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**The biology and ecology of *Valencia letourneuxi* Sauvage 1880 (VALENCIIDAE)
- Prospects for conservation**

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Abstract

Data are provided on the distribution, abundance, early development and biology of the endangered Greek endemic species *Valencia letourneuxi* Sauvage 1880, along with a record of its occurrence at new localities. *V. letourneuxi* is a small-bodied and short-lived insectivorous species, exhibiting cryptic colouration and sexual dimorphism. It matures in the first year of life, reproduces serially in late spring and summer, and deposits spherical eggs, around 2 mm, on aquatic plants. Most morphometric characters show size-specific trends, which complicate comparisons among populations or with other species. Of specific systematic importance is the relative position of the anal and dorsal fins, which remains almost unaltered throughout development, and allows safe distinction from *A. fasciatus*. The species was found mostly in deep areas with clean and slow running water, usually associated with freshwater springs. Rich submerged vegetation is the prominent ecological feature of all sites in which the species was found. Using as criteria of rarity the limited geographic distribution, the confinement of the species in few localities of each aquatic system and the low local densities, *V. letourneuxi* can be characterised as a "restricted and locally rare species". The restricted distribution, coupled with the narrow ecological requirements, makes the species vulnerable to extinction. Its disappearance from at least four aquatic systems and the serious population decline in a number of other systems seems to be connected with habitat loss or degradation caused by human activities. The prospects of conservation are discussed.

Keywords: *Valencia letourneuxi*, Greece, Habitat, Development, Larvae, Conservation.

Introduction

The Mediterranean genus *Valencia* (MYERS 1928), formerly classified in the family Cyprinodontidae, is now recognised as belonging to the monogeneric family Valenciidae (PARENTI, 1981). The genus is represented by two species: *Valencia letourneuxi* SAUVAGE 1880, endemic to western Greece and Albania, and *V. hispanica* Valenciennes 1846, endemic to the eastern Iberi-

an Peninsula. Due to some uncertainty about the taxonomic relationship of the two taxa, *V. letourneuxi* is often assigned to *V. hispanica*. However, genetic evidence suggests that the two taxa should be given separate specific status (VILWOCK *et al.*, 1982; PERDICES *et al.*, 1996).

Historical records indicate the past occurrence of *V. hispanica* over wide areas of

Spain and France, but its present distribution is restricted to six localities of the Valencian region (PLANELLES, 1995; PLANELLES & REYNA, 1996). *V. letourneuxi* was once widely distributed in western Greece but is now extinct from some localities and is vulnerable in most others. Records of occurrence (summarised by DAS, 1985; BIANCO & MILLER, 1989) show that its present distribution is limited to aquatic systems of western Greece (rivers Alfios, Pinios, Louros, Acheron and Kalamas) and to one of south Albania (Lake Butrino). Probable reasons for the decline have been stated as engineering, water pollution, water abstraction and ecological pressures from alien species (DAS, 1985; ECONOMIDIS, 1991).

V. letourneuxi (under the name *V. hispanica*) has been listed as "endangered" and a priority species for conservation in Annexes II and IV of the Directive of the European Union relating to the protection of natural habitats and wild fauna and flora. It is also considered in the IUCN report as one of the 24 most endangered species in Europe, and by the Bern Convention as a strictly protected species. By far, research on this species has focussed mainly on issues of taxonomy and distribution (STEPHANIDIS, 1974; ECONOMIDIS, 1991, 1995; PERDICES *et al.*, 1996). Nothing is known of its ecology and life-history except that it prefers clean stagnant freshwaters rich in vegetation, probably breeds in spring, and deposits adhesive eggs of about 2.2 mm diameter on aquatic plants (DAS, 1985; BIANCO & MILLER, 1989). Here we provide the first data on the biology and development of *V. letourneuxi* with a short description of its larvae. We also describe the distribution of the species in Greece and we announce its occurrence in four new localities.

Materials and Methods

Field data were collected during two large-scale ichthyological investigations of

the inland waters of western Greece and Peloponnese. The first investigation involved the study of ascending eels (*Anguilla anguilla*) and lasted from February 1991 to March 1994. Large riverine systems were routinely sampled on a usually monthly basis, smaller ones during infrequent visits. The second investigation studied some endemic freshwater fishes of Greece and lasted from March 1997 to September 1998. Three additional samples containing *V. letourneuxi* were taken from the Chiliadou spring (estuarine area of River Mornos) in April, May and July 1999. Two more samples were collected in a drainage channel of the estuarine area of River Acheloos in January 2000. In total, 324 samples were collected from more than 100 localities (stations). The positions of the sampling stations are shown in Fig. 1. All data obtained from field sampling and laboratory analyses were added to a database, which was designed to accommodate the results of ichthyological and ecological investigations.

Larvae were sampled with beakers and small scoop-nets, juveniles and adults with larger scoop nets, dragged scoop-nets, fry nets, a small seine and electrofishing. The presence of *V. letourneuxi* in the samples was always recorded but the sample size was kept small to avoid depleting the populations. Samples were normally preserved in 4% formalin, neutralized with sodium phosphate.

All sampled specimens were measured to the nearest 0.1 mm, as Total Length (L_T) and Standard Length (L_S). Unless otherwise stated, lengths are given as L_S . Observations of reproductive state were made in 25 specimens sampled in the Chiliadou area in May 1999. The guts of these fish were subsequently dissected and the contents of the alimentary canal were examined for diet composition. Total Weight (TW), Net Weight (NW) and Gonad Weight (GW) were measured in g. The Gonadosomatic Index (GSI) was measured as the percent-

