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Occurrence of non-native red cherry shrimp in European temperate waterbodies: a case study from Hungary

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Abstract – The international pet trade has caused numerous introductions of non-native species globally. This is also true for freshwater decapod crustaceans including the red cherry shrimp. This tiny creature has been previously found in thermally polluted waters in Europe (Germany and Poland). Here, we present its first occurrence in Hungary and in the entire Carpathian Basin. The species was sampled once per month over 1 yr, from November 2017 to November 2018 in a natural thermal pond (spa) and also in an adjoining non-thermal brook in Miskolctapolca, Hungary. Shrimps were preyed upon by adult fishes in the locality but many individuals, including ovigerous females and juveniles, were recorded within the survey continuously. The density of shrimps was positively correlated with the water temperature, despite some individuals being found in the non-thermal stream and also in winter. We consider that the population of this species in Hungary is now well-established and self-sustaining.

Keywords: *Neocaridina* / Europe / ornamental species / pet trade / Atyidae

Résumé – Présence de crevettes rouge cerise non indigènes dans les eaux tempérées d'Europe : une étude de cas en Hongrie. Le commerce international des animaux de compagnie a provoqué de nombreuses introductions d'espèces non indigènes dans le monde. C'est également vrai pour les crustacés décapodes d'eau douce, y compris la crevette rouge cerise. Cette minuscule créature a déjà été trouvée dans des eaux thermiquement polluées en Europe (Allemagne et Pologne). Nous présentons ici sa première observation en Hongrie et dans l'ensemble du bassin des Carpates. L'espèce a été échantillonnée une fois par mois sur une période d'un an, de novembre 2017 à novembre 2018, dans un étang thermal naturel (spa) ainsi que dans un ruisseau non thermique adjacent à Miskolctapolca, en Hongrie. Les crevettes étaient la proie des poissons adultes dans la localité, mais de nombreux individus, y compris des femelles ovigères et des juvéniles, ont été échantillonnés de façon continue pendant le suivi. La densité des crevettes était corrélée positivement avec la température de l'eau, malgré la présence de certains individus dans le cours d'eau non thermique et aussi en hiver. Nous considérons que la population de cette espèce en Hongrie est maintenant bien établie et autosuffisante.

Mots-clés: *Neocaridina* / Europe / espèce ornementales commerce des animaux de compagnie / Atyidae

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1 Introduction

The international pet trade in aquatic species is known to be one of the main vectors of non-native species introduction and spread into new localities (Padilla and Williams, 2004; Duggan, 2010; Chucholl, 2013; Patoka *et al.*, 2016b, 2018b). In contrast to ornamental fish, decapod crustaceans are relatively new to the pet trade and have grown in popularity in the aquarium industry in recent years (Papavlasopoulou *et al.*, 2014; Faulkes, 2015; Kotovska *et al.*, 2016; Lipták *et al.*, 2017; Vodovsky *et al.*, 2017). Tropical and subtropical decapods usually have a low probability of establishing populations within the temperate zone except in thermal waterbodies, as documented in crayfish *Cambarellus patzcuarensis* and *Cherax quadricarinatus* (Jaklič and Vrezec, 2011; Weiperth *et al.*, 2017), and shrimps *Macrobrachium dayanum* and *Neocaridina denticulata* (Klotz *et al.*, 2013; Jabłońska *et al.*, 2018). On the other hand, certain warm-water species such as crayfish *Cherax destructor* pose the potential to overwinter in temperate climatic conditions (Veselý *et al.*, 2015).

