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# Life history of invasive Ponto-Caspian mysids (Crustacea: Mysida): A comparative study

Péter Borza\*

Danube Research Institute, Centre for Ecological Research, Hungarian Academy of Sciences, Jávorka Sándor utca 14, H-2131 Göd, Hungary

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## ABSTRACT

In recent decades the Ponto-Caspian mysids *Limnomysis benedeni*, *Hemimysis anomala*, and *Katamysis warpachowskyi* expanded their ranges throughout the North Atlantic region and proved to have profound ecological impacts in the invaded waters. The aim of this study was to (1) provide a comprehensive description about the life history of the previously least known *K. warpachowskyi*, (2) reveal the number of generations produced annually by the three invasive Ponto-Caspian mysids, and to (3) compare the life history traits of the three species directly for the first time based on a simultaneous sampling. To obtain a high-resolution picture about their body length–frequency distributions, a very intensive (approximately weekly) sampling was carried out in an artificial embayment of the Danube River (in Hungary), where the three species coexist. The relatively large *L. benedeni* had five generations per year and produced comparatively low numbers of young, while the similar sized *H. anomala* completed only four generations, but compensated for this with a higher fecundity. The smaller sized *K. warpachowskyi* was able to produce more than five (probably 6) generations per year owing to its short maturation time and long reproductive season, and attained brood sizes close to those of *L. benedeni*. The generation numbers revealed by the study can be regarded as extraordinarily high considering the body size of the animals and the temperate climatic conditions, which might contribute to their invasion success by increasing the chance of establishment, especially in the course of jump dispersal events at which Ponto-Caspian mysids have proved very successful.

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## Introduction

Ponto-Caspian mysids got into the focus of scientific interest on account of their range expansions throughout the North Atlantic region and their profound ecological impacts in the invaded waters. Intentional introductions played a significant role in their spread especially in the former Soviet Union (e.g., Grigorovich et al., 2002), but three species (*Hemimysis anomala* G.O. Sars, 1907; *Katamysis warpachowskyi* G.O. Sars, 1893; and *Limnomysis benedeni* Czerniavsky, 1882) invaded European and North American inland waters without deliberate human assistance. At present, *H. anomala* has the widest distribution; in the last two decades it colonized the Baltic Sea basin, almost every major waterway in Central and Western Europe, and recently it also appeared in the British Isles as well as in the North American Great Lakes (summarized by Audzijonytė et al., 2008). *L. benedeni* is widely distributed in continental Europe (summarized by Audzijonytė et al., 2009), and it is also considered as a likely future invader of the Great Lakes (Ricciardi and Rasmussen, 1998). *K. warpachowskyi* expanded its range first of all in the River Danube basin (Wittmann, 2002, 2008); however,

recently it was also found in Lake Constance (Hanselmann, 2010), projecting its further spread in the River Rhine and other connected catchments. In Hungary, *L. benedeni* was first detected in the middle of the 20th century (Wojnárovich, 1954), whilst the other two species appeared around the millennium (Borza et al., 2011; Wittmann, 2002, 2007).

The ecological impact of the species is mainly associated with their trophic linkages. They represent prime food source for fish (e.g., Borcharding et al., 2006; Lantry et al., 2012; Specziár et al., 1997), so their presence may be beneficial for certain fish species, which served as the reason for their deliberate introductions into reservoirs and lakes. However, their diet also includes zooplankton, which may have unanticipated ecosystem-level implications; i.e., lengthened food chains may entail increased bioaccumulation of contaminants, new pathways of parasite transmission to fishes, and altered nutrient cycling, as observed in *Mysis* spp. introductions (Borcharding et al., 2006; Fink et al., 2012; Ketelaars et al., 1999; Lasenby et al., 1986; Ricciardi et al., 2012). As the latest Ponto-Caspian invader, *H. anomala* is anticipated to have especially significant impacts on the nearshore food web of the North American Great Lakes (Marty et al., 2010; Ricciardi et al., 2012).

Reliable information on the life history of invasive species is of primary importance in various fundamental and applied research issues; e.g., in revealing and modeling their population

\* Tel.: +36 30754 5381; fax: +36 27 345 023.

E-mail addresses: [borza.peter@okologia.mta.hu](mailto:borza.peter@okologia.mta.hu), [borzap@gmail.com](mailto:borzap@gmail.com)

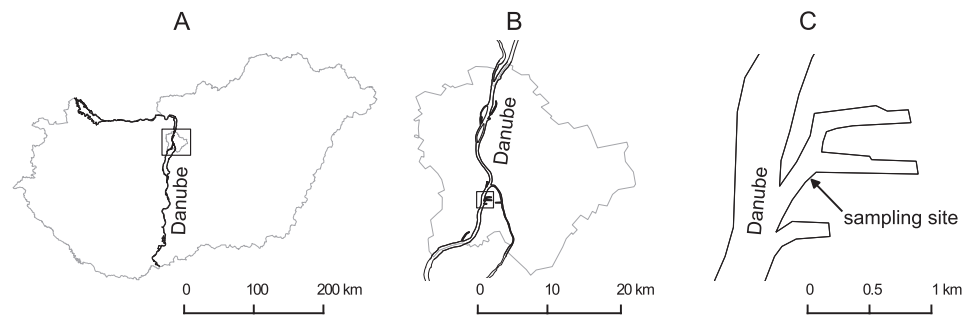


Fig. 1. The location of the sampling site: (A) Hungary, (B) Budapest and (C) Freeport of Csepel.

dynamics, assessing their production and biomass, and quantifying their impacts. Our current knowledge on the life history of invasive Ponto-Caspian mysids cannot be regarded as satisfying; I identified three gaps, which I tried to address in the present study.

