

Embryonic development time of the freshwater mysid *Limnomysis benedeni* Czerniavsky as a function of water temperature

Almut J. Hanselmann · René Gergs ·
Karl-Otto Rothhaupt

Abstract The numbers of alien species in freshwater systems and their detrimental impacts on the stability of ecosystems and global species diversity are increasing. To predict and assess such impacts, a thorough knowledge of the autecology and life cycle of the alien species is required. *Limnomysis benedeni* is common and one of the most invasive mysids in Europe. Here we show a clear dependency of the development time of the brood of *L. benedeni* on water temperature. In laboratory experiments (one in spring 2008 and two in 2009, in spring and summer) under controlled conditions, we determined embryonic development times and the probability of survival of the females and juveniles at water temperatures ranging from 4 to 25°C. At 6.5 and 25°C, the probability of survival of both the females and the larvae was lower than at 10, 15, or 20°C. Since the development time is one of the key characteristics of the life cycle and is therefore

necessary to calculate, for example, birth and mortality rates, we determined an equation for the development time as a function of the water temperature. This information will be useful to understand the distribution potential of this invasive species.

Keywords Population dynamics · Larvae · Climate change · Marsupium

Introduction

With increasing numbers of alien species in freshwater systems such as the River Rhine (Leuven et al. 2009), their detrimental impact on both the stability of ecosystems and the global diversity of species is also increasing (Sala et al. 2000). To be able to predict the impacts of alien species on resident species and communities, detailed information on their autecology and life cycle is required. The expansion of an invasive species is often enabled or limited by the temperature, especially with a possible influence of climate change (Vermonden et al. 2010).

Many common alien species in Central Europe are freshwater Crustacea, including Mysida (Leuven et al. 2009). In contrast to marine mysids, many life-cycle properties of freshwater mysids, e.g., development times of the brood, growth rates, and mortality rates, are still unknown. This information is important to determine the influence of an alien species on the

Handling Editor: Piet Spaak.

A. J. Hanselmann (✉) · K. O. Rothhaupt
Limnological Institute, University of Konstanz,
78457 Constance, Germany
e mail: almut.hanselmann@gmx.de

R. Gergs
Institute for Environmental Sciences, University
of Koblenz Landau, 76829 Landau, Germany

invaded ecosystem and to understand the distribution potential of an invasive species. The generation time is related to the duration of the reproduction period, the age at maturity, the number of broods, the brood size, and the body length of the adults (Ishikawa and Oshima 1951; Murano 1964; Davis 1966; Mauchline 1973; Wittmann 1984).

Limnomysis benedeni Czerniavsky, 1882, is one of the most invasive mysids in Europe; it is a common invasive species, originates in the brackish estuaries of the Black Sea and Caspian Sea, and has spread nearly throughout Eastern and Central Europe since the early twentieth century (Bij De Vaate et al. 2002; Wittmann and Ariani 2008; Audzijonyte et al. 2009). The mysid was found for the first time in Lake Constance in 2006 (Fritz et al. 2006). The species has been characterized as a necto-benthic (Porter et al. 2008), phyto-lithophilic (Dediu 1966), suspensions feeder (Gergs et al. 2008; Aßmann et al. 2009). In winter, in Lake Constance, the population does not reproduce, and the adults invest in body growth. In spring and summer, the species reproduces continuously. In spring, the large overwintering females produce large egg clutches. In summer, adults reach maturity at a size smaller than that in winter and produce smaller clutches (Hanselmann et al. 2011).

In general, the life cycle of a mysid is strongly correlated with the climate regime in its environment (Wittmann 1984). Within the order Mysida, the life-history trades, including, e.g., development time, generation time, and clutch size, vary widely. In temperate zones, as in Central Europe, with seasonal fluctuating water temperature, species of the family Mysidae, including *L. benedeni*, normally reproduce only during the summer (Wittmann 1984). The common attribute of the superorder Peracarida, to which the family belongs, is that brood development takes place in a brood pouch of the females, the marsupium, which is built by the sternites of the thorax and a pair of oostegites (Westheide and Rieger 1996). Larval development occurs inside the marsupium. Eggs are fertilized by the male directly after the female lays them in the marsupium and embryonic development and all larval stages except the last larval molt take place inside the marsupium. Just before the last larval molt, the female sets the larvae free and the last larval molt takes place directly after the larvae leave the marsupium. This development is similar for all mysid species (Mauchline 1973; Wittmann 1984). The

adult females protect their brood, provide it with oxygen by pumping movements of the oostegites, and are to a small extent responsible for the spatial arrangement of the larvae in the brood pouch (Wittmann 1978; Mauchline 1980). Each developing larva feeds on yolk invested by the mother in its egg (Morgan 1980), but the diameter of the eggs, as a proxy of the yolk mass, plays a subordinate role for the development time of other mysid species in comparison with the water temperature (Wittmann 1981b).

