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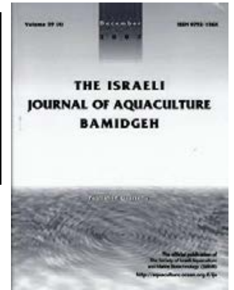

Genetic Stock Identification of the Flathead Grey Mullet (*Mugil cephalus*) in Lake Tiberias Based on Parent-Offspring Relationship

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Abstract

The Lake Tiberias (Sea of Galilee) grey mullet (*Mugil cephalus*) population originates from translocated wild-caught, or hatchery-reared, fish. The aim of the study was to identify the taxonomic status and stock origin of a sample of 32 mullet individuals caught in the Sea of Galilee, based on the mitochondrial Cytochrome Oxidase I (COI) sequence analysis and nuclear microsatellite markers. A total of 13 microsatellite markers were selected from nine different linkage groups with a number of alleles ranging from 5-23, with an average of 9.85 alleles/locus. By using COI sequences and the Barcode of Life Data (BOLD) identification system, seven individuals were taxonomically classified as Thinlip grey mullet (*Liza ramda*) and the remaining 25 as *M. cephalus*. Of 663 nucleotides, 122 (18.4%) differed between the COI sequences of the two distinct mullet species. A preliminary parentage analysis of the hatchery-reared stocking batch, based on 13 markers, assigned them as progeny of three mating pairs. These three mating pairs only were tested as potential parents of the 25 *M. cephalus* individuals captured from the lake. Marker-based comparison showed that a parent-offspring relationship was rejected for 17 *M. cephalus* individuals by at least four genetic markers. Eight of 25 *M. cephalus* individuals (32%), were identified as progeny of the three parental pairs with the overall probability of 3.88×10^{-7} for correspondence by chance to any of these three parental pairs. The 13 markers used has high statistical power to reject a putative parent-offspring relationship obtained by chance thus resulting in familial identification. This approach represents an accurate method of genetic stock identification and should also be applicable to populations of other species. The results confirm that the mullet hatchery-reared fingerlings survived in the Sea of Galilee when released alongside wild-caught fingerlings.

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Introduction

Lake Tiberias, also known as the 'Sea of Galilee' has a surface area of 166 square km and is located in the northeast region of Israel. Its maximum depth, measured in the northeast, is 48 meters. Extending 21 km from north to south and 11 km from east to west, it is pear-shaped. Its surface elevation has been assumed to be 209 meters below sea level, but for decades the actual water level has been 2 to 4 meters below the assumed level. The Sea of Galilee is fed primarily by the Jordan River. Other streams and wadis (seasonal watercourses) flow into the lake from the hills of Galilee. Fish diversity in the lake includes more than 20 native, exotic, and introduced populations: bleaks (*Mirogrex terraesanctae terraesanctae* and *Mirogrex lissneri*), cichlids (*Sarotherodon galilaeus*, *Oreochromis aureus, Coptodon zillii, Astatotilapia flaviijosephi, Tristramella sacra* and *Tristramella simonis simonis*), catfish (*Clarias gariepinus*), cyprinids (*Capoeta damascina, Barbus longiceps, Carassius carassius, Cyprinus carpio, Tor canis and Hypophthalmichthys molitrix*), mullets (*Mugil cephalus* and *Liza ramada*), salmonids (*Oncorhynchus mykiss*), Poeciliids (*Gambusia affinis*) and others. This latter group includes frequent escapees from commercial fishponds and hobbyists' aquariums of purebred and hybrid species (Golani and Mires, 2000; Roll *et al.,* 2007).

