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Changes of the Ichthyofauna in the Impoundment of the Aoos Springs, Greece

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Abstract: The impoundment of the Aoos Springs, which was created in 1990 for the needs of hydroelectric production, is characterised by the lack of studies on its fish fauna across years. Seasonal sampling efforts were conducted with Nordic gillnets and an electrofishing device to describe the fish fauna after 30 years from the construction of the impoundment. Our results revealed the presence of establish species in the impoundment and highlighted their connectivity with the inflow streams. The fish fauna has been enriched throughout the years, starting from four observed species few years after the formation of the impoundment (during 1996-1997) to nine species 20 years later (2015-2019). This increase in the number of species across years was mainly attributed to human impacts through unintentional or intentional stocking conducted to support the local recreational fishery. In any case, the importance of each species' ecological niche and the complexity of freshwater ecosystems should be considered in future initiatives aimed at maintaining fish biodiversity.

Key words: Reservoirs, non-native species, freshwaters, Greece

Introduction

Aiming towards the improvement of the ecological status of continental waters, the European Water Framework Directive considers impoundments as parts of the «lake-type water bodies». These systems, which are frequently termed as artificial lakes, are considered as intermediate ecosystems between riverine and lake environments exhibiting a remarkable fishery dynamic, both in terms of socio-economy and ecology (Kaczkowski et al. 2018). A fundamental issue confronting native fish species is the adaptation to the new ecological status generated by the impoundment's construction (Gido et al. 2000, Kruk et al. 2017). The problem of the fragmentation of rivers is not usually recognised on its real basis (Martinez et al. 1994, Marmulla 2001) and enrichment stocking trials is considered to be the solution (Bobori & Economidis 2006) in order to enhance fish yields.

The aim of the present study is the study of the changes of the fish fauna of the impoundment of the Aoos Springs (Northern Greece) across decades. The impoundment was constructed in 1988 and formed up to 1990 for the needs of hydroelectric production. This impoundment was characterised by the lack of recent studies on its fish fauna and on the impacts induced. A seasonal monitoring of the fauna took place 25 years ago (1996-1997, see Economou et al. 1998), whereas recent information on fish species inhabiting the impoundment could be found only after 2015 through monitoring conducted by the Management Agency of the Northern Pindos National Park (November 2014 – August 2015, see Leonardos et al. 2016, Korakis et al. 2016) and in 2016-2019 (unpublished data of the Management Agency) as well as sporadic short-term studies (Tsionki et al. 2019). Emphasis was also given to the presence of non-native and introduced fish

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species that could be found in the impoundment, introduced either by accident or not, thus, raising the need for management of these species (Arthington et al. 2016, Uzunova et al. 2020).

Materials and Methods

The impoundment of the Aoos Springs is located in the northern part of Greece at an altitude of 1343 m a.s.l., with a total surface of 11.5 km^2, a maximum depth of 54 m, a total water volume of 260 million m^3 and annual water-level fluctuations ranging between 6 and 8 m (Zacharias et al. 2000). The water column is thermally stratified during summer, whereas it appears to be quite uniform, from the surface to the bottom, throughout the rest of the year (Zacharias et al. 2000). Annual water temperature ranges from 4-6^{o}C in March to 5-21^{o}C in July, with the creation of thermal beds at depths of 5-15 m during summer (Zacharias et al. 2000). Mountain springs and river run off discharge from adjacent mountains are limit the fish assembles with the characteristics of a natural lake (Economou et al. 1998).

A random stratified seasonal fish survey was conducted in July and September 2019, following the European standards (EN14757) for fish monitoring in lake water bodies (CEN 2005). In particular, monofilament multi-mesh benthic Nordic type gill nets (12 panels of 1.5 × 30 m, height × length nets with mesh size from 5 to 55 mm) were used for fish sampling at each station (Fig. 1). Overall, 42 panels of multi-mesh benthic Nordic type gill nets were set at 2-10 m depths (16 trials) and 10-30 m depths (26 trials) (Fig. 1) before sunset and hauled after dawn, ensuring a stable sampling time of approximately 12 h.

Although the use of multi-mesh benthic Nordic type gill nets is proposed as suitable gear for the monitoring of fishing data (CEN 2005), additional information could also be collected by the complementary use of other fishing gear, such as the electrofishing devices (Sutela et al. 2008). A backpack electrofishing device (Hans-Grassl GmbH battery, Model IG200-2, DC (pulsed), 1.5 KW output power, 35-100 Hz, max. 850V) was also used to sample 14 stations in July and September 2019 and January 2020 (Fig. 1). In the peripheral discharges, sampling was done once during the rise of the flow covering (if available) at least a distance of about 100 m. The type of the sampled stations is presented on Table 1.