Firstly, the information available on their life history is different among the species. In relation to its wide non-native range, *H. anomala* is the most thoroughly investigated of them (Borcherding et al., 2006; Dumont and Muller, 2010; Ioffe et al., 1968; Ketelaars et al., 1999; Marty et al., 2010; Nunn and Cowx, 2012; Taraborelli et al., 2012). The life history of *L. benedeni* is also relatively well-studied (Gergs et al., 2008; Hanselmann, 2008; Szalontai, 2008); however, in the case of the most recent invader, *K. warpachowskyi*, no focused investigations have been conducted; only some general descriptions and ad hoc observations on some characters have been published (Cărăușu et al., 1955; Daneliya, 2001; Hanselmann, 2010; Wittmann, 2002).

Secondly, the information available on different life history traits is also variable. Some characteristics, such as body length and fecundity are relatively easy to determine even from single samples. In contrast, assessing the number of generations produced per year – or generation time – of these seasonally breeding multivoltine iteroparous species is an inherently difficult task (Mauchline, 1980). Due to the natural scatter in the timing of reproduction among individuals and the production of more than one broods per female, generation structure becomes more and more obscure during the breeding season. Accordingly, information on this parameter of the invasive Ponto-Caspian mysids is scarce; most of the recent studies on the autecology of species did not deal with it, or were admittedly not suitable to assess it (Borcherding et al., 2006; Hanselmann, 2008; Taraborelli et al., 2012).

Thirdly, almost all the information published derives from single-species studies. Since the traits vary with environmental conditions, these studies do not allow the direct comparison of the results, which could lead to a characterization of the species in relative terms.

Accordingly, my goals in the present study were to (1) provide a comprehensive description about the life history of *K. warpachowskyi*, (2) reveal the number of generations produced annually by the three invasive Ponto-Caspian mysids, and to (3) compare their life history traits directly for the first time based on a simultaneous sampling. The Hungarian section of the River Danube offered fortunate circumstances for an investigation aimed at achieving these goals; in artificial inlets of the river the three species coexist in high abundance. Another key element of the study design was the sampling frequency. Most life history studies on mysids are based on monthly or biweekly sampling, which may be suitable for species living under colder climatic conditions, but apparently does not allow the identification of generations in species with more rapid life cycles. To overcome this, a very intensive (weekly) sampling was planned, revealing a high-resolution picture about the length–frequency distributions of the species.

## Materials and methods

### Study site

The samples were taken in an artificial inlet of the River Danube in Budapest (Hungary), the Freeport of Csepel ( $47^{\circ}26'31.18''N$ ,  $19^{\circ}3'21.46''E$ ). The sampling site was located in the  $\sim 300$  m long,  $\sim 60$  m wide entrance canal of the Y-shaped embayment (Fig. 1). The bank of this section is reinforced with homogeneous rip-rap embankments (composed of stones measuring  $\sim 20$ – $80$  cm in diameter). It serves busy ship traffic and it is dredged from time to time to impede sedimentation. Since the embayment is connected directly to the main channel of the river, its water level fluctuates accordingly; however, currents are negligible.

### Sampling

The samples were taken with an approximately weekly frequency between 02.03.2009 and 20.05.2010, except for the winter months (altogether 50 samples). The sampling was carried out after sunset (by the onset of complete darkness) to make the effective collection of the nocturnally active *H. anomala* possible. Depending on the abundance of the species, 1 to 12 uniform hauls were made above the stones by hand net (aperture  $40$  cm  $\times$   $30$  cm, mesh size  $450$   $\mu$ m, handle length  $3.9$  m). Unfortunately, the complex, uneven surface formed by the stones presumably did not allow the collection of all the animals in the sampled area; therefore, the method cannot be regarded as quantitative. The proportion of the specimens caught might also have been different among the species owing to their different affinities to the surface; therefore, their contribution in the samples is not directly informative of their relative abundance.

As the species were collected simultaneously, it would have been ideal, if they had occurred in approximately equal density. If one of the species was rarer than the others by more than an order of magnitude, it was not possible to collect a sufficient amount of it. This was the case in late spring/early summer, when *K. warpachowskyi* did not seem to follow the high water levels, and in the autumn/spring, when the bulk of *H. anomala* probably migrated to the deeper parts of the water. *L. benedeni* could be collected efficiently in most of the cases (Appendix 1).

### Sample processing

The samples were preserved in 70% ethanol in the field. Mysids were sorted out and identified in the laboratory using stereomicroscope. Direct measurement of body length in mysids is impractical due to the usually bent posture of preserved specimens. To avoid this problem, other parameters showing strong correlation with body length can be measured (Mauchline, 1980). In this study the lateral carapax length (CL) of the animals was measured from dorsal view using ocular micrometer by  $20\times$  magnification (allowing

**Table 1**

Total lengths of mature males and ovigerous females (mm). Ranges derived from individual measurements; mean  $\pm$  SD values are based on total length–carapax length regressions.

		Overwintering generation		Spring–summer generations					Range
		Mean $\pm$ SD (n)	Range	Mean $\pm$ SD (n)					
				May–June	July	August	September	October	
<i>L. benedeni</i>	♂	9.4 $\pm$ 0.6 (167)	7.7–10.3	6.8 $\pm$ 0.5 (51)	6.8 $\pm$ 0.4 (79)	6.6 $\pm$ 0.5 (35)	6.5 $\pm$ 0.5 (29)	6.7 $\pm$ 0.4 (39)	6.0–7.7
	♀	9.6 $\pm$ 0.5 (233)	8.0–10.8	7.1 $\pm$ 0.3 (132)	6.9 $\pm$ 0.4 (101)	6.6 $\pm$ 0.4 (101)	6.3 $\pm$ 0.3 (73)		6.0–8.1
<i>H. anomala</i>	♂	9.5 $\pm$ 0.8 (263)	7.8–11.2	6.8 $\pm$ 0.6 (135)	6.8 $\pm$ 0.7 (172)	6.5 $\pm$ 0.7 (159)	6.8 $\pm$ 0.6 (30)	No data	5.9–8.4
	♀	10.1 $\pm$ 0.7 (31)	8.8–11.1	7.7 $\pm$ 0.3 (14)	7.9 $\pm$ 0.4 (49)	7.4 $\pm$ 0.4 (39)	7.2 $\pm$ 0.3 (6)	No data	6.4–8.7
<i>K. warpachowskyi</i>	♂	6.5 $\pm$ 0.4 (125)	5.2–7.6	4.7 $\pm$ 0.2 (21)	4.7 $\pm$ 0.4 (36)	4.4 $\pm$ 0.3 (29)	4.4 $\pm$ 0.3 (24)	5.1 $\pm$ 0.3 (152)	3.8–5.1
	♀	7.2 $\pm$ 0.4 (192)	6.3–8.6	5.4 $\pm$ 0.3 (71)	5.3 $\pm$ 0.5 (47)	4.9 $\pm$ 0.4 (89)	4.8 $\pm$ 0.3 (134)		4.1–6.8

