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OSSERVAZIONI E STUDI PRELIMINARI SU DI UNA POPOLAZIONE DI TROTA MARMORATA (SALMO TRUTTA MARMORATUS) MIGRATRICE DEL FIUME TOCE E DEL LAGO MAGGIORE

PRESENCE OF A MIGRATORY LACUSTRINE LIFE-HISTORY STRATEGY IN THE MARBLE TROUT (*Salmo marmoratus*): THE CASE OF THE NATIVE TROUT POPULATION OF LAKE MAGGIORE SPAWNING IN THE TOCE RIVER (ITALY)

PIERPAOLO GIBERTONI[1], MAURIZIO PENSERINI[1], STEFANO ESPOSITO[1], ALESSANDRA FOGLIA[1], DIEGO DAGANI[2], PAOLO BAZZONI[3], JEAN-FRANÇOIS RUBIN[4] AND LUCA FUMAGALLI[2]

[1]*Mediterranean Trout Research Group, Via Porali 1/A – 42037, Collagna, (RE), Italy*
[2]*Laboratoire de Biologie de la Conservation, Département d'Ecologie et d'Evolution, Université de Lausanne, Switzerland*
[4] *Ecole d'ingénieurs HES, 150 route de Presinge, 1254 Jussy, Genève,Switzerland*
[3] *Ossolana Acque – Ornavasso (VB), Italy*

Corresponding author: PierPaolo Gibertoni e.mail adress: gibertoni@igiardinidellacqua.com

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Riassunto

Nel corso dell'ultimo triennio, nell'ambito di un progetto di tutela ed incremento delle popolazioni salmonicole native del bacino idrografico del Fiume Toce (VB), si è provveduto a monitorare i parametri ambientali delle acque ed a studiare le popolazioni salmonicole in esse presenti. Oltre a popolazioni di Trota Marmorata residente è stata osservata una popolazione di salmonidi con abitudini migratorie. In effetti la continuità longitudinale del F.Toce, grazie anche alla recente attivazione di passaggi per pesci e all'applicazione dei deflussi minimi vitali dalle opere di derivazione idrica presenti in vari tratti del suo corso, garantisce la possibilità migratoria dalla foce nel Golfo Borromeo del Lago Maggiore sino a Crevoladossola. Tale continuità longitudinale è estesa anche ad importanti affluenti del F.Toce stesso, garantendo così numerosi e diversificati siti idonei per la riproduzione naturale ed abbondanti aree di caccia e di rifugio. Le caratteristiche fenotipiche e morfologiche permettono chiaramente di distinguere la popolazione residente da quella migratrice, avendo quest'ultima evidenti analogie con le popolazioni "lacustri" presenti nel Lago Maggiore. Anche in questo quadrante idrografico le attività di preservazione delle popolazioni native incontrano l'ormai noto problema dell'introgressione genetica causata dall'introduzione di trote fario e "lacustri" atlantiche, immesse da più di un secolo per incrementare la pescosità. Per studiare questo fenomeno si è proceduto all'investigazione biomolecolare applicando due tecniche: gli RFLP (Restriction Fragment Length Polymorphism) su regioni del DNA mitocondriale (aplotipi del gene 16s rDNA) e nucleare (genotipi del gene LDH-C1*) e 6 marcatori microsatelliti. I dati sono stati analizzati tramite il software Structure per l'assegnazione individuale alle popolazioni. Le due tecniche di indagine hanno evidenziato la presenza di individui ibridi con diverso grado di ibridazione nel 45% dei campioni analizzati. Dalla scalimetria è inoltre emerso che gli accrescimenti medi annui si attestano intorno ai 170-180 mm con raggiungimento della maturità sessuale alla classe di età 1+/2+ per i maschi e 3+ per le femmine. Il ritrovamento di una popolazione salmonicola che gode delle ricche acque del lago Maggiore per fini trofici e che utilizza letti di frega a 50 km di distanza risalendo il F.Toce e i suoi affluenti comporta fondamentali implicazioni sul fronte conservazionistico-gestionale e sulle politiche a sostegno della pesca ricreativa e professionale in acque fluviali e lacuali, interessate tra l'altro da giurisdizioni transprovinciali, transregionali e transfrontaliere, essendo le acque del Lago Maggiore normate da accordi particolari italo-elvetici. Inderogabili provvedimenti di tutela e salvaguardia si devono ora attuare al fine di conservare la Trota Marmorata lacustre del Verbano, una specializzazione ecologica (life history form) unica nel panorama dei salmonidi mediterranei.

