

## Research Article

## Life-history traits and ecological characteristics of the ornamental shrimp *Neocaridina denticulata* (De Haan, 1844), recently introduced into the freshwater systems of Israel

Ya'arit Levitt-Barmats<sup>1,2,\*</sup>, Zohar Yanai<sup>3,4</sup>, Tali Magory Cohen<sup>1</sup> and Noa Shenkar<sup>1,2,\*</sup>

<sup>1</sup>School of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

<sup>2</sup>The Steinhardt Museum of Natural History, Israel National Center for Biodiversity Studies, Tel Aviv University, Tel Aviv, Israel

<sup>3</sup>Musée Cantonal de Zoologie, Palais de Rumine, Lausanne, Switzerland

<sup>4</sup>Department of Ecology and Evolution, Biophore, University of Lausanne, 1015 Lausanne, Switzerland

\*Corresponding authors

E-mails: [yaaritle@tauex.tau.ac.il](mailto:yaaritle@tauex.tau.ac.il) (YLB), [shenkarn@tauex.tau.ac.il](mailto:shenkarn@tauex.tau.ac.il) (NS)

**Citation:** Levitt-Barmats Y, Yanai Z, Magory Cohen T, Shenkar N (2019) Life-history traits and ecological characteristics of the ornamental shrimp *Neocaridina denticulata* (De Haan, 1844), recently introduced into the freshwater systems of Israel. *Aquatic Invasions* 14(4): 684–702, <https://doi.org/10.3391/ai.2019.14.4.08>

**Received:** 5 June 2019

**Accepted:** 23 August 2019

**Published:** 8 October 2019

**Thematic editor:** Ian Duggan

**Copyright:** © Levitt-Barmats et al.

This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International - CC BY 4.0).

## OPEN ACCESS

### Abstract

Introduction rates of alien freshwater organisms are increasing worldwide. In Israel, freshwater habitats suffer from heavy anthropogenic stress resulting in numerous records of introduction to date. During 2014 a non-native caridean shrimp was found in several locations in the Jordan River system and in the Yarqon Stream. These systems represent different basins, hydrologically disconnected. Using both DNA analysis (mitochondrial genes COI and 16S) and morphological tools the species was identified as *Neocaridina denticulata*, an ornamental species originating from South-East Asia. Phylogenetic analyses of 56 Israeli and 18 Japanese specimens revealed no geographical differentiation among Israeli specimens, affiliated to a single haplotype, in contrast to the division of two groups within Japanese specimens. It is therefore possible that this species had been released by aquarium hobbyists. Field and laboratory observations revealed a highly reproductive and environmentally tolerant populations, reaching a reproductive stage within few months and releasing more than 70 larvae per ovigerous female. In addition, *N. denticulata* was found to be significantly larger in comparison to the native Atyidae shrimp *Atyaephyra orientalis*. We suggest that *N. denticulata* may expand its geographical distribution into similar freshwater systems in Europe and other Mediterranean countries, where suitable environmental conditions exist. We further provide tools for taxonomic identification and field observations for effective detection and inspection protocols.

**Key words:** alien shrimp, exotics, ornamental invertebrates, inland water, Caridea, Atyidae

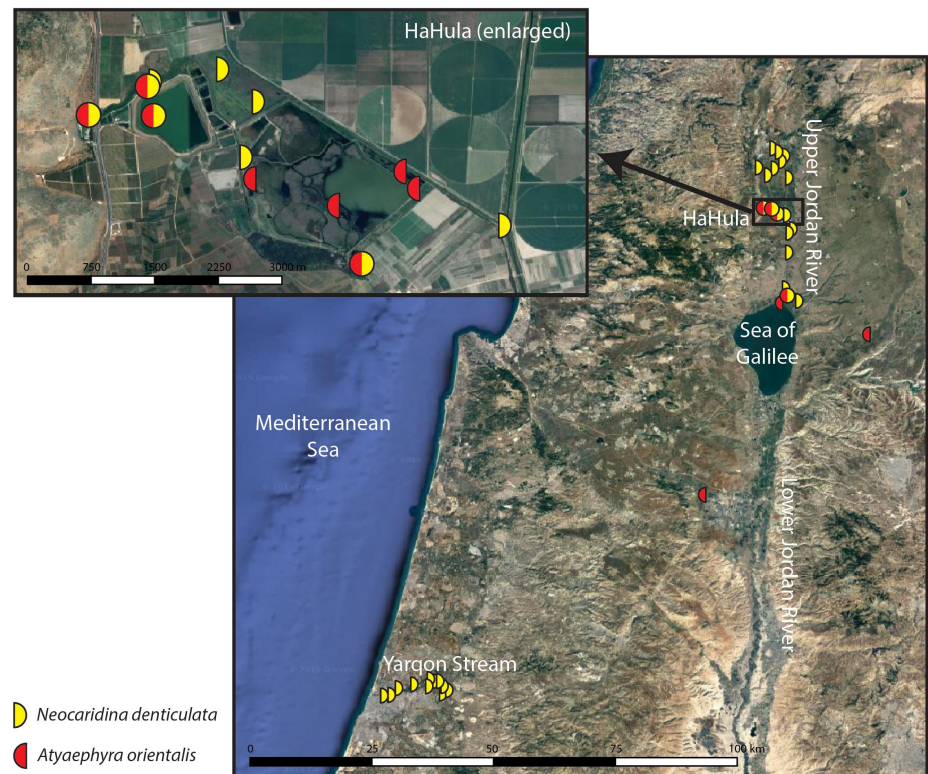
### Introduction

Non-native species in freshwater systems are widespread and some species are well-known for their ability to rapidly expand their geographical range (Strayer 2010; Nunes et al. 2015). Various stressors on freshwater ecosystems, caused by anthropogenic pressure and pollution of water due to domestic, agricultural, and industrial needs, may facilitate introductions of non-native species in these systems (Ricciardi 2001; Gleick 2003; Strayer 2010). Climate change also accelerates the successful establishment of non-

native species by facilitating the spreading and establishment of generalist and more tolerance species, in contrast to more specialists and environmental-limited native species (Rahel and Olden 2008; Strayer 2010). Considering the rapidly growing popularity of the aquarium industry, it has become a major introduction pathway and a significant risk of organisms being deliberately released or spread unintentionally as hitchhikers on aquarium plants (Duggan 2010; Strayer 2010; Turkmen and Karadal 2012; Patoka et al. 2015; Passarelli and Pernet 2019).

The freshwater shrimp from the family Atyidae De Haan, 1849 play a significant role in inland water bodies as primary consumers in the food web. Furthermore, they are involved in marked sedimentation reduction, increased algal production, and leaf-litter decomposition (Pringle et al. 1993; Covich et al. 1999; Crowl et al. 2001). In addition, these shrimp have become highly popular in the aquarium industry as pets and aquatic plant cleaners, where the worldwide major sources are Indonesia and Taiwan, as well as for fish bait or as a hitchhiker in live fish stock (Hung et al. 1993; Englund and Cai 1999; Niwa et al. 2005; Heerbrandt and Lin 2006; Niwa and Ohtaka 2006; Nur and Christianus 2013; Patoka et al. 2015; Mitsugi et al. 2017; Pantaleão et al. 2017; Maciaszek et al. 2018). Another source of introduction may be by outdoor garden ponds, though currently it is known only as a spreading ability of crayfish (Peay 2009; Patoka et al. 2014, 2017). The genus *Neocaridina* Kubo, 1938 comprises 31 species and subspecies (De Grave and Fransen 2011; Shih et al. 2017, 2019), originating from South-East Asia (Cai 1996). As a result of taxonomic difficulties, this genus is constantly under revision and the validation of a few species is currently questionable (e.g. Hung et al. 1993; Cai 1996; Liang 2004; Shih and Cai 2007; De Grave and Fransen 2011; Klotz et al. 2013). An ornamental shrimp, which is very common within aquarium hobbyists, was first reported as an introduced species in Hawaii in 1991, as *Neocaridina denticulata sinensis* (Kemp, 1918) and since then additional introductions were reported from Germany, Poland, Hungary, and possibly even Japan where native populations coexists (Englund and Cai 1999; Niwa et al. 2005; Klotz et al. 2013; Shih et al. 2017; Jabłońska et al. 2018; Weiperth et al. 2019).

In the freshwater system of Israel there is only one known native species of the family Atyidae, *Atyaephyra orientalis* Bouvier, 1913, which is native to the Jordan River and HaHula Nature Reserve (Christodoulou et al. 2012) (Supplementary material Table S1, Figure 1). Recently, populations of the genus *Neocaridina* have been recorded in Israel (Table S1, Figure 1). In addition to the relatively short winter season, the Israeli freshwater habitats suffer from heavy anthropogenic stress due to the combination of industrial life-style and dense population. Although there have been previous records of non-native species in natural systems, such as those on fish (Roll et al. 2007), gastropods (Roll et al. 2009), and crayfish (Snovsky and Galil 2011), this is the first report of a shrimp species introduced into Israel's inland water systems.