<sup>a</sup> The animals belonging to these groups (late survivors of the last summer-type generations) grew considerably during that period; therefore, mean values do not characterize their sizes adequately.

0.05 mm accuracy). Then digital pictures were made from lateral view of specimens comprising a representative sample of each species, and total length (TL, from the tip of the rostrum to the end of the telson without spines) of the specimens of known CL was determined using tpsDig2.14 digital picture analysing software (Rohlf, 2009). TL and CL showed a strong linear relationship in all of the three species (*L. benedeni*: TL = 2.12658CL – 0.17702,  $R^2 = 0.977$ ,  $n = 116$ ; *H. anomala*: TL = 1.58867CL – 0.06585,  $R^2 = 0.992$ ,  $n = 96$ ; *K. warpachowskyi*: TL = 1.51109CL – 0.03242,  $R^2 = 0.974$ ,  $n = 98$ ). For comparison, standard lengths can be calculated by subtracting the length of the telson, which constituted on average 11.11 (SD = 0.69), 12.76 (SD = 0.77), and 11.67 (SD = 0.84) percent of the TL of *L. benedeni*, *H. anomala*, and *K. warpachowskyi*, respectively. In *L. benedeni* a slight difference between the two genders could be observed, females being somewhat larger at a given CL. This, however, had little effect on the overall relationship, as the high  $R^2$  value shows. Nonetheless, in Table 1 and in the brood size–body length regressions the difference between genders was considered. In the body length distribution diagrams (Fig. 2) this difference, having no influence on the conclusions, was neglected for simplicity.

In addition to body length, gender and development stage (juvenile, male: immature/mature, female: immature/mature/ovigerous/empty) of the specimens were determined following the definitions of Mauchline (1980). In ovigerous females the brood was counted (presumably intact brood pouches only), and the main development stage (embryonic/nauplioid/postnauplioid) was identified, as well, based on Wittmann (1981). The largest diameter of 4–6 eggs (embryonic stage) per female was measured by ocular micrometer (by 50 $\times$  magnification, corresponding to 0.02 mm accuracy). In some cases, when juveniles were extremely abundant, subsamples were taken from them to obtain a manageable amount. Altogether 36,139 specimens were examined, of which 27,328 were individually measured (Appendix 1).

#### Data analysis

The statistical analysis was performed using R 2.11.0 (R Development Core Team, 2010) supplemented with the packages “multcomp” for multiple comparisons and confidence bands (Hothorn et al., 2008) and “nlme” for mixed effect models (Pinheiro et al., 2009). The annual generation numbers of the species was assessed based on (1) the visual identification of diagonal structures in the length–frequency histograms aligned together (Fig. 2), (2) the qualitative composition of the samples (Fig. 3), (3) the expectable generation times (based on the relatively well identifiable first and second generations), and (4) the appearance of the first individuals of delayed maturation in the autumn, marking the birth of the overwintering generation. Brood sizes were analyzed by fitting Poisson regression models. In the egg size analysis, linear mixed effect models were applied with the identifiers of individual

females as a random variable. The reliability of the gender ratio values was illustrated by Wilson binomial confidence intervals.