Summary
Most of native salmonid stocks in the Italian prealpine lakes are considered to be extinct, due to the massive introduction of domesticated trout and loss of suitable spawning sites. Little is known about the behavior and taxonomical status of these indigenous trout populations. During a project for restoring the local native population of marble trout in the Toce River Basin (Piedmont, Italy), we identified very large marble trout specimens that were phenotypically different from the resident population. Morphological characters and color pattern exhibited by these trout were indicative of lacustrine adaptation. The presence of these lake migratory trout is possible because of the longitudinal continuity of the Toce River and the main tributaries with the Lake Maggiore. Genetic investigations were performed using LDH-C1* and 16S-rDNA markers and six microsatellite loci in order to clarify the taxonomical status of this migratory population and to estimate the level of introgression by hybridization with domesticated introduced strains. The results clearly demonstrated that trout of Lake Maggiore spawning in Toce River Basin belong to the marble trout group and that hybridization with allochthonous stocks has taken place at various degrees. Length, sex and age measurements showed that migration life-history strategy is adopted by only a part of the population and migrants were almost exclusively females. Currently, fishing activities in the lake are regulated by an Italian-Swiss Convention that sets the legal minimum size detain for lake trout at only 30 cm. We suggest that,cooperation between national governments and local administrations is necessary to save this important unique life history form of marble trout.

Introduction
Salmonidae is a fish family that exhibits a significant ecological plasticity and a broad panel of life history traits. One of the most interesting behavioural aspects regarding salmonid populations is migration, which is carried out within rivers (riverine forms), between rivers and lakes (lacustrine forms) and between fresh- and saltwater (anadromous forms) (Klemetsen et al., 2003). Migrations have been extensively characterized in brown trout (*Salmo trutta* complex), but most studies concerned anadromous or lacustrine populations of North Europe. There are only a few studies on the adoption of different life-history strategies in trout inhabiting the Mediterranean area that also included migratory tactic (Sorić, 1990; Launey at al., 2003; Glamuzina and Bartulović, 2006; Snoj et al., 2010). Nowadays, there are no anadromous populations in the Mediterranean rivers because of the high temperatures of the sea. However, the wide distribution of trout within the Mediterranean basins suggests that trout were able to colonize Mediterranean rivers extensively by dispersion by sea during glacial periods, when sea temperatures were lower (Gibertoni et al. 2010). The finding of skeletal remains belonging to large-size salmonids in a cave near the sea at Praia a Mare (South Italy) confirms that Mediterranean trout formed anadromous populations during favourable periods (Durante, 1978). Praia a Mare cave was inhabited by *Homo sapiens* in a period between 7,590 ± 100 years and 12,130 ± 150 years BP; therefore, it is possible that these trout populations provided an important source of food for Mediterranean *H. sapiens*

during adverse climate periods, as the anadromous salmonid populations did in North Spain during the same periods (Adán et al., 2008).

It is possible to envisage that resident isolated populations formed during the warm inter-glacial periods, and that secondary contacts occurred several times during a number of colder periods because of migratory strategies. Bernatchez (2001) identified 5 mitochondrial genetic lineages in brown trout: Atlantic (AT), Danubian (DA), Adriatic (AD), Mediterranean (ME) and marmoratus (MA). The latter three lineages (AD, ME and MA) occupy the Mediterranean basin and correspond to the three refugial areas of potential origin: South France for the ME lineage, the Po basin for the MA lineage and the Anatolian-Balkan region (Bernatchez, 2001) or the Iberian Peninsula (Cortey et al., 2004, Susnik et al., 2007) for the AD lineage.