The red cherry shrimp *Neocaridina denticulata* is a small prolific and truthful freshwater species belonging to the family Atyidae and is usually traded as the red cherry shrimp (Cai, 1996; Weber and Traunspurger, 2016). The taxonomical name of this species is often confusing: some authors use different synonyms such as *N. heteropoda* and *N. davidi* or suggest a species complex (see Klotz *et al.*, 2013 and citation herein). Hence, this species will be further mentioned under its common name in this study. The red cherry shrimp is one of the most popular pet-traded freshwater crustacean species due to its tiny size (adults 15–30 mm long), attractive coloration, and because it is an algae-eater (Turkmen and Karadal, 2012; Uderbayev *et al.*, 2017; Vazquez *et al.*, 2017). It is native to inland waterbodies in East Asia. It reproduces exclusively in freshwaters without any pelagic larval stage (Tropea *et al.*, 2015). Therefore, the red cherry shrimp is common on the market and frequently kept in hobby aquaria (Lipták and Vitázková, 2015; Magalhães and Andrade, 2015; Patoka *et al.*, 2016a).

There are many examples of decapods intentionally or unintentionally introduced from aquaria to the wild (*e.g.* Chucholl and Pfeiffer, 2010; Novitsky and Son, 2016; Patoka *et al.*, 2016c). Also, private outdoor garden ponds may serve as a source for the subsequent spread of ornamental decapods to adjoining localities (Peay, 2009; Patoka *et al.*, 2014, 2017). In the case of the red cherry shrimp, the alternative pathway for new introductions is also unintentional transport together with live fish stock, as reported in China (Englund and Cai, 1999). However, this species is mainly introduced via the pet trade in new localities and has been reported in the wild in Germany, Poland, and Japan (Nishino and Niwa, 2004; Klotz *et al.*, 2013; Jabłońska *et al.*, 2018).

Once established, the red cherry shrimp is a highly productive species (Schoolmann and Arndt, 2018) with possible impacts on the ecosystem and associated biota. Oh *et al.* (2003) noted that this shrimp is able to carry more than batch of eggs within the season with optimal conditions. Weber and Traunspurger (2016) found that foraging and predation by these omnivorous shrimps resulted in an overall reduction in abundance, biomass, and secondary production of meiobenthos assemblages (benthic fauna larger than microfauna but smaller than macrofauna, size 44 µm−0.5 mm). Moreover, it has been reported that the red cherry shrimp is a host of numerous commensals which are transported in huge quantities via the pet trade together with their host as "hitchhikers" (Patoka *et al.*, 2016a).

Both current international and local legislative regulations focused on biological invasions in general and on the pet trade in particular seem to be ineffective in many cases, and paradoxically, often have the opposite effect than was intended (Patoka *et al.*, 2018a and citation herein). Thus, new introductions of non-native ornamental species are likely to be common, and data about their spread is crucial for improving the management of affected freshwater ecosystems. Schoolmann and Arndt (2018) predicted that the risk of further spreading of the red cherry shrimp throughout European waterbodies is possible. Therefore, to find potentially established population, we surveyed selected thermal and non-thermal waterbodies in Hungary, a country where the red cherry shrimp has been previously reported as a commonly traded ornamental species and rated as medium risk in terms of its potential invasiveness (Weiperth *et al.*, 2018).

2 Material and methods

2.1 Locality

The locality in Miskolctapolca (a suburb of Miskolc, Hungary, Fig. 1) was selected according to the information from the Facebook group of Hungarian shrimp fans: https://www.facebook.com/groups/125067571204548/. The thermal pond is used by humans in the area as a public spa and Hejő brook is a regulated stream. We established five sampling points, two in thermal and three in non-thermal waterbody (Tab. 1). Detailed characteristics of the locality and sampling points are given in Table 2.

2.2 Data collecting

The locality was initially surveyed in November 2017. Shrimps were sampled using five baited (fish meat and halibut pellets) plastic bottle traps at the first sampling point, where they were left overnight. Subsequently, all five sampling points were surveyed by trapping once per month for 1 yr. Shrimps were also collected using handling nets and using a backpack power generator electrofisher (DEKA 3000 Lord) along a 150-m transect downstream from each of three sampling points in the brook and a 10-m transect at both sampling points in thermal pond. All individuals from the brook and all individuals from first two samplings in thermal water were preserved in pure ethanol (96%) for later determination, while other individuals were released back to the respective sampling points following identification. Ichthyofauna in the locality (brook) was also surveyed by electrofishing in November 2017. Fish species were recorded with some individuals euthanized for later dissection to examine their stomach contents. The remaining fish were released immediately following identification.

