Research Article

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First Report of an Established Population of *Oncorhynchus mykiss* (Walbaum, 1792) (Salmonidae) on the Island of Crete, Greece

Maria Th. Stoumboudi, Roberta Barbieri & Eleni Kalogianni

Institute of Marine Biological Resources and Inland Waters, Hellenic Centre for Marine Research, 46.7 km Athens - Sounio Ave., 19013 Anavissos Attikis, Greece; E-mails: mstoum@hcmr.gr, robertab@hcmr.gr, ekalog@hcmr.gr

Abstract: In the present study, we provide evidence on the first established, self-sustaining population of *Oncorhynchus mykiss* in Greece, which is also the southernmost population reported for Europe. This population was found in an upland section of a spring-fed stream (Kissano Stream) in Southern Crete. During this study, in October 2012, rainbow trout of various sizes, ranging from 5 cm to 30 cm of total length, were caught at a riffle-pool section of the stream, located near the village of Spili. According to local inhabitants, the fish population in the Kissano Stream originated from a single introduction of rainbow trout juveniles in the mid-1980s, transferred from a trout farm in mainland Greece. A comparison of the population size-structure of *O. mykiss* in the Kissano Stream and the environmental features of its habitat to those of two other locations in Greece with *O. mykiss* presence (in Crete and in northern Greece) further supports the contention of a self-reproducing population in the Kissano Stream.

Key words: Rainbow trout, alien species, established population, Crete, Greece

Introduction

The rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) is a fish species widely introduced and farmed globally, outside its native range (Woynarovich et al. 2011). In 1992, it was reported that the species had been introduced in 87 countries (Welcomme 1992), while in 2010 the number of countries rose to 99, in 53 of which it appears to become established (Gherardi 2010). This broad introduction of *O. mykiss* is due to its high commercial market value and the interest it presents for sport angling. The success of its introduction is due to the fact that it is more tolerant than other trout species to a wide range of environmental conditions, while particular strains seem to be tolerant to temperate environments as well (Woynarovich et al. 2011, Chen et al. 2015).

However, reports for established self-sustaining populations of *O. mykiss* in nature, outside the species native range that comprises cold-water rivers and lakes of the Pacific coasts of North America and Asia, are rather limited. Fausch et al. (2001) reported the establishment of highly successful reproducing populations of *O. mykiss* in the southern Appalachian Mountains and in only a few locations in Scandinavia, Central Europe, the United Kingdom, and Japan. These authors also reported the establishment of moderately successful reproducing populations only at certain locations of the central Rocky Mountains, the Andes Mountains, New Zealand and Australia. In the review of Stankovic et al. (2015) on *O. mykiss* in Europe, more than 130 populations are listed as confirmed or potentially self-sustaining, across 16 European countries, which, notably, did not include Greece, with the highest abundance of these populations occurring on the foothills of the Alps in central Europe.

Oncorhynchus mykiss was first introduced to Greece in the 1950s as eggs from Switzerland and the fingerlings (which were produced mainly

by state hatcheries in northern Greece) were dispersed widely into several rivers and oligotrophic lakes, due to frequent stocking and aquaculture escapes (Economidis et al. 2000, Zenetos et al. 2009, Barbieri et al. 2015). *O. mykiss* is the most extensively farmed species in Greek freshwaters; nowadays, there are about 100 *O. mykiss* farms in Greece, with an annual estimated production of approximately 5,000 tonnes (Perdikaris et al. 2010). In the study of Economou et al. (2007), *O. mykiss* was reported to be the second most widespread alien species in Greek freshwaters, with the first being the Eastern mosquitofish *Gambusia holbrooki* Girard, 1859. According to the same study, populations of *O. mykiss* have been recorded in 27 hydrographic basins of Greece, and more specifically, in seven basins of northern Greece, eight in western Greece, six of the Peloponnese, two in central-eastern Greece and four of the Aegean Islands (and, in particular, of Crete). Four additional occurrences were reported recently, two in northern Greece and the Peloponnese (Koutsikos et al. 2012), and two more in Crete (Barbieri et al. 2013), thus raising the total number of *O. mykiss* records for Greece to 31, and for Crete to six. However, *O. mykiss* has not yet been reported as having established reproducing populations in Greece and its occurrence in different basins is attributed to the stocking programmes and recurrent aquaculture escapes (Economou et al. 2007). Only one population in south-eastern Peloponnese has been hypothesised so far to be potentially self-sustaining (Koutsikos et al. 2012).

In the present study, the first population of *O. mykiss* in Greece, as well as the southernmost population in Europe, is reported as established for the island of Crete, in the Kissano Stream. The population size-structure of *O. mykiss* in the Kissano Stream and the environmental features of its habitat are examined and compared with two other locations in Greece where similar data exist.

