

Short Note

# On the Status of Threespine Stickleback (*Gasterosteus aculeatus* Linnaeus 1758) in Lake Bracciano, Italy

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**Abstract:** For many species, the Mediterranean region harbors distinct lineages that are of conservation concerns. However, many of these are threatened by habitat degradation and by the introduction of non-native species. Here, we assess the status of the native threespine stickleback (*Gasterosteus aculeatus*) in the Lake Bracciano region in Italy, where stickleback have been historically present. During a dedicated sampling campaign in summer 2015, surveying the potential habitats that sticklebacks commonly occupy, we could not confirm the presence of this species but found introduced species to be often most abundant. Stickleback are thus likely to either have become extinct over the last decades or be on the verge to extinction in the Lake Bracciano region.

**Keywords:** *Gasterosteus aculeatus*; Mediterranean biota; conservation; ichthyofauna; biogeography

## 1. Introduction

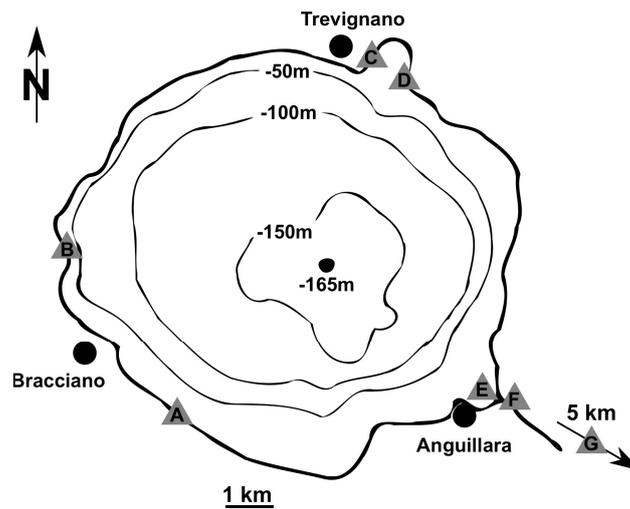
The Mediterranean region is a biodiversity hotspot for freshwater ecosystems, harbouring many endemic species as well as genetically distinct lineages of conservation concerns [1]. In Europe, freshwater threespine stickleback (*Gasterosteus aculeatus* species complex) occur from Iceland to the European Mediterranean coast (e.g., [2,3]) with rare occurrences in the Mediterranean part of Turkey [4] and Syria [5]. Whereas North European freshwater populations emerged since the last glacial retreat ~12,000 years ago, many Mediterranean populations are remnants of former glacial refugia [2,3,6]. Gene flow between the latter is absent because of the lack of marine stickleback in the Mediterranean Sea, resulting in high genetic differentiation among even geographically close populations [7,8].

Conservation-related assessments suggest that many Mediterranean stickleback populations represent separate evolutionary significant units [3,6,8]. Most Mediterranean populations occur in streams, and only very few lake-dwelling cases are known. Albeit stickleback are listed as a species of least concern for conservation by the International Union for Conservation of Nature (IUCN), they have a local status of being threatened in the Mediterranean region [7] but not in Italy [9]. However, despite recent phylogeographic studies on Mediterranean stickleback [3,6,8], little is known about its status in Italy.

Lake Bracciano (42°07' N, 12°14' E; Figure 1) is a deep, oligo-mesotrophic volcanic lake in Central Italy. The lake has several small permanent inlets and a major outlet—the Arrone River draining into the Mediterranean Sea. The larger part of the lake water is, however, used to supply freshwater to the nearby city of Rome. The ichthyofauna of the Lake Bracciano catchment consists of

13 native species and 11 species that were introduced over the last 150 years and have in some cases become highly abundant (whitefish—*Coregonus lavaretus*, Eastern mosquito fish—*Gambusia affinis*, and pumpkinseed—*Lepomis gibbosus* [10]).

Albeit threespine stickleback were historically reported in Lake Bracciano [11] and commonly caught by local fishermen until the 1980s [12], a contemporary assessment of the current stickleback population is lacking. By sampling the available littoral habitat, combined with stomach content data from a predatory species, we aimed to determine the presence and abundance of stickleback in the Lake Bracciano system.



**Figure 1.** Bathymetric map of Lake Bracciano (Italy), including the sampling sites (triangles A–G) and the water depth from the surface (after [13]).

## 2. Results

We did not capture any stickleback; however, ten other fish species were captured with a total trapping time of 1747 h within the Lake Bracciano region (Table 1). The most abundant species was pumpkinseed (*L. gibbosus*— $N = 322$ ) occurring at all lake sites. Similarly, common but less abundant was the freshwater blenny (*Salaria fluviatilis*), occurring at one inflowing stream and at all lake sites. Local high abundances of specific fish species occurred, e.g., the Eastern mosquito fish (*G. affinis*) at site D and the big-scale sand smelt (*Atherina boyeri*) at the stream site B. In the outflowing river, we found only three fish species (Table 1).

