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Microsatellite polymorphism in Italian populations of northern pike (*Esox lucius* L.)

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This work is dedicated to the memory of our friend Mario Panico that with patience and dedication has helped us with the treatment of Pike specimens.

Abstract

Northern pike (*Esox lucius*) is not considered an endangered species in Italy, but since recent studies indicate the decline of this population, conservation and management strategies based on the genetic differentiation of natural northern pike populations are needed. In this paper, genetic diversity was analysed in 10 Italian and 2 East European northern pike populations by means of seven microsatellite loci. Data indicated an appreciable genetic differentiation, in spite of a low genetic variation, and agreed with the low level of genetic polymorphism already observed for this species in North America and North Europe. Results of statistical tests revealed genetic peculiarities of the Italian populations, even though signals of recent contact between populations were found and discussed in relation to anthropic impacts, particularly to the stocking practice. This investigation represents the first approach to the knowledge of the genetic variability of Italian pike populations using microsatellite markers, and reported results could be of interest for future management and conservation programmes of this species in Italy.

Keywords: *Esox lucius*; Northern pike; Microsatellite; Fish management; Conservation genetics; Genetic diversity

1. Introduction

The taxonomy of Esocidae, mainly based on morphological characters, indicates the existence of five species distributed in Eurasia and North America. Only northern pike (*Esox lucius* Linnaeus, 1758) is present in Europe (Ladiges and Vogt, 1979) with a broad geographical and environmental distribution, including most of the northern temperate zones, above 40° latitude up to the Arctic zone, and Italy, Greece and Turkey. Northern pike being a big, edible, top predator is one of the most economically important freshwater fish for recreational and commercial fisheries and freshwater ecosystems management (Senanan and Kapuscinski, 2000; Kovàcs et al., 2001). Although pike is not yet considered an endangered species in Italy, and does not appear in the IUNC Red List of Threatened Species (2003 IUNC: www.redlist.org/), recent studies indicate a population decline (Lorenzoni et al., 2002). Similar conclusions on other North-European populations have been reported (Kovàcs et al., 2001; Launey et al., 2003). To date, genetic studies on pike populations have been carried out through polymorphism analyses at allozyme loci (Healy and Mulcay, 1980; Seeb et al., 1987), VNTR markers (Hansen et al., 1999) and mitochondrial DNA (Brzuzan et al., 1998; Maes et al., 2003; Nicod et al., 2004). Recently microsatellite loci were identified for pike, allowing the assessment of genetic variations within the same hydrographic system and between different ones (Miller and Kapuscinski, 1996, 1997; Hansen et al., 1999; Senanan and Kapuscinski, 2000; Laikre et al., 2005; Jacobsen et al., 2005; Aguilar et al., 2005), and they seemed able to detect a higher variation as compared to other techniques (Laikre et al., 2005). Nevertheless, microsatellites also pointed out very low values of genetic polymorphism for this species (Laikre et al., 2005).

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Besides, some authors (Nicod et al., 2004; Jacobsen et al., 2005; Larsen et al., 2005) underlined the importance of evolution in relation to either glacial bottlenecks or post-glacial founder phenomena. However, to date no genetic studies have been published concerning Italian pike populations, although the geographical position of the Italian peninsula might have rendered it an important fish refugium during the last glaciation (Garcia-Marin et al., 1999; Hewitt, 2000). Furthermore, public authorities started special management programmes of this species in some Italian freshwater ecosystems, using local breeders and juvenile dissemination. The success of these programmes depends on the knowledge of the genetic structure of the fish populations to be managed (Nielsen and Kenchington, 2001) and on the breeders selection. It follows that the genetic monitoring of natural pike populations is fundamental to assess genetic diversity and to identify patterns that must be avoided in the breeders selection. On the basis of these considerations, and in order to extend our knowledge to the arctic (South Europe) region, this paper addresses the microsatellite differentiation of eight pike populations inhabiting Central (Tiber drainage) and Northern Italy (Po drainage) compared with two populations from the Danube drainage. Some considerations on conservation and management of Italian pike populations are also reported.