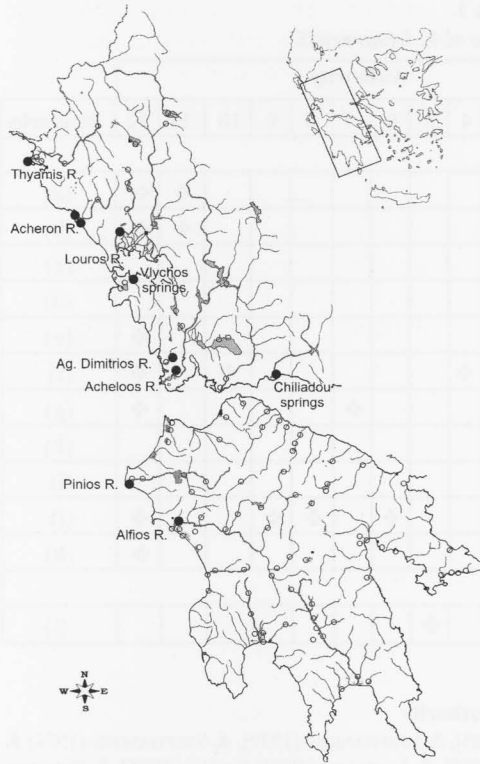


Fig. 1: Sampling stations in western Greece and Peloponnese (o) and localities where *Valencia letourneuxi* was recorded (•).

age of GW to NW. Morphological descriptions were based on preserved specimens. Illustrations were drawn with the aid of a camera lucida.

For a morphometric description of *V. letourneuxi*, the following characters were recorded from 91 individuals (abbreviations are given in parenthesis):

- total length (L_T)
- standard length (L_S)
- preanus length (between snout and anus) (lpr)
- postanus length (lpo)
- maximum body height (hma)
- body height at the caudal penducle (hcp)
- head height (hhe)
- head length (lhe)
- preorbital (snout) length (lpro)

- eye diameter (eyd)
- predorsal fin length (between snout and origin of dorsal fin) (pD)
- preanal fin length (pA)
- preventral length (pV)

In addition to the morphometric, three meristic characters were also recorded, the number of anal, dorsal and branched caudal fin rays (rA, rD and rC respectively).

Results

Distribution

Adequate information on the composition of fish fauna exists for 34 riverine systems of western Greece and Peloponnese (including the associated springs and lagoons), 23 natural and artificial lakes, and about 24 smaller water bodies (springs, marshes, etc.). Hydrological, biological and ecological descriptions of most of these aquatic systems have been provided by ECONOMOU *et al.* (1999). So far, *V. letourneuxi* has been reported from 9 aquatic systems of Greece, but probably it is now extinct from at least four systems. Here we announce the presence of the species in four more systems. Table 1 summarises all records of occurrence of *V. letourneuxi*. Figure 1 portrays the distribution of the species in the areas covered by the present investigation.

The known localities of *V. letourneuxi* are few indeed. The species is absent from all Greek lakes and is present in only few rivers and littoral springs. Moreover, the riverine populations are usually restricted to one or two sites of the river system, usually near springs. To some extent, the apparent sparseness of *V. letourneuxi* can be attributed to difficulty in detecting the species due to low local densities. However, since many aquatic systems have been sampled extensively, it may be a true reflection of rarity.

Table 1
Records of occurrence of *V. letourneuxi*.

Aquatic system	Authority												Remarks
	1	2	3	4	5	6	7	8	9	10	11	12	
Greece													
Alfios river	✦										✦	✦	(a)
Pinios river	✦										✦	✦	(b)
Acheloos river estuarine area*	✦												(c)
Ag. Dimitrios river*	✦												(d)
Louros river	✦		✦									✦	(e)
Acheron river	✦			✦						✦		✦	(f)
Thyamis river estuarine area	✦						✦					✦	(g)
Ditch of the Vlychos springs*	✦												(h)
Ditch of the Chiliadou springs*	✦												(i)
2 springs and 1 lagoon in Corfu island		✦				✦		✦	✦			✦	(j)
Zoodochos Pigi springs in Lefkas island												✦	(k)
Albania													
Butrinto lake					✦								(l)

* First record

Index to Authority

1. Present investigation, **2.** SAUVAGE (1880) [in DAS 1985], **3.** STEPHANIDIS (1939), **4.** STEPHANIDIS (1974) **5.** OLIVA 1961 [in DAS 1985], **6.** OLIVA (1965) [in DAS 1985], **7.** LABHART (1980) [in DAS 1985], **8.** SEEGER (1980) [in DAS 1985], **9.** WOELTJES (1982) [in DAS 1985], **10.** Das (1985), **11.** BIANCO & MILLER (1989), **12.** ECONOMIDIS (1991) [own observations and synopsis of past records]

Remarks on the distribution

(a) Found by [11] in one site near Epitalio village. Found once by [1] (May 1992) in a deep creek discharging about 150 m upstream of the river mouth. **(b)** Found by [11] at one site of the Pinios river near Stafidokampos village and by [1] at one deep site of the river near Kalyvia village. **(c)** Found by [1] at two sites (near ancient Ainias and near Fraxos forest) of the peripheral channel of the Lessini estate, in the estuarine area of the Acheloos river. Also found by I. Leonardos in stream Ag. Symeon near the Technological School of Messolongi, and by Th. Athanassopoulos in a marsh near the estuaries (personal communications). **(d)** Found by [1] in the springs of the short river Ag. Dimitrios (near Lessini village) and at one site 200 m downstream of the spring area. Also found by P. Economidis in the spring area (personal communication). **(e)** Found by [3], [12] and [1] in the Barbanakos (or Stephani) springs, near Stephani village, and by [12] in springs and marshes near Petra village. **(f)** Found by [4], [10], [12] and [1] in a creek running through a marshy area (near Ammoudia village) into the sea at the right of the mouth of the Acheron river. Found by [4] in springs near Kastri village, and by [4] and [10] in a spring west of Kypseli village. Found by [1] in a creek running through a marshy area (near Valanidorachi village) into the sea at the left of the mouth of the Acheron river. **(g)** Found by [7], [12] and [1] in a marsh near Drepano village, in the broader estuarine area of the Thyamis river. In September 1997 the marsh was almost completely dry. **(h)** Found by [1] in the deep ditch that discharges from the littoral spring Vlychos (near Vonitsa) into the sea. **(i)** Found by [1] in the deep ditch that discharges the littoral spring Chiliadou (near Chiliadou village, close to the estuaries of the Mornos river) into the sea. **(j)** Found by [2], [6], [8], [9] and [12] in two springs associated with lagoons in central (Chalikiopoulou, Dasia) and in one spring in southern (Lefkimm) of Corfu. Most of these populations are probably extinct. **(k)** Found by [12] in Zoodochos Pigi (or Megali Vrysi) spring on Lefkas. The spring is now dry and the population of *V. letourneuxi* is extinct. **(l)** Found by [5] in Lake Butrinto of south Albania, near the Greek borders, mostly in ditches draining into the lake.