The population of the lake's mullet species is the result of approximately 100,000-1,000,000 juvenile fish that are stocked annually into the lake (Zohary *et al.,* 2014). Mullet introduction is an important biotic component of the lake ecosystem. It contributes to water quality and enhances fishery (Whitfield *et al.*, 2012; Gophen and Snovsky, 2015). Young fingerlings are captured in estuaries along the Israeli Mediterranean coast and are introduced into the Sea of Galilee. Additionally, following the establishment of breeding management protocols for flathead grey mullet (*Mugil cephalus*) reared in captivity (Aizen *et al.*, 2005), fingerlings are produced in commercial hatcheries in Israel and stocked in the lake for enhancement of the local fishery industry. The mullets thrive in fish ponds and in the lake and are highly valued by local fishermen, yet they cannot reproduce in the Sea of Galilee (Abraham *et al.*, 1966; Bar-Ilan, 1975). Therefore, the survival of this closed population depends on restocking. The question of whether hatchery-reared fingerlings can survive when released alongside wild-caught fingerlings has not been studied to date.

Genetic stock identification, which is an important tool for fisheries management, relies on the ability to differentiate stocks of interest based on divergent allele frequencies of natural genetic tags such as Single Nucleotide Polymorphisms (SNPs) and microsatellites (Glover *et al.*, 2010, 2011; Zhang *et al.*, 2013; Narum *et al.*, 2004, Larson *et al.*, 2014). Verification of relationships in segregating populations is generally based on the 'exclusion principle' e.g., the probability that an unrelated candidate parent will be eliminated from consideration as a true parent based on segregation of alleles for multiple polymorphic markers (Jamieson and Taylor 1997). In the 1990s, microsatellite markers became the markers of choice, chiefly because of their high polymorphic information content (PIC) and ease of genotyping (Glowatzki-Mullis *et al.* 1995). Several microsatellite markers for *M. cephalus* were developed and published by the National Center for Biotechnology Information (NCBI) prior to our study in 2014 (Miggano *et al.*, 2005; Xu *et al.*, 2010). We redesigned microsatellites markers from these sequences (Dor *et al.*, 2016) and assembled a panel of 13 genetic markers, which is sufficient for parentage analysis. Fin clips of fish parents of the fingerlings stocked in the lake are routinely sampled for DNA analysis.

The conserved sequence of the 5' region of the mitochondrial cytochrome oxidase subunit I (COI) gene was proposed as a platform for the universal DNA barcoding of life (Hebert *et al.* 2003). Currently, about 275,000 of the estimated 4.5 million species (6.1%) are formally characterized with DNA barcodes and registered in the Barcode of Life Data system (BOLD, www.barcodinglife.org). BOLD was developed as a collaborative online workbench that has evolved into a resource for the DNA barcoding community, including tools for bioinformatics and taxonomy identification based on cluster analysis. Under this system, the Fish Barcode of Life initiative (FISH-BOL, www.fishbol.org) focuses on fish, which comprise half of the global vertebrate species. Rapid growth of the FISH-BOL database is facilitated by a universal primer cocktail that is capable of amplifying COI fragment in more than 93% of fish species (Ivanova *et al.*, 2007), and more than 13,250 out of the estimated 32,000 fish species have so far been barcoded

(41.4%). An important prerequisite for protection and conservation of marine biodiversity is building an inventory of taxonomic references that relies on genetic measurements. Most fish species exhibit profound phenotypical changes during development and juveniles of related species are hard to distinguish morphologically. In this context, the identification of fish species using DNA barcoding provides new perspectives in ecology and systematics of fish (Hanner and Gregory, 2007; Costa and Carvahlo, 2007), and biodiversity surveys, phylogenetic and evolutionary history of life (Hebert and Gregory, 2005).

The objectives of this study were to identify the genetic stock of *M. cephalus* in the Sea of Galilee, to assess the efficiency of its stocking, and survival in the Sea of Galilee. A molecular analysis based on both COI sequence and microsatellites was used to validate the authenticity of *M. cephalus* samples and identify their origin.