**Figure 1.** Sampling sites map of the freshwater systems of Israel: HaHula Nature Reserve, Jordan River, Yarqon Stream. Red symbol – *Atyaephyra orientalis*; yellow symbol – *Neocaridina denticulata*; red-yellow symbol – both species. Map originated from Google.

The aim of the current study was to examine certain life-history traits of the Asian atyid shrimp newly introduced into the freshwater systems of Israel; to study its occurrence in relation to the native species; and to investigate the genetic structure of the introduced populations in Israel in comparison with the Japanese atyid specimens.

## Materials and methods

### *Sample collection and taxonomic identification*

Specimens of the non-native species *Neocaridina* sp. and the native species *Atyaephyra orientalis* were collected between 2014–2018 from 30 different locations across freshwater systems in Israel: HaHula Nature Reserve, Jordan River, and Yarqon Stream (Table S1, Figure 1). At each site, individuals were sampled using hand-nets (400  $\mu$ m mesh-size) and transported to the laboratory at Tel Aviv University. A few of the live individuals were kept in a freshwater aquarium for several weeks under laboratory observation (see below). Finally, all specimens were identified and preserved in 70% ethanol and vouchered in the Crustacea collection at the Steinhardt Museum of Natural History, Israel National Center for Biodiversity Studies, Tel Aviv University, Israel (SMNH). One representative specimen was illustrated using a Leitz Ortholux II stereomicroscope with a drawing tube, under the guidance of Dr. Charles H.J.M. Franssen, from the Naturalis Biodiversity Center, Leiden, The Netherlands. In addition, 42

individuals of *N. denticulata* (De Haan, 1844) were sent from Japan to be compared with the Israeli population (Table S1). Tissue samples from 80 *Neocaridina* specimens (Israeli and Japanese), comprising a single swimming leg (pleopod) each, were taken and preserved in 100% ethanol for DNA analysis.

Morphological and meristic characters were taken and measured from the Japanese and Israeli sampled species, as detailed in Table S2, using a Canon SMZ18 and Leica M205C stereomicroscopes. Size was expressed as post-orbital carapace length (cl.), measured with a digital caliper to the nearest 0.1 mm, comprising the distance from the posterior orbital edge to the mid-dorsal posterior border of the carapace. The number of embryos was counted for 23 ovigerous females.

#### *Life-cycle traits under laboratory conditions*

Larvae of two ovigerous females from the Yarqon Stream were released into a freshwater aquarium at the end of May 2015. The shrimp larvae were kept under observation for two years in the lab at Tel Aviv University, and ultimately produced three subsequent generations. These were kept together with the freshwater plant *Ceratophyllum demersum* Linnaeus, 1753, in small aquaria (30 × 25 × 30 cm) in a water-table, maintained with an aeration system. All shrimp were held under artificial light conditions, according to the natural sunlight cycle. Temperature varied from 20–25 °C, and the shrimp were fed commercial fish food pellets (Tetra Marine XL Granules). Reproduction parameters such as sexual maturity and number of larvae, eggs, and embryos, were recorded. Concurrently, 18 of the third-generation larvae were separated into small individual jars (8.5 × 6.5 cm) to collect their exuviae and observe their growth rate. Water in all aquaria and jars was replaced every two weeks.

#### *DNA extraction and amplification*

DNA was extracted using a DNA Isolation Kit (Geneaid Biotech Ltd., Taiwan) following the manufacturer's protocol. A barcoding fragment of the mitochondrial cytochrome *c* oxydase subunit I (COI; 658 bp) was amplified using the universal primers LCO-HCO (Folmer et al. 1994); fragments of the mitochondrial 16S ribosomal DNA (16S; 543 bp) were amplified using the primers F-Car (5'-TGCCTGTTTATCAAAAACATGTC-3') and R-Car1 (5'-GAAAGATAGAACTAACCTGGCT-3') (von Rintelen et al. 2012). Polymerase chain reaction amplifications (PCR) were conducted using the 2X BlueStar™ PCR Master Mix (#BSPM-100, BSPM-200). Each was composed in a reaction volume containing 1 µl of DNA, 2 µl of primer (10 µM each), 25 µl of 2x BlueStar™ PCR Master Mix and H<sub>2</sub>O to complete to a total volume of 50 µl. Amplification conditions comprised of an initial denaturation at 95 °C for 1 min, followed by 32 cycles of 95 °C for 30 sec, 5 °C below T<sub>m</sub> for 30 sec, 72 °C for 30 sec, and a final elongation of



**Table 1.** The number of COI and 16S sequences used for the phylogenetic analyses in this study, together with the sequences retrieved from GenBank Specimens arranged alphabetically.

Species	COI			16S		
	Localities	N	Accession number	Localities	N	Accession number
<i>Atyaephyra orientalis</i> *	Turkey	1	JX289930	Turkey	1	JX469105
<i>Caridina cantonensis</i> *	China	1	AB300190	China	1	AB300176
<i>Caridina formosae</i> *	Taiwan	1	AB300189	Taiwan	1	AB300175
<i>Caridina pseudodenticulata</i> *	Taiwan	1	KY552467	Taiwan	1	DQ478511
<i>Neocaridina davidi</i> / <i>N. denticulata davidi</i>	Japan	23	LC324766–LC324767, MG734280–MG734300	Taiwan	4	KP168775–KP168776, KP168777–KP168778
<i>Neocaridina denticulata</i> / <i>N. denticulata denticulata</i>	Japan	13	AB300191,	Japan	5	DQ681268,
	Japan		AB524964–AB524973,	Hawaii		EF490005- EF490006,
	Japan		LC324764–LC324765	Taiwan		EU493134, China KP168774
<i>N. denticulata sinensis</i>	Israel**	42	MN336443–MN336484	Israel**	55	MN336370–MN336424
	Japan**	15	MN336485–MN336499	Japan**	18	MN336425–MN336442
<i>N. denticulata sinensis</i>	Taiwan	5	AB300183–AB300184,	Taiwan	5	AB300170–AB300171,
	Hawaii		AB300185–AB300186,	Hawaii		AB300172,
	Taiwan		AB300187	Taiwan		AB300173, Hawaii FN995377
<i>N. heteropoda koreana</i>	South Korea	1	LC324768	NA		NA
<i>Neocaridina ikiensis</i>	Japan	5	LC324775–LC324771	NA		NA
<i>Neocaridina ketagalan</i>	Taiwan	10	AB300180–AB300182,	Taiwan	3	AB300167–AB300169
	Taiwan		MG734250–MG734256	NA		NA
<i>Neocaridina palmata</i>	China	2	LC324769–LC324770	China	3	KP168779, KP168780, KP168781
				Hong Kong		
				Vietnam		
<i>Neocaridina saccam</i>	Taiwan	11	AB300177–AB300179, MG734222–MG734229	Taiwan	4	AB300164–AB300166, DQ681270
	Taiwan			Taiwan		
<i>Neocaridina spinosa</i>	China	1	AB300188	China	1	AB300174
<b>Total</b>		<b>133</b>			<b>102</b>	

\* – species used as outgroup

\*\* – sequences newly obtained in this study

2 min at 72 °C. PCR products were cleansed of residual primers and other residuals from amplicons and sequenced using an ABI 3730XL sequencer (Applied Biosystems). Out of the 80 specimens sampled, COI and 16S sequences were obtained for 58 and 73 samples, respectively (Table 1). Sequences were aligned and examined using Geneious R7 (v.7.1.5; <http://www.geneious.com>; Kearse et al. 2012). Sequences are available in GenBank (accession numbers MN336370–MN336499).

### Data analysis

For the molecular identification the sequences generated in this study were complemented with data from previous studies that included representatives of nine species and subspecies belonging to the genus *Neocaridina* (Table 1). Additionally, the species *Atyaephyra orientalis*, *Caridina cantonensis* Yu, 1938, *Caridina formosae* Hung, Chan and Yu, 1993, and *Caridina pseudodenticulata* Hung, Chan and Yu, 1993 were utilized as outgroups (Table 1). In total, 11 species were analyzed for both 16S and COI markers. Phylogeny was inferred by means of the Bayesian analysis method implemented in MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001;

Ronquist and Huelsenbeck 2003). The models chosen as having the lowest BIC scores in jModeltest 2.1.10 (Guindon and Gascuel 2003; Darriba et al. 2012) were TPM2uf + G for 16S and HKY + I + G for COI.

Two independent runs with four chains for ten million generations were made for each gene with sampling every 100 generations. Additionally, a concatenated tree was run for both genes combined using the same parameters. Convergence was assessed by examining stationarity in log-likelihood scores as the correlation of split frequencies between runs (Nylander et al. 2008). The first 1,250 trees (1,250,000 generations) were discarded as burn-in, and the remainders were used to estimate tree parameters and topology.