Materials and Methods

This study was conducted at three sites: Kaminia site at the Kaminia Stream (N 35°19'00.31", E 24°22'22.78", Crete), Spili site at the Kissano Stream (N 35°12'45.40", E 24°31'57.80", Crete), and Mega Lakko site at the Mega Lakko Stream (N 39°49'33.39", E 20°53'34.20", north-western Greece, Fig. 1). The Kaminia Stream is a small independent stream in northern Crete flowing into the Sea of Crete (sub-basin area 73.44 km^2). The Kissano Stream is also an independent stream in southern Crete, flowing into the Libyan Sea (sub-basin area 17.00 km^2), while the Mega Lakko Stream (sub-basin area 14.46 km^2) is a tributary of the Arachthos River, flowing into the Amvrakikos Gulf and the Ionian Sea (Fig. 1). These are all streams with permanent flow. The section of the Kaminia Stream sampled in this study is relatively degraded, receiving the wastewaters of the nearby villages, whereas the sections sampled of both the Kissano and the Mega Lakko Streams are relatively pristine.

Fish and environmental data acquisition

In the course of this study, the fish data collection and measurements of environmental parameters were conducted in autumn (03.10.2012 and 29.09.2013), within the frame of the national monitoring program of Greek rivers and streams (2012-2015) for the implementation of the WFD (2000/60/EC). An additional sampling was performed at Mega Lakko in spring (26.04.2013) and a survey was conducted at Spili in spring 2014.

The fish samplings were performed during day time (10:00 am – 17:00 pm), using a battery-powered backpack electrofishing device by Hans-Grassl GmbH (Model IG200-2, DC pulsed, 1.5 KW output power, 35-100 Hz, max. 850, according to CEN's specifications). In all occasions, a single pass within a 80-140 m stretch of the stream was conducted at each site by the electrofishing crew, which consisted of one operator and two netters, moving in a zigzag pattern upstream and covering all available fish habitats. The sampling started and ended at shallow riffles, which acted as physical barriers that minimised fish run away. The stunned fish were identified to species level on the spot, counted and their size class was recorded at 5 cm intervals. Then the specimens were released alive to the water. Care was taken to minimise any negative effects of electricity on fish, by adjusting output voltage at the lowest possible level, thus no mortality occurred among all the fish specimens that were captured in the frame of this study. At the end of each sampling, the fished area was estimated (fished length X mean wetted width), to calculate density (ind./m^2), which gives an estimation of the abundance of the target species.

At each site, the physicochemical parameters, i.e. conductivity (μS/cm), pH, dissolved oxygen content (D.O., mg/l), and water temperature (°C) were measured, using a Portable Multiparameter Aquaprobe AP-200 with a GPS Aquameter (Aquaread). The habitat parameters of the fished stream stretch, i.e. width (m), depth (m), flow (m/s), bottom aquatic vegetation (%), such as moss and submerged helophytes, and canopy cover (%), were recorded based on visual estimation. The substrate

