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Rapid population collapse of the critically endangered *Valencia letourneuxi* in Kalamas basin of Northwest Greece

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Abstract. In order to assess the current status of the critically endangered *Valencia letourneuxi* in Kalamas River basin, an extensive field survey including twelve sampling stations was undertaken using back-pack electrofishing device, D-shaped frame net and small mesh size nets in October 2008. In total, 552 fish specimens were collected, belonging to ten freshwater fish species, plus mullets (collectively grouped as *Mugil* spp.). *V. letourneuxi* was found at one station (Anakoli ditch) (8.3% of sampling stations), at very low relative density (0.95%), where it co-occurred with the loach *Cobitis hellenica*, the goby *Economidichthys pygmaeus* and the introduced Eastern mosquitofish *Gambusia holbrooki*, with the last at very high relative densities. Moreover, water samples from selected stations were phasmatophotometrically analysed for NH_3-N, NH_3, NH_4, NO_2-N, NO_2, $NaNO_2$, NO_3-N, NO_3, PO_4, P and P_2O_5. Overall, *V. letourneuxi* was extirpated from one of its two previously known populations in this basin and its single surviving population was characterized by low local relative density and steep decline in relation to recent records (2005). Moreover, the presence at very high densities of the *G. holbrooki* as well as the increased eutrophication (due to high ammonia and phosphorus inputs) in its highly modified habitat, suggest a rapid population collapse with significant risk of extirpation from Kalamas basin in the near future. The necessary conservation measures are outlined.
Key Words: *Valencia letourneuxi*, population status, extirpation risk, Kalamas river, Greece.

Abstract (In Greek). Με στόχο την αξιολόγηση της υφιστάμενης κατάστασης του κρίσιμα απειλούμενου είδους *Valencia letourneuxi* στο Ποταμό Καλαμά, διενεργήθηκε εκτεταμένη έρευνα πεδίου σε δώδεκα σταθμούς δειγματοληψίας με τη χρήση ηλεκτραλιείας και αποχών τον Οκτώβριο του 2008. Συνολικά αλιεύτηκαν 552 άτομα ψαριών που ανήκαν σε δέκα είδη των εσωτερικών υδάτων καθώς επίσης και κεφαλοειδή (ομαδοποιημένα ως *Mugil* spp.). Το είδος *V. letourneuxi* βρέθηκε σε έναν σταθμό (κανάλι της Ανάκολης) (στο 8.3% των σταθμών δειγματοληψίας), σε ιδιαίτερα χαμηλή σχετική πυκνότητα (0.95%) συμπατρικό με τα είδη *Cobitis hellenica*, *Economidichthys pygmaeus* και το μη ενδημικό *Gambusia holbrooki*, το οποίο και βρέθηκε σε πολύ υψηλές σχετικές πυκνότητες. Επιπλέον, δείγματα νερού από επιλεγμένους σταθμούς αναλύθηκαν φασματοφωτομετρικά για NH_3-N, NH_3, NH_4, NO_2-N, NO_2, $NaNO_2$, NO_3-N, NO_3, PO_4, P and P_2O_5. Συνολικά διαπιστώθηκε ότι ένας εκ των δύο πληθυσμών του *V. letourneuxi* είχε εξαλειφθεί και ο μοναδικός εναπομείναν πληθυσμός χαρακτηρίζεται από μειωμένη σχετική πυκνότητα και απότομη μείωση σε σχέση με παλαιότερη δειγματοληψία (2005). Η παρουσία σε πολύ υψηλές πυκνότητες του αλλόχθονου *G. holbrooki* καθώς και ο ευτροφισμός (λόγω των υψηλών επιπέδων αμμωνίας και φωσφορικών) του υποβαθμισμένου βιότοπου υποδεικνύουν την ραγδαία κατάρρευση του πληθυσμού με σημαντική πιθανότητα εξάλειψης του είδους από την λεκάνη απορροής του Ποταμού Καλαμά στο άμεσο μέλλον. Στην παρούσα εργασία περιλαμβάνονται τέλος τα απαραίτητα μέτρα προστασίας του είδους.
Key Words: *Valencia letourneuxi*, πληθυσμιακή κατάσταση, πιθανότητα εξάλειψης, Ποταμός Καλαμάς, Ελλάδα.