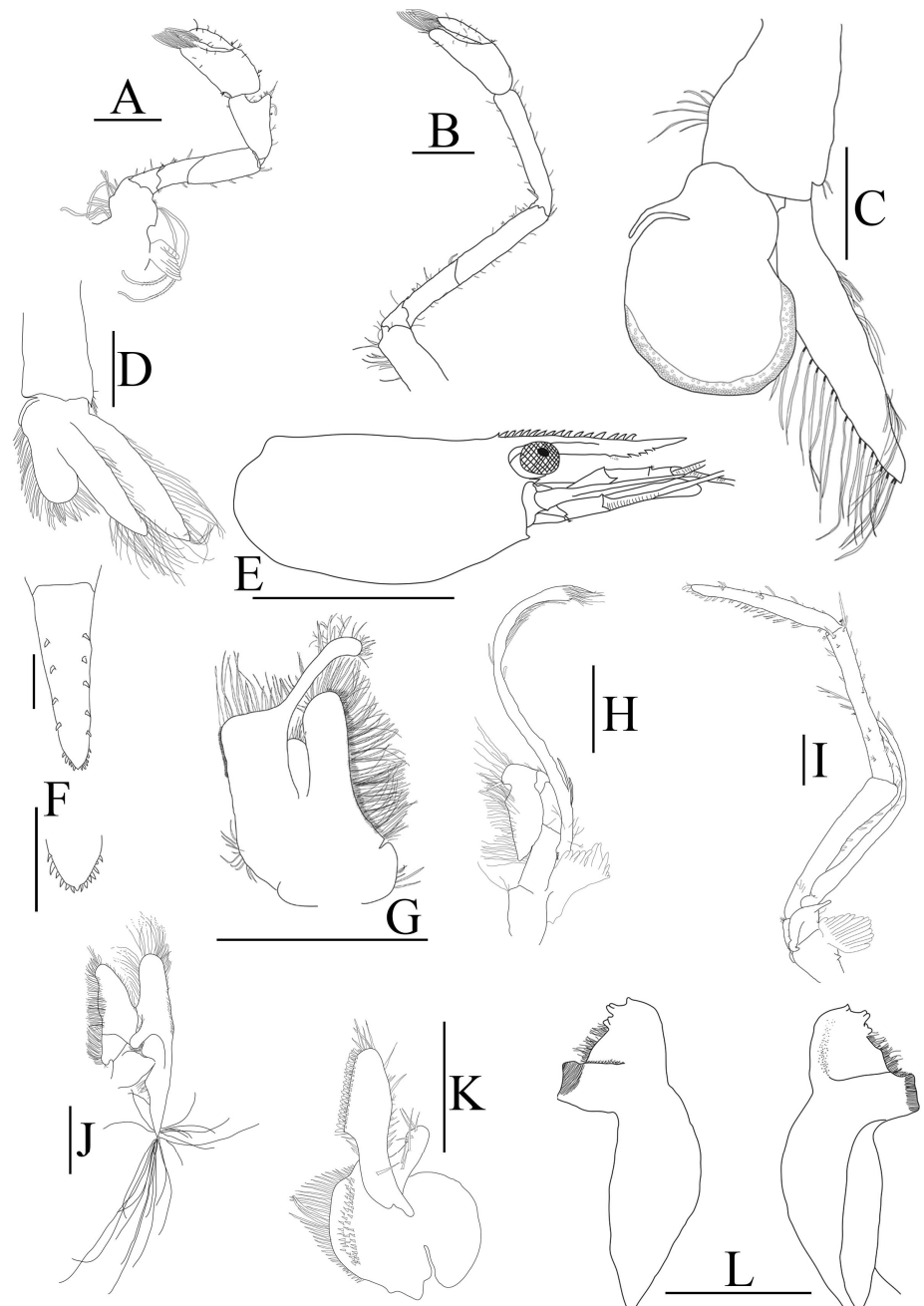
### *Statistical analysis*

A one-way analysis of variance (ANOVA) was used to determine any differences between carapace length (cl.) of the native species *Atyaephyra orientalis* and the alien species *Neocaridina* sp., as well as an associated male-female comparison of the alien species, with cl. as the dependent variable. Tests for homoscedasticity and normality assumptions were made for the parametric test (Sokal and Rohlf 2009). To determine whether a relationship exists between female cl. and the number of embryos she produces a generalized linear model (GLM) with a Poisson distribution analysis was implemented, using the lme4 package (version 1.1-19, Bates et al. 2015). For specimens lacking the first exuviae the data were completed using the average of other specimens: 0.8 for males and 0.7 for females. Calculations were based on one length measurement per individual per week; when a larva molted twice weekly (in the first molting stages) an average of both lengths was calculated. The previous exuviae measurement was listed as the same for all the following weeks until the next molting event in order to eliminate NAs in the data. To observe the growth rate of the alien shrimp and the differences between its sexes, a mixed effects model test was used with specimen as the random effect (Zuur et al. 2009). All statistical tests were conducted using R version 3.5.1 (R Development Core Team 2014).

## **Results**

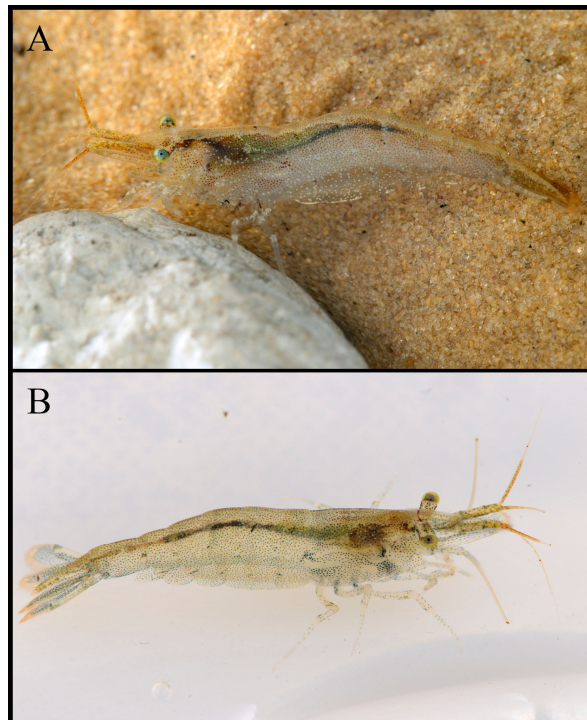
### *Taxonomic identification*

Identification of Israeli specimens corresponds to the morphological characters of the freshwater family Atyidae based on the first and second pair of pereopods carpus, which is excavated anteriorly, and fingers with dense brushes of setae (Figure 2A–B). Within the family, based on the endopod of first pair of pleopods in the male which is strongly widened, oval or subcircular, and the appendix masculina of second pair of pleopods in the male, which is also widened, with relatively long setae (Figure 2C–D),



**Figure 2.** *Neocaridina denticulata*. AR29250. A. first pereiopod; B. second pereiopod; C. first pleopod; D. second pleopod; E. carapace and anterior appendages, lateral view; F. telson, dorsal view; G. first maxilliped; H. second maxilliped; I. third maxilliped; J. maxilla; K. maxillula; L. mandibles. A–B, E–L, ovigerous female, cl. 5.8 mm; C–D, male, cl. 4.9 mm. Scales bar: 1 mm, except E = 5 mm.

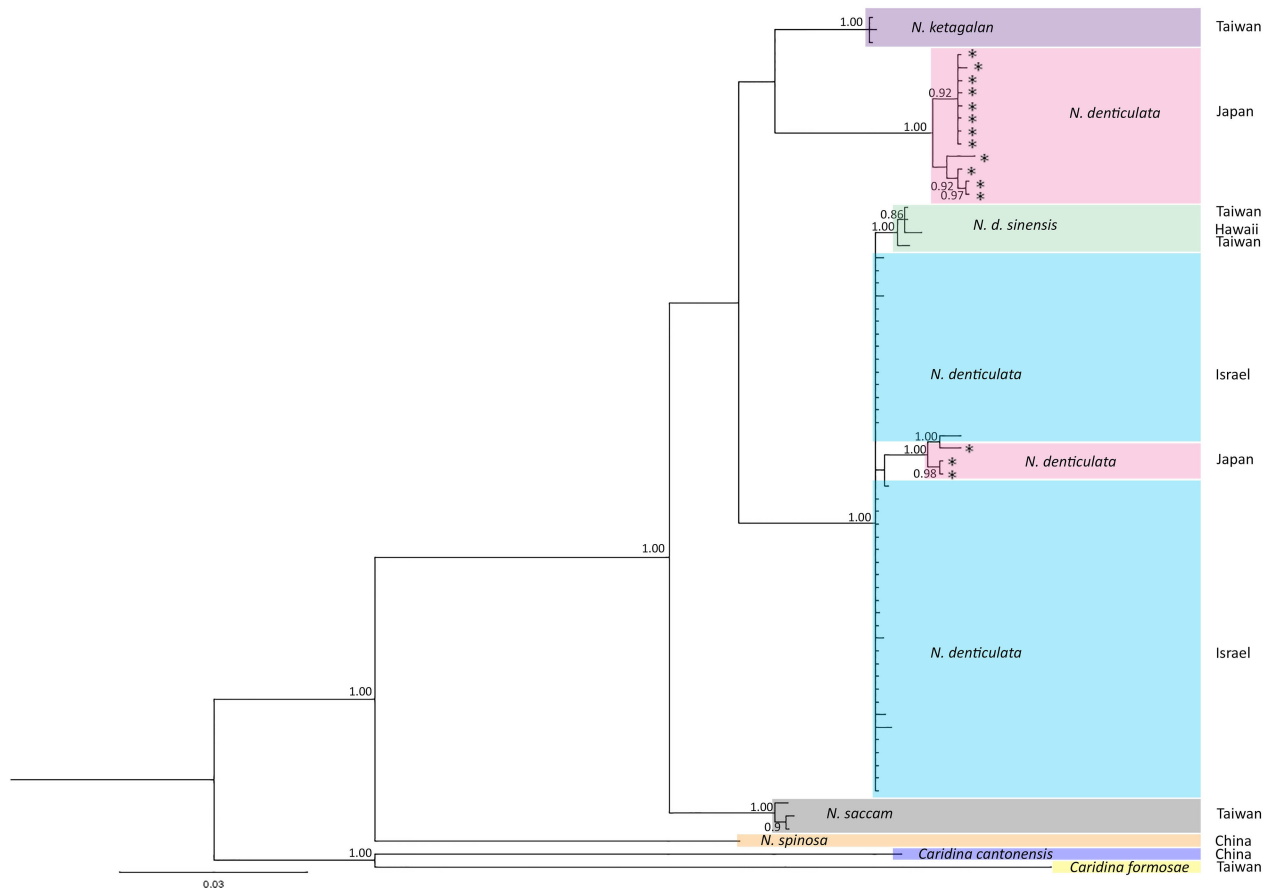
the Israeli specimens belong to the genus *Neocaridina* Kubo, 1938 (Kubo 1938; Holthuis 1993; Cai 1996). Within the genus *Neocaridina*, the Israeli specimens correspond to the following morphological characters (Figure 2): carapace with nearly horizontal rostrum in dorsal view, reaching or overreaching the tip of antennular peduncle, though five specimens possess a rostrum which is not reaching to the end of second segment of antennular peduncle. Rostrum dorsal margin with 13–25 (mostly 14–20) teeth, 3–4 teeth behind posterior edge of orbit; ventral margin with 1–9