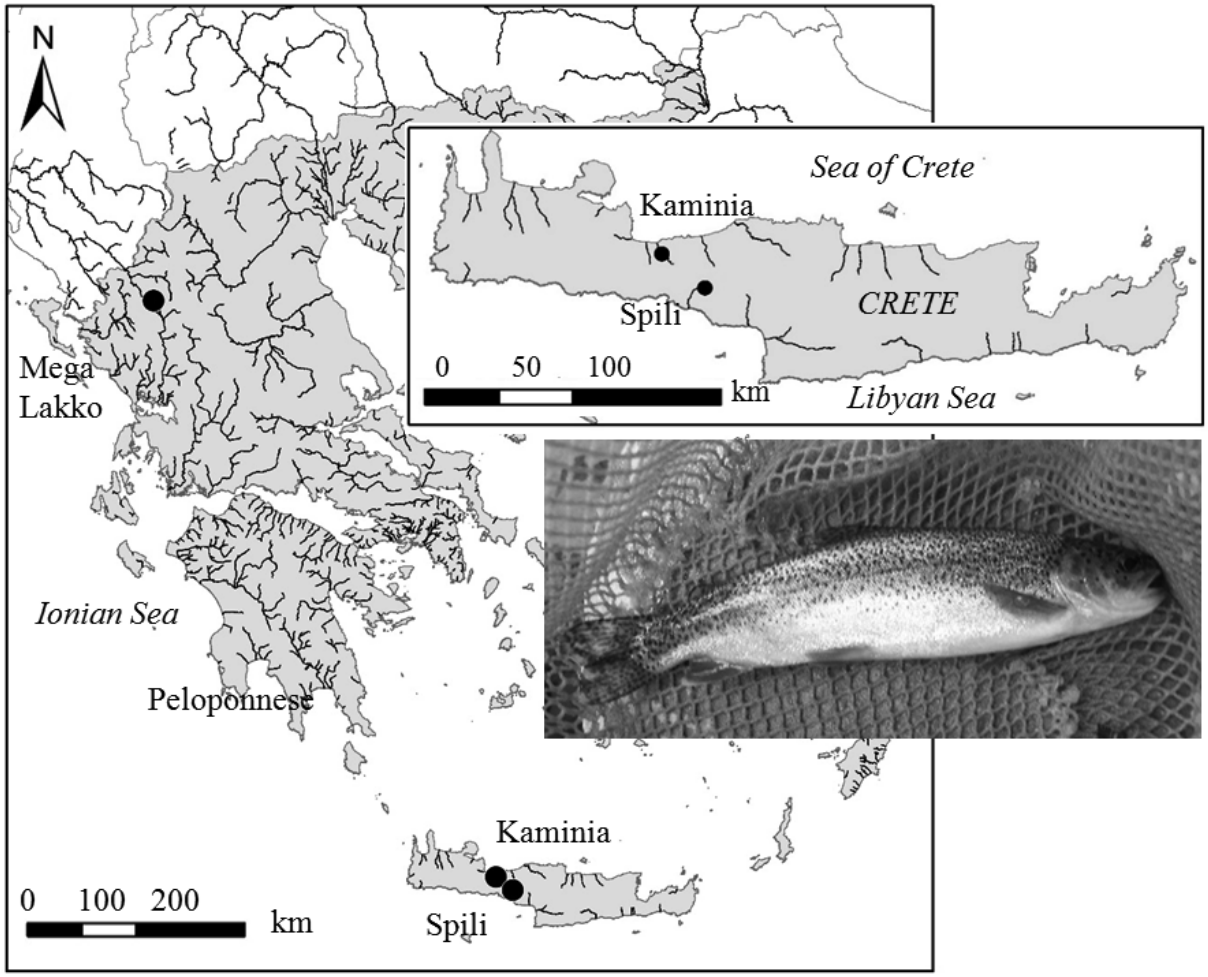

Fig. 1. Map of Greece with major rivers and location of the three study sites. Inset: Magnification of Crete with the two Cretan sites. Inset: Photo of rainbow trout caught during this study

coarseness was defined by using a modified Wentworth scale, whereas the coarse substrate was defined as the substrate consisting of particles > 63 mm (rock, boulders and cobbles). The dominant habitat (%) was also estimated at each site, by distinguishing between four habitat types, based on depth, flow and substrate, i.e. riffles (shallow/ fast flowing with coarse substrate), runs (deep/ moderately fast flow), glides (shallow/ slow moving) and pools (deep/ low flow/ fine substrate). For the habitat types, see Platts et al. (1983) and Aadland (1993). Finally, concerning spatial data, the elevation (m) was recorded in the field with a GPS Aquameter, while the distance from source and the catchment area upstream of the site were calculated based on 25x25 DEM of Greece.

Results and Discussion

Environmental characteristics of *O. mykiss* habitats

Table 1 summarises the environmental characteristics of the studied sites. Spili and Mega Lakko, which hosted larger numbers of *O. mykiss* than the Kaminia site, were sites with moderate to high elevation and with much shorter distance from source than the more lowland Kaminia site. The catchment areas of these two sites were also much smaller than that of the Kaminia site.

Regarding habitat characteristics, all three sites were relatively shallow with coarse substrate and sparse bottom vegetation. However, they differed in their habitat morphology, namely Kaminia site was dominated by slow flowing, shallow glides, while at Spili the dominant habitat was fast flowing, shallow riffles with some interspersed pools, and the Mega Lakko site consisted of a sequence of glides and riffles, with some rapids as well.

Finally, the dissolved oxygen content was lower and water temperature was higher at Kaminia than at Spili and Mega Lakko, although all sites fell within the range of temperatures tolerated by the species (Froese & Pauly 2009). The higher water temperature at Kaminia agrees with the lowland location of the site, as well as the larger distance

Table 1. Environmental data of the three study sites in autumn 2012 (Kaminia and Spili) and in autumn 2013 (Mega Lakko)

Spatial data	Kaminia	Spili	Mega Lakko
Elevation (m)	120	381	836
Distance from source (km)	16.7	5.7	4.9
Habitat data			
Width (mean, m)	4.5	2.5	3
Depth (mean, m)	0.35	0.25	0.3
Flow (mean, m/s)	0.1-0.25	0.25-0.5	0.25-0.5
Coarse substrate (> 63 mm, %)	70	90	70
Bottom vegetation	sparse	sparse	sparse
Canopy cover (%)	70	90	30
Dominant habitats (%)	glide	riffle	glide/ riffle
Physicochemical data			
Conductivity	524	469	321
Temperature (°C)	21.3	19	14.2
D.O. (mg/l)	9.2	10.4	10.13
pH	8.69	8.71	8.03

from source and the larger catchment area than the other two sites.