Rezumat. În vederea investigării situației actuale a speciei critic periclitate *Valencia letourneuxi* în bazinul râului Kalamas, au fost demarate largi studii pe teren, care au implicat 12 stațiuni de colectare prin ”electrofishing” (echipament ”back-pack”, mincioage cu cadru D şi plasă deasă), desfăşurat în luna octombrie a anului 2008. A fost capturat un total de 552 exemplare de pești, aparținând la zece specii de apă dulce și chefal (specii încadrate în genul *Mugil*). *V. letourneuxi* a fost identificată într-o singură stațiune (canalul Anakoli) (8.3% din stațiunile de colectare), la o densitate relativă foarte scăzută (0.95%), unde ea coabitează cu o specie de grindel, *Cobitis hellenica*, un gobiid *Economidichthys pygmaeus* și cu o specie introdusă, *Gambusia holbrooki*, ultima fiind estimată la o foarte mare densitate

relativă. Chimismul apei a fost analizat pentru NH_3-N, NH_3, NH_4, NO_2-N, NO_2, $NaNO_2$, NO_3-N, NO_3, PO_4, P și P_2O_5. Per ansamblu, din cele două populații de *V. letourneuxi* cunoscute în bazinul respectiv, una a dispărut, iar singura populație supraviețuitoare a fost caracterizată prin densitate locală scăzută și declin abrupt comparativ cu înregistrările recente din 2005. Mai mult, prezența la densități foarte mari a speciei *G. holbrooki*, precum și eutrofizarea crescută (pe fondul creșterii nivelului de amoniac și fosfor) a habitatului său puternic modificat, preconizează o prăbușire a numărului de indivizi și un risc semnificativ de dispariție a speciei din bazinul Kalamas în viitorul apropiat. În prezenta lucrare sunt subliniate măsurile de conservare care se impun.
Cuvinte cheie: *Valencia letourneuxi*, statutul populației, risc de dispariție, râul Kalamas, Grecia.

Introduction. The Greek valencia, *Valencia letourneuxi* (Sauvage, 1880) is a distinctive endemic species of the south Adriatic-Ionian ichthyo-geographical region (Economidis & Bănărescu 1991; Criveli 1996). It is a temperate, short-living species distributed mainly in freshwater habitats close to the coast, but tolerates salinity up to 4‰ in the wild and 46‰ in laboratory conditions (Kottelat & Freyhof 2007; Bianco & Nordlie 2008). Typical microhabitats are spring-fed streams with deep, clear and slow running waters and adequate aquatic vegetation to provide food, shelter and spawning substrate (Barbieri et al 2000).

The historical range of the species extended from southern Albania to the north Peloponnese peninsula, with a fragmented distribution within Greece. Populations occurred in thirteen systems according to past records, but recent assessments indicate the extirpation of its two known westernmost (in the Ionian Islands) and its southernmost (Alfios drainage) populations, with most of the remaining ones having very low local densities (Kalogianni et al 2010a). According to Crivelli (2006), current geographical range is restricted to 2,250 km^2 with the total habitat area less than 15 km^2. Due to its deteriorating status, caused by habitat modifications and competition with and aggression by the introduced eastern mosquitofish *Gambusia holbrooki* Girard, 1859, it has been registered as critically endangered in the IUCN International Red List, as a priority species in Annex II of the EU Habitats Directive (92/43/EEC) and as a strictly protected species in the Bern Convention (Kottelat & Freyhof 2007).