**Figure 3.** *Neocaridina denticulata*. A. Red-spotted appearance, female, cl. 5.8 mm, AR29363; B. Blue-spotted appearance, female, cl. 6.0 mm, AR29376. Photos: O. Rittner.

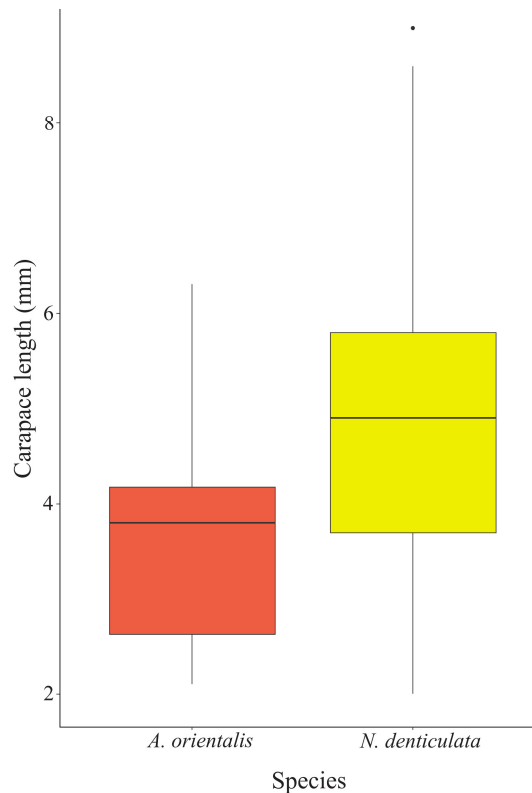
(mostly 3–7) teeth (as detailed in Table S2). Antennal and pterygostomian spines are present. Telson with 3–6 (mostly 4–5) pairs of dorsal spinules, though the number of spines can occasionally differ on each side, and 1 pair of dorsolateral spines near distal end; 4–5 (mostly 4, one female with 3) pairs of spines on distal margin. Eyes well-developed. Palp of first maxilliped rounded; ultimate segment of third maxilliped as long as penultimate segment; mandible without palp, molar process truncated. First pair of pereopods short; chela 2.0–2.2 times as long as broad; carpus shorter than chela, 1.7–1.9 times as long as high. Second pair of pereopods much longer and slender than the first pair; chela 3.0–3.8 (mostly 3.2–3.8) times as long as broad; carpus much longer than chela, 5.0–5.3 (four females were found with a ratio of 4.5) times as long as high. Third pair of pereopods with 4–8 (one male and one female were found with 3 and one female with 9) accessory spines on flexor margin. Endopod of male first pleopod is piriform. Uropodal diaeresis with 10–14 (seven specimens were found with fewer than 10 and one female with 15) movable spinules. Body color mostly white-translucent, though some individuals displayed red or dark blue dots (Figure 3). The above description correspond mostly to the Japanese swamp shrimp *Neocaridina denticulata* (Kubo, 1938) with the exception of the short rostrum specimens. On the contrary, *N. davidi* (Bouvier, 1904) is characterized with shorter rostrum which is not reaching to the end of second segment of antennular peduncle, however it differs from the Israeli specimens by its deeply excavated carpus of first cheliped and the distinct sexual dimorphism in the third pair of pereopods (Klotz et al. 2013).





**Figure 4.** Bayesian inference concatenated mitochondrial phylogenetic tree of both cytochrome *c* oxidase I (COI) phylogenetic tree and 16S ribosomal DNA reconstructed in this study. Values above the nodes indicate posterior probability in the Bayesian analysis ( $\geq 0.85$ ). \* – sequences newly obtained in this study.

The molecular analyses of COI and 16S comprised a total of 55 Israeli specimens and 18 Japanese *N. denticulata* specimens obtained in this study (as detailed in Table 1); as well as nine *Neocaridina* species and subspecies and four outgroups retrieved from GenBank (as detailed in Table 1). The phylogenetic trees (Figures 4, S1) confirm the morphological identification and similarity between the Israeli and Japanese specimens of the species *N. denticulata*. However, the phylogenetic relationships within the *N. denticulata* species complex are still unclear (e.g. Cai 1996; Liang 2004; Shih and Cai 2007) and specimens which were identified as *N. sinensis* and *N. davidi* were also found to be on the same cluster. The Israeli specimens cluster together with no apparent geographical structure, including the shorter rostrum specimens. The Japanese specimens seem to be separated into two groups based on the morphological characters and the phylogenetic trees: three of the specimens (Oka 2, 10, 19) were found with a short rostrum, which is not reaching the end of second segment of antennular peduncle. These three specimens were phylogenetically closely related to the Israeli specimens in both COI and 16S trees. The genetic distance between these groups was 2.2% in COI (exhibiting differences at 10 sites along the sequences) and none in 16S (the sequences were identical). The 15 remaining Japanese specimens were found with a longer



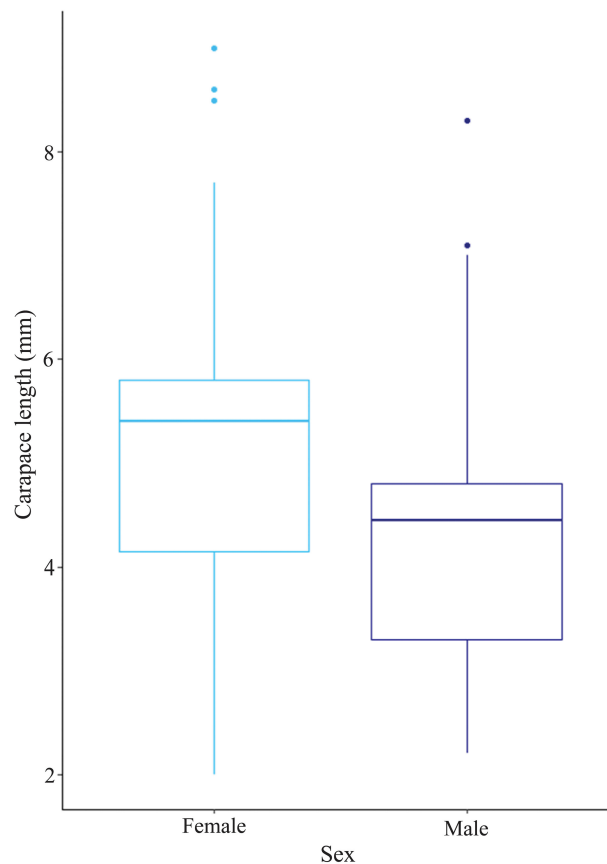
**Figure 5.** Carapace length of the native species *Atyaephyra orientalis* ( $n = 36$ ) and the alien species *Neocaridina denticulata* ( $n = 85$ ) collected in the freshwater systems of Israel between March 2014–April 2018; one-way ANOVA,  $F = 9.5$ ,  $P < 0.001$ .

rostrum and were genetically distinct, forming a monophyletic group. The genetic distance between these specimens to the Israeli and the three Japanese specimens was 8.2–8.3% in COI and 1% in 16S. Additional morphological characters of all Japanese specimens mostly corresponded to the description of the species (as detailed in Table S2).

*Atyaephyra orientalis* was identified following Christodoulou et al. (2012).