Abundance

One rough indicator of rarity is the abundance of a species in comparison to the abundance of other species having a similar geographic distribution. We used as an index of abundance of *V. letourneuxi* the number of individuals caught during routine ichthyological investigations in western Greece and Peloponnese (Feb. 1991 - March 1994, March 1997 - Sept. 1998) and on three sampling occasions in 1999. On the basis of abundance, *V. letourneuxi* appears to be a rare species. Only 182 individuals of *V. letourneuxi* were caught out of about 31600 fish in total. Since, however, *V. letourneuxi* was not the exclusive target of the investigations, many of the sampled localities were atypical for this species.

Species with a restricted or patchy distribution may be confined to few sites but be locally abundant. Therefore, local densities are a complementary criterion of rarity. For some of the systems with *V. letourneuxi* we estimated: (a) the percentage of samples containing *V. letourneuxi* in the total number of samples taken from these systems, and (b) the percentage of individuals of *V. letourneuxi* in the total number of fish found in the samples (Table 2). In all systems except in the Chiliadou spring, *V. letourneuxi* was encountered at low densities and can be characterised as "locally rare".

Ontogenetic development

A fertilized egg attached to a plant was detected accidentally during the cleaning

procedure in a rearing tank containing a small stock of *V. letourneuxi*. Another egg was collected from the Chiliadou spring among a mass of aquatic plants. Both eggs had well formed embryos and were spherical, yellowish, 1.98 - 2 mm in diameter, with a large (about 1.77 mm in diameter) homogeneous yolk mass and a thin perivitelline space (Fig. 2a). Stellate melanophores were distributed on the yolk and the dorsal part of the embryo.

The description of larvae that follows is based on wild specimens sampled in the Barbanakos spring in May 1992. The smallest larva available was 6.7 mm long, having just initiated the formation of anal fin rays (Fig. 2b). The dorsal part of the primordial fin is extremely small. The pigmentation pattern consists of many small and more or less evenly distributed melanophores, which cover almost the whole body surface, with the exception of the abdomen and a portion of the ventrolateral region of the head. Pigment cells are also present on the primordial fin and along the rays of all fins. Larvae over 7.5 showed first signs of dorsal fin ray development and extended coverage of scales (Fig. 2c). Pelvics bud at ca. 9.5 mm (Fig. 2d). By 10 mm, pelvic fin rays had appeared and the primordial fin was completely absorbed. Fig. 2e shows a juvenile with a complete scale coverage and almost total loss of larval characters.

With growth, the melanophores progressively increase in number over the body and along the fin rays, without changes in general pigmentation. Specimens larger than 18 mm in length showed sexual dimorphism in

Table 2
Frequency of occurrence (%FO) in samples, and percentage (%N) in total fish caught, of *V. letourneuxi* at some sampling sites.

Sampling locality	Sampling period	N° of samples	%FO of <i>V. letourneuxi</i>	Total fish	%N of <i>V.letourneuxi</i>
Barbanakos springs (Louros R.)	Apr. 92 - Jan. 95	13	23.1	2060	1.5
Ag. Dimitrios R. (spring area)	Dec. 91 - Jan. 95	18	61.1	1732	1.3
Vlychos springs	Jun. 94 - Mar. 97	9	22.2	1669	0.1
Chiliadou springs	May 97 - July 99	3	100.0	139	56.8

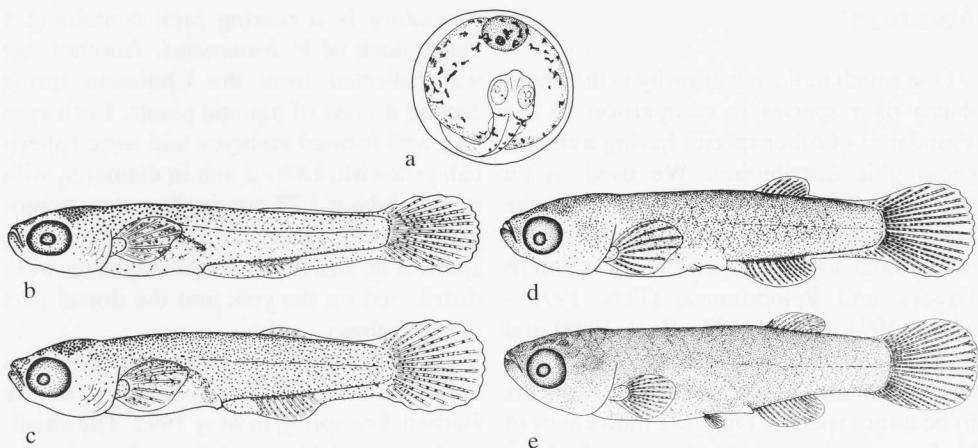


Fig. 2: Early life-history stages of *Valencia letourneuxi*. (a) Egg spawned in the aquarium. (b-e) wild-caught larvae; (b) 6.7 mm L_S; (c) 8.1 mm L_S; (d) 10.0 mm L_S; (e) 12.9 mm L_S.

colouration. In males there are 10-14 vertical stripes on the body, but in females these are very weakly pigmented and indistinct (Fig. 3). Additional differences between male and female fish are the more rounded margin of the caudal, anal and dorsal fins in females, and the dark margins of the dorsal and anal fins in males.

The number of transverse stripes in males is a character distinguishing *V. letourneuxi* and the morphologically similar species *Aphanius fasciatus* Nardo 1824, which has fewer stripes.