River Kalamas represents the northern distribution limit of *V. letourneuxi* within mainland Greece, but various anthropogenic pressures continuously pose serious threats to the fish fauna of this drainage area. The species was recorded there in the past in Drepano marsh (Labhart 1980; Barbieri et al 2000), but during a more recent survey (2005) it could not be detected there (Kalogianni et al 2010a). However, another population was first detected during the same survey at another location (Anakoli spring-fed system), but at very low densities (Kalogianni et al 2010a). The relatively recent extirpation event in Drepano marsh, the low densities of the only known surviving population at Anakoli in 2005, as well as the apparent extirpation of *V. letourneuxi* from all its known localities at the adjacent Ionian Island of Corfu (Villwock et al 1982; Economidis 1995; Kalogianni et al 2010a), with which the Kalamas populations share a similar genetic profile, distinct from that of populations of southern Greece (Maltagliati et al 2006), rendered essential a thorough and up-to-date monitoring of its status in Kalamas basin. The aim of the present work was to investigate various habitats of River Kalamas for the presence of *V. letourneuxi* and to assess its status and its prospects for conservation in a drainage-wide context.

Material and Method. River Kalamas (or Thyamis, Figure 2) springs forth from Mount Dousko (close to the Greek-Albanian borders) at 1,300 m altitude and drains in the Ionian Sea, after a course of 115 km. Catchment area is approx. 1,831 km^2 and the coastal zone is characterized by an extended (78 km^2) deltaic system. Field work took place in October 2008, using a portable (back-pack) electrofishing device (Hans Grassl GmbH IG200/1), as well as a D-shaped frame net at a right angle to the handle and small mesh size nets (Figure 1). Twelve sampling stations (both freshwater and brackish habitats) were thoroughly investigated (Figure 2, Table 1).

Figure 1. Electrofishing (**a**) and using D-shape frame net (**b**) during the field survey.

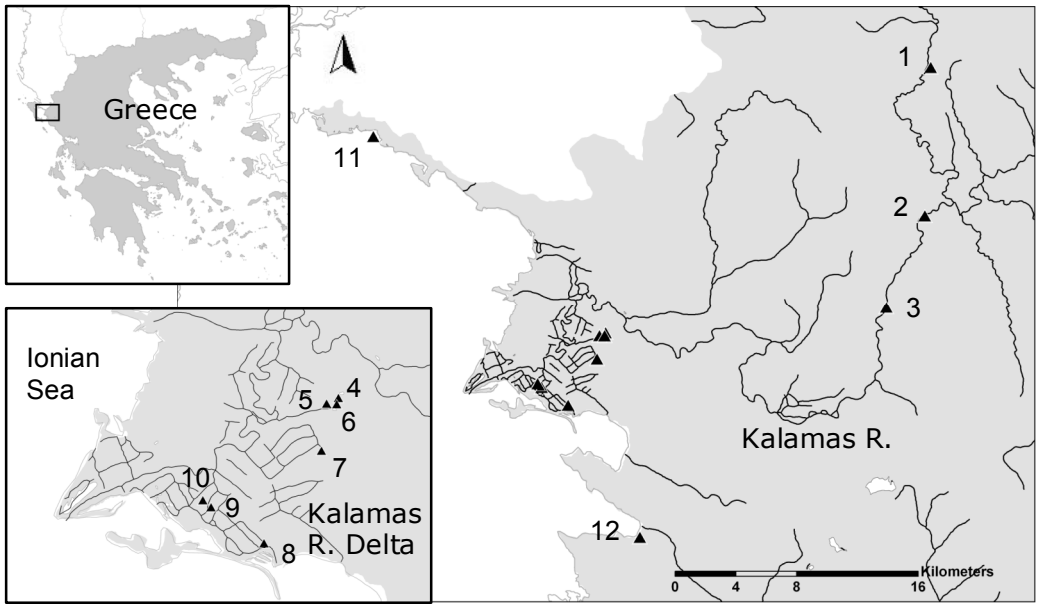

Figure 2. Sampling stations (1-12, ▲) in Kalamas River basin.