All fish specimens were identified to the species level (Kottelat & Freyhof 2007, Barbieri et al. 2015) and measured for total length (TL, cm) and weight (W, g). The samples caught by electrofishing device were released back to the lake after measurements had been taken, whereas the samples caught by nets were transferred to the laboratory. The number of specimens per species were measured in two length-classes (juveniles: smaller than 5 cm; and adults: larger than 5 cm). To test for differences of young to adult's composition per species and stations, a χ^2-test (χ^2, df, p=0.05) was used (Zar 2010). In this study we assume as young specimens those with TL lower than 5 cm, because for all the studied species the length at first maturity was observed for sizes larger than 5 cm (Froese & Pauly 2021). Even though we arbitrarily set this threshold length class, we consider that this categorisation would capture a generic pattern among juveniles and adult specimens for all the studied species.

To identify similarities on the species composition among stations, a clustering technique based on the Bray Curtis similarity index was used. The Shannon – Wiener diversity index (*H*) was used to measure the diversity between the species composition of zone depths (data set of Nordic gillnets) and among the station groups (data set of electrofishing). The standard error (*SE*) of *H* according to Hutcheson (1970) and the confidence interval at level 0.95 ($CL_{95\%}$) as $CL_{95\%} = 2SE$, were estimated. Hutcheson t-test (Ht t-test, df, p=0.05) was used to test for differences on the Shannon – Wiener diversity index between the catches with the two gears, between zone depths (data set of Nordic gillnets) and among the station groups (data set of electrofishing) (Hutcheson, 1970).

Results

The total number of fish specimens caught using gillnets was 195, with six species cumulatively contributing 86% to the total species caught and namely *Cyprinus carpio* Linnaeus, 1758, *Carassius gibelio* (Bloch, 1782), *Squalius* sp. (Aoos population), *Barbus prespensis* Karaman, 1924, *Alburnoides bipunctatus* (Bloch, 1782) and *Lepomis gibbosus* (Linnaeus, 1758) (Table 2). The *H* index, in terms of numerical abundance was 1.49 ± 0.09 ($H \pm CL_{95\%}$), while in terms of biomass it was 1.03 ± 0.01. In respect to depth zones, the *H* at 2-10 m depths was higher ($H \pm CL_{95\%}$: 1.61 ± 0.16 and 1.15 ± 0.02, respectively) than at depths 10-30m (1.36 ± 0.11 and 0.98 ± 0.01, respectively) (*Ht t*-test = 2.51, df = 118.54, $p < 0.05$ and *Ht t*-test = 16.8, df = 10062.95, $p < 0.05$, respectively). The species composition in terms of number of specimens showed a higher (χ^2-test, $P<0.05$) contribution (> 10%) of five species at depths 2-10 m than at depths 10-30 m (Fig. 2), with most representative species being *Squalius*