Morphometric-meristic characters

Table 3 provides basic morphometric and meristic data in relation to fish size for 91 individuals caught at 5 localities in western Greece (Barbanakos spring of R. Louros, 30 individuals; R. Ag. Dimitrios, 17; Ammoudia marsh of R. Acheron, 4; Chiliadou spring, 39, Vlychos spring, 2). Morphometric characters are expressed as ratios, usually to standard length. The most prominent morphometric changes occurring during development (Fig. 4) were: increase of body height relative to body length (indicating a progressive increase of robustness), decrease of postanal length (indicating a gradual shift of the anus backwards) and decrease of eye diameter. It is of interest that some of the morphometric trends established during the larval stage continued in the juvenile stage, making difficult to define the transitional interval from larvae to juveniles on the basis of morphometric characters. The relative position of fins remains almost constant during development. Of special diagnostic importance is the pA/pD ratio, which is always lower than 1, indicating that the dorsal fin is positioned slightly anteriorly to the anal fin. This character alone permits safe distinction from *A. fas-*

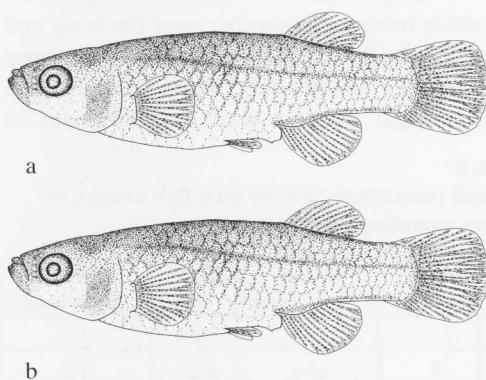


Fig. 3: Adult *Valencia letourneuxi*. (a) female 38 mm L_S; (b) adult 35 mm L_S.

Table 3
Synopsis of morphometric/meristic data for *Valencia letourneuxi* by size group
(see text for abbreviations).

Size groups (mm)											
Size (mm)	6-7	7-8	8-9	9-10	10-12	12-14	14-16	16-20	20-25	25-30	>30
Number	3	9	10	3	2	6	4	16	22	7	7
Morphometric data											
lpr/L _S	0.49	0.52	0.53	0.56	0.58	0.61	0.63	0.62	0.63	0.64	0.67
lpo/lpr	1.03	0.92	0.87	0.80	0.72	0.65	0.59	0.61	0.58	0.57	0.49
hma/L _S	0.16	0.17	0.18	0.20	0.21	0.23	0.24	0.25	0.25	0.27	0.26
hcp/L _S	0.11	0.12	0.12	0.13	0.14	0.14	0.15	0.15	0.15	0.15	0.15
hhe/L _S	0.19	0.19	0.20	0.20	0.21	0.21	0.22	0.21	0.21	0.22	0.21
lhe/L _S	0.27	0.28	0.29	0.29	0.29	0.29	0.30	0.30	0.29	0.30	0.28
pD/L _S		0.67	0.67	0.68	0.67	0.70	0.72	0.72	0.72	0.72	0.71
pA/L _S	0.62	0.63	0.63	0.63	0.62	0.65	0.67	0.66	0.66	0.67	0.68
pV/L _S				0.49	0.49	0.51	0.52	0.50	0.50	0.50	0.51
hhe/lhe	0.70	0.68	0.69	0.70	0.73	0.73	0.75	0.72	0.72	0.73	0.76
lpro/lhe	0.12	0.15	0.14	0.16	0.15	0.18	0.20	0.20	0.22	0.24	0.24
lpro/L _S	0.03	0.04	0.04	0.05	0.04	0.05	0.06	0.06	0.06	0.07	0.07
eyd/lhe	0.44	0.42	0.40	0.39	0.37	0.38	0.37	0.35	0.33	0.31	0.30
eyd/L _S	0.12	0.12	0.12	0.11	0.11	0.11	0.11	0.10	0.10	0.09	0.08
hhe/hma	1.20	1.13	1.11	1.03	1.03	0.92	0.92	0.87	0.86	0.81	0.82
lpro/eyd	0.82	0.35	0.34	0.42	0.41	0.47	0.53	0.59	0.66	0.77	0.83
pA/pD		0.94	0.94	0.93	0.92	0.93	0.93	0.92	0.93	0.93	0.95
pV/pD				0.72	0.73	0.73	0.72	0.70	0.69	0.70	0.72
pV/pA				0.79	0.79	0.79	0.77	0.76	0.76	0.74	0.75
Meristic data											
rD		1.89	5.70	8.67	9.00	9.50	9.50	9.14	9.00	9.33	9.00
rA	5.00	8.67	11.00	11.33	12.50	12.50	12.25	12.57	12.42	12.83	12.00
rC								12.50	13.73	14.50	15.00

ciatus, in which the anal fin is positioned anteriorly.

Male and female fish could not be distinguished safely on the basis of morphometric and meristic differences. The body height at the caudal peduncle was apparently different in the two groups of fish, but the difference was not statistically significant (t-test) (Fig. 5).

A comparison among populations was made for ascertaining morphometric-meristic differences that may possibly reflect

genetic differences. The comparison was complicated by the size specificity of some characters and the limited number of individuals of the same size available for analysis. Visual inspection of the plots indicated that most individuals from the Ag. Dimitrios spring have a higher lpr/L_S, pD/L_S, pA/L_S and rD than individuals from the Chiliadou spring. However, only the difference in rD was statistically significant at the 0.05 probability level (t-test).

