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Enemy at the gates: introduction potential of non-indigenous freshwater crayfish in Greece *via* the aquarium trade

Research Article

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Abstract: Indigenous freshwater crayfish species (ICS) are important biodiversity components and desirable fishery targets. However, ICS populations are increasingly threatened by various anthropogenic stressors. Moreover, established populations of non-indigenous freshwater crayfish species (NICS) and new 'waves' of NICS introductions exert significant pressure on ICS populations at a pan-European level. These effects include direct competition for space and resources as well as crayfish 'plague' transmission from introduced North American species. Given low public knowledge of this problematic, considerable risk of future introductions exist as a result of conventional and internet-based aquarium trade, which often lead to deliberate and/or accidental releases of NICS into the wild. In 2011, we conducted a survey of freshwater crayfish species in eleven large-size pet shops located in three major cities and in three large internet-based aquarium companies in Greece. Overall, eight species belonging to three genera (Procambarus, Cherax and Cambarellus) were recorded, originating from the USA, Australia, New Guinea and Mexico. The invasion potential of the three most popular species was assessed using the Freshwater Invertebrate Invasiveness Scoring Kit (FI-ISK). Two species were determined to constitute a 'very high risk' of invasion. As such, regulatory measures need to be implemented to monitor the ornamental trade of NICS in Greece and a national framework developed for protecting ICS.

Keywords: Aquarium trade • Biodiversity • FI-ISK • Non-indigenous freshwater crayfish

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1. Introduction

Several populations of indigenous freshwater crayfish species (ICS) in Europe are at risk of becoming endangered or extinct as a result of various anthropogenic stressors such as the crayfish 'plague' disease, other diseases, competition with invasive non-indigenous crayfish species (NICS), translocations of both ICS and NICS, loss of genetic diversity, pollution, water abstraction for irrigation and direct human use, habitat destruction,

eutrophication and overexploitation [1]. Furthermore, increasing information on NICS introductions into European freshwaters [2] indicates a serious risk of extinction for local populations in the near future through both direct (e.g. predation and crayfish 'plague') and indirect effects (e.g. grazing of aquatic macrophytes, alteration of biomass and species richness of macroinvertebrates, bank erosion and substrate disturbance) [3]. The latter could exert dramatic changes to the biodiversity of aquatic communities and the integrity of ecosystems in Europe.

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Recent efforts to review and record historical and current occurrences of ICS in Greece [4-6] showed that crayfish populations are present in at least 34 hydrographic systems (natural and man-made lakes, rivers and small streams). However, the distribution of crayfish is much more restricted nowadays than in previous decades, and many populations of ICS are isolated and confined to headwaters and lentic environments that remain pristine [4]. Although there is only one established population of invasive NICS (i.e. signal crayfish Pacifastacus Ieniusculus (Dana, 1852)), ICS (noble crayfish Astacus astacus (L.), narrowclawed crayfish Astacus leptodactylus Eschscholtz 1823, stone crayfish Austropotamobius torrentium (Schrank, 1803)) and their habitats are confronted to serious threats due to human-mediated stressors. These threats are expected to be exacerbated by globalization leading to increased trade and e-commerce of NICS, unrestricted movement/travelling of NICS, intense economic activity, demographic pressures [7,8] and climate change, which generally favour NICS with less strict ecological preferences and increased adaptive ability compared to ICS [3]. Recently, astacologists have determined that the aquarium trade is as one of the most important and plausible sources of NICS introductions in Europe and worldwide. However data on the Hellenic aquarium trade of NICS is lacking.

So far, aquatic ecologists have focused on studying and managing ecosystems already invaded and degraded by non-indigenous species. Precautionary measures are urgently needed for mitigating further introductions of non-indigenous species, one of the most important threats to biodiversity worldwide. Here, our objective was to record the presence of ornamental NICS in the Greek aquarium trade market and assess their invasion potential into the wild.