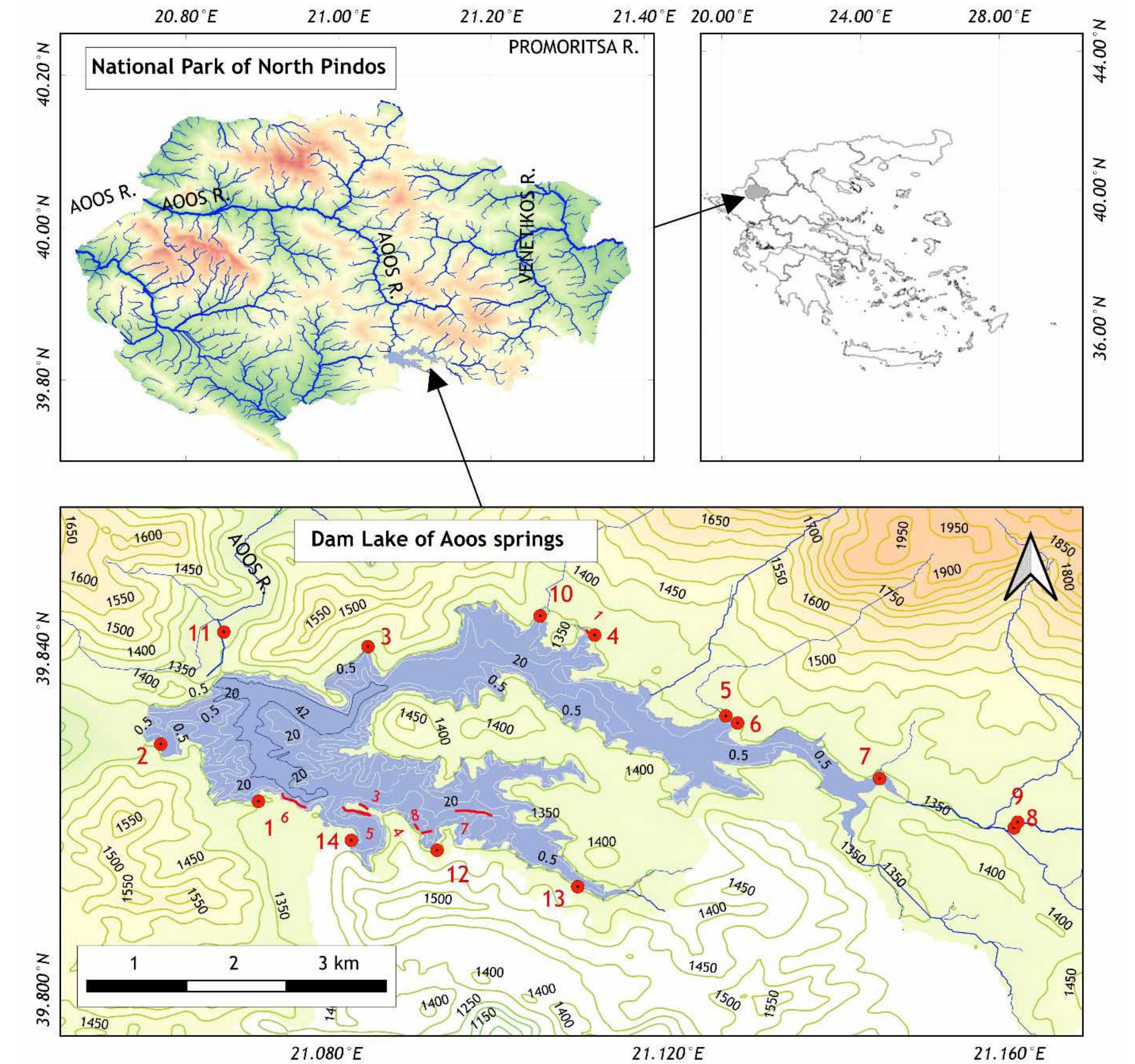

Fig. 1. Map of sampling stations using Nordic type benthic multi-mesh gillnets (red lines) and electric powered backpack device (red circles) conducted in the impoundment of the Aoos Springs between July 2019 and January 2020.

sp.(Aoos population) at depths 2-10 m, and *C. carpio* at depths 10-30 m (Fig. 2).

As a result of the samplings conducted with the electrofishing device, 1377 specimens of six species were caught (Table 2). More than half of the specimens caught were represented by *Squalius* sp. (Aoos population, 52.4%), which was observed at 13 stations, and to a lesser extent by *L. gibbosus* (26.6%) and *A. bipunctatus* (18.7%), which were observed at six and 11 stations, respectively. A limited number of specimens (less than 3% of the cumulative contribution) belonged to *B. prespensis* (three stations), *C. gibelio* and *C. carpio* (both at one station) (Fig. 3). The *H* index based on numerical abundance was 1.10±0.035 (Fig. 3) and it was significantly (*Ht t*-test = 8.06, df = 255.31, P < 0.05) lower than the corresponding value estimated for the samples collected with the Nordic gillnets.

Specimens with TL lower than 5 cm (young individuals) represented 74%, 54%, 61% and 84% of the caught specimens of *Squalius* sp. (Aoos population), *L. gibbosus, B. prespensis* and *A. bipunctatus*, respectively (Fig. 3). Species composition between young and adults did not differ significantly (χ^2-test, P < 0.05) among stations. Clusters based on Bray-Curtis similarities among species composition at each station (the rare species *C. carpio* and *C. gibelio* were excluded from the analysis), identified three groups at the 55.7% similarity level (Fig. 4). Cluster A was characterised by the notable presence of *L. gibbosus* (proportion: Pr = 63%), the low presence of *Squalius* sp. (Aoos population, Pr = 28%)

Table 1. Type of sampled stations, SC: category of station (A = lake shore, B = stream, C = interface lake – stream), number of fishing trials and number of specimens per station caught by electrofishing in the impoundment of the Aoos Springs.