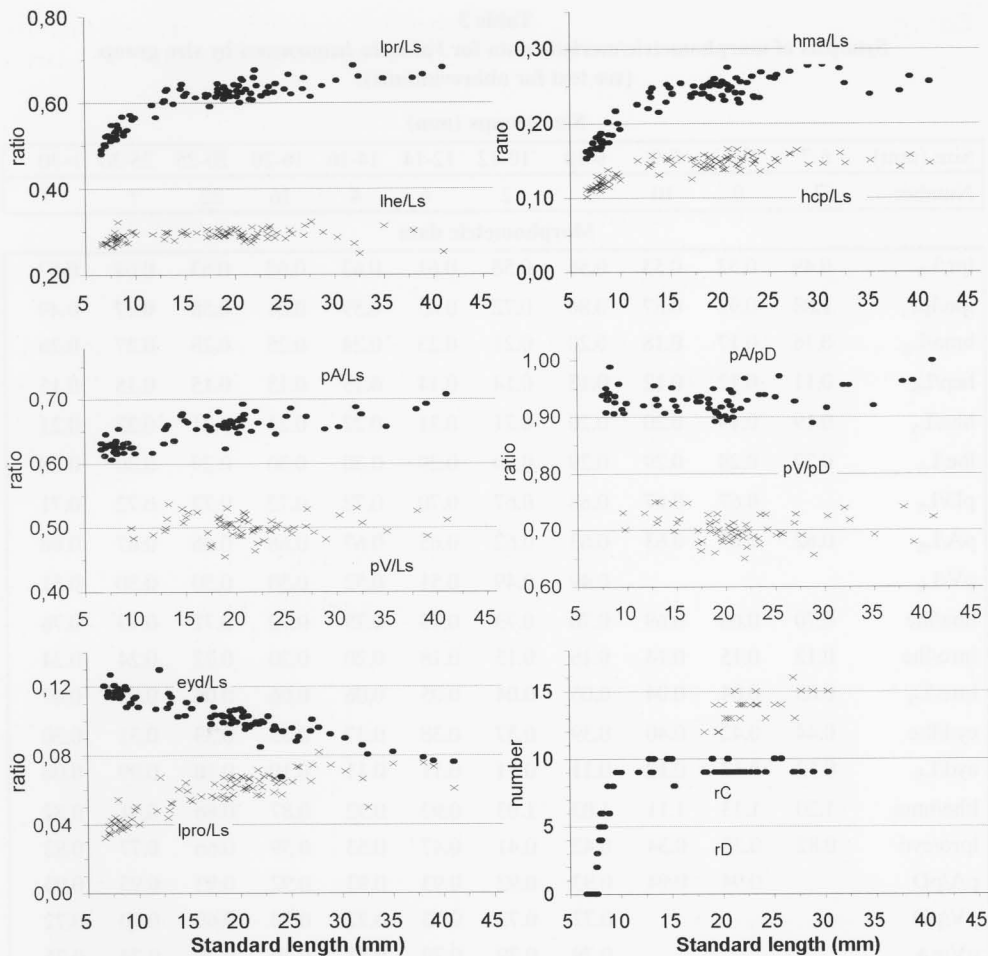


Fig. 4: Ontogenetic changes of some morphometric and meristic characters.

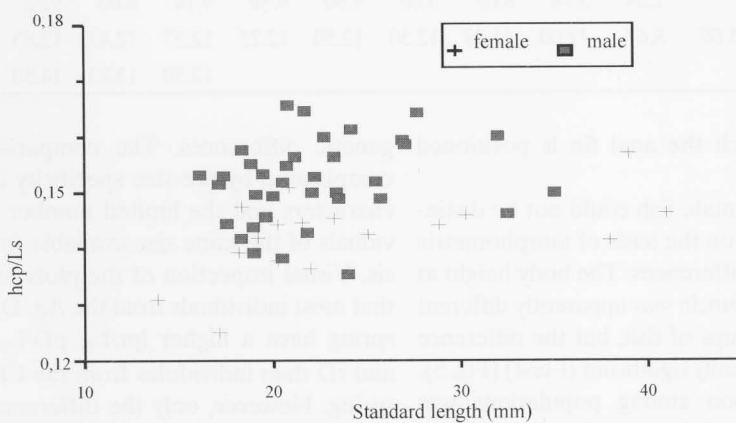


Fig. 5: The ratio of the body height at the caudal penducle (*hcp*) to the standard length (L_s) in male and female fish.

Ecology

V. letourneuxi was found mostly in deep sites with slow running or stagnant, but usually clean water. Most of its known habitats are associated with freshwater springs. However, four habitats can be characterised as slightly brackish, with salinity not exceeding 4‰ (Ammoudia, Valanidorachi, Drepano and Vlychos).

The common feature of all habitats is the very rich aquatic vegetation, which probably provides food, spawning substrate and protection from natural enemies. In the Chiliadou spring, the fish were associated

with a thick layer of floating plants that covered the surface of the water. The larvae were seen to swim in the surface layer, and were easily distinguishable from all other larval fishes by their strongly reflective eyes. Other fish species recorded with *V. letourneuxi* are shown in Table 4.

Biology

Of all areas investigated only the Chiliadou spring yielded a sufficient number of individuals for biological examination. Table 5 presents some biological parameters, and Figure 6 shows the length-

Table 4
Species occurring with *V. letourneuxi* in our sampling sites.

										Number of			Fish species associated with <i>Valencia letourneuxi</i>													
													Sampling area				samples			1	2	3				
4	5	6	7	8	9	10	11	12	13	14																
Alfios R.										1				+	+	+										
Pinios R.										1						+										
Ag. Dimitrios R. (spring area)										11				+		+				+				+		
Lourois R. (Barbanakos spring)										3				+		+					+	+				
Ammoudia marsh (Acheron R. estuarine area)										2				+						+		+				
Valanidorachi marsh (Acheron R. estuarine area)										1										+		+			+	+
Drepano marsh (Thyamis R. estuarine area)										1											+					
Vlychos springs (coasts of Amvrakikos gulf)										2				+		+				+						
Chiliadou springs																										

Index to species

Cyprinidae

- (1) *Pseudophoxinus stymphalicus*
- (2) *Leuciscus cephalus*
- (3) *Barbus peloponnesius*
- (4) *Phoxinellus pleurobipunctatus*
- (5) *Tropidophoxinellus hellenicus*

Gobiidae

- (6) *Economidichthys pygmaeus*
- (7) *Knipowitschia milleri*
- (8) *Knipowitschia* sp.

Poeciliidae

- (9) *Gambusia affinis*

Gasterosteidae

- (10) *Gasterosteus aculeatus*

Cobitidae

- (11) *Cobitis hellenica*
- (12) *Cobitis* sp.