## Results

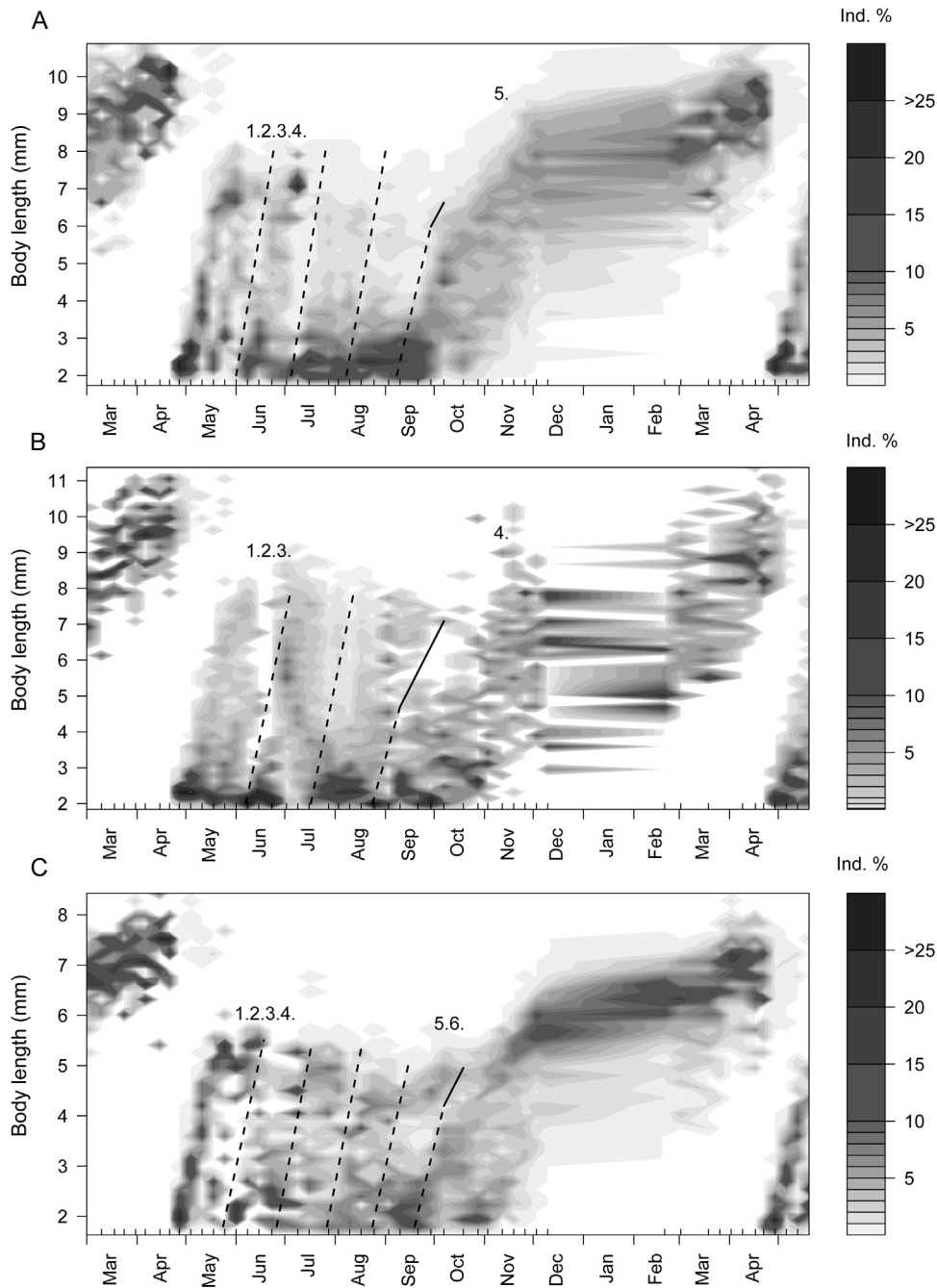
### Body lengths

There was a marked difference in body length between the overwintering and the spring–summer generations in all of the three species (Table 1). In the spring–summer generations a gradual decrease in body length could be observed; the largest sizes occurred in late spring/early summer, whilst the smallest mature specimens were found in late summer/early autumn. At any given time, *L. benedeni* and *H. anomala* were very similar in size, whereas *K. warpachowskyi* was considerably smaller than the other two.

### Number of generations

The life cycle of the species is demonstrated by the length frequency distributions (Fig. 2) and the qualitative compositions of the samples (Fig. 3). All of the three species can be characterized as warm season breeders (Wittmann, 1984); juveniles were produced between the spring and autumn, and the autumn-born offspring formed the overwintering generation, which became fertile only by next spring.

In *L. benedeni* (Fig. 2A, 3A) the first juveniles were born in the middle of April. The first generation consisted of two relatively distinct cohorts. The second cohort, born in the middle of May, can be attributed to the repeated breeding of the overwintering generation; the first cohort was not fertile yet that time. The second generation, produced by the first one, appeared in early June, indicating a generation time of approximately 6 weeks. In this generation, the cohort structure of the previous one was more-or-less still observable, at least in the 25 May and 2 July samples, before the flood. In the sequel, asynchrony escalated and reproduction became more and more continuous, but two further generations – born from early July and early August on, respectively – could be identified with high certainty. As indicated by the delayed appearance of secondary sexual characteristics, the specimens born from early September on (produced by the fourth generation) belonged to the fifth, overwintering generation. These specimens have not become mature until next spring; however, breeding specimens deriving from the last summer-type (fourth) generation were still present in the autumn, producing offspring right until early November. This late cohort cannot be regarded as a sixth generation, since it is not the product of the fifth one; it is part of the fifth generation. Breeding specimens in the autumn apparently survived after releasing their brood, and began to grow in size. They comprised 2.3–5.2% of the entire population between 28 October and 3 December 2009. By spring, differences in the state of development of the secondary sexual characteristics between representatives of the fourth and fifth



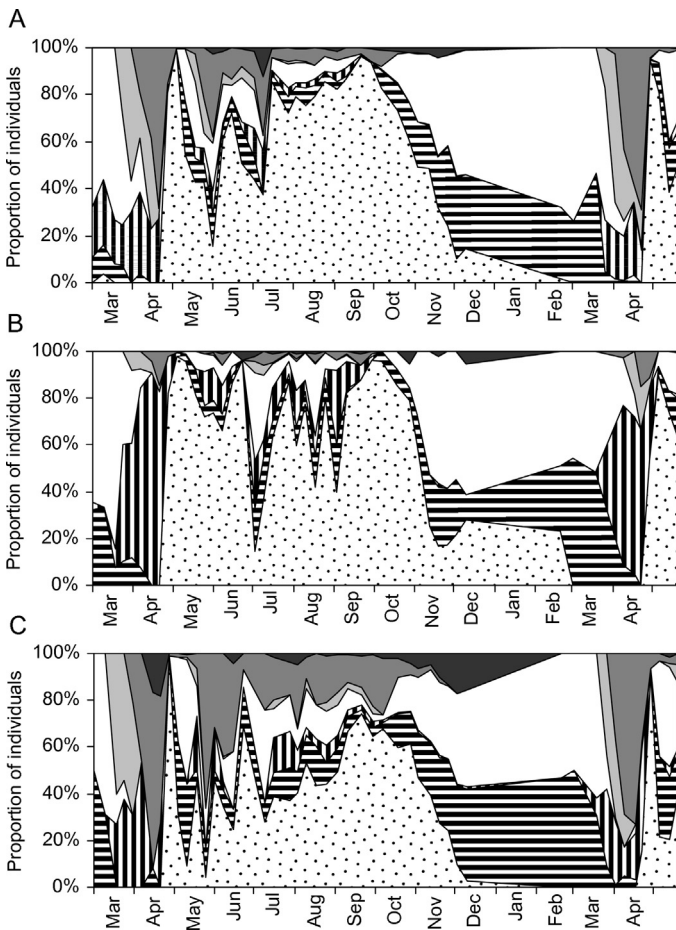
**Fig. 2.** Length–frequency distributions of (A) *L. benedeni*, (B) *H. anomala*, and (C) *K. warpachowskyi*. Solid lines: borderlines between summer-type and overwintering-type individuals. Dashed lines: presumed borderlines between generations (numbered). Upward ticks on the horizontal axis show the sampling dates. *Note:* additional levels are used in *H. anomala* at 0.1% and 0.5% to reveal the structure of the abundant samples of August. Samples with <math>\leq 10</math> specimens were not included.

generations vanished; therefore, the contribution of the former in producing next year's first generation cannot be quantified.