Out of the 15 pikes, three individuals had empty stomachs, whereas the stomachs of all other specimens contained, on average, 2.3 *A. boyeri* (range 1–7). In addition, one stomach contained three freshwater shrimps (*Palaemonetes antennarius*).

**Table 1.** The number of minnow traps and hours set for each sampling where applicable. Given, are the number of specimens captured per species at each site where minnow traps were used, whereas, for the outflowing stream, only the presence of a given species is indicated (i.e., “x”).

Site	No. Traps Set	No. Hours Set	Total Trapping Time (h)	<i>Anguilla anguilla</i>	<i>Atherina boyeri</i>	<i>Cobitis bilineata</i>	<i>Esox lucius</i>	<i>Gambusia affinis</i>	<i>Lepomis gibbosus</i>	<i>Leuciscus suffia</i>	<i>Padogobius bonelli</i>	<i>Rutilus robilio</i>	<i>Salaria fluviatilis</i>
A	12	15	180	1	-	2	-	-	24	-	-	-	11
B (Lake)	3	21	63	-	-	-	-	-	19	-	-	-	2
B (Stream)	12	21	252	-	62	1	-	-	-	-	5	1	4
C	22	13	286	-	-	-	-	-	179	-	-	-	1
D	9	14	126	-	-	-	1	51	-	3	-	-	-
E	35	24	840	-	-	13	-	-	100	-	-	-	3
F	-	-	-	-	-	x	-	x	x	-	-	-	-
G	-	-	-	-	-	x	-	-	-	-	-	-	-

### 3. Discussion

Despite being historically present in the Lake Bracciano catchment [11], we did not find a single threespine stickleback using capture techniques that are commonly used to collect stickleback [14] and that were successful to retrieve specimens in three other Italian watersheds (Tiber, Garigliano, Tagliamento) during the same sampling campaign. Adult stickleback may, however, have existed in parts of the lake that were not rigorously assessed, i.e., the pelagic and the deeper vegetated part of Lake Bracciano [15]. Capturing these would require increased sampling efforts using gill nets [16]. Nevertheless, combining our observation with (i) the absence of stickleback in a recent biodiversity assessment of the Bracciano region [10], (ii) the accounts of professional fishermen suggesting that stickleback were abundant until the 1980s but are not caught nowadays, and (iii) a comprehensive investigation of stomach contents for piscivorous species in Lake Bracciano failing to retrieve stickleback [17], an extinction of stickleback in the Lake Bracciano region seems yet likely.

Many Mediterranean stickleback populations have declined or became extinct as a result of habitat loss and the release of non-native fish [7,18,19]. Both of these factors may have affected the stickleback population in Lake Bracciano: The riparian vegetation has declined over the last decades [20] alongside a significant decrease in deepwater macrophytes over the last 5–8 years [15], both being important foraging and breeding habitats for stickleback [21]. In addition, several predatory species were introduced [10]. Particularly, the largemouth bass (*Micropterus salmoides*), introduced in 1998, has become an important predator [22]. Also, we found pumpkinseed to be highly abundant in the pelagic zone of the lake (Table 1), potentially competing with stickleback for food resources and nesting sites. The predominance of pumpkinseed and largemouth bass was similarly found to drive the extinction of lake-dwelling stickleback in Spain [18]. Interestingly, the freshwater blenny (*S. fluviatilis*) was the only native species whose population density was not affected in the latter case, which may also be true for Lake Bracciano, given its widespread presence (Table 1).

Taken together, it seems likely that the threespine stickleback has either become extinct or is on the verge of extinction in the Lake Bracciano system, potentially as a result of the introduction of non-native species and habitat deterioration. Further long-term direct sampling efforts combined with indirect assessment methods such as the meta-barcoding of environmental DNA from water samples are yet needed to confirm the extinction of this population. Combined with the restricted and fragmented distribution of stickleback populations in the adjacent water drainages [10], our observation nevertheless questions the current assessment by the IUCN of *G. aculeatus* being a species of least concern in Italy [9].

### 4. Materials and Methods

We used metallic minnow traps (Dynamic Aqua-Supply Ltd., Surrey, BC, Canada), which are commonly used to collect stickleback quantitatively [14], to probe the littoral zone of Lake Bracciano during breeding season. Each trap had a mesh size of 6.35 mm, being 42 cm long, maximum 22.9 cm wide with an entrance hole of 2 cm diameter. We set traps at four lake sites (A–C and E, Figure 1) and three inflowing streams (sites A, B, D) in June 2015. Because of the increased flow regime and the relatively low water level, we used hand nets in the outflowing Arrone river (sites F and G) to qualitatively assess the presence of stickleback. In addition, we extracted the stomachs of 15 adult pikes (*Esox lucius*), a top predator in the Lake Bracciano ecosystem, that were caught in the pelagic zone of the lake using gill nets by professional fishermen. All items found in the stomachs were counted and identified to the nearest taxonomic level.

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**Author Contributions:** S.S., E.A., and K.L. performed the sampling, S.S. and K.L. wrote the manuscript with input from E.A.

**Conflicts of Interest:** The authors declare no conflict of interest.

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