After species' identification [based on diagnostic keys in Kottelat & Freyhof (2007)], fish were returned to the water. Relative density (percentage of specimens out of total number of the sympatric species caught in each station) of all fish species was calculated. Water samples were obtained in sterile bottles of 1 L from three locations (stations 4, 5 and 6) at the Anakoli spring system (where *V. letourneuxi*'s presence was confirmed) and for purposes of comparison from station 7, also in the deltaic area. Samples were subsequently analysed (using standard analysing protocols of HACH DR-2000 phasmatophotometer) for NH_3-N, NH_3, NH_4, NO_2-N, NO_2, $NaNO_2$, NO_3-N, NO_3, PO_4, P and P_2O_5 in the analytical chemistry laboratory of the Department of Aquaculture & Fisheries, Technological Educational Institute of Epirus.

Results and Discussion. According to the IUCN Red List, *V. letourneuxi* is one of the twenty four most endangered species in Europe, due to various anthropogenic pressures. For example, water abstraction and pollution were the main reasons of its extirpation from the adjacent Ionian Islands (Corfu and Lefkas; Economidis 1995). Moreover, major threats to the surviving mainland populations are river bank erosion, aquatic vegetation damage and increased sedimentation, caused by irregular water release from dams, as well as land reclamation, hydraulic works and organic pollution, as in the case of River Kalamas (Barbieri et al 2000; Kalogianni et al 2010a).

During the course of this survey, 552 fish specimens were collected in Kalamas River basin, belonging to ten freshwater fish species, plus mullets (collectively grouped as *Mugil* spp., Figure 3; Table 1). *G. holbrooki* was by far the most abundant species mainly in the lowland areas of the basin (stations 4-10) followed by mullets. *V. letourneuxi,* in contrast, was found at only one (Anakoli ditch, station 6, Table 1) out of twelve stations (8.3 %), at very low relative density (0.95%), where it co-occurred with the loach *Cobitis hellenica* Economidis & Nalbant, 1996 (3.81%), the goby *Economidichthys pygmaeus* (Holly, 1929) (18.1%) and *G. holbrooki* at very high relative densities (77.14%).

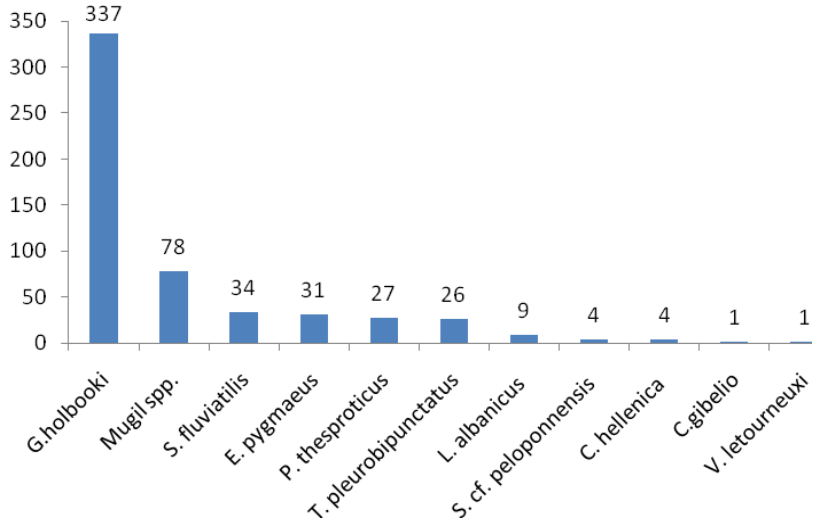

Figure 3. Total number of specimens of each fish species caught in Kalamas basin during the October 2008 survey.