2. Experimental Procedures

We recorded the presence of NICS in eleven large-size aquarium stores in Greece's two largest cities (Athens and Thessaloniki) and one large provincial city (Ioannina) between January and December 2011. We also conducted an online-based research of three major internet-based companies with stores in the Greek pet market simultaneously. Data on the number of sales per store were not available. However, we anecdotally recorded that nine specimens of the most ornamental and large-size *C. quardicarinatus* were sold during 2011 in one store in the city of Ioannina with a population of 100,000 inhabitants. We used the Freshwater Invertebrate Invasiveness Scoring Kit, v1.19

(FI-ISK) tool developed by the Centre for Environment Fisheries and Aquaculture Science (CEFAS) (http://cefas.defra.gov.uk/our-science/ecosystems-and-biodiversity/non-native-species/decision-support-tools. aspx) to assess the invasion potential of the three most popular aquarium species (*Procambarus clarkii*, *Cherax destructor* and *Cherax quadricarinatus*). The same tool was also applied to ICS and NICS in Greek freshwaters systems for comparison purposes.

3. Results

Overall, we recorded eight species of NICS belonging to three genera (Table 1). Available data for four species (*Procambarus clarkii, Cherax quardicarinatus, Cherax peknyi* and *Cambarellus patzcuarensis*) suggested that they were imported from Singapore. *C. quardicarinatus* may also have been imported from an alternative location in Italy. The species most advertised for sale in the e-commerce were *P. clarkii* and *C. quadricarinatus*.

The FI-ISK revealed that invasion potential was particularly high for *P. clarkii* and *C. destructor*. The analysis rejected these species for introduction in the aquarium trade based on a 16 points threshold. *C. quadricarinatus* had moderate score, requiring further assessment for introduction. The FI-ISK scores are presented in Figure 1 against: (a) the scores for the three ICS and the only NICS established in Greece; and (b) the scores generated by Tricarico *et al.* [9] for the same species in Italy. Accurate scoring for *C. peknyi*, *P. alleni*, *C. shufeldtii*, *C. montezuma* and *C. patzcuarensis* was not possible due to the limited bibliographic data on their biology and invasion ecology.

4. Discussion

European ICS populations have suffered from serious declines during the last century, mainly due to the spread of the crayfish 'plague' disease via introduced American species. All crayfish of Northern and Central American origin, such as species from the genera Pacifastacus, Procambarus, Cambarellus and Orconectes are suspected vectors of crayfish 'plague'. Freshwater crayfish are particularly good invaders compared to other groups of aquatic organisms. Currently, ten NICS are established in the European freshwaters with establishment success record approaching 100%, compared to the number of introduced species to the wild [10]. Although NICS are still absent from neighbouring countries to Greece (i.e., Albania, the former Yugoslav Republic of Macedonia, Bulgaria, and Turkey) [2,5],

Source	[3,20,25,26]	[27]	[3,9,20,25,26]	[3,9,20]
Documented/ possible impacts	Very highly invasive ability, carrier of the 'pague', competition with ICS for space and resources, structural changes in food webs, predation, bioaccumulation, elimination/reduction of abundance and richness of indigenous species, damages to agricultural crops, ditches and river banks, increased water turbidity.	Unknown but possible carrier of the 'plague'.	Invasive. Impacts in the area of origin (altering the cycling of nutrients and the structure of the food webs, competition with other endemic freshwater crayfish species, possible carrier of pathogens), but not yet documented in Europe. It is expected to become a major threat to ICS (i.e. outcompete and displace them due to tolerance of environmental fluctuations, rapid growth and reproductive advantages).	Unknown but a variety of pathogens is associated with the species. Rapid growth could be an advantage over the ICS in warm-water conditions.
Habitat preferences	Lentic including saline waters (swamps, marshes seasonally flooded wetlands, slow flowing water, reservoirs, irrigation systems, rice fields).	Lentic (stagnant waters, pools and flooded marshes).	Lentic-Lotic (slow-moving water with heavy vegetation,ponds, ditches and swamps, streams, rivers).	Primarily lotic (rivers and water holes).
Basic biological features	Reproduction > 10°C, temperature tolerant ≤35°C (best growth: 22-30°C), r-selected, breeding capacity over 600 eggs, rapid growth, life span: 4 years, max length: 15 cm TL, tolerant of brackish water (12 psu) and adverse environmental conditions (e.g. lower dissolved oxygen lethal limit of 0.4 mg/L), burrowing ability, omnivorous-polytrophic.	Max size up to 7 cm, breeding capacity up to 300 eggs, kept in 20-26°C in aquaria (apparently wider tolerance in nature), moderate salinity tolerance, blue-color mutation, burrowing ability, omnivorous-polytrophic.	Reproduction > 15°C, breeding capacity of up to 450 eggs, life span: 3-6 years, breeding before 1st year, tolerant of 1-35°C (best growth: 22-30), medium salinity tolerance (up to 12 psu), lower dissolved oxygen lethal limit of 1.0 mg/L), max length: 15 cm TL, burrowing ability, omnivorouspolytrophic.	Reproduction above 23°C, maturing at 7 months, breeding capacity of up to 1500 eggs, tolerant of 4-35°C (best growth: 25-30°C), medium salinity tolerance (12 psu), lower dissolved oxygen lethal limit of 0.5 mg/L), max length: 35 cm TL, life span: 4-5 years, omnivorous-polytrophic.
Native origin	S.E United States	Florida state	Eastern Australia	Northern Australia & Papua New Guinea
Abbreviation	PCC	PCA	Q XO	Ö
Common name	Red swamp crayfish	Florida blue crayfish	Yabby	Redolaw
Authority	Girard, 1852	Faxon, 1884	Clark, 1936	von Martens, 1868
Species	Procambarus clarkii	Procambarus alleni	Cherax destructor	Cherax quardicarinatus

