FIRST RECORD OF THE CISALPINE PIKE *ESOX CISALPINUS* BIANCO & DELMASTRO, 2011 IN LIGURIA (NW ITALY): FUTURE STUDIES AND STORAGE PERSPECTIVES.

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ABSTRACT

The first record of the cisalpine pike *Esox cisalpinus* Bianco & Delmastro 2011 in the lower Magra River Basin (E Liguria, NW Italy) is reported. The finding of a dead pike specimen in a fyke net in Laghi Gemelli inside the Montemarcello-Magra-Vara Natural Park holds conservation and management implications for the distribution and definition of native fish communities within ichthyological districts in Italy in view of application of the European Water Framework Directive.

KEY WORDS

Cisalpine esox, Esocidae, Liguria, genetic species identification, management

INTRODUCTION

Habitat alterations, pollution, and water withdrawal endanger nearly all native freshwater species (Jenkins, 2003; Dudgeon et al., 2006). Under the pressure of the ubiquitous spread of exotic species, interspecies relationships (predation, competition for ecological niches or trophic resources, hybridization) between native and non-native taxa have become a growing threat to species conservation (Kolar & Lodge,

2001; Leprieur et al., 2008; Pysek et al., 2010). Impoverishment of phenotypic and genetic diversity of native population consequent to these interactions is well documented for several freshwater fish species mainly within the Salmonidae family (Susnik et al., 2007; Meraner et al., 2010).

Genus *Esox* (Esocidae, Esociformes, Actinopterygii), the only one still alive in the small Esocidae family (Lucentini et al., 2011), is currently represented by two species found in Italian waters. The northern pike *Esox lucius* Linnaeus, 1758, an endemic species in North America and Eurasia, is allochthonous to Italian basins (Lucentini et al., 2011). The recently described cisalpine pike *Esox cisalpinus* Bianco & Delmastro, 2011, is instead autochthonous and native in some Italian rivers and lakes (Bianco & Delmastro, 2011; Denys et al., 2014). It often cohabits with *E. lucius* since the species has undergone numerous introductions (Bianco & Delmastro, 2011).

Here we report the first record of the cisalpine pike *E. cisalpinus* within the Montemarcello-Magra-Vara Natural Park (Liguria, NW Italy) in the north-western limit of the Tuscany-Latium district. The aim is to improve our knowledge on freshwater fish fauna inhabiting Ligurian Alps – Apennines System basins so that targeted actions can be undertaken to counteract the extinction or the depletion of the genetic heritage of autochthonous species.

MATERIAL AND METHODS

During the LIFEEMYS "Ligurian Invasive Fauna Eradication pro Indigenous *Emys orbicularis* restocking" (LIFE12 NAT/IT/000395) Project, one dead pike specimen was found in a fyke net in the Laghi Gemelli (44°07'20" N, 9°55'59" E), an artificial lake created from a disused gravel and clay quarry within the Magra River catchment. The lake (surface area 2600 m^2 and average depth approximately 0.50 m) has no tributaries; it is primarily fed by the main aquifer or by flooding from the nearby Magra River during intense rainfall events.

Poor specimen conservation did not allow correct interpretation of flank skin color pattern or other meristic characters; therefore, a muscle and a right pelvic fin snippet were taken from the specimen and placed separately in polypropylene tubes containing ethanol 100%. Genomic DNA was extracted from tissue samples using a commercial silica-based kit (GE Healthcare). In order to identify the species, we used the mitochondrial gene coding for the cytochrome oxidase subunit 1 (*COI*) as a genetic marker which taxonomic studies have employed because its nucleotide sequence is identical in individuals of the same species but different in members of dissimilar species. A specific PCR protocol for fish was used (Ward et al., 2005) and the amplicon was sequenced on both strands using Sanger's method. The consensus sequence obtained after alignment of the two strands was compared with those deposited in GenBank and BOLD (Barcode of Life) databases. A neighbor-joining tree based on Kimura 2 parameter distance matrices was built to better assign the species, followed by a confidence test (bootstrap test) to determine the statistical validity of species allocation.

RESULTS AND DISCUSSION

Comparison of the *COI* sequence with those present in GenBank showed that the best total score was obtained with the sequences belonging to *E. cisalpinus*. However, values of similarity above 99% were also found with sequences of *E. cisalpinus* and *E. lucius*. Since BOLD returned the same result, making it impossible to unambiguously assign the species, we analyzed the genetic distances using the bootstrap confidence test.

Analysis of the distance matrices placed the individual in the cluster of *E. cisalpinus* with statistically significant values for the nodes for both genetic markers, since the bootstrap rate of >70% corresponded to a probability >95% that it is a true cluster and that the species assignment is correct (Figure 1). The *COI* sequence of the specimen was then deposited in Genbank (accession number KU197015).