#### *General characters and geographic distribution*

A total of 85 individuals of *N. denticulata* and 36 individuals of *A. orientalis* from the freshwater systems in Israel were studied morphologically. The native species *A. orientalis* was found in the HaHula Nature Reserve and northern Jordan River, while the alien species was found also in the Yarqon Stream, a coastal stream pouring in the Mediterranean Sea, thus hydrologically disconnected from the Jordan River and HaHula system (Figure 1). Both species were found in similar habitat types, namely ponds in a natural marshland area (HaHula Nature Reserve) and *N. denticulata* was also found near the banks of water bodies, from small brooks to medium-size streams, where there is submerged vegetation and the water flows slowly. The average cl. of *A. orientalis* and *N. denticulata* was  $3.5 \pm 1.1$  ( $n = 36$ ) and  $4.9 \pm 1.6$  ( $n = 85$ ) mm, respectively, with the alien species being significantly larger than the native one (ANOVA,  $F = 9.5$ ,  $P < 0.001$ ; Figure 5), based on the collected specimens. Similar results were obtained

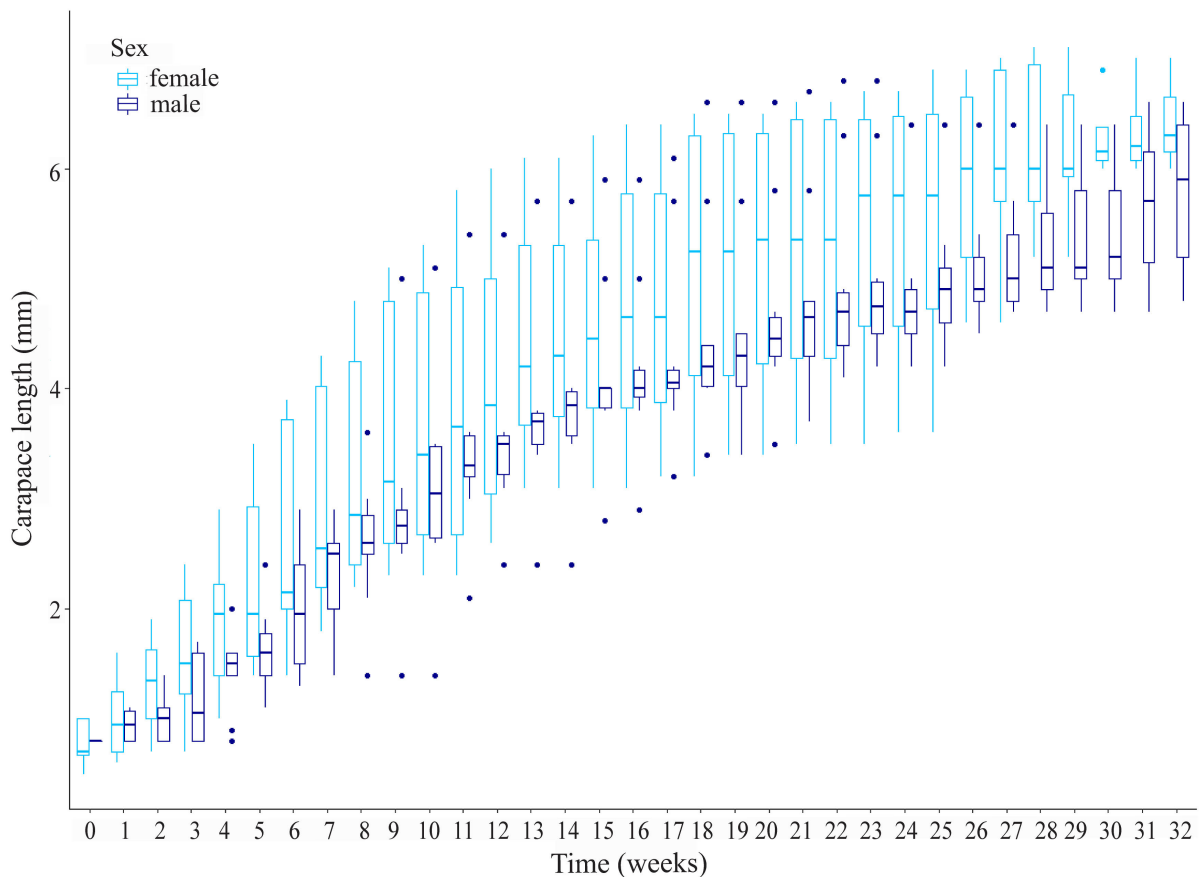


**Figure 6.** *Neocaridina denticulata*. Carapace length of males ( $n = 26$ ) and females ( $n = 58$ ) collected in the freshwater systems of Israel between March 2014–April 2018; one-way ANOVA,  $F = 4.0$ ,  $P < 0.05$ .

for HaHula Nature Reserve, where the native species was more abundant ( $n = 36$  for *A. orientalis* and  $n = 20$  for *N. denticulata*; ANOVA,  $F = 25.7$ ,  $P < 0.001$ ). *Neocaridina denticulata* average cl. of females and males was  $5.1 \pm 1.6$  ( $n = 58$ ) and  $4.4 \pm 1.5$  ( $n = 26$ ) mm, respectively, with the females being significantly larger than males (ANOVA,  $F = 4.0$ ,  $P < 0.05$ ; Figure 6). For each site separately, a similar trend is revealed, though not statistically significant.

#### *Life-cycle traits under laboratory conditions*

Approximately 65 larvae were released by the Yarqon Stream females into the aquarium in the lab in May 2015. Five months later ovigerous females appeared, carrying their embryos for about 30 days. The larvae were released during 2–3 hatching events, with the last event including molting of the female. In the second generation, the first ovigerous female was found after three months. The young ovigerous females had fewer embryos, the majority of their first reproduction efforts failed, and occasionally no live larvae were released. The correlation between female size and number of embryos was found to be significant ( $n = 23$ , GLM;  $P < 0.001$ ,  $y = 1.9x + 0.27$ ,  $r^2 = 0.2$ ), although the third largest ovigerous female bore the highest number of embryos ( $n = 70$ ). The smallest ovigerous female bore 17 embryos



**Figure 7.** Growth-rate curve of *Neocaridina denticulata* larvae observed in the laboratory at Tel-Aviv University for 32 weeks, for males ( $n = 10$ ) and females ( $n = 8$ ). Whiskers represent borders of first and fourth quartiles.

(cl. 2.3, body length 12.5 mm). From the third reproduction effort on, the number of released larvae was more than 70 per ovigerous female. The young shrimp started to eat after the fifth or sixth molt, and the male's oval appendix masculine on the second pleopod could be distinguished after about a month; male body length was 7–8 mm (cl. 2–3 mm).

The exuviae of 18 larvae were collected for 49 weeks. The growth rate of the larvae demonstrated a log shape, with the shrimp initially growing rapidly, until their molting decreased to once or twice a month (Figure 7). As the number of females declined to below half the initial number before the end of the observation, the figure presents the results up to week 32. The growth rate of males and females was initially similar, but with time the males grew more slowly and by their last molt, they were smaller than females (GLM, interaction between time and sex:  $\beta = -0.02 \pm 0.004$ ,  $df = 579.24$ ,  $t = -3.99$ ,  $P < 0.001$ ), with the exception of one male that displayed a faster growth rate. By week 32 the three largest specimens were females (cl. 7 mm), while the largest male was shorter (cl. 6.6 mm). Females demonstrated a higher growth rate variation per week than males.

## Discussion

The introduction rate of alien freshwater organisms is globally increasing, and may be in relation to the rapidly growing international aquarium trade



worldwide (Karatayev et al. 2007; Duggan 2010; Strayer 2010; Turkmen and Karadal 2012; Mazza et al. 2015; Nunes et al. 2015). The current study examines the introduction of the Asian freshwater shrimp *Neocaridina denticulata* in the freshwater system of Israel, including certain ecological aspects regarding its possible influence on the native fauna. *Neocaridina denticulata* has been found in the majority of freshwater systems in Israel, i.e. coastal, Jordan Valley and Dead Sea systems, as defined by Goren and Ortal (1999). These systems represent different basins, therefore are hydrologically disconnected, and water-restricted organisms (e.g., crustaceans) are not expected to cross them easily. It is alarming that the introduced species has been found across all systems in such a short period, pointing at its successful dispersal and establishment rates. Field and laboratory observations revealed a highly reproductive, environmentally tolerant, and relatively large-sized species, potentially providing the alien with some advantages over the native species in establishing sustainable populations. The impact of this generalist species on the native fauna and especially on *Atyaephyra orientalis*, is yet unknown, but it appears to be spreading along the Jordan River system and is constantly being reported from new habitats. In addition to the potential competition with the native species, *N. denticulata* individuals may be accompanied by symbiotic worms (Niwa et al. 2005; Niwa and Ohtaka 2006; Klotz et al. 2013), which increase the potential of these worms also being introduced into the freshwater systems of Israel.