Because of its central geographical position, the Italian Peninsula played a fundamental role in the distribution of the Mediterranean trout. All three Mediterranean haplotypes (AD, ME and MA) can be found in the Po basin (Giuffra et al., 1994). The MA haplotype characterizes the marble trout *Salmo marmoratus*, an ecologically specialized species inhabiting the medium and lower part of alpine rivers. Marble trout typically occupies high-productivity waters and is characterized by a large size, a prevalently piscivorous diet and the distinctive marbled colours pattern. The Mediterranean brown trout belonging to the AD and ME lineages inhabits the Apennine streams and the headwaters of west alpine rivers. Thus, in the Po basin both marble and brown trout coexist with different geographical distributions due to different ecological niches and, probably, distinct dispersal histories (Gibertoni e al., 2010). Pre-alpine lakes are located at the border between the distribution areas of marble and native Mediterranean brown trout, making the taxonomic distinction of the autochthonous stocks uncertain. Lacustrine trout in pre-alpine lakes have been reported already in the 16th century by P. Giovio. Cuvier described the marble trout for the first time in 1817 studying specimens from lakes of Lombardy (Gridelli, 1937). Cuvier and Valencienne (1848) then confirmed the previous finding analysing specimens from Po River and Lake Maggiore. Notably, the first descriptions of a trout variety exhibiting "dark marks of irregular shapes, combined and mixed to form a kind of mottled pattern" have been obtained on specimens collected from Alpine lakes. Later in 1940, Pomini studied lacustrine migratory trout in pre-alpine lakes and wrote: "Furthermore, I am certain that *Salmo* from Verbano Lake (Lake Maggiore) and Lario Lake (Lake of Como), …, are just *marmoratus* phenotypes because of the many common characters (*e.g.*, vomerine teeth, vertebral column, general shape) that are identical to the trout found in Po basin". Despite the presence of historical sources that suggest the existence of a lake form of *S. marmoratus* in recent times, marble trout is not found in lacustrine habitats any longer (Sommani, 1960; Gandolfi et al., 1991; Mojetta, 1995). The most important historical account of lake migratory trout is again from Pomini's work, that described two different lake population spawning in Sarca River and Mincio River (respectively inlet and emissary of the Lake Garda). Pomini wrote that the trout from Lake Garda is very different from *S. marmoratus* both for its general morphology and for vertebral elements suggesting that the autochthonous trout for this lake probably belong to the native brown trout lineage (mediterranean *Salmo trutta*). Native lake migratory form of Lake Garda is probably extinct (Betti 2001). Despite this, *Salmo carpio,* another autochthonous salmonid, still lives in Lake Garda. *S. carpio is* a planktivorous-pelagic endemic that completes its entire biological cycle within the lake. *Salmo carpio* is currently reproductively isolated and genetic analysis traced the possible origin of this species to an ancient hybridisation event between Mediterranean brown trout and marble trout (Giuffra et al., 1996; Gandolfi et al., 2006).

Salmo dentex, inhabiting rivers of the Balkan region is another example of trout with a Mediterranean distribution that exhibits adaptation to lacustrine conditions. Genetic analyses revealed that this taxon is not a valid species, but represents a peculiar life history form of *S. marmoratus* in the river Neretva and of Mediterranean brown trout *S. trutta* in the basin of Lake Skadar (Snoj et al., 2010). The situation of lacustrine form in the Balkan region is analogous to that of the Po Basin, where pre-alpine lakes could be inhabited alternatively by migratory lacustrine form of marble and Mediterranean brown trout.

The identification of autochthonous trout populations is even more confounded by the progressive replacement of the native European stocks with domesticated brown trout stemming from the Atlantic lineage. Indeed, decades of stocking activities in European countries have seriously endangered native stocks by introgressive hybridization (Largiader & Scholl 1995; Poteaux *et al*., 1999; Hansen *et al*., 2002; Jug *et al*., 2005; Sonstebo *et al*., 2008 a, b). Large amounts of domesticated stocks have also been introduced in the alpine lakes of Italy over the last century. Introgressive hybridization, water pollution, river antropization, construction of dams and horizontal artefacts that obstructs migratory routes towards spawning sites caused the depletion, probably irreversible, of almost all native populations in pre-alpine lakes.

In this work, we report on the discovery of a migratory marble trout population in Lake Maggiore that was found during a project of recovery of the local population of resident marble trout in the Toce river. We report the first data on its genetic status and its life-history traits such as the presence of partial migration. Knowledge of these traits is important to plan optimal conservation strategies for the protection of the last critically endangered native trout that can be found in Italian pre-alpine lakes.

Materials And Methods

Trout were captured by electrofishing during the reproductive season from November 2008 to November 2010 in the Toce River and in its main tributaries, including the Anza Stream. Toce River flows into the Borromean Gulf, the arm of Lake Maggiore extending from Verbania to Stresa in its south-western area (Fig. 1). Capture strategy was performed to obtain local spawners of resident marble trout with the aim to breed them artificially. During these standard sampling sessions, we identified very large marble trout that were phenotypically different from the resident population of Toce River. The larger dimensions, the silvering colour, the posterior blunt "swallow-tail" margin of the caudal fin were indicative of lacustrine adaptation (Fig. 2). Eggs obtained from these specimens were incubated and reared separately from the resident marble trout.