In the case of *H. anomala* (Fig. 2B, 3B) the first juveniles were also born in the middle of April. In contrast to *L. benedeni*, recruitment was more continuous in the first generation, although two less pronounced peaks could be observed. The second generation emerged in the middle of June, somewhat later than that of *L. benedeni*, marking a generation time of about 7 weeks. The high water levels in early July had a significant effect on size distribution of the species; juveniles seemed to be under-represented in the flooding water. The third generation began to rise in the middle of July. Overwintering-type specimens of 4.5 mm TL were found on 10 September, much earlier than in *L. benedeni*,

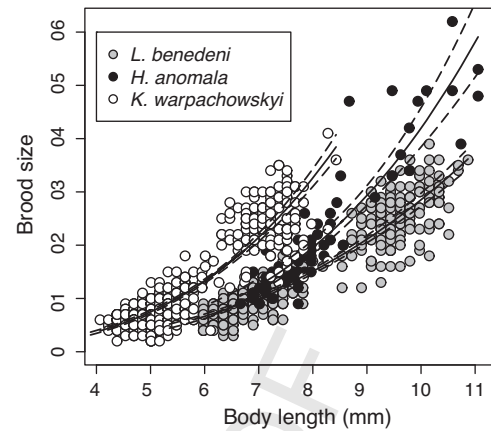
denoting that the animals born in late August already belonged to fourth and last, overwintering generation. Newborn juveniles were found until late October. Some large (>10 mm TL) and well developed specimens found in late October–November indicate that the last summer-type generation may have involved in the overwintering population, but their numeric contribution cannot be evaluated due to the scarcity of the material available (owing to the very low density of the species in the period in question).

The spring reproduction of *K. warpachowskyi* (Fig. 2C, 3C) began somewhat earlier than that of the other two species examined; in 2010, juveniles were found a week earlier, although in low numbers. In 2009 this period was missed, denoting that the actual difference was probably less than a week that year. The first



**Fig. 3.** Qualitative compositions of the samples of (A) *L. benedeni*, (B) *H. anomala*, and (C) *K. warpachowskyi*. Dotted: juveniles; horizontal stripes: immature males; vertical stripes: mature males; white: immature females; light gray: mature females; dark gray: ovigerous females; black: empty females. Samples with <math><10</math> specimens were not included.

generation consisted of two distinct cohorts, similarly to *L. benedeni*. Due to its smaller body size its generation time was the shortest of the three species (about 5 weeks); the second generation appeared already in late May, at which time gravid females of the overwintering and the first generation co-occurred. Due to the relatively scarce material available (owing to the high water levels which the species did not follow), the generation structure is rather blurred in the following period. According to the most likely scenario based on the expected generation times, the relatively distinct cohort of late July-early August corresponds to the fourth generation. The appearance of overwintering-type juveniles can be placed to the middle of September, based on the sizes attained by them in



**Fig. 4.** Relationship between the total lengths and brood sizes of ovigerous females. Dashed lines represent the 95% family-wise confidence bands of the fitted models.

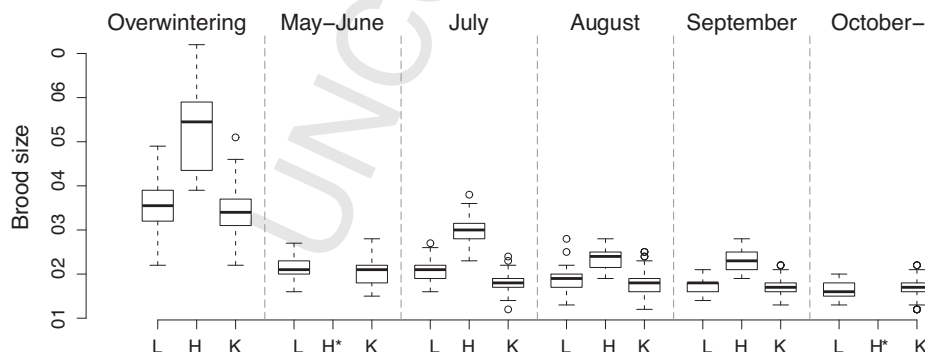
early October, so a fifth summer-type generation can be assumed born from late August on. Accordingly, *K. warpachowskyi* probably produced six complete generations; however, uncertainty is relatively high due to the fast life cycle of the species.

Summer-type individuals contributed with 7.3-17.8% to the entire population between 19 October and 10 December 2009.

#### Fecundity

There was a strong positive relationship between the fecundity ( $F$ ) and body length (TL) of ovigerous females in all of the three species (Fig. 4, *L. benedeni*:  $F=0.040TL^{2.853}$ ,  $n=440$ , null dev.=2021.5, residual dev.=251.8; *H. anomala*:  $F=0.016TL^{3.417}$ ,  $n=72$ , null dev.=426.5, residual dev.=67.5; *K. warpachowskyi*:  $F=0.048TL^{3.135}$ ,  $n=581$ , null dev.=2806.0, residual dev.=492.6). The effect of body length manifested in seasonally varying fecundity with considerably higher values in the overwintering generation than in the spring-summer generations (Fig. 5). In the overwintering generation the mean brood size of *H. anomala* ( $42.75 \pm 9.15$  SD,  $n=16$ ) was significantly higher than that of *L. benedeni* ( $25.39 \pm 5.29$  SD,  $n=152$ ,  $P<0.0001$ ) and *K. warpachowskyi* ( $24.38 \pm 4.86$  SD,  $n=154$ ,  $P<0.0001$ ). The difference between the two latter species was not significant ( $P=0.108$ ).