Considering the taxonomic complexity within the species complex of *N. denticulata* (e.g. Cai 1996; Liang 2004; Shih and Cai 2007), and the inconclusive results of the morphological and molecular analyses in this study, the Israeli populations are presented here without denoting subspecific names. The large variation in some of the morphological characters of the Israeli specimens, together with the similarity within the *N. denticulata* complex revealed in the phylogeny tree, make the identification of the alien species uncertain. Following De Grave and Franssen (2011) suggesting *N. sinensis* is a synonym of *N. denticulata* together with the suggestion of Klotz et al. (2013) of which *N. sinensis* is a synonym of *N. davidi* may conclude that the three mentioned species are all *N. denticulata*, with some variation within different populations. The previous studies which considered *N. d. denticulata* and *N. d. sinensis* as native species also in Taiwan, China, and Korea (Hung et al. 1993; Cai 1996; Oh et al. 2003; Shih and Cai 2007) may support this hypothesis. The co-occurrence of non-native population with native populations in Japan (e.g. Klotz and Von Rintelen 2014) may also lead to hybridization though it has not yet been shown under laboratory conditions (Mitsugi and Suzuki 2018). In addition, there is constantly crossbreeding of ornamental *Neocaridina* species for achieving different colored specimens (e.g. <https://www.aquariacentral.com/forums/threads/shrimp-crossbreeding.248565/>).

The molecular analyses revealed no geographical differentiation, indicating that all the Israeli specimens from the freshwater systems are of the same haplotype. Complementing the phylogenetic results of separation within the Japanese shrimp, is the rostrum length, in which the three specimens with shorter rostrum (Oka 2, 10, 19) were genetically closer to the Israeli specimens than to the rest of the Japanese specimens which have a longer rostrum. Therefore, both morphological and molecular findings, indicate that the Israeli specimens are genetically related to Japanese populations, and may have originated from the same source.

We suggest that the introduction vector of the alien shrimp may be via direct or indirect release by aquaria hobbyists or the shrimp farming industry (Niwa and Ohtaka 2006; Turkmen and Karadal 2012; Patoka et al. 2015; Mitsugi et al. 2017; Yanai et al. 2017). However, as known from other invertebrates (Strayer 2010; Duggan 2010), another possibility is the unwitting release of the shrimp as hitchhikers on aquarium plants. An interview with two Israeli importers of ornamental aquatic animal equipment revealed that most of the *Neocaridina* stock in Israel is imported from Indonesia, or grown in a few locations in Israel during the last decade; while the freshwater plants are still mostly imported from Europe in moisture vacuum bags (Levitt-Barmats *pers. obs.* 2019), in which a few-days-old larvae can cling unobserved to the leaves and survive for 2–3 days. The native atyid shrimp *Atyaephyra orientalis* is not found in the Yarqon Stream, so the appearance of the alien shrimp is presumably noticeable, as the river is relatively-well monitored. However, in the HaHula water system the native atyid species is very common, as it is in several habitats throughout the Jordan River system, and could easily be confused by non-specialists with the alien species. As collected material prior to the year 2000 was inaccessible to this study, the identification of the atyid specimens collected from these habitats is uncertain, hindering estimation of the alien species' introduction period. The reduced rainfall during the last decades in the northern part of Israel (Ziv et al. 2014; Hochman et al. 2018), had a severe impact on inland water ecosystem biodiversity, which may increase the possibility of a successful introduction and establishment of opportunistic alien species (Chapin et al. 2000; Rahel and Olden 2008).

A literature survey of studies on *N. denticulata* habitats in Japan, Taiwan, and South Korea reveals that this species inhabits both small and large rivers (Niwa and Yokoyama 1993; Oh et al. 2003; Kasai et al. 2010; Yatsuya et al. 2012), and large lakes (up to 104 m deep, Shih and Cai 2007). Such large rivers and lakes do not occur in Israel. However, in both its native and newly-introduced regions, *N. denticulata* demonstrates a preference for slow water flow or standing waters, and generally avoids high current velocities, emphasizing the wide range of habitats suitable for this generalist species. It occurs mostly in vegetated patches near the water surface and can be collected using hand-nets (Hung et al. 1993; Oh et al.

2003; Hamano et al. 2005; Yatsuya et al. 2012; Mitsugi et al. 2017). The available data on the chemical and physical characters of its native habitats are scarce, but it appears that the variable water conditions in the studied Israeli habitats (conductivity 341–3150  $\mu\text{S}$ , water temperature up to 31 °C) do not limit *N. denticulata*. Submerged vegetation along the banks, which probably provides shelter for *N. denticulata*, seems to be important for its establishment at all sites. With no large lakes around, they establish populations in calm, vegetated riversides. *Atyaephyra orientalis* is considered to be a relatively small species (Christodoulou et al. 2012), while *N. denticulata* is significantly larger. As both species may compete for food and niches in the same habitats, the larger body size of the alien may provide it with an advantage over the native species. The females of *N. denticulata* were found to be significantly larger than males as known from the literature (Kubo 1938; Mizue and Iwamoto 1961; Ogawa et al. 1987; Oh et al. 2003; Mitsugi and Suzuki 2018), though more females were collected than males, probably as a result of the slower-swimming ovigerous females.

During the current study three generations of *N. denticulata* were grown under laboratory conditions for two years. As long as the conditions and temperature in the aquaria were stable, they were reproductive throughout the year, with larger females producing more embryos, supporting the findings of earlier studies (Mizue and Iwamoto 1961; Oh et al. 2003; Nur and Christianus 2013). This also correlates with female age, with their first reproduction efforts being less successful in regard to number of embryos and live larvae compared to their later efforts.

Based on previous studies, the life-span of *N. denticulata* is less than 1.5 years, and maximum cl. and body length are 9.4 and 30 mm, respectively (Mizue and Iwamoto 1961; Ogawa et al. 1987; Oh et al. 2003; Mitsugi and Suzuki 2018). The laboratory grown larvae showed greater growth rate in females, while both sexes slowed around 32 weeks. The increased female growth rate creates a reproductive advantage as larger females bear more embryos, though do not molt until after the larvae are released. The overall growth rate is slower when compared to *N. denticulata* from Japan is slower. Mizue and Iwamoto (1961) larvae were 16 mm in body length after two months, compared to 10–13 mm in the current study. Ogawa et al. (1987) reported larvae that were 17–19 mm in body length after three months, compared to 15–17 mm in the current study. The slower growth rate of the Israeli specimens may explain their longer life-span under the laboratory conditions.

In conclusion, on a local level, even though the Yarqon Stream and the Jordan River system (including HaHula) are separate systems, the alien shrimp was recorded for the first time across the country during the same year. As the time of introduction of *N. denticulata* into the Jordan River system is unknown, there is a possibility it may have entered this system

previously. The decrease in the stream flow and other ecological condition changes, may have led to a population explosion of the alien shrimp and its subsequent expansion to the HaHula Nature Reserve, where the system is more stable. Two introduced watercourses, the upper Jordan River and Yehudiyya Stream, both spill into the Lake of Galilee, the only large freshwater lake in Israel. Considering the favored habitat of *N. denticulata* in East Asia, it is very likely that it will eventually establish large population in the Sea of Galilee. In addition, during the 1990s, in order to increase the stream flow of the Yarqon Stream, water from the Sea of Galilee was diverted to sustain the fauna and flora of the Yarqon (<https://www.yarqon.org.il/en/drainage/water-sources/>). This may be what established a connection among the separated freshwater systems, which allowed the spread of the alien shrimp, but this theory certainly requires further investigation.

On a wider geographical scale, although the current study does not provide direct evidence of the introduction of *N. denticulata* by the aquarium trade, it seems the most likely explanation. In the future, therefore, *N. denticulata* may also expand its geographical distribution into similar freshwater systems in Europe and other Mediterranean countries, where the freshwater aquarium trade flourishes, such as in Turkey, Germany, and Italy (Turkmen and Karadal 2012; Klotz et al. 2013; Mazza et al. 2015; Patoka et al. 2015), and where suitable environmental conditions exist. Future introductions can be prevented with effective detection and inspection protocols (see Hulme et al. 2008; Nunes et al. 2015; Yanai et al. 2017). Moreover, the possibility of introduced species to escape into natural systems emphasizes the importance of continuously monitoring the freshwater systems of Israel, in order to rapidly detect new alien species in the future.

## Acknowledgements

This research could not have been carried out without the support and help of the Steinhardt Museum of Natural History, Israel National Center for Biodiversity Studies, Tel Aviv University. We are very grateful to N. Niwa for collecting and sending the specimens from Japan, and also to D. Milstein, L. Goren, and the Aquatic Ecology Center for collecting some of the material in Israel. Special thanks to C. Fransen for the valuable guidance; D. Huchon, M. Hadas-Sason, and K. Tamar for the help with the molecular work; H. Barmats for the help with the figures; N. Dorchin for the use of her facilities; and N. Paz for editorial assistance. We would like to deeply thank the two anonymous reviewers for their constructive suggestions that greatly improved the manuscript. This study was supported by the Israel Taxonomy Initiative PhD fellowship to YLB.