Station	Type	SC	Fishing trials	Number of individuals
1	Lake, rocky shore, standing waters	A	3	192
2	Lake, intense aquatic vegetation, standing waters	A	1	11
3	Lake, mud, gravel, calm water	A	2	25
4	Lake, mud, gravel, calm water	A	2	110
5	Stream, very coarse gravel, low flow	B	3	240
6	Lake, mud, gravel, calm water	A	2	58
7	Lake-Stream, mud, low flow	C	3	55
8	Stream, coarse gravel, flow	B	2	163
9	Stream, coarse gravel, flow	B	2	117
10	Stream, gravel, low flow	B	3	54
11	Stream, gravel, low flow	B	1	43
12	Lake, mud, gravel, calm water	A	3	67
13	Lake-Stream, mud, gravel, calm water	C	2	59
14	Lake, rocky shore, calm water	A	3	183
	Total		32	1377

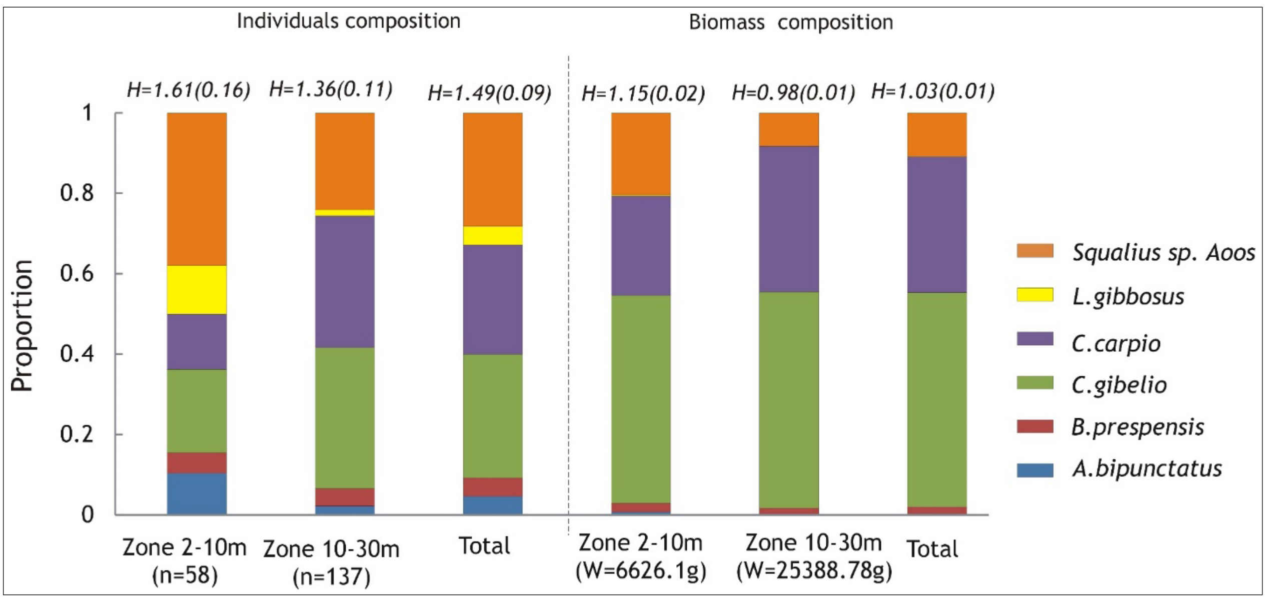

Fig. 2. Composition of fish species in terms of number of specimens and biomass caught by Nordic type benthic multi-mesh gillnets in two depth zones (2-10m and 10-30m) of the impoundment of the Aoos Springs between July 2019 and January 2020. *H*: Shannon Wiener index ($CL_{95\%}$).

Table 2. Number of individuals (n), total weight (TW; g), mean total length (TL; cm) and weight (W; g) of each specimen, respectively, per species caught by Nordic gillnets in the impoundment of the Aoos Springs. Min: minimum TW or TL; max: maximum TW or TL.

Species	n	TW	TL (min-max)	TW (min-max)
Alburnoides bipunctatus	9	72.8	7.08 (6.40-8.00)	8.08 (5.86-11.60)
Barbus prespensis	9	561.0	16.05 (13.00-19.50)	62.32 (30.09-110.13)
Carassius gibelio	60	17065.2	23.29 (14.60-33.50)	284.41 (59.81-778.69)
Cyprinus carpio	53	10840.0	21.68 (16.60-26.70)	204.52 (90.33-356.75)
Lepomis gibbosus	9	24.4	5.66 (4.70-6.40)	2.70 (1.44-3.84)
Squalius sp. (Aoos population)	55	3451.6	16.11(7.00-27.50)	62.75 (3.61-227.43)
Total	195	32014.9		