Anguillidae

- (13) *Anguilla anguilla*

Seranidae

- (14) *Dicentrarchus labrax*

Table 5
Some biological data of *V. letourneuxi* from the spring of Chiliadou.

Length (mm) and weight (g) variables		
TL - SL	TW - TL	TW - SL
TL = 0.78 + 1.20 SL	TW = 0.000014 TL ^{3.002}	TW = 0.000035 SL ^{2.892}
n = 76, r ² = 0.998	n = 74, r ² = 0.993	n = 74, r ² = 0.994

Body size and sex		
	male	female
Max SL (mm)	36.2	34.0
Max TW (g)	1.40	1.18

	m/f
Sex ratio	0.48

GSI (May 1999)		
	Male	Female
Average	1.47	3.40
Number	25	11
range of GSI	0.33 - 2.44	0.80 - 10.38
range of SL (mm)	16.1 - 36.2	17.2 - 34.0

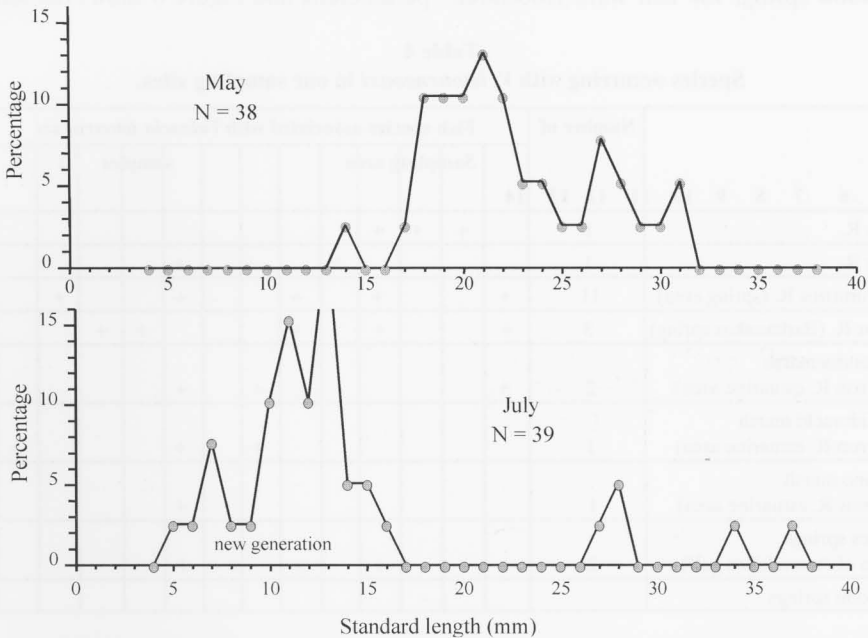


Fig. 6: The length-frequency distribution of *V. letourneuxi* in May and July 1999 in the Chiliadou spring.

frequency distribution of *V. letourneuxi* in two samples from the Chiliadou spring taken in May and July 1999. Sampling in July 1999 was conducted with a small hand net (16 cm diameter, 1 mm mesh size), which was ineffective for catching adult fish and very small larvae. Nonetheless, the data show that the local population possibly consists of two adult age classes. The largest fish sampled was 36 mm L_S (44 mm L_T). No lar-

vae were caught in May, but the presence of ripe females and the finding of an egg at an advanced stage of development attached to a plant indicate that spawning had already started. The July sample contained mostly larvae and early juveniles. Judging from the presence of newly hatched larvae in this sample, spawning seems to extend at least to the end of July.

All females larger than 17 mm L_S sampled

in May had well developed ripening eggs, suggesting that maturity and spawning commence in the first year of life. The gonadosomatic index (GSI), taken as an index of reproductive effort, showed a clear tendency to increase with size, especially in females (Fig. 7). Ova of several sizes are present in the ovary at the same time, indicating a serial spawning mode. The length-frequency distribution of ova in one female (41 mm L_S , caught on 22.3.1994 in Drepano) is shown in Figure 8. This distribution could indicate daily spawning of a few eggs each time.

Figure 9 shows the composition of the diet of 28 fish from the Chiliadou spring sam-

pled in May 1999. The diet is expressed as %N (percentage of the x^{th} prey item in the total number of prey items recorded in all fish examined) and as %FO (percentage of fish containing at least once the x^{th} prey item). Insects and their developmental stages comprised the bulk of the diet. In numerical terms, species of Chironomidae were the most important prey items. In biomass terms, however, Coleoptera made the greatest contribution to diet, because of their large size. The alimentary canals of all individuals examined were parasitised by digenean trematodes.

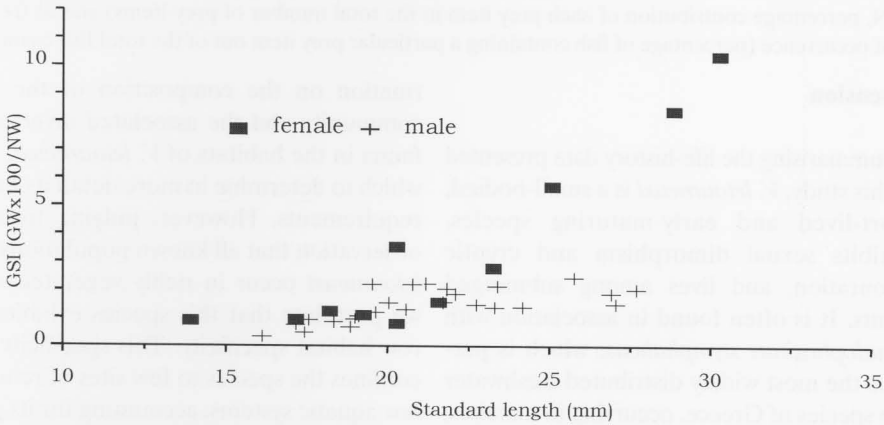


Fig. 7: Size-specific trends of the gonadosomatic index (GSI) in male and female fish.