Over the three spawning seasons (2008/09, 2009/10, 2010/11), a total of 44 lacustrine trout specimens were captured. The number of specimens captured each year gradually diminished because of the implementation of the Minimum Instream Flow (MIF; or DMV, *Deflusso Minimo Vitale*) that currently guarantee the river with 2500 l/s more water than 2008. The increased flow has hindered electrofishing activities and has permitted the spawning lake trout to spread over all the medium course of the Toce River. Before the application of MIF, trout were preferentially found at artificial horizontal barriers making the capture of specimens comparatively simple. Furthermore, we limited fishing with the aim of reaching the production goal of only 15,000-20,000 lacustrine marble trout eggs, in order to reduce the impact on natural spawning while awaiting for additional information about the mating and migratory biology of this life-history form. Lacustrine trout were distinguished and selected on the basis of the aforementioned characters (silvering colours, swallow-tail).

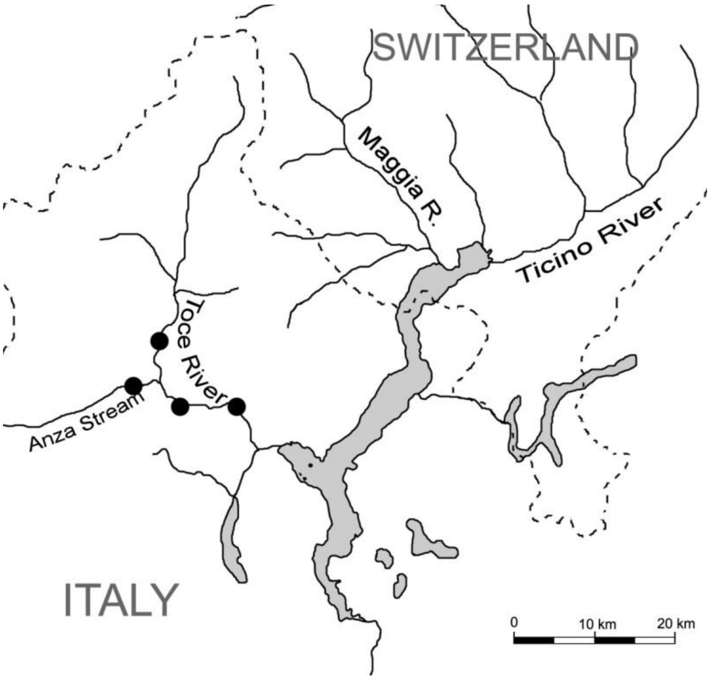

Figure 1 - Drainage basin of Lake Maggiore. Black dots indicate the localization of sampling stations where migratory lacustrine marble trout were caught

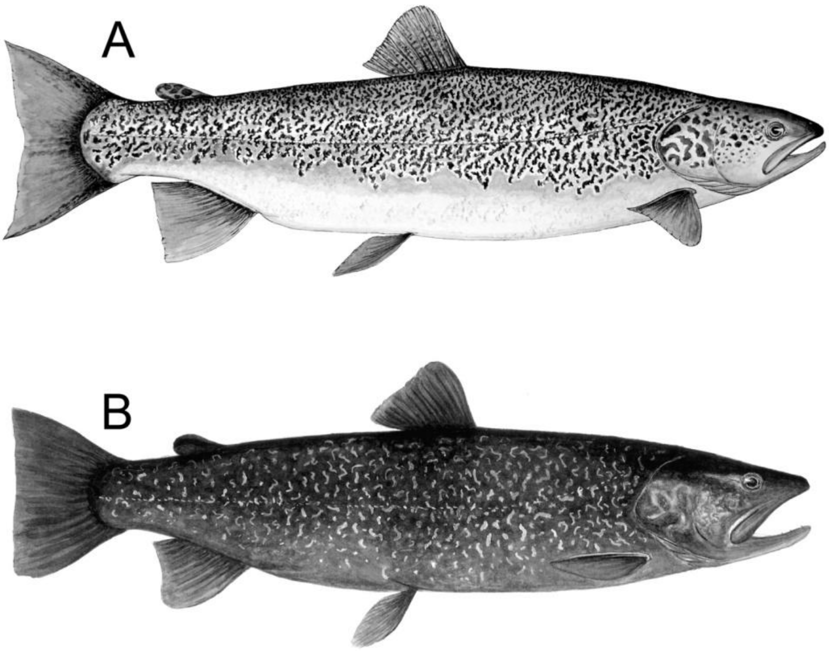

Figura 2 - The drawings show the main phenotypic characteristics used to discriminate between lacustrine migratory (A) and riverine resident (B) marble trout. A) Migratory trout are characterized by the silvery pigmentation and the “swallow-tail” margin of the caudal fin. Lake marble trout also exhibit a finer vermiculate pattern on the back . B) For resident marble trout, note the general dark coloration and the caudal fin shape. The posterior margin is nearly straight and the lower border is consumed by the continuous rubbing on the riverbed. Original drawings by Stefano Esposito