and of *A. bipunctatus* (Pr = 8%) and the rare *B. prespensis* (Pr = 1%). Cluster B was characterised by the notable presence of *Squalius* sp. (Aoos population, Pr = 73%), the low presence of *A. bipunctatus* (Pr = 27%), the presence of the rare *B. prespensis* (Pr = 1%) and the absence of *L. gibbosus*. Cluster C was characterised by the equivalent presence of *Squalius* sp. (Aoos population), *A. bipunctatus* and *B. prespensis* (Pr: 28% - 37%) and the absence of *L. gibbosus* (Fig. 4). The *H* index ($H \pm CL_{95\%}$) significantly differed among stations in terms of biomass (Hutcheson t-test > 4.65, df > 100.5, P < 0.05) following the order: Cluster B (0.63 ± 0.04) < Cluster A (0.98 ± 0.04) < Cluster C (1.09 ± 0.07) (Fig. 4).

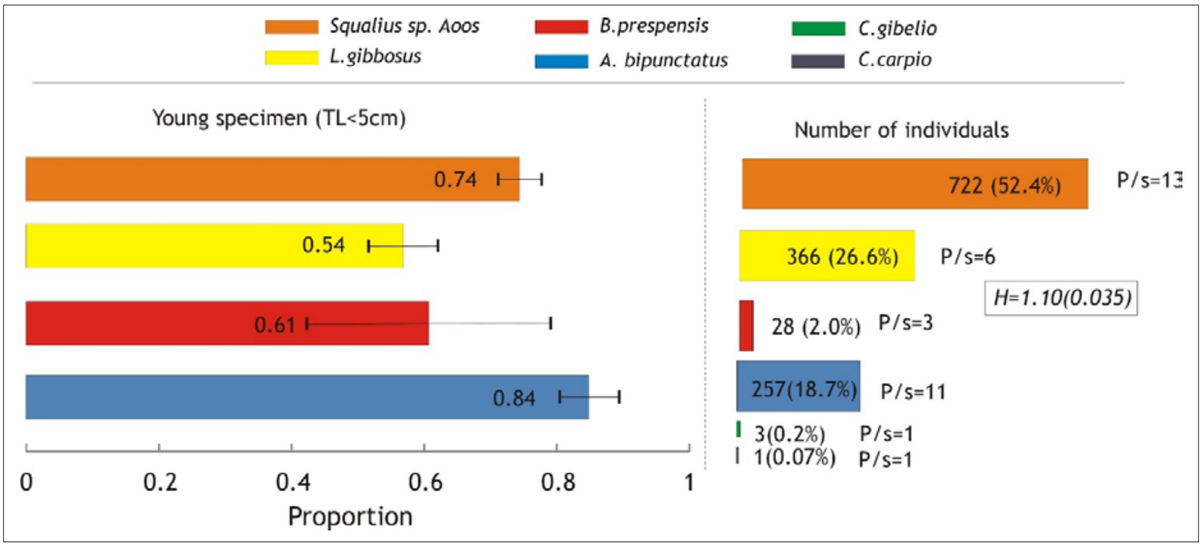

Fig. 3. Fish species composition (right) and proportion of young specimen per species (left) caught by electrofishing at 14 stations in the impoundment of the Aoos Springs between July 2019 and January 2020. Bars represent the $CL_{95\%}$; P/s: the number of stations where each species is present; *H*: Shannon-Wiener index ($CL_{95\%}$).