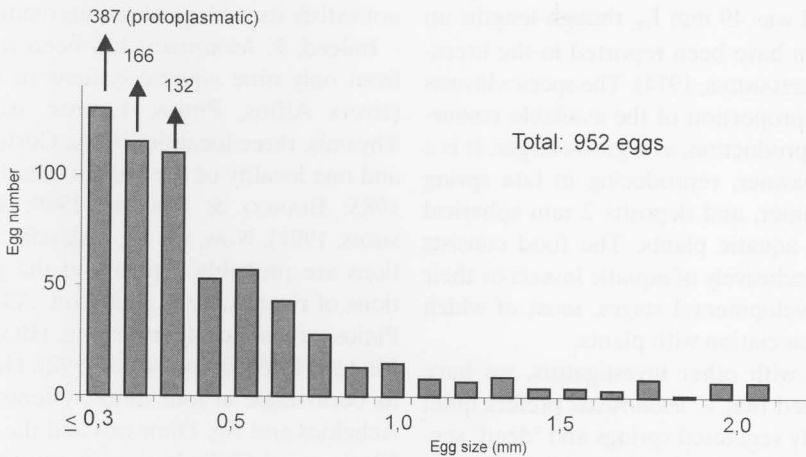


Fig. 8: Length-frequency distribution of ova in the ovary of a single female (41 mm L_S) sampled in Drepano (Thyamis R.).

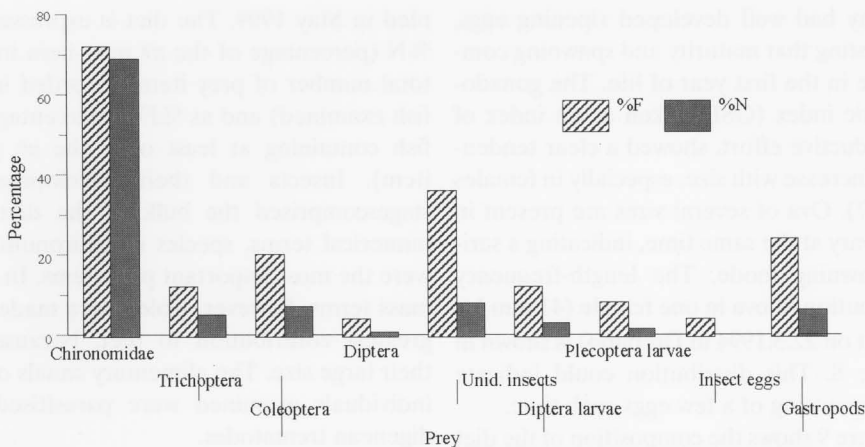


Fig. 9: The diet of *V. letourneuxi* in May 1999 in the Chiliadou spring expressed in numerical terms (%N, percentage contribution of each prey item in the total number of prey items) and as frequency of occurrence (percentage of fish containing a particular prey item out of the total fish examined).

Discussion

Summarising the life-history data presented in this study, *V. letourneuxi* is a small-bodied, short-lived and early-maturing species, exhibits sexual dimorphism and cryptic colouration, and lives among submerged plants. It is often found in association with *Pseudophoxinus stymphalicus*, which is perhaps the most widely distributed freshwater fish species of Greece, occurring in marshes, springs and riverine sites with stagnant water. In our samples, the maximum length recorded was 49 mm L_T , though lengths up to 67 mm have been reported in the literature (STEPHANIDIS, 1974). The species invests a larger proportion of the available resources to reproduction, as it grows larger. It is a serial spawner, reproducing in late spring and summer, and deposits 2 mm spherical eggs on aquatic plants. The food consists almost exclusively of aquatic insects or their early developmental stages, most of which live in association with plants.

Along with other investigators, we have established that *V. letourneuxi* prefers quiet and richly vegetated springs and "dead" segments of rivers banks (DAS, 1985; ECONOMIDIS, 1992). There is lack of adequate info-

rmation on the composition of the plant community and the associated invertebrate fauna in the habitats of *V. letourneuxi*, upon which to determine in more detail its habitat requirements. However, judging from the observation that all known populations of *V. letourneuxi* occur in richly vegetated areas, we postulate that this species exhibits narrow habitat specificity. This specificity both confines the species to few sites of relatively few aquatic systems, accounting for its punctuated distribution, and restricts local population densities, because most localities do not satisfy its ecological requirements.

Indeed, *V. letourneuxi* has been reported from only nine aquatic systems of Greece (rivers Alfios, Pinios, Louros, Acheron, Thyamis, three localities of the Corfu island and one locality of the Lefkas island) (DAS, 1985; BIANCO & MILLER, 1989; ECONOMIDIS, 1991). Now, the four islandic populations are probably extinct and the populations of rivers Louros, Acheron, Alfios and Pinios are seriously recessing (BIANCO & MILLER, 1989; ECONOMIDIS 1992). However, its occurrence in four more systems (rivers Acheloos and Ag. Dimitrios and the springs Vlychos and Chiliadou) is announced here. Our data on densities indicate that only the

Chiliadou population maintains a satisfactory level of abundance.

Although the present distribution of *V. letourneuxi* has been determined by historical factors and past extinction events, the limits on abundance are probably set by ecological factors. Using as criteria of rarity the limited range of geographic distribution, the confinement of the species in few localities of each aquatic system and the low local densities (GASTON & LAWTON, 1990), *V. letourneuxi* can be characterised as a "restricted and locally rare species".

Causes of population decline

The reduction in abundance and geographic range of *V. letourneuxi* is alarming evidence that the species is critically endangered. The decline of populations could well be a natural process that all species failing to adapt to their changing environments may pass before extinction. However, the decline could also have been caused, or at least accelerated, by habitat destruction or degradation caused by human activities. We hypothesise that the most important impacts on the species habitats have resulted from the operation of dams. Hydro-electric and irrigation dams have been constructed in almost all large rivers of western Greece (Alfios, Pinios, Acheloos, Mornos, Louros, Arachthos, Thyamis). The irregular release of water through the dams modifies the flow pattern and causes erosion of the banks and damage to the vegetation and benthic fauna. It is postulated that some riverine populations have been lost permanently due to destruction of the specialised habitats required by *V. letourneuxi*.