## References

- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67: 1–48, <https://doi.org/10.18637/jss.v067.i01>
- Cai Y (1996) A revision of the genus *Neocaridina* (Crustacea, Decapoda, Atyidae). *Acta Zootaxonomica Sinica* 21(2): 129–160
- Chapin FS, Zaveleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, Hooper DU, Lavorel S, Sala OE, Hobbie SE, Mack MC, Diaz S (2000) Consequences of changing biodiversity. *Nature* 405: 234–242, <https://doi.org/10.1038/35012241>
- Christodoulou M, Antoniou A, Magoulas A, Koukouras A (2012) Revision of the freshwater genus *Atyaephyra* (Crustacea, Decapoda, Atyidae) based on morphological and molecular data. *ZooKeys* 229: 53–110, <https://doi.org/10.3897/zookeys.229.3919>



- Covich AP, Palmer MA, Crowl TA (1999) The role of benthic invertebrate species in freshwater ecosystems. *BioScience* 49: 119–127, <https://doi.org/10.2307/1313537>
- Crowl TA, McDowell, WH, Covich, AP, Johnson SL (2001) Freshwater shrimp effects on detrital processing and nutrients in a tropical headwater stream. *Ecology* 82: 775–783, <https://doi.org/10.2307/2680196>
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature methods* 9: 772, <https://doi.org/10.1038/nmeth.2109>
- De Grave S, Fransen CHJM (2011) Carideorum Catalogus: The Recent Species of the Dendrobranchiate, Stenopodidean, Procarididean and Caridean Shrimps (Crustacea: Decapoda). *Zoologische Mededelingen, Leiden* 85: 195–588
- Dufour-Dror JM (2012) Alien Invasive Plants in Israel. Middle East Nature Conservation Promotion Association, Jerusalem, Israel, 214 pp
- Duggan IC (2010) The freshwater aquarium trade as a vector for incidental invertebrate fauna. *Biological Invasions* 12: 3757–3770, <https://doi.org/10.1007/s10530-010-9768-x>
- Englund RA, Cai Y (1999) The occurrence and description of *Neocaridina denticulata sinensis* (Kemp, 1918) (Crustacea: Decapoda: Atyidae), a new introduction to the Hawaiian Islands. *Bishop Museum Occasional Paper* 58: 58–65
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3(5): 294–299
- Gleick PH (2003) Global Freshwater Resources: Soft-Path Solutions for the 21<sup>st</sup> Century. *Science* 302: 1524–1528, <https://doi.org/10.1126/science.1089967>
- Goren M, Ortal R (1999) Biogeography, diversity and conservation of the inland water fish communities in Israel. *Biological Conservation* 89: 1–9, [https://doi.org/10.1016/S0006-3207\(98\)00127-X](https://doi.org/10.1016/S0006-3207(98)00127-X)
- Guindon S, Gascuel O (2003) A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. *Systematic Biology* 52: 696–704, <https://doi.org/10.1080/10635150390235520>
- Hamano T, Ideguchi K, Nakata K (2005) Larval drift and juvenile recruitment of amphidromous freshwater shrimps (Decapoda: Caridea) in the Nishida River, western Japan. *Aquaculture Science* 53(4): 439–446
- Heerbrandt TC, Lin J (2006) Larviculture of Red Front Shrimp, *Caridina gracilirostris* (Atyidae, Decapoda). *Journal of the World Aquaculture Society* 37: 186–190, <https://doi.org/10.1111/j.1749-7345.2006.00025.x>
- Hochman A, Mercogliano P, Alpert P, Saaroni H, Bucchignani E (2018) High-resolution projection of climate change and extremity over Israel using COSMO-CLM. *International Journal of Climatology* 38: 5095–5106, <https://doi.org/10.1002/joc.5714>
- Holthuis LB (1993) The recent genera of the caridean and stenopodidean shrimps (Crustacea, Decapoda) with an appendix on the order Amphionidacea. Nationaal Natuurhistorisch Museum, Leiden, 328 pp, <https://doi.org/10.5962/bhl.title.152891>
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755, <https://doi.org/10.1093/bioinformatics/17.8.754>
- Hulme E, Bacher S, Kenis M, Klotz S, Kühn I, Minchin D, Nentwig W, Olenin S, Panov V, Pergl J, Pysek P, Sol D, Solarz W, Vila M (2008) Grasping at the routes of biological invasions. *Journal of Applied Ecology* 45: 403–414, <https://doi.org/10.1111/j.1365-2664.2007.01442.x>
- Hung M-S, Chan TY, Yu HP (1993) Atyid Shrimps (Decapoda: Caridea) of Taiwan, with descriptions of three new species. *Journal of Crustacean Biology* 13: 481–503, <https://doi.org/10.2307/1548789>
- Jabłońska A, Mamos T, Gruszka P, Szlauer-Lukaszewska A, Grabowski M (2018) First record and DNA barcodes of the aquarium shrimp, *Neocaridina davidi*, in Central Europe from thermally polluted River Oder canal, Poland. *Knowledge & Management of Aquatic Ecosystems* 419: 14, <https://doi.org/10.1051/kmae/2018004>
- Karatayev AY, Padilla DK, Minchin D, Boltovskoy D, Burlakova LE (2007) Changes in Global Economies and Trade: the Potential Spread of Exotic Freshwater Bivalves. *Biological Invasions* 9: 161–180, <https://doi.org/10.1007/s10530-006-9013-9>
- Kasai A, Kurikawa Y, Ueno M, Robert D, Yamashita Y (2010) Salt-wedge intrusion of seawater and its implication for phytoplankton dynamics in the Yura Estuary, Japan. *Estuarine, Coastal and Shelf Science* 86: 408–414, <https://doi.org/10.1016/j.ecss.2009.06.001>
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649, <https://doi.org/10.1093/bioinformatics/bts199>
- Klotz W, Von Rintelen T (2014) To “bee” or not to be - On some ornamental shrimp from Guangdong Province, Southern China and Hong Kong SAR, with descriptions of three new species. *Zootaxa* 3889: 151–184, <https://doi.org/10.11646/zootaxa.3889.2.1>
- Klotz W, Miesen FW, Hüllen S, Herder F (2013) Two Asian fresh water shrimp species found in a thermally polluted stream system in north Rhine-Westphalia, Germany. *Aquatic Invasions* 8: 333–339, <https://doi.org/10.3391/ai.2013.8.3.09>
- Kubo I (1938) On the Japanese atyid shrimps. *Journal of the Imperial Fisheries Institute, Tokyo* 33: 67–100
- Liang XQ (2004) Fauna Sinica. Invertebrata Vol. 36. Crustacea. Decapoda. Atyidae. Science Press, Beijing, China, 375 pp [In Chinese with English abstract]