2. Materials and methods

2.1. Pike populations sampled

Sample locations were selected on the basis of Italian geomorphology, showing a clear geographic barrier, the Apennines, separating the peninsula into a central zone, where the Tiber hydrographic system is located, and in a northern zone, where the larger Po basin is located. In addition, Italian fish populations, including northern pike, were separated from the Central Europe ones by the Alps barrier (Fig. 1). The North Italian pike populations studied were located in the upstream trait of Po and Adda Rivers and in Maggiore and Segrino Lakes (Po drainage). Specimens from Central Italian

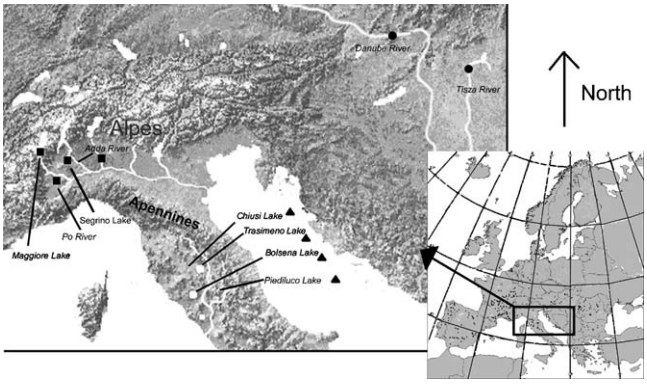

Fig. 1. Map reporting the collection sites of the 10 analysed northern pike populations. Danube drainage (●), Po drainage (■), Tiber drainage (▲).

populations were sampled from Trasimeno, Chiusi, Piediluco and Bolsena Lakes (Tiber drainage). The two Danube basin populations were sampled from the Danube River (Slovak Republic) and from its Hungarian tributary (Tisza River) (Fig. 1). Fish samples were provided by local authorities, fisheries co-operatives or colleagues. Fresh scales specimens or a small piece of caudal fin (10 mg) were removed and stored at −20 °C in absolute ethanol until processing. Sample size ranged from 11 to 50 individuals per population for a total 303 individuals (Table 1). For a sub-sample of 10 individuals, tests were performed also through a comparison with control DNA extracted from internal tissues (liver and muscles) of animals destined to human nutrition.

2.2. Microsatellite polymorphism analysis

DNA was extracted from the distal portion of the caudal fin or from dried scales using Wizard Genomic DNA purification Kit (Promega). For the fin tissue, a standard tissue Promega protocol was used; for scales, an over-night pre-lysis in the Nuclei Lysis Solution (Promega) was followed by processing through a standard Promega protocol (Lucentini et al., 2006b). The final DNA preparation was resuspended in the DNA rehydration solution supplied with the extraction kit. DNA concentration was estimated by 1.0% agarose gel electrophoresis, compared with lambda DNA standards (Invitrogen) and by spectrophotometric assay (GeneQuant, Amersham Biosciences). Only high-quality DNA was used for PCR amplification of seven microsatellite loci: Elu19, Elu51, Elu76, Elu78, Elu87, Elu276 (Miller and Kapuscinski, 1996, 1997) and EluB38INRA (Launey et al., 2003). The PCR programme (Lucentini et al., 2006a) had an initial phase of 94 °C for 3 min, 30 cycles carried out at 94 °C for 30 s, annealing temperature for 40 s (58 or 60 °C), 72 °C for 30 s and a final extension phase of 72 °C for 20 min in a Biometra T-Personal Thermal Cycler. A 25 ng of DNA were amplified using Ready-To-Go PCR Beads (Amersham Pharmacia), 25 pmol of each primer in a total volume of 25 μl. Forward primers were marked using fluorochromes NED, FAM and VIC (Applied Biosystems). The amplicons were run on a ABI377 sequencer (Applied Biosystems) in presence of 400-ROX HD (Applied Biosystems). Data were analysed through Genographer software (http://hordeum.oscs.montana.edu/genographer). Alleles were designated according to their sizes and were filed in a Microsoft Excel sheet as a binary two-columns format. The data matrix was transformed in GENEPOP 3.3 (Raymond and Rousset, 1995), Arlequin 2000 (Schneider et al., 2000) and F-STAT 2.9.3.2 (Goudet, 1995) data format through the Microsatellites toolkit, a specific patch for Microsoft Excel (Park, 2001).

2.3. Data analysis

To evaluate genotyping errors, and to characterize allelic dropout or misprinting, all the experiments were replicated as