This spring-fed ditch (depth 1.5 m), with dense riparian vegetation (mostly *Phragmites australis*), low flow and silty substrate, was covered by a thick layer of aquatic vegetation (mostly *Ceratophyllum* spp. and *P. australis*) and was thus typical of *V. letourneuxi* known habitats (Barbieri et al 2000). The water temperature was 15.7 °C, DO 8 mg L^{-1}, pH 7.16, conductivity 1,180 μS cm^{-1} and salinity <2‰. Indicative of its highly restricted presence in this spring-fed system is the fact that the species was not found either at the actual spring area, nor further downstream (stations 4 and 5 respectively). Population relative density (0.95 %), however, was very low, and even reduced by 66.1% compared to that of 2.8% recorded at this station in a 2005 survey (Kalogianni et al 2010a). In addition, summer droughts (especially that of September 1997) are probably the main reason for the extirpation of its other known population in this basin (Drepano marsh), first reported in Labhart (1980).

In contrast to *V. letourneuxi* that exhibits habitat specificity and narrow ecological requirements (Kalogianni et al 2010a), its sympatric species at the Anakoli ditch, are able to exploit less stable aquatic ecosystems. In addition, competition with and aggression by *G. holbrooki,* that has been introduced in the past to control malaria, has been proposed as a main reason of *V. letourneuxi*'s population decline (Economidis 1995; Kottelat & Freyhof 2007), as it has also been suggested for its related species *Valencia hispanica* (Valenciennes, 1846) (Rincón et al 2002). Interestingly, in the only known locality in Greece that *V. letourneuxi* is relatively abundant (in River Mornos basin) the target species does not co-occur with *G. holbrooki* (Kalogianni et al 2010ab).

Table 1

Sampling stations and relative density of fish fauna in Kalamas basin; n is number of fish

Station	*Location*	*n*	*Species – relative density (%)*
1	Lista springs	22	*Telestes pleurobipunctatus* (Stephanidis, 1939) (90.9) *Squalius* cf. *peloponnensis* (Valenciennes, 1844) (9.1)
2	Raveni	33	*Pelasgus thesproticus* (Stephanidis, 1939) (81.8) *Luciobarbus albanicus* (Steindachner, 1870) (18.2)
3	Pente Ekklisies	11	*T. pleurobipunctatus* (54,5) *S.* cf. *peloponnensis* (18,2) *L. albanicus* (27.3)
4	Anakoli spring	96[a]	*E. pygmaeus* (3.125) *Salaria fluviatilis* (Asso, 1801) (3.125) *Mugil* spp.(10.42) *G. holbrooki* (83.33)
5	Anakoli brook		
6	Anakoli ditch	105	*C. hellenica* (3.81) *E. pygmaeus* (18.1) *G. holbrooki* (77.14) ***V. letourneuxi* (0.95)**
7	Kestrini bridge	47	*Mugil* spp. (68.1) *E. pygmaeus* (8.5) *G. holbrooki* (23.4)
8	Riho estuary Drepano marsh	62[b]	*Mugil* spp. (19.6) *G. holbrooki* (80.4)
9	Vatatsa estuary	29	*E. pygmaeus* (17.3) *G. holbrooki* (82.7)
10	Vatatsa channel	70	*Mugil* spp. (20.0) *Carassius gibelio* (Bloch, 1782) (1.4) *G. holbrooki* (78.6)
11	Kato Aetos swamp	32	*G. holbrooki* (100.0)
12	Plataria brook	45	*S. fluviatilis* (68.9) *G. holbrooki* (8.9) *Mugil* spp. (22.2)

[a] Data from stations 4 and 5 were pooled

[b] Number refers to Riho est.; Drepano marsh (n=0) was partially dried out

Finally, concerning the water quality of the single station (station 6) hosting the *V. letourneuxi* population, NH_3-N, NH_3 and NH_4 levels were higher than neighboring stations 4 and 5 of the Anakoli spring system, where they were practically negligible (Fig. 4a). These levels were even higher than those of station 7 (Kestrini bridge), which was originally thought to be highly polluted, due to its proximity to agricultural land. In contrast, nitrite and nitrate levels were lower in station 6, compared to the other stations (Fig. 4b, c). Finally, phosphorus levels were also increased in station 6, in relation to 4 and 5 (Fig. 4d). In regard to the maximum limits set by the EC Freshwater Fish Directive (78/659/EEC) for the protection of cyprinids, non ionized NH_3 at station 6 exceeds by far the maximum concentration of 0.025 mg L^{-1} of EC guidelines. The high ammonia and phosphorus levels detected in the Anakoli habitat hosting *V. letourneuxi*, suggest increased eutrophication and thus habitat degradation, probably due to inputs from nearby agricultural and livestock activities.