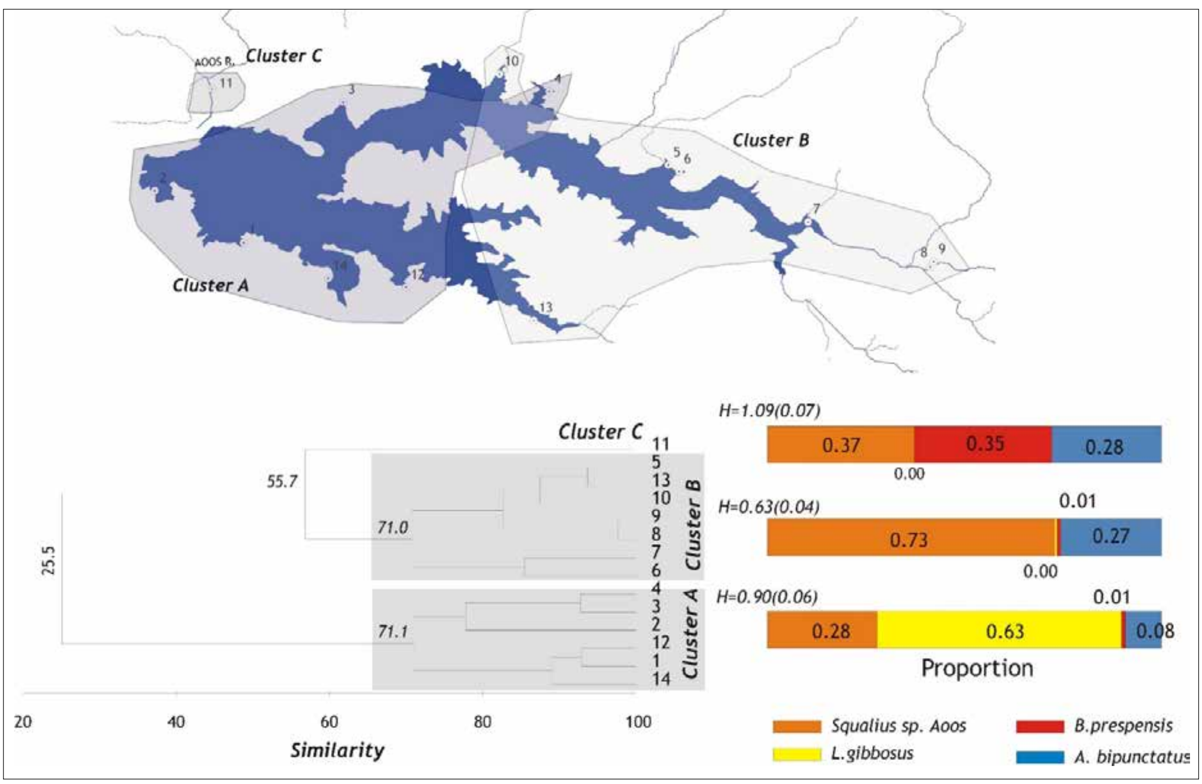

Fig. 4. Dendrogram of the cluster analysis (Left-down), species composition per cluster (Right-down) and spatial distribution of clusters (Top). *H*: Shannon – Wiener index ($CL_{95\%}$).

Discussion

The study updates our knowledge about the fish fauna in the impoundment of the Aoos Springs and sets a reference base for its systematic monitoring with special emphasis given to the management of the non-native species. The changes of the fish fauna observed in the studied impoundment (Fig. 5) revealed that fish fauna has been enriched across years starting from four species observed few years after the formation of the impoundment (during 1996-1997: Economou et al. 1998) to nine species found 20 years later (2015-2019). Apart from the six species found in the present study (Table 2), one more species was also reported from a recent study – *Oncorhynchus mykiss* (Walbaum, 1792) (Tsionki et al. 2019). Recreational fishers confirmed the presence of one specimen of Russian sturgeon *Acipenser gueldenstaedtii* Brandt & Ratzeburg, 1833, caught in 2019 (photo available by Ioannina Fishing club) and of Adriatic brown trout *Salmo farioides* Karaman, 1938. Hence, the fish fauna of the impoundment of the Aoos Springs consists of the autochthonous species *S. farioides*, *B. prespensis*, *Squalius* sp. (Aoos population) and *A. bipunctatus* and the introduced species *C. carpio*, *O. mykiss, A. gueldenstaedtii*, *C. gibelio* and *L. gibbosus*. Six out of the nine above-mentioned species found in the impoundment are considered threatened according to the IUCN evaluation (Fig. 5).

Intentional stockings with species of commercial interest have been systematically conducted in the impoundment of the Aoos Springs from the first years of its construction (Economou et al. 2007, Korakis et al. 2016, Leonardos et al. 2016). These efforts are a widespread strategy used to increase fish yields all around the world (Cucherousset & Olden 2011), however, the necessity to manage non-native species is also critical (Arthington et al. 2016). Rainbow trout *O. mykiss* have been systematically introduced into the system since its inception, as they are a target species for the recreational fishing. Data attained from the local freshwater fish farm (Terovo, Ioannina) revealed that during 2003-2018 between 30,000 and 200,000 individuals were annually introduced into the impoundment to support the local recreational fishery. The few spotted introductions that were conducted in the mid-1990s, justify the presence of this species during the initial sampling in the lake (1996-1997: Economou et al. 1998); the same applies for 20 years after that (Tsionki et al. 2019). *Oncorhynchus mykiss* exhibits low population persistence, implying that recruitment is highly limited in Greek freshwaters (Koutsikos et al. 2018). Likely, its notable decline from the studied impoundment followed the end of the stockings' trials. Paschos et al (2008) also reported the introduction of 5,000 individuals of *A. gueldenstaedtii* during 1997-1999, with two individuals exceeding 20 kg being caught by recreational fishers (Ioannina Fishing club) after two decades.