In the spring-summer generations brood sizes decreased significantly with time ( $\chi^2=467.2$ ,  $df=764$ ,  $P<0.0001$ ). In this comparison, *H. anomala* had the highest fecundity again, attaining  $19.93 \pm 3.71$  SD ( $n=27$ ) in July and  $13.20 \pm 3.49$  SD ( $n=5$ ) in September. Brood sizes of *L. benedeni* and *K. warpachowskyi* decreased from  $11.26 \pm 2.41$  SD ( $n=77$ ) and  $10.42 \pm 2.70$  SD ( $n=62$ ) in May-June to  $6.32 \pm 1.96$  SD ( $n=31$ ) and  $6.58 \pm 1.86$  SD ( $n=137$ ) after September, respectively. Interspecific differences proved significant in all pairwise comparisons ( $P<0.001$  in all cases; Tukey corrected).



**Fig. 5.** Boxplots of the brood sizes of *L. benedeni* (L), *H. anomala* (H), and *K. warpachowskyi* (K) in different periods. \*: insufficient data.

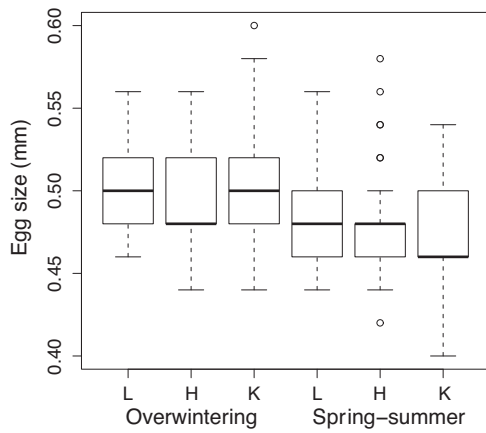


Fig. 6. Boxplots of the egg sizes of *L. benedeni* (L), *H. anomala* (H), and *K. warpachowskyi* (K) in the different generation types.

Egg sizes

Egg sizes varied between 0.40 and 0.60 mm in the material (Fig. 6). Eggs were somewhat larger in the overwintering generation than in the spring-summer generations in all of the three species ( $\Delta 0.023 \pm 0.004$  mm SE,  $F_{1,99} = 38.12$ ,  $P < 0.0001$ ), and "crowdedness", expressed as the percentage deviance from the expected number of young for the size of the female, also had a significant negative influence ( $-0.0035 \pm 0.0008$  mm SE for each 10% difference,  $F_{1,99} = 20.61$ ,  $P < 0.0001$ ). Differences among the species were less considerable, as compared to the effect of the generations; only the difference between *L. benedeni* and *K. warpachowskyi* proved significant ( $\Delta 0.009 \pm 0.004$  mm SE,  $P = 0.03$  with Tukey correction). Individual variations were not negligible (SD = 0.0135, residual SD = 0.0235).

Gender ratios

*L. benedeni* and *K. warpachowskyi* showed similar patterns with regard to gender ratios; females outnumbered males in most of the cases (Fig. 7). The proportion of males was especially low in the autumn, before the sex of the abundant overwintering cohort became identifiable, and in the spring, before the first breeding generation attained that size. The late survivors of the last breeding generation were also predominantly females (Fig. 3,  $90.5 \pm 5.6\%$  SD for *L. benedeni*,  $94.2 \pm 2.2\%$  SD for *K. warpachowskyi* in October-December).

In *H. anomala* no such pattern could be observed; gender ratios varied within a wide range, and male dominance was also common, especially among the overwintering specimens.

Discussion

Interspecific comparison

Studying the three species simultaneously allows the direct comparison of their life history traits. Some characteristics could be demonstrated for all of the three species (e.g., larger overwintering specimens, strong correlation between female body length and brood size, and the contribution of the last breeding generation to the overwintering generation). These represent the general observations for mysids (Mauchline, 1980), and are in accordance with published results. However, with regard to gender ratios considerable interspecific differences could be observed. In *L. benedeni* and *K. warpachowskyi* females outnumbered males in most of the cases, which is in agreement with the general patterns in mysids (Mauchline, 1980), as well as with the results published for *L. benedeni* (Gergs et al., 2008; Szalontai, 2008). The underlying mechanism is probably the differential mortality between sexes with most of the males dying shortly after breeding, best reflected in the