All the specified above indicate that both the Spili and Mega Lakko sites had relatively more favourable environmental conditions for *O. mykiss*, than the Kaminia site.

Fish assemblage composition

Table 2 shows fish species composition and densities at the three sampling sites.

At Kaminia and Spili (Crete), the alien *O. mykiss* was the only species caught, i.e. one individual at Kaminia and a total of 17 individuals at Spili. The absence of any sympatric species in the two aquatic systems of Crete is not surprising. During a survey of 52 riverine, lake and brackish water sites of Crete Island in late summer and early autumn of 2012, 17 were dry and 13 were fishless (Barbieri et al. 2013). The remaining hosted a total of five native and five introduced species, confirming earlier reports on the depauperate native fish fauna of the island (Bianco et al. 1996). Of the five native species mentioned above, only two were found in freshwater stream habitats of Crete, namely the peri-Mediterranean blenny *Salaria fluviatilis* (Asso, 1801) and the European eel *Anguilla anguilla* (Linnaeus, 1758) (Barbieri et al. 2013).

In contrast to the Cretan sites, at the Mega Lakko site during the autumn sampling, 56 individuals of *O. mykiss* were caught in association with 10 individuals of the Peloponnese barbel *Barbus peloponnesius* Valenciennes, 1842, and 10 individuals of the Epiros riffle dace *Telestes pleurobipunctatus* (Stephanidis, 1939). At the same site, in the preceding spring sampling, only two individuals of *O. mykiss* were caught, along with 13 individuals of *B. peloponnesius* and eight of *T. pleurobipunctatus* (Table 2). These two cyprinid species are endemic to Greece and Southern Albania, rheophilic and quite widespread in western Greece (Barbieri et al. 2015).

At the three study sites, the population density of *O. mykiss* ranged from 0.002 to 0.190 ind./m^2 (Table 2). Overall, the highest abundance of *O. mykiss* was observed at the Mega Lakko in autumn 2013, while the lowest at Kaminia (autumn 2012) and at Mega Lako in spring 2013.

Size-structure of *O. mykiss* populations

An examination of the population size-structure of *O. mykiss* at the Kaminia, Spili and Mega Lakko sites in autumn and at Mega Lakko in the preceding spring, provides evidence on the provenance and reproduction status of these populations (Fig. 2).

At Kaminia, the finding of a single, large individual (30-35 cm) in autumn is probably the result of accidental escaping to this system of *O. mykiss* individuals from the taverns of Argyroupoli Village (found in the proximity of Kaminia Stream), which keep live fish in ponds, originating from the rainbow trout farm in Almyros-Heraklion (Tingilis 2000a, b).

At Spili, the *O. mykiss* population consisted of individuals of all size classes between 6 and 30 cm, with individuals of 11-15 cm dominating the population, and had an overall balanced size distribution, that indicated a self-reproducing population. The self-sustaining status of the Kissano Stream population is further supported by information obtained by inhabitants of the nearby village of Spili, concerning the origin of the local *O. mykiss* population. The interviewees at Spili informed the research team that in the mid-1980s there was a single stocking with *O. mykiss* fingerlings, originating from mainland Greece (northern Peloponnese) in the village's Venetian fountain that is collecting cool (about 13°C) spring water, which feeds the Kissano Stream. There were several independent confirmations of this stocking, as well as statements that this had never been repeated since then. Furthermore, the existing trout farms in Crete, i.e. the one in Almyros-Heraklion and the two more in the Zaros area (central Crete, Tingilis 2000b) are not in the proximity of the Kissano Stream. In the report of Tingilis (2000b) several past introductions of *O. mykiss* in Cretan reservoirs, springs and streams

Table 2. Species composition at the three study sites (Kaminia, Spili and Mega Lakko), with total number of fish caught (n), frequency of occurrence (FO, %), and values of fish species density (ind./m^2)