The non-native species *C. gibelio* has been one of the most common introduced fishes in Greece for over a century (Moutopoulos et al. 2022), often unintentionally introduced together with stockings of *C. carpio*. The latter, which is a native species in the Greek freshwaters (Barberi et al. 2015), introduced into the impoundment during the period 1996-2000 (Paschos et al. 2008), whereas occasional stocking trails with other cyprinids, also including *C. gibelio*, have been taken place, likely by recreational fishers. *Lepomis gibbosus* is a non-native species, reported for first time from the impoundment of the Aoos Springs at 2015 (Korakis et al. 2016). Information from the local social network indicated that the perceivable presence of the species was dated back as early as 2010. According to the statements provided by recreational fishers and also confirmed by other studies in Greek freshwaters (Zogaris 2017), this species can be used as live bait for recreational fishing, because of its rainbow flashing skin and small body size and, therefore, it could be unintentionally introduced as bait for catching *A. gueldenstaedtii* and *O. mykiss*. Other potential hypotheses of its introduction include the accidental release from aquariums (Perdikaris et al. 2010). In this context, public education on the consequences of non-native species is critical for the sustainability of freshwater fish fauna. The expansion of non-invasive species may lead to several local extirpations and even extinctions of species with a narrow range (Darwall & Freyhof 2016). Recently, a field study from a natural lake in Greece has shown that *L. gibbosus* may pose a competitive threat to local populations, especially endemics that share habitat and compete for food resources (Bobori et al. 2019).

The reduction and the potential extinction of the native trout *S. faroides*, which used to be abundant 20 years ago (Economou et al. 1998), is likely to occur due to one or more of the following reasons: (a) river fragmentation (Copp et al. 2020), (b) the limitation of the available reproductive fields offered by the new system (Encina et al. 2006) and (c) the inter-species competition, for habitats or predation, by *O. mykiss* (Blanchet et al. 2007, Houde et al. 2016). Recreational fishers stated that *S. faroides*, together with *O. mykiss*, were also caught in large numbers 20 years ago (Economou et al. 1998), while regarding *S. faroides* their catches have been