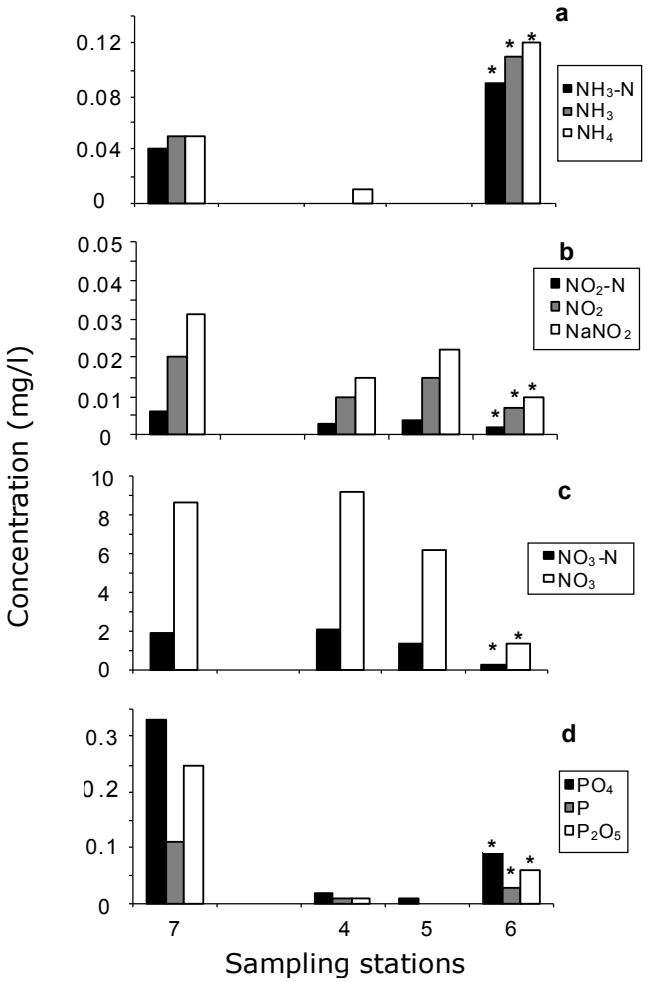

Figure 4. Ammonia (**a**), nitrite (**b**), nitrate (**c**) and phosphorus levels (**d**) of selected stations, including the locality where *V. letourneuxi* was recorded (station 6, marked with asterisks).

Conclusion. The extirpation of the Drepano marsh population and the rapid decline of the Anakoli population due to habitat degradation and possibly *G. holbrooki* competition suggest the possibility of extirpation of the species from the Kalamas basin in the immediate future, unless conservation and/or rehabilitation measures are implemented. More specifically, since the species is not recruitment-limited and population density limits are partially set by the carrying capacity of the habitat (Barbieri et al 2000), a habitat management, restoration and preservation plan is deemed necessary. This should include habitat protection actions, such as securing the necessary water volumes by controlling water abstraction, reducing nutrient inputs by translocation of nearby livestock activities and adopting organic farming methods at the neighbouring agricultural land, water quality monitoring, as well as actions of enhancing natural reproduction in controlled environments (artificial ponds or aquaria). Restocking using specimens with similar genetic profile is also an option (a genetic study of the various *V. letourneuxi* populations is currently underway). Finally, sensitizing local community on the issues above and designating this particular habitat as species protection area within the "Natura" framework are also an immediate priority.

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