Fish Species	Kaminia	Spili	Mega Lakko	
	Autumn 2012 (n = 1)	Autumn 2012 (n = 17)	Autumn 2013 (n = 76)	Spring 2013 (n = 23)
	FO % (ind./m^2)	FO % (ind./m^2)	FO % (ind./m^2)	FO % (ind./m^2)
O. mykiss	100 % (0.002)	100 % (0.133)	74 % (0.190)	9 % (0.008)
B. peloponnesius			13 % (0.034)	56 % (0.051)
T. pleurobipunctatus			13 % (0.034)	35 % (0.031)

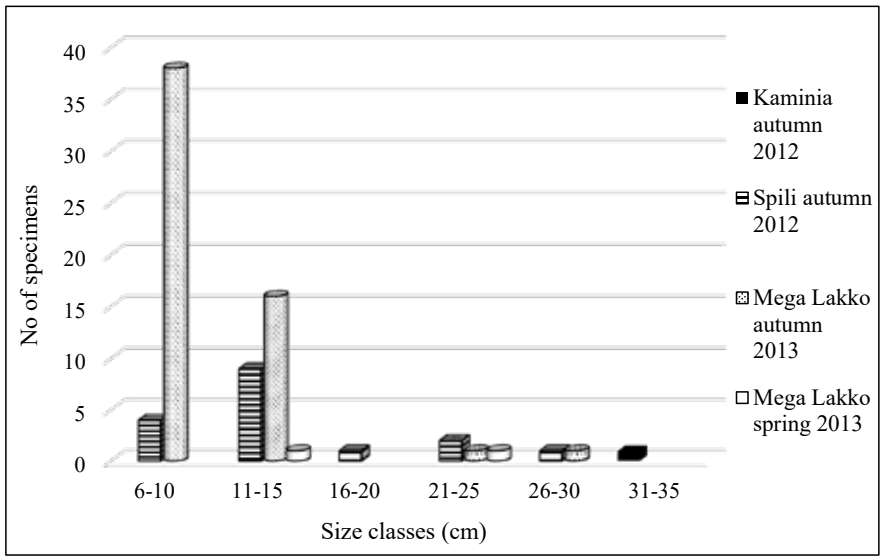

Fig. 2. Population structure of *O. mykiss* at the sites of Kaminia, Spili and Mega Lakko

are mentioned, including the single stocking at Spili, however, the only introduction date provided is for Kourna Lake (1978).

At Mega Lakko in autumn, the *O. mykiss* population was severely skewed towards small individuals (6-10 cm), while individuals of the size group 16-20 cm were absent. The dominance of small individuals in early autumn, combined with the complete absence of juveniles and small individuals in the preceding spring, when only two specimens of *O. mykiss* >11 m were caught, proves a stocking event in the area that occurred between the two samplings (April and October 2013). Indeed, in a report from 2001, repeated stockings with *O. mykiss* (every year from 1992 up to 2000) in the upland section of Arachthos basin are documented (PESCA 2001), and there are currently several rainbow trout farms operating in this area, as well as along the neighbouring Louros River. In addition, during the course of this study, locals reported at least one recent introduction with 30,000 *O. mykiss* fingerlings from a Louros River hatchery.

Establishment of an *O. mykiss* population and conservation implications

Our data support the contention of a self-reproducing *O. mykiss* population at the Kissano Stream, near the village of Spili in Crete Island. Regarding the reasons for the successful spawning of the Crete population, they include some favourable habitat characteristics, such as moderate winter temperatures during the reproductive period and favourable flow regime with winter flooding and low summer flows (Fausch et al. 2001, Candiotto et al. 2011). Another reason may be some differentiation in the reproductive traits of the specific strain of *O. mykiss*, whereas the lack of competition from native salmonids in the streams of Crete, and thus of interspecific competition, also plays a role, as hypothesised elsewhere (Gatz et al. 1987). Further research is obviously required to confirm the self-sustaining status of the population at Spili in Crete Island, by acquiring data on gonad maturity and the development of eggs.

When exploring the consequences of the

possible establishment of *O. mykiss* population at Spili, it appears that since there are no native trout populations in Crete, there is no potential competition with native salmonids and thus no deleterious effects on native trout, in the streams that are now devoid of fish, such as the Kissano Stream. However, under the climate change scenarios, an increase in water temperatures may facilitate the self-reproduction of *O. mykiss* populations in mainland Greece, where it may constitute a local threat, mainly due to trophic competition with native trout. This is especially relevant for northern Greece that hosts some stenoendemic native trout species (e.g., the Prespa trout *Salmo peristericus* Karaman, 1938, the Pelagonian trout *Salmo pelagonicus* Karaman, 1938, and the Louros trout *Salmo lourosensis* Delling, 2010, Barbieri et al. 2015).

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