Length, sex and age (as determined by scalimetry) were recorded for each lacustrine trout. Moreover, samples of adipose fin were collected and stored in 95% ethanol at -20°C for subsequent genetic analysis. Scalimetry, length measurement and collection of tissue samples were performed only on spawners after transfer to the hatchery.

The nuclear LDH-C1* and the mitochondrial 16S-rDNA markers were analysed with the RFLP technique (McMeel et al., 2001; Nonnis Marzano et al., 2003) in order to genetically identify specimens captured during the first season (2008/09). This methodology was performed with standard procedures that were already described in Penserini et al. 2006.

During the next season (2009/10), 13 lacustrine trout fished in the Toce River were genotyped at six microsatellite loci (BFRO001: Snoj et al., 1997; BFRO002: Susnik et al., 1997; Str85INRA: Presa & Guyomard, 1996; Ssa197: O'Reilly et al., 1996; Str541 and Str591: Estoup et al., 2000). We used the software STRUCTURE v2.3.1 (Pritchard et al., 2000), with a burn-in of 200,000 steps and 1,000,000 MCMC replicates, to assign each individual to a population,,using 21 samples of pure Slovenian marble trout (Fumagalli et al. 2002) and 15 Atlantic brown trout from Sweden as reference. Individuals were then assigned to three categories: pure marble trout (admixture coefficient (q) ≥ 0.9), pure Atlantic brown trout ($q \leq 0.1$) and hybrids ($0.1 > q < 0.9$). Moreover, the population-level admixture was estimated following the method proposed by Hansen et al. 2001.

From February 2009 to February 2010, we carried out a phenotypic characterization of trout fished in lake waters by professional fisherman. Specimens were assigned to two phenotypic classes: "*marmoratus*" and "*lacustris*". Classification criteria were, for the "*marmoratus*"" phenotype, marbled colours pattern extended on the side, back and operculum; for the *lacustris"* phenotype, "x"-shaped isolated black spots on sides and spotted opercula, the typical habitus of *Salmo trutta lacustris*. In Lake Maggiore, the *lacustris* phenotype is linked to the frequent and massive introduction of domesticated stocks of Atlantic origin.

Results

During the spawning season in 2008, when lacustrine trout were identified phenotypically for the first time, 10 migrating lake trout were sampled. In order to confirm our visual observations, analysis of LDH-C1* nuclear and 16S mitochondrial marker was carried out. The results are shown in Table 1. The combined analysis of both markers permitted the identification of 4 lacustrine marble trout that can be considered potentially pure. The other specimens were hybrids with a varying degree of introgression by the Atlantic strain. The introgression level was estimated calculating the frequencies of native and non-native alleles for both nuclear and mitochondrial markers, resulting in 0.25 of non-indigenous alleles for LDH-C1* and 0.3 of non-indigenous alleles for 16s rDNA, respectively. Results are shown in Fig. 3.

During the second spawning season, 13 specimens were collected. Tissue samples from these trout were genotyped at six microsatellite loci in order to confirm the observations of the previous year with a more thorough analysis. Nine specimens were assigned to the pure marble trout cluster and 4 were hybrids with different introgression degree. All reference individuals were correctly assigned to their class of origin (Fig. 4). The estimated population-level admixture (Hansen et al. 2001) was 0.82 (95% confidence intervals (CI) = 0.72-0.92)

In order to further characterize the phenotype of this lacustrine population, length, sex and age of all captured samples was recorded. First, all captured spawners were assigned to two age classes representing 3+ and 4+ cohorts. Lacustrine immature females were also captured. These specimens, belonging to the 2+ cohort, exhibited the typical silvered phenotype.

Aiming to minimize the impact on the reproductive cycle of this population, immature females were released immediately in their home river. These specimens were not included in our study because scalimetry, length measurement and collection of tissue samples were performed only on spawners after transfer to the hatchery.