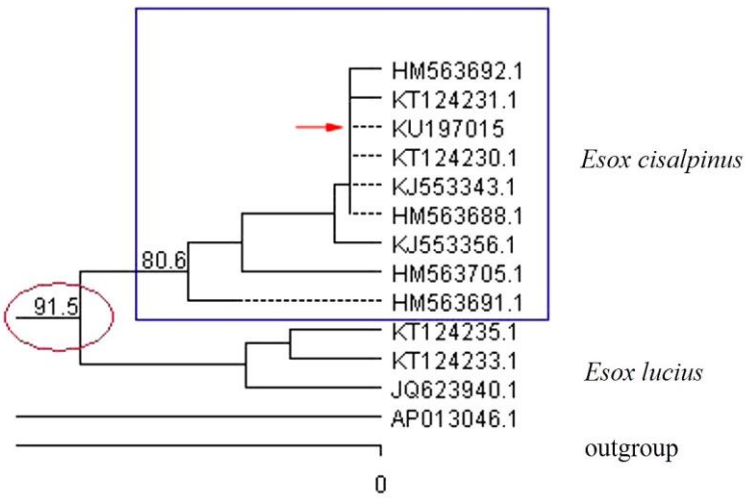

Fig. 1. Neighbor-Joining tree of *Esox spp*. with bootstrap test obtained from the analysis of *COI* sequence. Red arrow indicates *Esox* spp. specimen from Laghi Gemelli Montemarcello-Magra-Vara Natural Park, E Liguria, NW Italy).

Although the mitochondrial markers for matrilineal inheritance did not completely rule out the possibility that the species detected was actually a hybrid, the result did reveal the presence of *E. cisalpinus* in the study area. Because this species has been identified fairly recently (Bianco & Delmastro, 2011), its real distribution is still largely unknown. The present record partly fills this gap. Previous findings from molecular studies conducted at the pan-European level (Nicod et al., 2004) revealed unique characteristics about the pike populations inhabiting Lago Maggiore (Lombardy and Piedmont, N Italy; Ticino canton, S Switzerland) and Lago Trasimeno (Umbria, C Italy). As the result of at least Pleistocene isolation events, such features would clearly differentiate Italian *E. lucius* populations from transalpine populations (Bianco, 2014). Cisalpine pike is a native species of the Padano-Veneto and the Tuscany-Latium districts (Bianco & Delmastro, 2011; Bianco, 2014¸ Denys et al., 2014). As a commercial species, it has

been bred and introduced to inland Italian and European waters suitable for its survival (Bianco, 2014¸ Denys et al., 2014). Since this species is considered native to the Tuscany-Latium district, there is evidence for the hypothesis that *E. cisalpinus* is autochthonous to the Magra-Vara basin, as Ciuffardi et al. (2015) has recently speculated.

Over-fishing and poaching, habitat loss and degradation of reproductive substrates, as well as the introduction of exotic species and hybridization with the northern pike, constitute the main threats to *E. cisalpinus* conservation. Lorenzoni et al. (2002, 2007) reported that besides diet overlap with largemouth bass *Micropterus salmoides*, interactions with other species inhabiting the same habitat could have negative impacts on *E. lucius*. The same line of reasoning could be equally extended to the cisalpine pike, too.

The International Union for Conservation of Nature (IUCN) category for this species in Italy is still classified as "data deficient" (Bianco et al., 2013). Instead, it would be more accurately classified at least as "vulnerable" owing to hybridization phenomena and competition between native and *E. lucius* populations (Bianco, 2014). A focus of future research is to improve our knowledge about *E. cisalpinus* inhabiting the Montemarcello-Magra-Vara Natural Park in order to better understand its distribution within Magra River basin, population size and its possible co-existence or degree of hybridization with *E. lucius*. Information on how the species can be reached in Laghi Gemelli will be important as well.

Conservation actions such as control and prohibition of the introduction of exotic species, in addition to environmental improvements such as the use of artificial spawning beds where aquatic and riparian vegetation is scarce (Gillet & Dubois, 1995; Pedicillo et al., 2008) and projects for the reintroduction of genetically selected specimens need to be undertaken. Finally, off-limit areas for fishing activities should be designated at sites where species occurrence has been ascertained and the use of barbless hooks and bait for predator

fishing in the surrounding areas recommended Trapped specimens should be carefully handled with wet hands and released without damaging or impairing their viability.

Irrespective of whether the fish in question was a pure specimen or a hybrid, the present record is relevant to the debate over the application of monitoring plans required by the European Water Framework Directive 2000/60/CE. Documentation on the conditions of native freshwater fish communities, based on their composition, abundance, and the presence of sensitive taxa that act as sentinels of the status water bodies should constitute one of the main measures of the health of lotic inland waters (Oberdorf et al., 2002).

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