Materials and methods

Fish samplings

In the current study, stocking of fry in the Sea of Galilee was carried out using two batches. The first batch, on January 2013, consisted of 89,000 wild fry and 11,000 farmed fry that were hatchery-reared from June 2012. The second batch, on December 2013, consisted of 140,000 farmed fry that were hatchery-reared from October 2013. A sample of 25 fry from the latter batch was collected prior to introduction to the lake and stored in absolute ethanol for future genetic analysis.

The hatchery-reared fry that were introduced into the Sea of Galilee in January 2013, originated from a single mating group consisting of one female and 4 males, whereas those of the second batch (December 2013) originated from two mating groups: the first included one pair, a male and female, and the second included two females and eight males. Both mating groups were held separately in 5 m^3 tanks at the National Center for Mariculture (NCM; Eilat, Israel) and induced to spawn following the method of Aizen *et al.* (2005).

A preliminary parentage analysis of the 25 fry sample from NCM, based on 13 markers, assigned them as progeny of the pair of parents from the first mating group, and two pairs of parents from the second mating group. Thus, only these three mating pairs were tested as potential parents for fish captured in the lake. Parents' tail fins were sampled for DNA extraction following Zilberman *et al.* (2006) and their gender was determined by type of gametes (either oocytes or sperm cells) obtained by gonadal biopsy. Prior to all handling procedures, fish were anesthetized in 0.07% clove oil.

In January 2016 fishermen from the Sea of Galilee net-captured 11 mullets of approximately 60-200 gr, 15-25 cm (Capture 1). In January 2017 they net-captured an additional batch of 21 mullets of approximately 700-3000 gr, 40-70 cm (Capture 2). Thus, a total of 32 fish were captured, designated as "Mullet" with a serial number of 5-15 and 21-41 according to the two captures (Table 3), and analyzed for genetic stock identification.

DNA extraction and genetic analysis

DNA was extracted from the fins and the whole body of the hatchery fry samples using the MasterPure™ DNA Purification Kit (Epicentre® Biotechnologies, WI, USA) following the manufacturer's recommended protocol. DNA samples were diluted to a concentration of 30 µg/µl and 1 µl was used as the DNA template for PCR amplification of the mitochondrial gene Cytochrome Oxidase I (COI) reference fragment. Primer cocktail COI-3 and M13 nested primers were used for amplification and sequencing, respectively, according to Ivanova *et al.* (2007). The PCR products were subjected to 1.5% agarose gel electrophoresis. The band of relevant size was excised from the gel and purified with DNA Montage Gel Extraction Kit (Millipore, Bedford, MA, USA). This step is necessary to provide a DNA template at high quality for sequencing, without residues of unutilized primers from the PCR reaction. For each sample of the captured 32 fish, the forward and reverse trace files of the sequenced COI PCR product were assembled using the *GAP4* program (Staden 1996). The resulting sequences of 663 bp were compared to the DNA barcoding data base version 4 (BOLD, http://v4.boldsystems.org) for taxonomic classification (Shirak *et al.*, 2009; 2016). Nucleotide sequences were aligned using ClustalW, using the default parameters and the DNA ClustalW weight matrix (http://clustalw.genome.jp/). The sequences were deposited in GeneBank via the BOLD

system (BankIt1996190 TLM002-17.COI-5P KY683175; BankIt1996190 TLM001-17.COI-5P KY683176).

A total of 13 microsatellite genetic markers were selected from the mullet linkage map based on their polymorphism rate (Dor *et al.*, 2016). The primer sequences are presented in Table 1. The captured fish, their siblings and their putative parents were genotyped for the genetic markers. PCR products of all tested markers were detected by an ABI3130 Genetic analyzer and automatically genotyped using GeneMapper software v.4.0 (Applied Biosystems, CA, USA). The identity of the 13 markers, their linkage group (LG) position, the number of alleles, and polymorphic information content (PIC) are presented in Table 2. The 13 markers are scattered in 9 different linkage groups and two unassigned linkage groups, thus displaying independent segregation. The number of alleles per genetic marker ranged from 5 to 23 with an average of 9.85. PIC of markers varied from 0.29-0.91 with a mean of 0.64. The genotypic data for the 13 genetic microsatellite markers were used for parent-offspring identification following Weller *et al.* (2010).

