software (Van Oosterhout et al., 2004). Linkage disequilibrium and conformation to *HWE* were estimated between loci and at each locus by exact tests with 1000 Markov Chain iterations under the null hypothesis of identical allelic distribution between samples using GENEPOP 4.2.1 (Raymond and Rousset, 1995). Deviations from *HWE* for each locus and each site were calculated as a chi-square test of observed and expected heterozygosity.

To assess the level of genetic diversity, mean number of alleles per locus (A), observed heterozygosity (H_o), and expected heterozygosity (H_e) (Nei, 1978) were determined with GENETIX 4.05 (Belkhir et al., 2004). The allelic richness (Ar) and private alleles (Ap) were estimated with HP-RARE 1.1 a program that compensates for sampling disparity using rarefaction (Kalinowski, 2005). Global and specific Wright's F-statistics between sites (F_{IS}, F_{ST} and F_{IT}) were calculated according to

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