Smaller rivers, streams and springs have been affected mainly by water abstraction. Water is a limited resource in many areas of Greece and the exploitation of rivers is advanced, mainly for irrigation purposes. Some springs, especially in the Louros area, are now completely tapped for their water

supply. Water from the Ag. Dimitrios spring is used for irrigation purposes, while the Chiliadou spring supplies water to a nearby community. It is therefore not surprising that most of the presently known populations are in the vicinity of carstic springs, which provide a relatively constant supply of water and stable physicochemical conditions.

Other major impacts on the species' habitats have resulted from engineering works and swamp drainage. We have evidence of the probable disappearance of the species from the Drepano marsh (Thyamis river), where the species was recorded by earlier investigators and was detected by us in 1994. Now, the marsh does not maintain a satisfactory water level. In three sampling occasions during 1997 and 1998 not even one individual was captured there, despite considerable effort.

The impact of pollution is difficult to evaluate, and we have no evidence of extinction events that may be connected with organic and chemical contamination. Theoretically, pollution may seriously degrade water quality, especially in small bodies of water that cannot buffer the harmful effects of pollutants in the way that large bodies can.

In the future, the threats to the species are likely to increase. Scheduled plans for agricultural and industrial development will increase the magnitude of fluctuations in water supply and quality with a consequence the destruction and degradation of more habitats. For instance, the projected increase of the irrigation area in the Louros R. basin from 77 to 206 km² by the year 2015 will dramatically reduce the water supply. The entire riverine ecosystem, which is one of the richest in western Greece in terms of endemic species, can be considered as endangered. Similar projects exist for almost all rivers of western Greece. Unfortunately, freshwater fish conservation has hardly featured in the management of the water. Public water policy continues to encourage the creation of dams and water

delivery systems at the expense of free-flowing rivers and natural fish communities.

Nonetheless, habitat destruction does not seem to be the only reason for the recession of *V. letourneuxi*. The uncontrolled introduction of alien species is another potential threat. BIANCO & MILLER (1989) have stated as a probable reason of the decline of *V. letourneuxi* in Greek rivers the presence of *Gambusia affinis* (BAIRD & GIRARD, 1853), which was introduced for mosquito control. PLANELLES & REYNA (1996) have recorded a number of fish species introduced to Spanish fresh waters, which are potential predators or competitors to *V. hispanica*, and stressed the importance of creating or enforcing suitable legislation for preventing uncontrolled introductions. None of these species has been introduced to the fresh waters of western Greece, but the existing legal mechanisms are either insufficient or not adequately implemented to prevent harmful introductions.

The prospects of conservation

The reasons for the discontinuous distribution and low densities of *V. letourneuxi* seem to be biological. Being a niche-specialist, the species is confined to a limited range of habitats and becomes locally scarce. However, the causes of reduction of populations and decrease of population size seem to be anthropogenic, probably connected with habitat degradation due to dam operation, water abstraction and engineering.

In agreement with MAITLAND (1995), we consider that the major conservation objective must be habitat restoration and management. However, habitat protection has costs associated with it, as for example depression of economic activities that rely on the use of water. While scientifically sound principles ought to guide the efforts for conservation, the decisions for conservation actions are political, and depend both on economic interests and on the value

given to biodiversity by the society (BIEBER-KLEMN 1995). Bearing in mind that the agricultural and industrial development of the country is heavily dependent on stream and river water, priorities must be decided. Large riverine systems are unsuitable for broad-scale conservation, because the magnitude of economic activities become prohibitive for actions addressed to a single species. It would be more realistic to develop and apply conservation plans in few small undisturbed water bodies, with little economic activity in the surrounding lands.

On the basis of these general principles, we recommend as suitable systems for the application of conservation plans the springs of Chiliadou, the river Ag. Dimitrios, and the Ammoudia marsh (Acheron R.). The Barbanakos spring (Louros R.) and the Kypseli spring (Acheron R.) could also be candidate systems, because these two springs are independent from the main body of the rivers and may not be affected by the scheduled reclamation works. We suggest as a minimum conservation plan the "no-action" strategy, i.e. do nothing that would affect the actual habitat or the aquifers that feed the springs. This strategy is acceptable from the ecological point of view, and is also preferable from the aspect of cost.

Ideally, the selection of conservation areas should be based on genetic criteria, in addition to the ecological ones. Unfortunately, the genetic architecture of the *V. letourneuxi* populations has not been studied yet. The morphological, morphometric and life-history data provided in this study did not show differences of possibly genetic importance. However, our data are limited and were derived from closely situated populations. Moreover, it is not possible to ascertain to which extent the differences identified are genetically-based or reflect environmental effects. Other data provided by BIANCO & MILLER (1989) indicate that the specimens of Peloponnese have a lower number of dorsal fin rays than

the specimens from Epiros. Here again it is unclear if the different number of rays should be assigned to genetic factors or represents expressions of plasticity. Because traditional analytical techniques may fail to reveal genetic differentiation, research into allozyme loci or mitochondrial DNA would be helpful for identifying priority populations for conservation.

We do not mean to imply that habitat protection and conservation must be the only conservation objective. A variety of other conservation options are available, including artificial propagation, translocation and construction of local refugia. At present, however, the long-term effects of some of these options are difficult to evaluate. For instance, the effects of artificial propagation as a means of reinforcing local populations are doubtful, for it is not known if the population decline is due to failure of reproduction and high early mortality, or to the deterioration of the conditions of existence of adults. In the latter case, introduction of fry to a site with *V. letourneuxi* will not increase the population size beyond the level that the "adult" habitat is prepared to accept. Translocation to safe areas offers a more promising conservation approach. At present, we have identified two suitable habitats for translocation. In the face of escalating problems of habitat destruction, the option of artificial manipulation of the local flow regimes and topography, in order to increase ecological stability, also offers a long-term conservation perspective. However, such actions require costs for land purchasing and technical works, which become prohibitive for small-scale conservation.

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