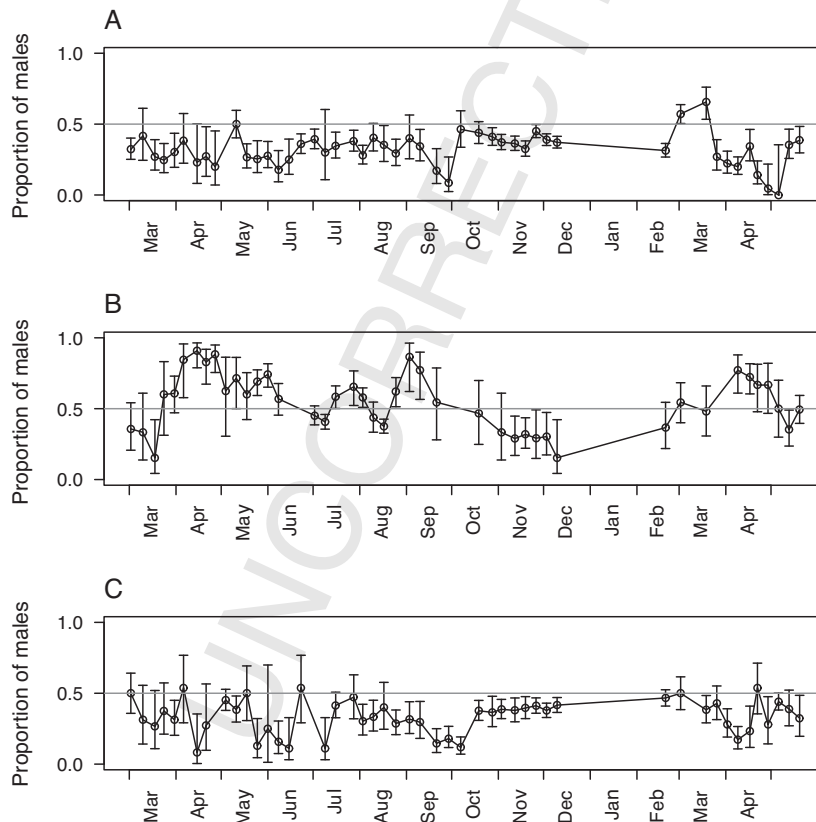


Fig. 7. Gender ratios (proportions of males) of (A) *L. benedeni*, (B) *H. anomala*, and (C) *K. warpachowskyi*. Whiskers correspond to the Wilson binomial confidence intervals of the values.

present data by the female dominance in the overwintering generations before the gender of the first generation became identifiable. The results for *H. anomala* do not fit into this concept. Although similar observations (i.e., male dominance especially in the spring) have been reported in other publications (Borcherding et al., 2006; Ioffe et al., 1968), conclusions should be drawn carefully, since the migratory behavior of the species may imply biases. According to the observations of Ketelaars et al. (1999) there is a difference between the vertical distributions of the genders, mature females preferring deeper regions of the water. Consequently, the apparent male dominance in the present and other studies may be an artifact attributable to the more hiding behavior of females.

Body length is a strong determinant of various reproductive traits in mysids (Mauchline, 1980; Wittmann, 1984). With regard to egg size, a significant positive relationship has been demonstrated with female body lengths; however, this could not be observed within the context of the three species investigated. There is also a positive correlation between female body lengths and brood sizes (Mauchline, 1980; Wittmann, 1984), which could be evinced in the present study. However, the difference between *H. anomala* and *L. benedeni* was much greater than it could be expected based on the model of Wittmann (1984) for epipelagic and coastal species; the slightly larger *H. anomala* had much larger brood sizes. The fecundity of *K. warpachowskyi* – approximating the values of the significantly larger *L. benedeni* – also deviated from the expectations.

Body length, fecundity, and number of generations varied among the three species in a consistent manner, which – given their approximately equal egg sizes – might be indicative of their resource allocation strategy. The traits of the two larger species indicated an apparent trade-off relationship; *H. anomala* produced a relatively high number of eggs, which apparently had a cost on its generation times, whereas *L. benedeni* had smaller brood sizes allowing a more rapid life cycle. On the other hand, its relatively small body size allowed *K. warpachowskyi* to produce more generations per year than its relatives, while it was able to maintain numbers of offspring close to those of *L. benedeni*, indicating a higher reproductive (vs. somatic) allocation.

Whether these results can actually be connected to the resource allocation strategy of the species needs to be tested by further investigations revealing the quantitative relations of their energy budget (as in e.g., Clutter and Theilacker, 1971). Since these traits can be assumed to be subject to contemporary selective forces – possibly related to the invasion process in part –, this should be preferably done in a combined intra- and interspecific framework.

#### Annual generation numbers

The approach of increasing the sampling frequency has proved efficient at revealing the annual generation numbers of the species. Combining it with level plot visualization techniques, high-resolution length-frequency distribution maps can be created, which allow the visual interconnection of components in individual length-frequency distributions. Of course, this approach has its own limitations as shown by the uncertainty about *K. warpachowskyi*. In this case, further increasing the sampling frequency cannot be assumed to provide better results, since the strong noise in the distributions is a natural consequence of the fast life cycle. However, the present dataset also suffers from the occasionally insufficient numbers of specimens, so a steadier sampling focused on this species could perhaps provide a clearer picture. For this, a sampling site devoid of water level fluctuations could be recommended. Similarly, more coherent results could be obtained for *H. anomala*, if the biases arising from the vertical migration of the species could be entirely avoided. For this, a vertical sampling seems appropriate, as exemplified by the method of Taraborelli et al. (2012).

The generation numbers revealed in the present study can be regarded as extraordinarily high. Review publications on the reproductive biology of mysids do not mention more than four generations in warm season breeding species; Wittmann (1984) indicated 2 to 4 generations for this group, while according to Mauchline (1980), species having more than three generations per year could only be found among continuous breeders. In contrast, similar numbers can be found in the Russian literature; 4 generations for *H. anomala* (Ioffe et al., 1968) and 4 to 5 generations for *L. benedeni* (Ioffe, 1973). Although the exact generation number of *K. warpachowskyi* could not be determined with full certainty, it is evident that it has more than five generations, which has never been demonstrated for warm season breeding mysids. What is more, the generation numbers evinced in the present study may not be the highest possible. The breeding season of the species shows significant latitudinal variation (e.g., Băcescu, 1954; Komarova, 1991); for example, in the southern occurrences of *L. benedeni*, breeding may start as early as February (Komarova, 1991), denoting that the species may produce even more generations warmer areas.

These results invoke the question, whether these species have uniquely rapid life cycles, or the prolificacy of warm season breeding mysids has been generally underestimated, because most of the studies on species with rapid life cycles were not conclusive, or the authors provided a conservative estimation of generation numbers due to the inadequate sampling routines applied. Answering this question, however, would require further similarly intensive studies on other representatives of the group.

Unique or not among mysids, these numbers can be considered extremely high among similar sized aquatic macroinvertebrates under comparable climatic conditions. Many of these have much slower, often multiannual life cycles (e.g., Hury and Wallace, 2000), but even the closely related Ponto-Caspian amphipods do not produce more than three generations per year (Grabowski et al., 2007). Similar generation numbers can only be found among the notoriously prolific dipterans (e.g., chironomid midges; Armitage et al., 1995).