- Maciaszek R, Kamaszewski M, Strużyński W, Łapa P (2018) Epibionts of ornamental freshwater shrimps bred in Taiwan. *Annals of Warsaw University of Life Sciences - SGGW* 57: 133–142, <https://doi.org/10.22630/AAS.2018.57.2.13>
- Mazza G, Lastrucci L, Ferretti G, Lazzaro L, Aquiloni L, Giuliani C, Tricarico E, Foggi B, Inghilesi A (2015) Aliens just a click away: the online aquarium trade in Italy. *Management of Biological Invasions* 6: 253–261, <https://doi.org/10.3391/mbi.2015.6.3.04>
- Mitsugi M, Suzuki H (2018) Life history of an invasive freshwater shrimp *Neocaridina davidi* (Bouvier, 1904), (Decapoda: Caridea: Atyidae) in the Tomoe River, the Boso Peninsula, eastern Japan. *Crustacean Research* 47: 9–16, [https://doi.org/10.18353/crustacea.47.0\\_9](https://doi.org/10.18353/crustacea.47.0_9)
- Mitsugi M, Hisamoto Y, Suzuki H (2017) An invasive freshwater shrimp of the genus *Neocaridina* Kubo, 1938 (Decapoda: Caridea: Atyidae) collected from Boso Peninsula, Tateyama City, Chiba Prefecture, eastern Japan. *Crustacean Research* 46: 83–94, [https://doi.org/10.18353/crustacea.46.0\\_83](https://doi.org/10.18353/crustacea.46.0_83)
- Mizue K, Iwamoto Y (1961) On the Development and Growth of *Neocaridina denticulata* de Haan. *Bulletin of the Faculty of Fisheries Nagasaki University* 10: 15–24
- Niwa N, Ohtaka A (2006) Accidental Introduction of Symbionts with Imported Freshwater Shrimps. In: Koike F, Clout MN, Kawamichi M, De Poorter M, Iwatsuki K (eds), *Assessment and Control of Biological Invasion Risk*. World Conservation Union, Switzerland, pp 182–186
- Niwa N, Yokoyama T (1993) Distribution of *Neocaridina denticulata* in the Yumesaki River System, Japan. *Aquaculture Science* 41(4): 529–534
- Niwa N, Ohtomi J, Ohtaka A, Gelder SR (2005) The first record of the ectosymbiotic branchiobdellidan *Holtodrilus truncatus* (Annelida, Clitellata) and on the freshwater shrimp *Neocaridina denticulata denticulata* (Caridea, Atyidae) in Japan. *Fisheries Science* 71: 685–687, <https://doi.org/10.1111/j.1444-2906.2005.01017.x>
- Nunes AL, Tricarico E, Panov VE, Cardoso AC, Katsanevakis S (2015) Pathways and gateways of freshwater invasions in Europe. *Aquatic Invasions* 10: 359–370, <https://doi.org/10.3391/ai.2015.10.4.01>
- Nur FAH, Christianus A (2013) Breeding and life cycle of *Neocaridina denticulata sinensis* (Kemp, 1918). *Asian Journal of Animal and Veterinary Advances* 8: 108–115, <https://doi.org/10.3923/ajava.2013.108.115>
- Nylander JAA, Olsson U, Alström P, Sanmartín I (2008) Accounting for Phylogenetic Uncertainty in Biogeography: A Bayesian Approach to Dispersal-Vicariance Analysis of the Thrushes (Aves: Turdus). *Systematic Biology* 57: 257–268, <https://doi.org/10.1080/10635150802044003>
- Ogawa Y, Kakuda S, Wakashita F (1987) On the Ecology of *Neocardina denticulata* (Decapoda, Atyidae) inhabiting the Ashida River, Eastern Region of the Hiroshima Prefecture. *Aquaculture Science* 35(1): 33–41
- Oh CW, Ma CW, Hartnoll RG (2003) Reproduction and population dynamics of the temperate freshwater shrimp, *Neocaridina denticulata denticulata* (De Haan, 1844), in a Korean stream. *Crustaceana* 76: 993–1015, <https://doi.org/10.1163/156854003771997864>
- Pantaleão JAF, Gregati RA, da Costa RC, López-Greco LS, Negreiros-Fransozo ML (2017) Post-hatching development of the ornamental ‘Red Cherry Shrimp’ *Neocaridina davidi* (Bouvier, 1904) (Crustacea, Caridea, Atyidae) under laboratorial conditions. *Aquaculture Research* 48: 553–569, <https://doi.org/10.1111/are.12903>
- Passarelli B, Pernet B (2019) The marine live bait trade as a pathway for the introduction of non-indigenous species into California: patterns of importation and thermal tolerances of imported specimens. *Management of Biological Invasions* 10: 80–95, <https://doi.org/10.3391/mbi.2019.10.1.05>
- Patoka J, Petrtyl M, Kalous L (2014) Garden ponds as potential introduction pathway of ornamental crayfish. *Knowledge and Management of Aquatic Ecosystems* 414: 13, <https://doi.org/10.1051/kmae/2014019>
- Patoka J, Bláha M, Devetter M, Rylková K, Čadková Z, Kalous L (2015) Aquarium hitchhikers: attached commensals imported with freshwater shrimps via the pet trade. *Biological Invasions* 18: 457–461, <https://doi.org/10.1007/s10530-015-1018-9>
- Patoka J, Bláha M, Kalous L, Kouba A (2017) Irresponsible vendors: Non-native, invasive and threatened animals offered for garden pond stocking. *Aquatic Conservation: Marine and Freshwater Ecosystems* 27: 692–697, <https://doi.org/10.1002/aqc.2719>
- Peay S (2009) Invasive non-indigenous crayfish species in Europe: Recommendations on managing them. *Knowledge and Management of Aquatic Ecosystems* 394–395: 3, <https://doi.org/10.1051/kmae/2010009>
- Pringle CM, Blake GA, Covich AP, Buzby KM, Finley A (1993) Effects of omnivorous shrimp in a montane tropical stream: sediment removal, disturbance of sessile invertebrates and enhancement of understory algal biomass. *Oecologia* 93: 1–11, <https://doi.org/10.1007/BF00321183>
- R Development Core Team (2014) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria
- Rahel FJ, Olden JD (2008) Assessing the Effects of Climate Change on Aquatic Invasive Species. *Conservation Biology* 22: 521–533, <https://doi.org/10.1111/j.1523-1739.2008.00950.x>
- Ricciardi A (2001) Facilitative interactions among aquatic invaders: is an “invasion meltdown” occurring in the Great Lakes? *Canadian Journal of Fisheries and Aquatic Sciences* 58: 2513–2525, <https://doi.org/10.1139/f01-178>
- Roll U, Dayan T, Simberloff D, Goren M (2007) Characteristics of the introduced fish fauna of Israel. *Biological Invasions* 9: 813–824, <https://doi.org/10.1007/s10530-006-9083-8>
- Roll U, Dayan T, Simberloff D, Mienis HK (2009) Non-indigenous land and freshwater gastropods in Israel. *Biological Invasions* 11: 1963–1972, <https://doi.org/10.1007/s10530-008-9373-4>

- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574, <https://doi.org/10.1093/bioinformatics/btg180>
- Shih HT, Cai Y (2007) Two new species of the land-locked freshwater shrimps genus, *Neocaridina* Kubo, 1938 (Decapoda: Caridea: Atyidae), from Taiwan, with notes on speciation on the island. *Zoological Studies* 46(6): 680–694
- Shih HT, Cai Y, Niwa N, Nakahara Y (2017) A New Species of Land-locked Freshwater Shrimp of the Genus *Neocaridina* (Decapoda: Caridea: Atyidae) from Iki Island, Kyushu, Japan. *Zoological Studies* 56: 30, <https://doi.org/10.6620/ZS.2017.56-30>
- Shih HT, Cai Y, Chiu YW (2019) *Neocaridina fonticulata*, a new land-locked freshwater shrimp from Hengchun Peninsula, Taiwan (Decapoda, Caridea, Atyidae). *ZooKeys* 817: 11–23, <https://doi.org/10.3897/zookeys.817.29332>
- Snovsky G, Galil BS (2011) The australian redclaw crayfish *Cherax quadricarinatus* (von Martens, 1868) (Crustacea: Decapoda: Parastactidae) in the Sea of galilee, Israel. *Aquatic Invasions* 6: S29–S31, <https://doi.org/10.3391/ai.2011.6.S1.007>
- Sokal RR, Rohlf JF (2009) Introduction to biostatistics, 2<sup>nd</sup> ed. Dover Publications Incorporation, New York, 363 pp
- Strayer DL (2010) Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology* 55: 152–174, <https://doi.org/10.1111/j.1365-2427.2009.02380.x>
- Turkmen G, Karadal O (2012) The survey of the imported freshwater decapod species via the ornamental aquarium trade in Turkey. *Journal of Animal and Veterinary Advances* 11: 2824–2827, <https://doi.org/10.3923/javaa.2012.2824.2827>
- von Rintelen K, Page TJ, Cai Y, Roe K, Stelbrink B, Kuhajda BR, Iliffe TM, Hughes J, von Rintelen T (2012) Drawn to the dark side: A molecular phylogeny of freshwater shrimps (Crustacea: Decapoda: Caridea: Atyidae) reveals frequent cave invasions and challenges current taxonomic hypotheses. *Molecular Phylogenetics and Evolution* 63: 82–96, <https://doi.org/10.1016/j.ympev.2011.12.015>
- Weiperth A, Gábris V, Danyik T, Farkas A, Kuříková P, Kouba A, Patoka J (2019) Occurrence of non-native red cherry shrimp in European temperate waterbodies: a case study from Hungary. *Knowledge & Management of Aquatic Ecosystems* 420: 9, <https://doi.org/10.1051/kmae/2019002>
- Yanai Z, Dayan T, Mienis H, Gasith A (2017) The pet and horticultural trades as introduction and dispersal agents of non-indigenous freshwater molluscs. *Management of Biological Invasions* 8: 523–532, <https://doi.org/10.3391/mbi.2017.8.4.07>
- Yatsuya M, Ueno M, Yamashita Y (2012) Occurrence and distribution of freshwater shrimp in the Isazu and Yura Rivers, Kyoto, western Japan. *Plankton and Benthos Research* 7: 175–187, <https://doi.org/10.3800/pbr.7.175>
- Ziv B, Saaroni H, Pargament R, Harpaz T, Alpert P (2014) Trends in rainfall regime over Israel, 1975–2010, and their relationship to large-scale variability. *Regional Environmental Change* 14: 1751–1764, <https://doi.org/10.1007/s10113-013-0414-x>
- Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Statistics for Biology and Health. Springer-Verlag, New York, 574 pp, <https://doi.org/10.1007/978-0-387-87458-6>

### Supplementary material

The following supplementary material is available for this article:

**Table S1.** Summary of sampling sites of *Neocaridina denticulata* and *Atyaephyra orientalis* during March 2014–April 2018 in the freshwater systems of Israel and Japan.

**Table S2.** Summary of morphological and meristic characters of *Neocaridina denticulata* collected from the freshwater systems of Israel and Japan.

**Figure S1.** Bayesian inference mitochondrial phylogenetic trees reconstructed in this study.

This material is available as part of online article from:

[http://www.reabic.net/aquaticinvasions/2019/Supplements/AI\\_2019\\_Levitt-Barmats\\_etal\\_SupplementaryTables.xlsx](http://www.reabic.net/aquaticinvasions/2019/Supplements/AI_2019_Levitt-Barmats_etal_SupplementaryTables.xlsx)

[http://www.reabic.net/aquaticinvasions/2019/Supplements/AI\\_2019\\_Levitt-Barmats\\_etal\\_SupplementaryFigure.pdf](http://www.reabic.net/aquaticinvasions/2019/Supplements/AI_2019_Levitt-Barmats_etal_SupplementaryFigure.pdf)