Table 1. List of non-indigenous freshwater crayfish species in the Greek aquarium market with their basic biological features, habitat preferences and impacts.

Species	Authority	Common name	Abbreviation	Native origin	Basic biological features	Habitat preferences	Documented/ possible impacts	Source
Cherax peknyi	Lukhaup & Herbert, 2008	Zebra crayfish	CXP	Papua, New Guinea	Tolerate 18-29°C, persist where habitat modification has been severe, burrowing ability, omnivorous-polytrophic, life-span: 5-7 years.	Lotic-Lentic (slow flowing, still waters).	Unknown. Invasive. Impacts in the area	[28,29]
Cambarellus shufeldtii	Faxon, 1884	Cajun dwarf crayfish	CAS	Southern US states	Short-lived (2-3 years), small-sized (up to 3.8 cm), temperature preference of 18-24°C, breeding capacity of 30-40 eggs, burrowing ability, omnivorouspolytrophic.	Primarily lentic (swamps, ditches, lakes, ponds, oxbow lakes and sluggish streams).	of origin, (aggressiveness, dominance and range expansion over other native crayfish species, intermediate host of parasites) and possible	[9,30-32]
Cambarellus montezumae	Saussure, 1857	Dwarf crayfish	CAM	Mexico	Short-lived (2 years), small-sized (up to 4 cm), temperature tolerance of 10-25°C, breeding capacity up to 120 eggs, omnivorous-polytrophic. Short-lived (18 months), small-sized	Primanly lentic (swamps, ponds, sluggish streams, rivers, lakes, reservoirs and canal banks).	carrier of the 'plague'. Unknown but possible carrier of the 'plague'	[33,34]
Cambarellus patzcuarensis	Villalobos 1943	Dwarf orange crayfish	CAP	Mexico	(up to 4 cm), orange-color mutation in aquarium market, temperature tolerance of 10-28°C, breeding capacity up to 60 eggs omnivorous-polytrophic. Conservation status according to IUCN: Endangered.	Lentic (lakes, springs).	Unknown but possible carrier of the 'plague'	[35,36]

continued Table 1. List of non-indigenous freshwater crayfish species in the Greek aquarium market with their basic biological features, habitat preferences and impacts.

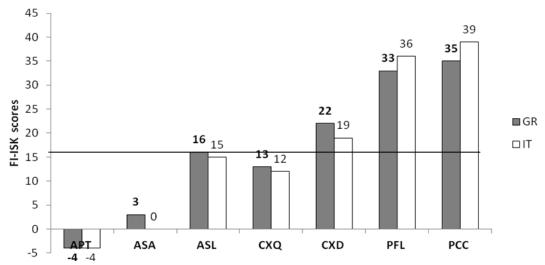


Figure 1. FI-ISK invasiveness score for selected aquarium NICS (CXQ, CXD and PCC), established NICS (PFL) and ICS (APT, ASA and ASL) in Greece (current study) and Italy (data from Tricarico et al. [9]). Solid line indicates the threshold level of 'high risk' species. APT:

Austropotamobius torrentium, ASA: Astacus astacus, ASL: Astacus leptodactylus, CXQ: Cherax quadricarinatus, CXD: Cherax destructor, PFL: Pacifastacus leniusculus, PCC: Procambarus clarkii.

various human-mediated vectors of introduction may threaten existing ICS populations in these countries. Such vectors include legal or illegal stocking in natural water bodies, aquaculture, live food trade, aquarium and pond trade and, to a lesser extent, biological supply trade for experimental purposes and live bait [11]. The main threats assessed for ICS in Greece in this study are summarised in Table 2.