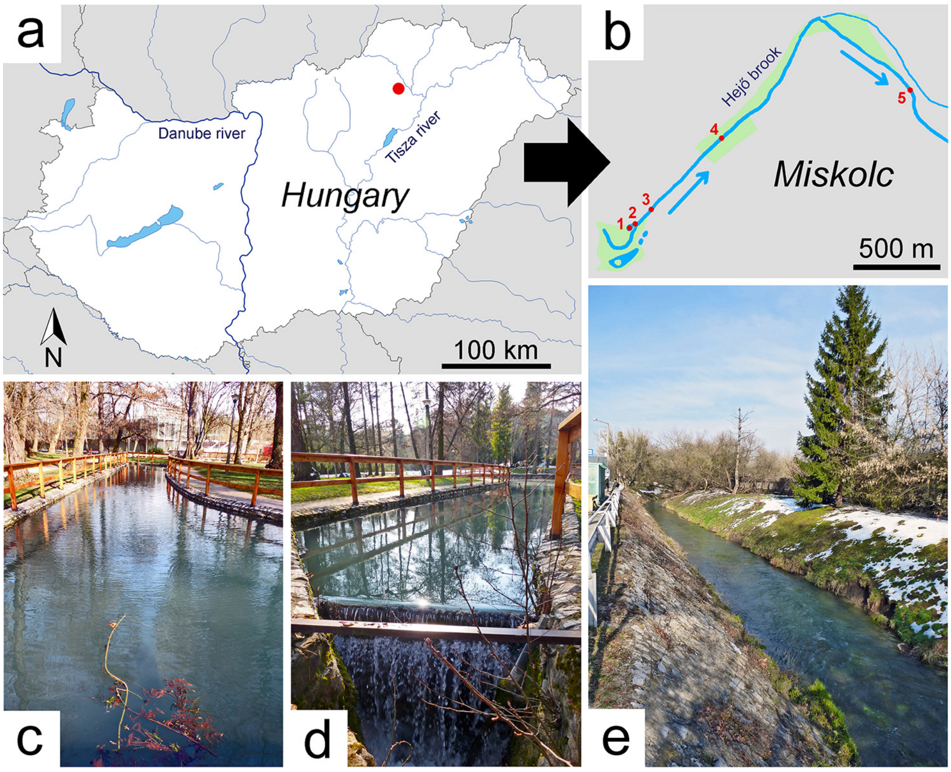

Fig. 1. Map showing the locality in Miskolctapolca, Hungary (indicated by red dot) (a), positions of five sampling points (indicated by red dots and numbers) (b), the sampling point 1 (thermal pond) (c), the sampling point 2 (outflow from the thermal pond) (d), and sampling point 4 (Hejő brook) (e).

Table 1. Name of the locality, number of the sampling point, type of the waterbody (thermal or non-thermal), and GPS coordinates.

Locality	No. of sampling point	Type	GPS
Miskolctapolca (pond)	1	Thermal	48°3′44.5″N, 20°44′52.2″E
Miskolctapolca (outflow)	2	Thermal	48°3′44.8″N, 20°44′54.2″E
Hejő brook	3	Non-thermal	48°3′48.1″N, 20°44′58.6″E
Hejő brook	4	Non-thermal	48°4′5.5″N, 20°45′24.7″E
Hejő brook	5	Non-thermal	48°4′17.5″N, 20°46′33.0″E

2.3 Species determination

Preserved shrimps were morphologically examined following the characteristics in Englund and Cai (1999) and Klotz *et al.* (2013). Three individuals were used for DNA analysis. The initial morphological species identification was confirmed by a molecular marker amplified by polymerase chain reaction. A primer pair LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) was used for amplification of the COI gene (Folmer *et al.*, 1994). The DNA extraction and amplification was processed according to Patoka *et al.* (2016d). The samples were sequenced using the Macrogen sequencing service (www.macrogen.com). Chromatograms were assembled and checked for potential errors. Edited sequences were aligned using Clustal W, as implemented in the BioEdit software package (Hall, 1999) and compared in NCBI database in Basic Local Alignment Search Tools (BLAST) (Madden, 2013). The obtained DNA sequences were deposited in GenBank database.