Table 1. Sequences of primers for 13 microsatellites.

Marker	Forward primer	Reverse primer
FHM550	CGCCGACTTAAACTTCAGGA	ACAAACGCCCTACAAAGTGC
MCE6	TTACAGCTCAGTCGGCACAC	CAGAGGTTCCCCAAAGATGA
FHM255	ATGAACACATGGACCCACAG	ACATCCAACAAGAGGCGTTT
MSC4GH	CGTTCTAGGGCATTCTCTGC	ACGGCCCATATTAGGAAACC
MCE4	ACCAGGTGAGAGCAGGAATG	CGTTCGTTTCCTCCAAGATG
2D7GM	TGTATGCTTGGTGGAGTGGA	TGGGGTGGGAGTATGTTTTG
MUCE44	CATAGGGGAATGTGGAATGG	TCCGACAGCCACTGTTATGA
204HH	GGAGAGGCTAGAGGGGTGAA	AGGAACACACAGCCACACAG
2D15FM	CGTGTCTGCATTGACTCTCTG	TTTTATCTGTCAGGAGCCGTTT
MCE25	TCGGCATGTATATGAAAGCA	ACTCACACAGTGGGCTTCCT
MCE2	GAGGGTGGAGTGTGAAGCTC	AGGAAAATGGAAGCCACCTC
MCE3	GAGGGTGGAGTGTGAAGCTC	AGGAAAATGGAAGCCACCTC
MCE7	TCCTCTTCAGCCACCAAATC	CAAGAAAGGACGGGATTGAA

Table 2. Distribution of Linkage Group (LG) location, number of alleles, and polymorphism for 13 genetic markers.

Marker	fhm550	mce6	fhm 255	msc4 gh	mce4	2D7 Gm	muce 44	204 HH	2d1 5fm	mce 25	mce 2	mce 3	mce7
LG[1]	2	3	5	11	11	13	14	17	17	20	21	N/A	N/A
N.of alleles[2]	11	5	13	8	4	6	4	8	8	22	23	7	9
PIC[3]	0.83	0.57	0.82	0.74	0.39	0.59	0.57	0.65	0.58	0.88	0.91	0.29	0.55

[1]LG location according to Dor *et al.*, 2016. Not assigned (N/A).
[2]Based on 43 unrelated individuals (86 chromosomes)
[3]Polymorphic Information Content

Computation of Polymorphic Information Content (PIC) for microsatellites markers

The PIC value for each genetic marker was estimated by determining the frequency of alleles per locus using the following formula:

$PIC = 1 - \sum X_i^2$

Where X_i is the relative frequency of the ith allele of the genetic marker.

Evolutionary relationship of taxa

The evolutionary history based on COI sequence was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 0.39 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura-2 parameter method (Kimura 1980) and are in the units of the number of base substitutions per site. The rate variation among sites

was modeled with a gamma distribution (shape parameter = 2.53). The analysis involved 20 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$. All positions containing gaps and missing data were eliminated. A total of 615 positions were in the final dataset. Evolutionary analyses were conducted using MEGA6 (Tamura *et al.*, 2013).

In addition, the STRUCTURE 2.3.4 program was used to investigate the population structure based on multi-locus genotype data, to infer the presence of distinct populations, assign individuals to populations, and to estimate population allele frequencies. All runs were performed with 10,000 burn-in period and 50,000 Markov chain Monte Carlo (MCMC) repeats. The population number (K) was selected based on the "Best K estimation" from CLUMPACK software (Kopelman *et al.*, 2015).