The length (average ± standard deviation) of the specimens classified as 3+ and 4+ by scalimetry was equal to 49.6 ± 2.7 cm and to 62.8 ± 4.9 cm, respectively (Fig. 5). Sex ratio is significantly biased towards females. In fact, over three sampling seasons only 4 males over 44 specimens were captured (9% males - 91% females). Phenotypic analysis of trout captured by professional fisherman in the Lake Maggiore is reported in Table 2. Of the 85 trout analysed, 65% was assigned to the allochthonous *lacustris* phenotype and the remaining 35% to the native *marmoratus* phenotype.

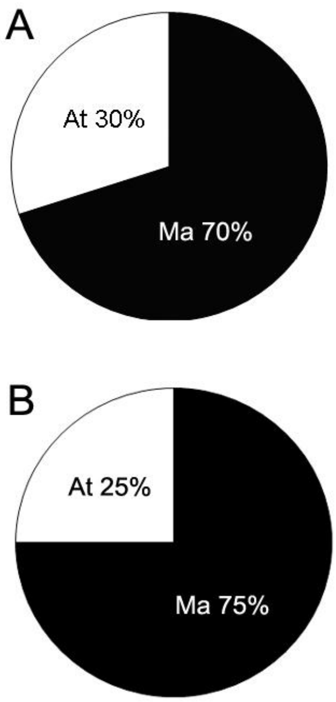

Figura 3 - Graphics illustrate the admixture between native and non-native genes in lacustrine marble trout sampled during 2008/09 spawning season. A) allelic frequencies of nuclear gene LDH-C1*; B) aplotype frequencies of 16s rDNA . Ma, native genes; At, non-native genes

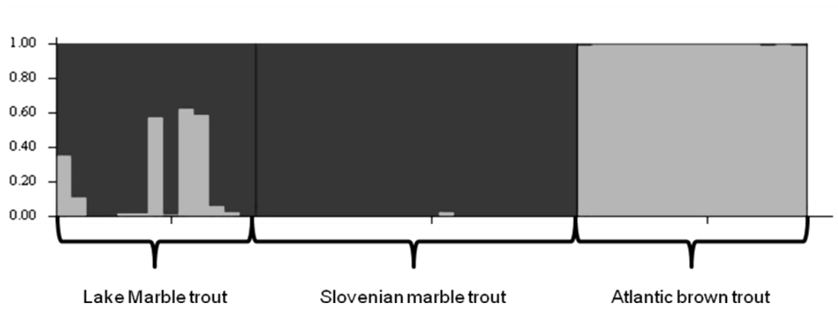

Figura 4 - Bayesian clustering inferred by STRUCTURE v2.3 (Pritchard et al., 2000) for 13 Lake marble trout from the Toce River during the 2009/10 spawning season, 21 pure Slovenian marble trout and 15 pure Swedish brown trout, assuming two different clusters of individuals (k=2), an admixture model and no prior population information

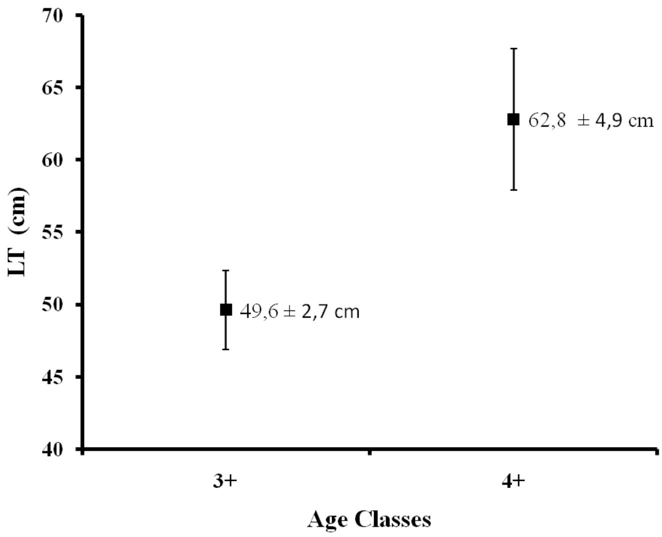

Figura 5 - Results of scalimetry analysis. Mean length values of the two studied cohorts (age classes) with respective Standard Deviations are shown

Table 1 - Results of genetic analysis on combined mitochondrial (16S-rDNA) and nuclear (LDH-C1*) markers. For 16S-rDNA: M= marble, A= atlantic; for nuclear LDH-C1: *100 coincides with the autochthonous alleles, *90 represents the allochthonous introduced allele