3 Results

We found that the red cherry shrimp was well-established in the locality, with many juveniles and ovigerous females captured during the surveys. The obtained three mitochondrial DNA sequences (COI gene) of length = 672 bp (GenBank acc. nos. MH780819, MH780820 and MH780821) confirmed the morphological identification of the captured shrimps as *N. denticulata* (GenBank acc. no. JX156333.1, Yu *et al.*, 2014). The density of shrimps was positively correlated with the water temperature. More than 1 km downstream from the mouth of the thermal tributary to Hejő brook, we sampled few shrimps in the six-degree water in the autumn and winter (sampling point 3). In the spring and summer, we found shrimps more than 3 km downstream from the thermal spring (sampling point 5). The majority of shrimps were captured among the roots of *Alnus* sp. and *Salix* sp. Details about seasonal variability in each sampling point including sex of captured shrimps are given in Table 3 and Figure 2.

Table 2. Detailed characteristics of the locality and sampling points with the range in each parameter over the complete survey.

Environmental parameters	Sampling point				
	1	2	3	4	5
Water temperature (°C)	24.1–31.6	21.9–27.6	11.8–27.9	6.8–25.6	3.2–24.7
Water depth (m)	>1.5	>1.5	0.1–0.7	0.3–0.6	0.2–0.9
Distance from the bank (m)	0.5–1.0	0.2–0.5	0.2–1.0	0.5	0.2–0.6
Water velocity (m/s)	0.3	0.3–1.0	1.2–2.3	0.9–1.8	0.7–1.6
Submerged vegetation (%)	5–15	<5	20–60	10–50	0–50
Emergent vegetation (%)	5–20	<5	<5	5–20	5–25
Woody debris (%)	<5	5–10	<5	10	5–20
Shading tree cover (%)	0–20	5–30	15–30	20	10–30
Depth of sediment (m)	0.3	0.1	0.2	0.2	0.2–0.4
Type of sediment	Rock, mud	Rock, mud	Clay, mud	Clay, mud	Mud

Table 3. Date of the sampling of red cherry shrimps (month and year) at five sampling points, with number of captured individuals: females (ovigerous females)/males/juveniles.

Date	Sampling point				
	1	2	3	4	5
XI. 2017	79(42)/29/106	15(11)/36/88	10/3/8	1/7/9	0/4/1
XII. 2017	59(16)/36/99	25(8)/25/102	1/1/4	1/0/2	0/0/0
I. 2018	77(10)/48/152	28(11)/38/119	0/4/1	0/0/0	0/0/0
II. 2018	59(7)/26/108	19(9)/38/51	0/2/0	0/0/0	0/0/0
III. 2018	44(11)/63/88	1(5)/47/51	3/8/11	0/0/6	0/0/0
IV. 2018	59(21)/68/114	61(28)/91/123	11(2)/11/29	4(1)/5/11	0/2/0
V. 2018	41(26)/87/125	75(46)/91/105	29(17)/11/25	10(4)/10/26	2(1)/2/7
VI. 2018	71(39)/102/105	81(51)/103/136	39(8)/36/75	14(2)/25/36	7(1)/7/11
VII. 2018	72(35)/99/185	88(49)/115/204	38(15)/45/95	21(9)/16/15	5/2/19
VIII. 2018	56(21)/67/201	69(38)/125/165	55(5)/28/77	10(2)/19/41	1/9/15
IX. 2018	71(17)/88/102	96(81)/65/124	12(4)/16/23	2/7/22	3/4/18
X. 2018	68(51)/91/115	75(19)/41/115	8(4)/4/10	3/8/13	2/1/21
XI. 2018	83(61)/95/135	68(55)/62/95	1/2/0	5/1/2	1/3/5