Estimation of the probability of inclusion

Allele frequencies for the 13 markers were computed based on unrelated individuals, and putative parents in mating groups (86 chromosomes). Exclusion of parent-offspring relationship was based on at least two genetic markers showing conflict in allele segregation from putative parent to offspring. Probability of inclusion for segregation of alleles in complete concordance to Mendelian segregation for all 13 markers for the putative three pairs of parents (P) was computed based on the population allele frequencies as follows:

$$P = \prod_{i=1}^{13}[(q_{1i}p_{f1i}p_{m1i}) + (q_{2i}p_{f1i}p_{m2i}) + (q_{3i}p_{f2i}p_{m1i}) + (q_{4i}p_{f2i}p_{m2i})]$$

Where Π is the multiplicative sum; p_{f1i}=probability of the first father allele for marker i, p_{f2i}=probability of the second father allele for marker I, p_{m1i}=probability of the first mother allele for marker i, p_{m2i}=probability of the second mother allele for marker I; and q_{1i}, q_{2i}, q_{3i} and q_{4i}, are coefficients that are equal to unity if the sire and dam alleles for each term were the same, and equal to 2 if the father and mother alleles were different. If the father or mother were homozygous, then p_{f2i}= 0 or p_{m2i}= 0, respectively. Probability of inclusion was computed for each of the three pairs of parents separately and then combined as the cumulative probability that a captured fish was progeny of any of the three parental pairs.

Prediction of the fraction of mullet of farmed origin in the lake

The fraction of mullet of farmed origin among all mullet in the lake (of farmed and wild origins) is expected to be within a range of the sample mean ± two standard deviations. Standard deviation of a Binomial distribution was calculated as the square root of npq, when n was sample size of *M. cephalus* capture (25), p was the probability that parentage was determined (8 out of 25, 0.32) and q was the probability that parentage was not determined (0.68), thus resulting in standard deviation of 2.33.

Results

The COI mitochondrial gene sequence of 663 bp was analyzed for all 32 individuals captured from the Sea of the Galilee. Six and one individuals from captures 1 and 2, respectively, showed complete similarity to those of *Liza ramada*. The sequences were compared to the existing seven most similar sequences in BOLD from Greece (HM208837 and DQ441608 for *L. labrosus* and *M. cephalus*, respectively), Turkey (KC500832 and KC500946 for *L. aurata* and *M. cephalus*, respectively), South Africa (JF493809 and JF493905 for *L. richardsonii* and *M. cephalus*, respectively) and Portugal (JQ775058 for *L. ramada*). The phylogenetic tree classification of a sample of 13 captured individuals (Mullet 5-15, 26, 27) is presented in Fig 1. Sequences of seven individuals (Mullet 5, 12-15, 26, 27) showed complete similarity to those of *M. cephalus* from Greece, with distant clustering to the respective *M. cephalus* sequences from Turkey and South Africa. The remaining sequences (Mullet 6-11) showed complete similarity to those of *L. ramada* from Portugal with distant clustering to the sequences of other Liza species. A total of 122 out of 663 nucleotides (18.4 %) were different between the COI sequences of our two distinct species defined as *M. cephalus* and *L. ramada* (Fig 1 and Table S1). No intra-specific variability was detected within these two species.