Generation time has been demonstrated to be connected with invasiveness in various organisms; e.g., amphipods (Grabowski et al., 2007) and plants (Williamson and Fitter, 1996). The explanation may lie in the ability of a species to establish populations when arriving at a location in small numbers (Crawley et al., 1986). The faster the life cycle of a species is, the shorter time it requires to increase its initial population size, avoiding the hardships small populations have to face (Allee effects; Taylor and Hastings, 2005). Invasive Ponto-Caspian mysids are indeed very successful at establishing new isolated populations, as indicated by the high incidence of jump dispersal events in their invasion record. This type of spread played a major role in their long distance range expansions mediated by ships, well exemplified by the appearance of *H. anomala* in the Great Lakes system presumably via ballast water (Pothoven et al., 2007), and the emergence of *K. warpachowskyi* in Lake Constance, for which overland transport of recreational ships can be blamed most likely (Hanselmann, 2010). In addition, *H. anomala* and *L. benedeni* have colonized several more-or-less isolated smaller waters (i.e., gravel-pit lakes, fishing ponds), which also implies jump dispersal (Borza et al., 2011; Wittmann and Ariani, 2009).

The key to invasion success might be determined by several factors playing different roles. The present study demonstrated that Ponto-Caspian mysids indeed have extraordinarily short generation times, which might act as a prerequisite of successful establishment. The potential possessed by all of the three species seems to be sufficient, enabling them to successfully colonize new waterbodies under the given conditions. The interspecific variations apparently do not account for the differences in their invasion success, which in fact shows the opposite order as their potentials



based on their generation times would suggest. In this context other factors, such as behavioral traits, habitat preference or historical factors might be decisive, masking the effect of the mere mathematical chance of establishment. On the other hand, it would be worthwhile to test whether the number of generations produced per year has some predictive power in discriminating between invasive and non-invasive Ponto-Caspian mysids.

#### *K. warpachowskyi*

The previously least known *K. warpachowskyi* excelled the other two species at reproductive capacity; it had the longest reproductive period, the shortest generation time, produced eggs of similar size as the two larger species, and its fecundity was also prominent relative to its size. These results do not necessarily indicate that it would be an invader superior to its counterparts, but clearly show that its currently narrower non-native range is not a result of limited potential, but of other factors, most likely merely the shorter time since it began to spread; in which case the species can be expected to make up its arrears rapidly.

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#### Appendix 1.

Water levels, water temperatures, number of net hauls, and number of specimens collected. L: *L. benedeni*; H: *H. anomala*; K: *K. warpachowskyi*; \*: values missing due to the high (6 April 2009, 2 July 2009) or low (7 October 2009) water levels not allowing the use of the standard sampling procedure; \*\*: values missing due to technical problems. The number of juvenile specimens not measured individually is given in parentheses. Water levels were recorded at Budapest, Vigadó square (rkm 1646.5). Source: VITUKI Environmental and Water Management Research Institute Non-profit Ltd. (2011).

Date	Water level (cm)	Water temperature (°C)	Number of net hauls	Number of specimens		
				L	H	K
02.03.2009	294	4.8	12	146	28	44
10.03.2009	472	5.3	6	25	12	16
18.03.2009	466	6.1	5	63	13	15
24.03.2009	326	6.1	5	65	10	24
31.03.2009	400	10.0	10	53	51	53
06.04.2009	543	10.6	**	26	13	13
15.04.2009	487	13.0	6 (H: 3)	13	44	12
21.04.2009	414	15.0	9 (H: 4)	22	35	11
27.04.2009	342	15.3	6	78	239	198
04.05.2009	319	16.8	7	594(428)	1449(1049)	250
11.05.2009	320	16.2	3	217	479	114
18.05.2009	397	18.5	4	176	159	40
25.05.2009	346	20.0	2 (H: 1)	97	344	25
01.06.2009	369	17.6	4	103	399	10
08.06.2009	259	19.1	5	115	214	54
15.06.2009	240	20.6	7	157	30	24
23.06.2009	378	19.5	6	366	25	42
02.07.2009	714	17.5	**	335	243	4
09.07.2009	518	20.5	2	16	542	25
16.07.2009	336	23.0	4	688(385)	387	181
28.07.2009	373	*	5	588	954(519)	58
03.08.2009	269	24.0	3	840(365)	1585(1099)	110
10.08.2009	321	*	3	382	2587(2230)	149
17.08.2009	277	22.8	1	246	1653(1022)	56
25.08.2009	214	*	3	621	412	183
03.09.2009	202	*	3	196	25	126
10.09.2009	250	19.7	4	501	124	129
21.09.2009	212	19.1	6	901(606)	88	268
29.09.2009	135	18.3	5	372	85	285
07.10.2009	101	17.2	**	319	22	342
19.10.2009	173	9.0	5	578	103	442
28.10.2009	240	10.3	4	603	19	192
03.11.2009	157	7.8	4	611	30	365
12.11.2009	186	7.5	3	672	51	205
19.11.2009	176	7.8	3	420	84	206
26.11.2009	171	7.4	4	583	29	421
03.12.2009	151	6.5	3	595	42	387
10.12.2009	172	5.7	3	610	19	345
20.02.2010	135	2.4	4	362	39	287
02.03.2010	392	4.1	8	121	44	68
19.03.2010	213	5.1	8	43	27	94
26.03.2010	349	8.0	8	64	2	64
02.04.2010	316	10.0	6	108	4	75
09.04.2010	236	10.8	8	160	35	87
17.04.2010	322	10.7	6	67	47	30
22.04.2010	263	12.1	8	71	27	32
29.04.2010	233	15.7	8	422(329)	52	289
06.05.2010	276	17.5	4	679(500)	434(279)	333
13.05.2010	283	17.1	3	129	201	68
20.05.2010	450	12.8	3	200	267	62

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