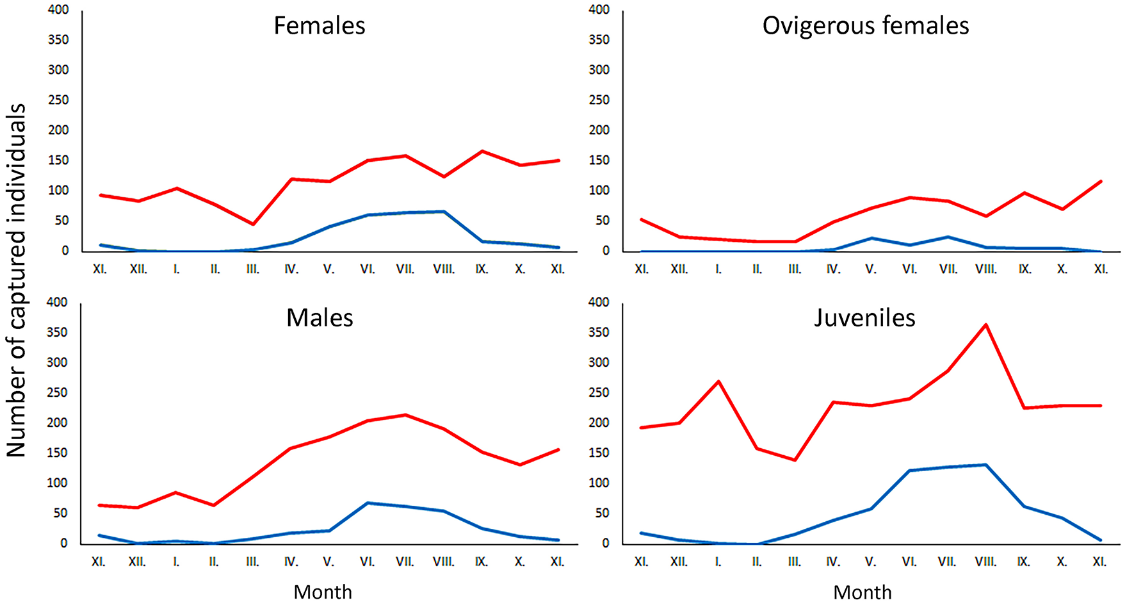

Fig. 2. Plots of captured red cherry shrimps (total numbers of females, ovigerous females, males, and juveniles) in thermal (red line) and non-thermal water within the complete survey (divided to months: from XI. 2017 to XI. 2018).

In the locality of the Hejő brooks, we also found the following macroinvertebrates: *Asellus aquaticus*, *Gammarus fossarum*, larvae of *Calopteryx splendens* and *C. virgo*; and fish species: *Alburnus alburnus*, *Cyprinus carpio*, *Gobio gobio*, *Lepomis gibbosus*, *Perca fluviatilis*, *Rhodeus sericeus*, *Rutilus rutilus*, *Scardinius erythrophthalmus*, *Squalius cephalus*, and *Pseudorasbora parva*. Juveniles of several fish species were observed but not captured. Five adult *G. gobio*, 7 *L. gibbosus*, 10 *P. parva*, 17 *R. rutilus*, and 21 *S. cephalus* were euthanized for the dissection of their stomach and shrimp remains were found in all dissected specimens.

4 Discussion

In this study, we report the occurrence of the red cherry shrimp for the first time in Hungary and from the entire Carpathian Basin. Contrary to previous records from the European territory (Klotz *et al.*, 2013; Jabłońska *et al.*, 2018), we found this non-native decapod occurring not only in thermal or thermally polluted waters, but also in adjacent brook with seasonal fluctuations in water temperature. The origin of the collected shrimps is unknown because this species is both directly imported from South-Eastern Asia, re-exported from other European countries such as the Czech Republic, and also produced locally in Hungary as an ornamental species (Weiperth *et al.*, 2018). Inasmuch as the release of unwanted animals from aquaria or unintentional escape is a frequent pathway for new introductions of non-native ornamental species, including decapod crustaceans, we assume that they were released by some local or spa-visiting hobby keeper(s). Even if there are no available data about previous occurrences of the red cherry shrimp in the locality, there is the possibility that this species may have been established in the locality for many years, as was case of this species' occurrence in Poland (Jabłońska *et al.*, 2018).