Although NICS introduced since the mid-19th century up to 1975 were introduced primarily to re-stock overfished European crayfish populations and for aquaculture, many recently introduced NICS were brought into Europe via the aquarium trade since the 1980s [2]. Of all the NICS assessed in our survey, P. clarkii is listed among the hundred worst invasive species in Europe and among the top 27 animal species introduced into Europe for aquaculture and related activities [12,13]. Although fishing of P. clarkii constitutes at least 50% of the European ICS fisheries production [3], there are examples of pronounced adverse effects of this species on native ecosystems in diverse locations around the world: negative impacts in littoral and semi-terrestrial marsh ecosystems in southern Spain [14]; destruction of reed-beds and intensive predation on native fish species in Lake Naivasha in Kenya [15]; decreased rice production in Portugal [16]; and damages to river banks and agricultural fields due to its burrowing activity in the USA [17]. Additionally, P. clarkii is one of the most important vector of crayfish 'plague', carrying the pathogen (Aphanomyces astaci Schikora) as a chronic infection [18] and may also be an intermediate host for helminth parasites which can

be transmitted to humans. Finally, P. clarkii is able to accumulate cyanobacterial toxins [19] and subsequently transfer them to their predators as well as to humans. Populations of P. clarkii are widespread in Europe, particularly in Spain, Italy and southern France, which have similar climatic conditions to Greece. P. clarkii is also present in various climatic zones (from Israel to Belgium), including several insular populations [2,14]. Similarly, C. destructor is thriving in Spain and an established population was recently found in central Italy [2,3,20]. Finally, the tropical C. quardicarinatus has recently established a self-sustaining population in thermal hot springs in Slovenia [21]. The introduction of C. quardicarinatus could also happen in warmer parts of southern Greece, since this species tolerates temperatures down to 4°C [22]. Currently, there are no available data on introduction/establishment of the rest of the NICS surveyed during the present work in the European aquatic ecosystems.

The aguarium trade of NICS is largely uncontrolled around the globe. As a result, the risk of further introductions freshwater cravfish in European waters is considerable. Based on recent studies of ornamental fish [23] and crayfish [24] species, there is a clear relationship between the frequency of species occurrence and size in the aquarium trade industry and the likelihood of introduction/establishment into the wild. Moreover, trade laws of the single European market (i.e. free movement of goods and services), need to consider the potential risks from aquarium trade. The most noteworthy example is the largely unregulated German aguarium trade market (regular and internet-based)