Cod.	**16s-rDNA**	**LDH-C1***
1	M	100/90
2	M	100/100
3	M	100/100
4	M	100/90
5	A	100/90
6	A	100/90
7	M	100/90
8	M	100/100
9	A	100/100
10	M	100/100

Table 2 - Results of the phenotypic analysis performed on trout caught in the lake from February 2009 to February 2010. Number (n) of sampled trout presenting a "*marmoratus*" or "*lacustris*" phenotypes and their respective percentage values are shown. No sample was collected in July because of the low fishiness during hot months. No data are available for October, November and December when fishing for trout is forbidden

	N	**"*marmoratus*" phenotype (n)**	**"*marmoratus*" phenotype%**	**"*lacustris*" phenotype (n)**	**"*lacustris*" phenotype %**
February 2009	34	15	0.44	19	0.56
March 2009	5	3	0.60	2	0.4
April 2009	1	0	0.00	1	1.00
May 2009	4	1	0.25	3	0.75
June 2009	2	0	0.00	2	1.00
August 2009	1	0	0.00	1	1.00
September 2009	34	11	0.32	23	0.68
February 2010	4	0	0.00	4	1.00
Total	85	30	0.35	55	0.65

Discussion

Genetic introgression between native and introduced stocks

The results of genetic and phenotypic analysis confirmed the existence in the Lake Maggiore, of a lacustrine migratory population of marble trout that spawn in Toce river. Genetic analysis, even if performed on a limited number of individuals, have pointed out that the native genotype is threatened by introgression with "Atlantic" genes derived from the massive inputs of zootechnical stocks carried in order to increase the abundance of trout population in the lake and support the activities of professional and recreational fishing. Despite the low number of available individuals, the analysis of LDH-C1* and 16s-rDNA markers on samples collected in 2008 and the analysis of six microsetillite loci on individuals caught in 2009 gave similar results, confirming an introgression rate between 18 and 30%.

Phenotypic analysis of fishes caught in Lake Maggiore has revealed a proportion of trout that can be assigned either to the *marmoratus* or *lacustris* phenotype showing a significant bias towards the "*lacustris* phenotype", distinctive of introduced livestock strains. The genetic introgression of lake trout sampled in Toce River is smaller than what we could have expected on the basis of phenotypic analysis performed on trout caught in the lake. During three years of breeder recovering in the Toce River and its tributaries, only two migrant lake trout were caught, both males assignable to the *lacustris* allochthonous phenotype unambiguously. This difference could be explained by the lower adaptability and breeding (Hansen, 2002; Muhlfeld et al., 2009; Lynch and O'Hely, 2001) of introduced strains, limiting the presence of atlantic spawners to few males attracted by native migrant females. The results of mitochondrial gene analysis revealed the existence of individuals with mitochondrial Atlantic genes. This implies that also non-native females are able to spawn successfully.
In Switzerland, in the Ticino River above Lake Maggiore, marble trout has disappeared during the 70s. Books dated half of the nineteenth century describe the abundant presence of large trout in the Lake Maggiore swimming up the Ticino River. Big spawning lake trout were also caught, during the autumn, in the tributaries Melezza Stream and Maggia River ensuring a significant economic return for small villages where intense fishing for trout was practiced using nets and permanent fish traps (Monti, 1864). Toce River is in communication with the Ticino River across the Lake Maggiore. The preservation of the residual migratory Toce River's marble trout population could allow a natural recolonization from downstream, if a longitudinal continuity of the main course of the Ticino River will be restored.

Partial migration and life-history traits
Out of the 44 trout caught in the river Toce, 40 (91%) were females. This observation is consistent with the trend for females to exhibit more migratory behaviour than males in brown trout (Campbell 1977, Jonsson 1985; Klemetsen et al., 2003). Salmonids have complex life histories that are frequently characterized by the coexistence of both a migratory and a resident population (Olsson and Greenberg, 2004; Wysujack et al., 2009). In these populations, typically, only a fraction of individuals adopts a migratory life history tactic (Jonsson and Jonsson, 1993). According to many authors, the decision for a migratory strategy is determined by a cost/benefit calculation (Hendry et al., 2004). Costs of migration such as energy, physiological changes and exposure to external risks (e.g. predation) would be compensated by greater benefits for females than for males (Klemetsen et al., 2003). This difference between sexes is explained considering that the fitness of females and egg production depends on growth rate and body mass (Fleming and Gross, 1991). Therefore, the benefits of emigrating toward more productive environments are more evident for females. Many of these observations were carried on anadromous or landlocked brown trout populations that can be spawn also in very small brooks (Jonsson et al., 2001). In these populations body mass and egg production of sea or lake trout females is very high compared to that of resident forms. In marble trout of the Lake Maggiore area, the size differences between resident and migratory individuals could be not so marked.
In the Toce River, studies on the fecundity of migratory trout of Lake Maggiore compared to that of resident marble trout have not been performed yet and, therefore, a similar analysis cannot be carried out. Lacustrine marble trout appear to be larger than residents on average (personal observations); however, marble trout are known to reach considerable size also within rivers due to a fish-eating diet specialization and an abundant supply of forage fishes.
Why marble trout migrate toward the lake? Why only females? The factor that controls the adoption of a migratory life history strategy may be the population density in their home rivers. Variation in local density may favor the coexistence of migratory and non migratory