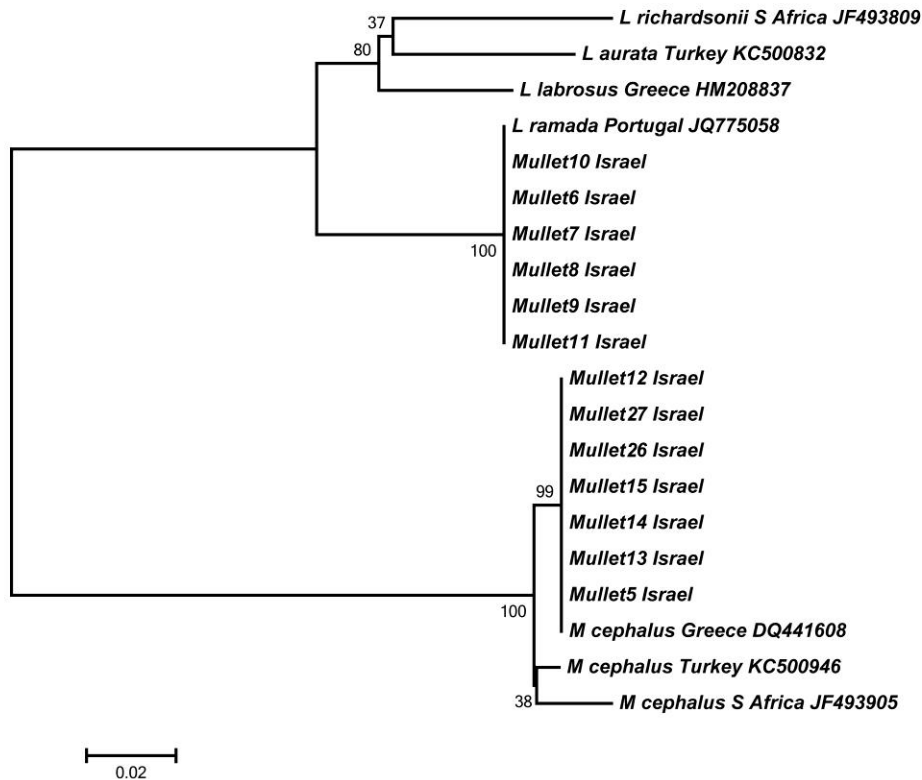

Fig 1. Phylogenetic tree based on the COI mitochondrial gene sequence of a sample of captured mullet fish and deposited sequences in BOLD was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Units are the number of base substitutions per site. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

The number of alleles for the 13 genetic markers analyzed for the 32 captured fish ranged from 5-23 with an average of 9.85. PIC of markers varied from 0.29-0.91 with a mean of 0.64. Analysis of the 32 captured fish and their putative parents for these markers with Best K estimation resulted in K=2 (Fig 2). Unsupervised analysis by STRUCTURE clustered the samples into distinct groups corresponding to *M. cephalus* (in green) and *L. ramada* (in red). Thus, the two different methods of analysis based on different molecular data; i.e., COI sequences and microsatellite data, assigned unequivocally the individuals to two distinct species: *M. cephalus* and *L. ramada*.

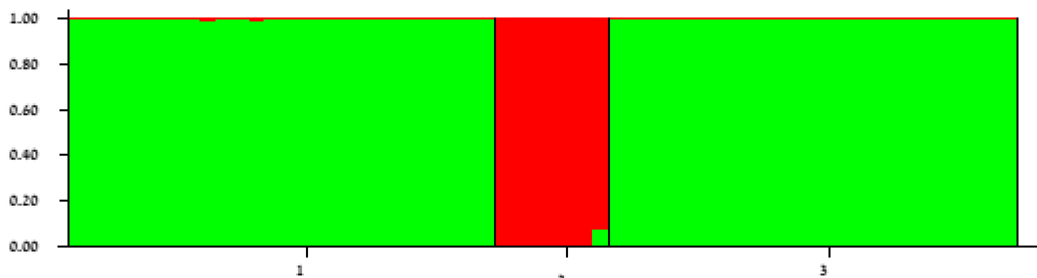

Fig 2. Population histogram of captured fish and their putative parents. Individual genotypes of 58 farmed and captured mullet fish for 13 genetic markers were assigned to genetically similar clusters (K) as inferred by STRUCTURE. At population level K=2, this software output was a Q matrix that divided the samples into 3 groups. Group numbers are indicated below the X axis: 26 individuals of the two mating groups originally obtained from the Israeli Mediterranean coast (group 1); 7 and 25 individuals collected from the Sea of Galilee and identified as *L. ramada* and *M. cephalus* by analysis of the COI gene (groups 2 and 3, respectively). Y axis is the population membership coefficient for two subdivisions represented by green (groups 1 and 3) and red (group 2) colors.