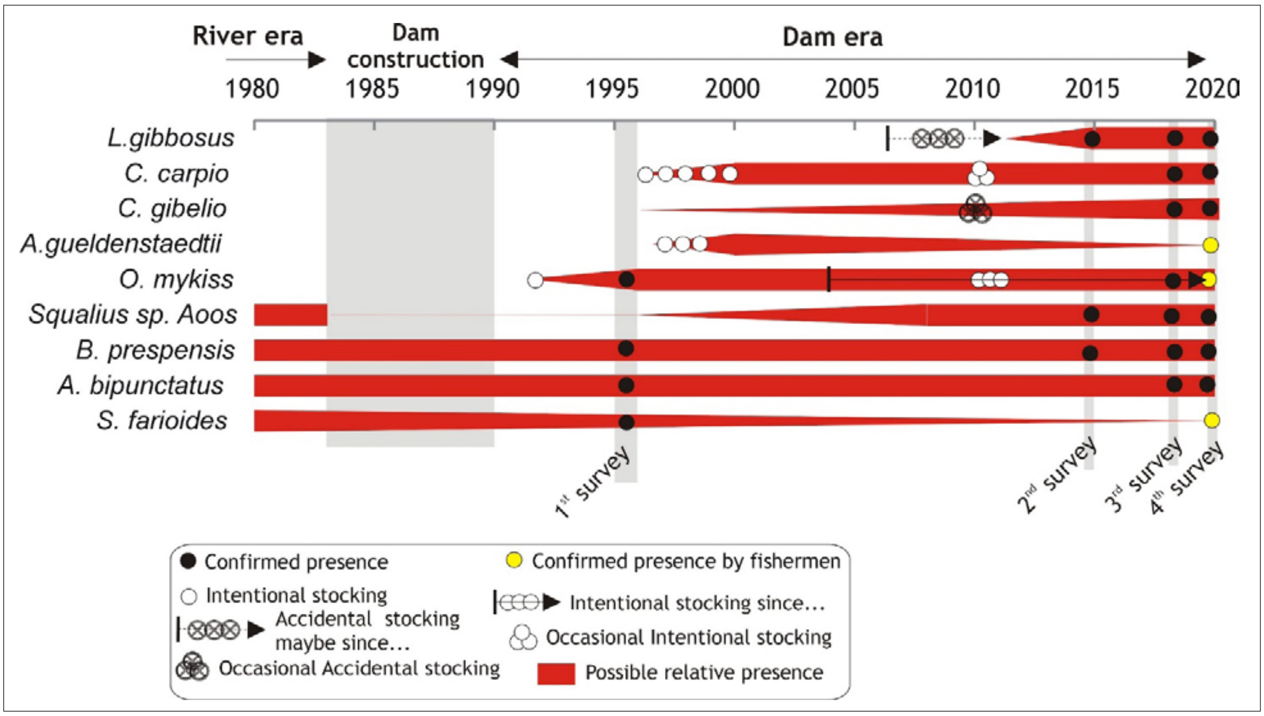

Fig. 5. Historical succession of the ichthyofauna observed in the impoundment of the Aoos Springs since its construction (1990-2020). *Acipenser gueldenstaedtii* is classified as Critically Endangered, *C. carpio* as Vulnerable, *Squalius* sp. (Aoos population) as Near Threatened and *B. prespensis*, *L. gibbosus* and *A. bipunctatus* as Least Concern.

severely declining through the years. Regarding the presence of *Squalius* sp. (Aoos population), which is a native species of the Aoos Basin that inhabits low and moderate altitudes (up to 900 m a.s.l.) (Barbieri et al. 2015), this species has been recorded in the impoundment only during recent years (Management Agency 2015, Tsionki et al. 2019, present study) and not during the years after the construction of the impoundment (Economou et al. 1998). The presence of young specimens of this species found approximately one km downstream of the impoundment cannot be attributed to escaped eggs or young specimens from the lake's fish population. This is because the hydrological link that used to exist between the two systems due to dam overflow has now been terminated. The fragmentation of the river, which prevented the free flow of the water and the movement of the fish, resulted in forming two distinct habitats with different species composition (Economou et al. 2007, Tsionki et al. 2019). This supports our hypothesis that the population of *Squalius* sp., which was likely untraceable during the initial sampling in 1996-1997 (Economou et al. 1998), developed from a small population trapped in the impoundment during the early years after its construction.

According to the sizes of the specimens caught with both gears, most of the specimens are at least in the first year of their life cycle (Froese & Pauly 2021). Size differences of the specimens caught when using the two fishing methods showed that young specimens of many species refuge in the shore line of the impoundment (Table 2, Fig. 3) (Sutela et al. 2008), whereas the adults of the same species inhabit the lower layers of the water column (Fig. 2) as it was observed previously for *C. carpio*. Fish fauna composition showed geographical variation in addition to the expected changes in specimen sizes between gears. (Table 2, Fig. 4). Although in the present study *L. gibbosus* was distributed in the south and south-western part of the lake (Cluster A in Fig. 4), this species has been also reported, though with low densities, at the north-eastern side of the lake (Management Agency unpublished data). It is noteworthy that the presence of *L. gibbosus* was detected in the central and western part of the impoundment and not in the flooded meadows before or after the impoundment. In contrast, despite the fact that sampling has been performed after the spawning period, the species that use gravel or sandy spawning grounds in standing waters, such as *Squalius* sp. (Aoos population), *A. bipunctatus* and *B. prespensis* (Froese & Pauly 2021) are distributed at the south-west part of the impoundment (Cluster B in Fig. 4). The impoundment offers also suitable spawning grounds, namely shallow waters with flooded meadows (Froese & Pauly 2021) for species such as *C. carpio* and *C. gibelio*, mainly observed in the western part of the impoundment. As a result, sampling with both fishing gears revealed an ontogenetic continuity of the species (juveniles and adults), indicating that the system supports species establishment and highlights the connectivity between lake and inflow streams.

In conclusion, human intervention in aquatic systems should largely focus on minimizing unintentionally or intentionally introduced non-native species that have a negative influence on populations of native species in order to achieve maximum biodiversity conservation benefits (Green & Grosholz 2020). In this context, the definition of national targets for detecting and controlling invasive alien species need to be of particular area of research interest (Koutsikos et al. 2021). Moreover, the examination of issues like intraspecific interactions with native biota when developing strategic plans, e.g. ecosystem-based models (Moutopoulos et al. 2020), can emphasize the necessity of an ecological niche approach and highlight the complexity of lake ecosystems. Decent monitoring schemes should be also adopted in order for the effects of the invasion to be evaluated timely and the driving forces in the environment and society to be identified, providing, thus, the necessary feedback to re-evaluate each management strategy.

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