life histories within populations (Bohlin et al. 2001). Accordingly, migratory tactic should be promoted during periods of high density, whereas non-migratory behavior should dominate during periods of low density (Morita et al. 2000). Studies on pure populations of Slovenian marble trout have shown that the average length of individuals is inversely proportional to the total density of trout during the first year of life (Vincenzi et al., 2008). Migration toward the lake during this early period of life could be the successful tactic to reach larger sizes and therefore produce more eggs.

For males, the adoption of this strategy may be less convenient. The fitness of a male depends not only on its size but also on the ability to fertilize eggs. The marble trout is a very territorial fish and a key factor for the reproductive success of males is the competition for territory. In this sense, the occupation of territories is important and provides the chance to spawn with the highest number of females. The adoption of a migratory tactic requires males to abandon the territory. Marble trout can reach large sizes even within their home river, providing them the ability to compete for spawning areas also against potential large males migrating from the lake. It is yet unclear at what age the migration toward the lake takes place, although the capture of one-year old individuals into the lacustrine environment suggests that it may also occur during the first year of life.

During the sampling, immature lacustrine females (2+ age class) were caught. It is not known if all the migrating individuals go back into the Toce River every year (for wintering as immature fishes or for spawning as mature trout) or whether this is an occasional phenomenon. It is still not clear why these non-breeding migration occur.

Conservation strategies

Evidently, further investigations on the behaviour of population trout in the lake are necessary in order to protect it. The longitudinal continuity of the Toce River and its main tributaries guarantees the possibility of migration from the river mouth in Borromean Gulf of Lake Maggiore until Crevoladossola. This was possible with the recent application of the Minimum Instream Flow (DMV) that was an important step to allow the native salmonid population to reach all available spawning sites. Despite this, the migratory marble trout population of Lake Maggiore seems to be still seriously threatened by i) hybridization with domesticated commercial strains and ii) inadequate fishing regulations. Therefore, the immediate halt of the release into the lake of zootechnical brown trout appears to be necessary to avoid interference with the reproduction of native population. Rendering fishing regulations more adequate could be a difficult issue to solve because Lake Maggiore is under the control of different central governments (Italy and Switzerland), regional (Piedmont, Lombardy (IT) and Ticino (CH)) and provincial-district administrations (Italian Provinces of Verbano-Cusio-Ossola, Novara, Varese and Swiss District of Locarno). Currently, fishing activities in the lake are regulated by an Italian-Swiss Convention that sets the legal minimum size detain for lake trout at only 30 cm. No mention is made for marble trout. This study showed that the first two reproductions of the Lake Maggiore marble trout occur at the achievement of about 49 cm (3+ age class) and 62 cm (4+ age class) of length. Therefore, a minimum legal length in excess of 60 cm for the marble trout would be necessary to preserve at least the first two spawns. The freshwaters of the Toce River basin are also regulated by the Provincial Regulation of Fishing of Verbano-Cusio-Ossola. For lake trout, the minimum size of 30 cm is set in continuity with the Italian-Swiss Convention. In the Provincial Regulation, the minimum legal size for marble trout is set to 35 cm. Our study shows that the original native migratory trout of Lake Maggiore is the marble trout. Therefore, the indigenous salmonid population of the lake is legally protected by a minimum retain size of 35 cm. This minimum size is evidently inadequate because native Lake Maggiore trout reach the 35 cm limit when just two years old,

not allowing, when legally captured and killed, the realization of even a single reproductive cycle.
Therefore, our study shows that urgent measures are needed to save this important unique life history form of marble trout.

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