The parentage test results from the two catches are shown in Table 3. For each individual, the number of genetic markers that were in conflict with Mendelian segregation of alleles from the three pairs of putative parents is presented. For 17 out of 25 individuals, parent-offspring relationships were rejected by at least four independent genetic markers. The summary of results is presented in Table 4. In both captures of fish, genotypes showed no conflict in segregation of genetic markers for four individuals with their putative parents. The coefficient of inclusion; i.e., the cumulative probability for segregation of microsatellites alleles that correspond to Mendelian segregation by chance with any of the three pairs of parents was 3.88×10^{-7}. This indicates that the eight individuals may be regarded as progeny of the three pairs of parents in NCM that were introduced to the lake. As expected, these individuals exhibited the *M. cephalus* COI sequence.

Table 3. Results of taxonomic classification based on the COI sequences, and parentage test using genetic markers.

Catch no.	*Sample ID*	*Taxonomic classification*	*No. of markers rejecting parentage*		
			Family 1	*Family 2*	*Family 3*
1	Mullet 5	*M. cephalus*	9	9	0*
	Mullet 6	*L. ramada*	12	12	10
	Mullet 7	*L. ramada*	12	12	12
	Mullet 8	*L. ramada*	12	12	13
	Mullet 9	*L. ramada*	13	13	12
	Mullet 10	*L. ramada*	13	13	12
	Mullet 11	*L. ramada*	13	13	12
	Mullet 12	*M. cephalus*	4	0	9
	Mullet 13	*M. cephalus*	6	4	7
	Mullet 14	*M. cephalus*	0	5	6
	Mullet 15	*M. cephalus*	0	5	9
2	Mullet 21	*L. ramada*	12	13	13
	Mullet 22	*M. cephalus*	8	11	5
	Mullet 23	*M. cephalus*	6	7	8
	Mullet 24	*M. cephalus*	0	4	8
	Mullet 25	*M. cephalus*	6	6	9
	Mullet 26	*M. cephalus*	7	9	8
	Mullet 27	*M. cephalus*	0	4	7
	Mullet 28	*M. cephalus*	6	7	8
	Mullet 29	*M. cephalus*	7	8	6
	Mullet 30	*M. cephalus*	5	6	9
	Mullet 31	*M. cephalus*	7	6	7
	Mullet 32	*M. cephalus*	6	7	7
	Mullet 33	*M. cephalus*	10	9	10
	Mullet 34	*M. cephalus*	9	10	9
	Mullet 35	*M. cephalus*	0	4	8
	Mullet 36	*M. cephalus*	7	7	10
	Mullet 37	*M. cephalus*	8	8	9
	Mullet 38	*M. cephalus*	10	10	8
	Mullet 39	*M. cephalus*	9	8	10
	Mullet 40	*M. cephalus*	5	0	7
	Mullet 41	*M. cephalus*	6	7	5

* The probability for segregation of alleles that fit Mendelian segregation by chance to any of the three pairs of parents was 3.88×10^{-7}.

Table 4. Summary of number of fish and relative frequency (in brackets) of *Mugil cephalus* and *Liza ramada* fish in captures from the Sea of Galilee.

	Total	*Liza ramada*	*Total*	*Mugil cephalus Origin*	
				Other[1]	*Farmed*[2]
Capture 1	11	6	5	1 (0.20)	4 (0.80)
Capture 2	21	1	20	16 (0.80)	4 (0.20)
Total	32	7 (0.22)	25	17 (0.68)	8 (0.32)

[1]Collected from the Israeli Mediterranean coast and introduced to the lake.
[2]Identified as progeny of three pairs of parents in NCM that was introduced to the lake.