Threats	ASA	APT	ASL	Cumulative Score	Justification/Comments
NICS	3	3	3	9	NICS in aquarium trade/Favourable climatic conditions. One established population of PFL neighbouring to ASA and APT populations.
Crayfish plague	3	3	3	9	All ICS susceptible to plague. Massive mortalities and suspected incidents in the past.
Fragmentation	2/3	3	2/3	7-9	Increased during the last decades for all species. More severe due to the already geographically isolated populations and augmented by anthropogenic impacts.
Toxicants	2/3	2/3	2/3	6-9	Serious problem by agricultural intensification. Irresponsible use and deposit practises.
Climate change	2	3	1/2	6-7	Expected to have a varied but increasingly important impact in inland waters. Directly related to the adaptability of each species (reduced for APT).
Habitat alterations	2	2/3	1/2	5-7	Significant and on-going (e.g. dams, public and hydraulic works).
Water abstraction and water level reduction	2	2	0/1	4-5	Water abstraction significant in many lowland areas/ lakes. However, ICS populations are currently constrained in less lowland and populated areas.
Eutrophication	2	1/2	1	4-5	Poor or medium quality of many lakes primarily affecting important ASA populations in the past. APT populations mainly in pristine brooks not close to agricultural fields.
Land-use	2	1	1	4	Moderate for lowland ASA populations.
Predators	1	1	1	3	Diminishing populations of top predators (e.g. eel, trout, European catfish, pike perch). Birds and other higher terrestrial or aquatic animals may be in some cases more important predators.
Exploitation	1	0/1	0	1-2	Low intensity by recreational fishermen being disputably harmful, except in slow growth populations in high altitudes. Crayfishing and cooking tradition in very limited places.
Other diseases	?	?	?	?	Data completely missing, except for commensal branchiobdellids.
Mean range of threat level	2.00-2.18	1.90-2.27	1.36-1.81	1.75-2.08 (5.27-6.27)	Values in parentheses refer to the mean range of the cumulative scores.
IUCN conservation status	VU	VU	Not listed		The ASL population is unique to the country. Accordingly, it could be handled as a threatened population, after a thorough assessment, given the transboundary nature of its habitat.
Protection frameworks	EU Habitats Directive 92/43/EEC (Appendix V)	EU Habitats Directive 92/43/EEC (Appendix II)	Not listed		EU Habitats Directive 92/43/EEC and NATURE 2000 framework are only marginally effective (e.g. no SPAs - Species Protection Areas - have been designated for APT).
Legislation	Royal Decree 142/71, FEK 49A/71 (open season from 15th of February to 15th of May and minimum size of 10 cm TL) No 147703/4.7.2002 Ministerial Act for import permission for alien species via a central commission 708/2007 (EC) Directive for the use of alien and locally absent species in aquaculture NATURE 2000 EU Water Directive (2000/60/EC).			m size of 10 rt permission ission and locally	Consolidated national legislation is urgently needed for the protection of ICS and their habitats. The 708/2007 (EC) Directive does not directly apply to the aquarium trade; however it introduces the 'white list' approach for the importation of species and provides the responsibility to the Member States to take the appropriate measures to protect biodiversity threatened by the movement of aquatic animals for aquaculture purposes. Greece may not fulfil the EU Water Directive (2000/60/EC) goals of 'good ecological status' and 'good chemical status' of surface and ground waters by the year 2015. Recent environmental-ecological studies on the larger lakes of the country showed that most of them are currently disturbed, falling into the 'poor', 'bad' or 'moderate' water quality classes based on pH, chlorophyll-α concentration, Secchi depth, total phosphorus, high proportion of cyanobacteria in phytoplankton communities and limited macrophyte coverage [37].

Table 2. Quantitative scoring of the significance of the main threats to ICS in Greece and available protection frameworks; 0=none, 1=low, 2=medium, 3=high.

of live ornamental freshwater crayfish with 120 NICS available in stores, including 105 species considered to be high-risk vectors of crayfish 'plague' [24].

Our results suggest that the number of NIFCS available in the Greek domestic market is relatively low compared both to other animal taxa, and to other European countries (e.g., Germany and Czech Republic) This trend may be due to the low popularity of freshwater crayfish among aquarium hobbyists and their higher prices compared to many popular aquarium fish (e.g., €5-50 for P. clarkii, C. destructor and C. quadricarinatus and about €15 for Cambarellus species). However, given the absence of public education and sensitization of local communities, NICS introductions still constitute a considerable risk to ICS in Greece via deliberate or accidental releases. Accordingly, efforts to protect the native freshwater biodiversity of Greek freshwater systems should concentrate on several management initiatives, including (a) the identification of the main import channels for NICS, banning the import of highly invasive NICS (e.g., P. clarkii and C. destructor), and banning translocations of invasive ICS after a thorough assessment within the context of the 'white list' approach of the 708/2007 (EC) Directive [3]; (b) setting up a contingency plan with mechanisms to promote rapid responses in the event of NICS and crayfish 'plague' introductions in Europe; and (c) strict enforcement of legislation. Moreover, several additional initiatives could support the biodiversity of freshwater aquatic species in Greece, including a framework to monitor and control of the signal crayfish population in northern Greece, the protection of ICS populations and their habitats through the designation of SPAs as well as re-introduction programs, the enhancement of public education on the risk of NICS introductions (e.g., through responsible aguarium management) and research support on applied invasion biology and management.

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