The red cherry shrimp inhabits small streams with rocky bottoms and dense aquatic vegetation (Viau *et al.*, 2016). The locality was therefore found suitable with the limiting environmental factor of water temperature. For the reason that the temperature in the thermal pond reaches 24.1–31.6 °C, which is an optimal temperature for reproduction of the red cherry shrimp (Nur and Christianus, 2013; Tropea *et al.*, 2015), we assume that this reservoir is a primary source of shrimps in the locality. Alternatively, the continual occurrence of individuals in the Hejő brook suggests that the adaptability of at least some of the red cherry shrimp population towards lower temperatures and annual temperature fluctuation is higher than previously expected; Mykles and Hui (2015) noted that the red cherry shrimp grows and reproduces at room temperature. Even if Tropea *et al.* (2015) experimentally found the highest proportion of ovigerous red shrimp females in water with a temperature of 28 °C and Mykles and Hui (2015) suggested 22–25 °C as optimum, we found some individuals in 11.8 °C water. Even if the water temperature influences the duration of the incubation period and the developmental time of embryos (Tropea *et al.*, 2015), we suggest that some individuals are also able to reproduce in non-thermal natural waters in the temperate zone, and successive generations could become adapted to these conditions. Mizue and Iwamoto (1961) briefly reported on a successful overwintering of this shrimp in Japanese freshwaters; however, neither water temperature nor other environmental conditions were specified in the publication; Oh *et al.* (2003) found this shrimp successfully reproducing and overwintering in one Korean temperate stream, and our data support this finding. Moreover, Serezli *et al.* (2017) suggested that lower water temperature (below 23 °C) causes a female-biased sex ratio in the population, which is crucial for population viability.

The trade and keeping of ornamental decapod crustaceans in freshwater aquaria are well-established in Hungary, and the unique hydrological features of this country with its numerous thermal springs and waterbodies serve as a perfect environment for exotic freshwater species to establish and flourish (Weiperth *et al.*, 2017, 2018). On the other hand, we found the red cherry shrimp also occurs in non-thermal streams. Hence, even if the red cherry shrimp is generally perceived as an invasive species (Serezli *et al.*, 2017), our findings potentially raise the predicted invasiveness of this species, which was assessed as a medium-risk in previously analyzed markets trading in ornamental decapods (Uderbayev *et al.*, 2017; Weiperth *et al.*, 2018). Although we have no data about any symbionts attached on the carapace surface of captured shrimps, the potential introduction of bdelloid rotifers, stalked protozoans, and scutariellid temnocephalidans previously found on shrimps imported from Indonesia (Patoka *et al.*, 2016a) cannot be excluded, and the probability of these symbionts establishing new populations via shrimp introductions is unknown.

Despite the documented predator–prey interaction between fish and the red cherry shrimp, the monitored population is considered well-established and self-sustaining. However, the red cherry shrimp has a great commercial potential as an ornamental species, it has a status of non-indigenous and thus undesirable species in the wild in Europe. Although the density of captured shrimps (non-surprisingly) positively correlated with the water temperature, some individuals were found in the non-thermal stream and also in winter. Since this shrimp has been mostly overlooked by policymakers and wildlife managers as an invasive species, the data we present in this study should change our approach to this species in the freshwaters of Central Europe. However, a potential ban on the trade of the red cherry shrimp, and related legislative restrictions, will be probably ineffective in halting its spread because it is a popular aquarium species. In line with the recommendations of Patoka *et al.* (2018a), we regard that the key to mitigating the risk of further spread and establishment of the red cherry shrimp is by educating the general public on the negative consequences of releasing aquarium species into freshwaters.

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