Discussion

The phylogenetic classification is an important test for the identification of the target species in the lake. Based on DNA barcoding identification, only seven out of 32 samples were not *M. cephalus* species, but of a morphologically similar species (*L. ramada)*. The results provide important data that these two mullet species only, exist in the lake and along the Israeli coast, as compared to the reported four mullet species in Western Greece (Katselis *et al.*, 2006), and several different mullet species previously found in the Israeli coastal waters (Herzberg and Pasteur, 1975). Moreover, it appears that *M. cephalus* is the predominant mullet species in the lake.

A total of 22% of the 32 samples was of *L. ramada* species, which differed by 18.4% in the COI sequences from *M. cephalus*. Thirteen microsatellite markers were selected based on high polymorphic information content to determine divergence between species and parent-offspring relationship. Based parenthood genetic markers only five of the 13 mullet were polymorphic in *L. ramada* individuals, showing clear divergence of allele frequencies from *M. cephalus*. As a result, assignment of the 32 individuals into the two species was identical based on either mitochondrial DNA barcoding or nuclear genetic markers analysis.

Seventeen out of the 25 *M. cephalus* individuals in the captures of Sea of Galilee were excluded from being fingerlings of the three pairs of putative parents by at least four genetic markers. The exclusion of genetic relationship is considered to be absolute if based on at least two genetic markers, accounting for genotyping error or the rare possibility of mutation (Weller *et al.*, 2010). These individuals showed partial genetic relatedness between themselves. The remaining eight individuals showed segregation of 13 genetic markers from their putative parents, in accordance to Mendelian rules. The coefficient of inclusion was virtually zero, indicating the low probability for correspondence to parentage by chance. Four individuals were from capture 1 (0.8) and the other four from capture 2 (0.2), indicating a 4-fold frequency of stocked fish (Chi squared $p<.0007$). The fish in capture 1 weighted 60-200 gr, while the fish in capture 2 weighted 700-3000 gr. As the farmed fish growth rate in the conditions of the lake is slow, most of the larger fish in the 2nd capture may originate from an earlier stocking than that of January 2013, and thus did not originate as progeny of the parents. Assuming a binomial distribution with a standard deviation of 2.33, the population mean is expected to be within a range of the sample mean of 8 $\pm$ two standard deviations. Thus, it can be estimated that the number of fish from the stocking batch is within the range of 3.3 to 12.7, based on a sample of 25 individuals, reflecting a mean frequency of 0.13 to 0.51. The results confirm that the mullet hatchery-reared fingerlings survived when released alongside translocated wild-caught fingerlings in the Sea of Galilee. In a previous study of genetic identification of wild and farmed cod in coastal Norway, molecular tools were implemented to identify the most likely farm(s) of origin for recaptured escapee cod (Glover *et al.*, 2011). A similar study has genetically identified escapees of commercially farmed Atlantic salmon in the wild (Zhang *et al.*, 2013). The genetic identification was based on the most likely clustering of individuals to populations with different genetic profiles. Correct self-assignment percentage at the range of 65 to 75% demonstrated relatively high probability of identifying individuals to their farm of origin (Glover *et al.*, 2010). In our study, exclusion of stock of origin of individuals is absolute, while inclusion of individuals as originating from a hatchery-reared grey mullet family virtually approached zero (3.88×10^{-7}). To the best of our knowledge, this is the

first documentation of individual genetic identification of introduced fish to a lake based on parent-offspring relationship.

In summary, the preliminary two captures of a total of 32 grey mullet individuals were sufficient to test the feasibility of genetic stock identification in the Sea of Galilee. The panel of 13 genetic markers has shown high statistical power to reject a putative parent-offspring relationship obtained by chance. Eight of 25 captured individuals of farmed origin (0.32), were determined to be progeny of three parental pairs, reflecting a mean frequency of 0.13 to 0.51. The results confirm that the mullet hatchery-reared fingerlings survived when released alongside translocated wild-caught fingerlings in the Sea of Galilee. This approach represents an accurate way for genetic stock identification and should be also applicable to populations of other species.

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