We determined to what extent mysid embryonic development time depends on water temperature in laboratory experiments under controlled conditions at different water temperatures. Furthermore, we determined the probability of survival of the females and juveniles.

Methods

Experimental set-ups

To measure the relationship between temperature and the development time of the brood in the marsupium, we chose a temperature range from 4 to 25°C. We ran three experiments, one in spring 2008 (run I, starting on April 24, at 4, 10, 15, 20, and 25°C) and two in 2009, in spring (run II, starting on April 30, at 6.5, 10, 15, and 20°C) and summer (run III, starting on July 30, at 15 and 20°C). Run III was carried out to see whether the eggs from the smaller summer clutches have development times comparable to the eggs from the larger spring clutches. Three climate chambers and two climate cabinets (plant growth chamber KBW 720, Binder GmbH, Tuttlingen) were used to provide a stable water temperature; the overall tolerance was $\pm 0.3^\circ\text{C}$. In all experiments, the same light conditions with a diurnal light rhythm of 12 h 12 h (day night) and the same experimental setup were used. Day length in the field was about 14 h in April and 15 h in July.

For each temperature, 50 brood-carrying females (except in run III, which had 35 at 15°C and 40 at 20°C because fewer breeding females were available) were kept individually in 1 l glass jars with 750 ml filtered (30 μm) and slightly aerated lake water. We used the green alga *Scenedesmus obliquus* (Turpin) (SAG 276-3a, Göttingen, Germany) as food, which was added *ad libitum* (4 ml suspension, ~ 1 mg/l carbon)

twice a week. During earlier experiments, we measured that this was more than the females were able to consume. Once a week, the glasses were cleaned carefully with a soft brush and the water was replaced.

The gravid females were freshly caught in the eastern part of Lake Constance in Austria near the confluence of the Rhine River at ~ 0.5 m depth. For determining the clutch size and categorizing the stages of the larvae in the marsupium at the beginning of each set of experiments, a sample of the population was caught via kick-sampling and directly fixed with 96% ethanol. The females caught for the experiments were adapted overnight to the respective temperature and placed into the glass jars the next morning. Because *L. benedeni* neonates are released during the night (Mauchline 1980), the water in the glass jars was checked for neonates once each morning; hatched juveniles and their mother were directly fixed in 96% ethanol and counted. If a female died, it was not removed unless the decomposition process inhibited larval development. Inhibiting conditions occurred when the decay of the dead mother started to pollute the water (visible pollution or smelling water).

Calculations and statistics

For the development time at each temperature (D_T), the number of remaining egg-carrying females was plotted against time. The x-axis intercept of the linear regression ($y = 0$) is the calculated development time for the respective temperature. These D_T values were then plotted against the temperature to obtain the relationship between development time and temperature. The probability of survival at each temperature was calculated for females and larvae. For larvae, the mean brood size of hatched larvae of each replicate was compared to the mean brood size at the beginning, which was determined in the field, as described above; the field brood sizes of the runs differed. The optimal temperatures were calculated from the first derivation of the nonlinear regression that described the correlation.

A linear-regression model was calculated for each temperature in each experiment, and a nonlinear-regression model was calculated for the relationship between development time and temperature and for the probabilities of survival (R Development Core Team 2008).

Results

Development time

At all temperatures except 4°C, juvenile mysids hatched, and we could determine the development times of *L. benedeni* in the marsupium (Fig. 1). For these resulting development times, the linear regression of time versus the number of remaining females was significant (Table 1). When the measured development times were plotted against the temperature, a significant negative exponential curve was obtained ($y = 122.549 \times e^{-0.127}$, $P < 0.001$; Fig. 2; Table 2). The validity of this equation ends below 6.5°C because at the next lower tested temperature at 4°C in run I, no juvenile *L. benedeni* were found, all females died within 60 days, and the experiment was stopped after 80 days. For temperatures above 25°C, the equation is not tested. In the summer experiments (run III), the hatching of the juveniles started immediately, whereas in the spring experiments (run I and II), the first juveniles were found after several days (Fig. 1).

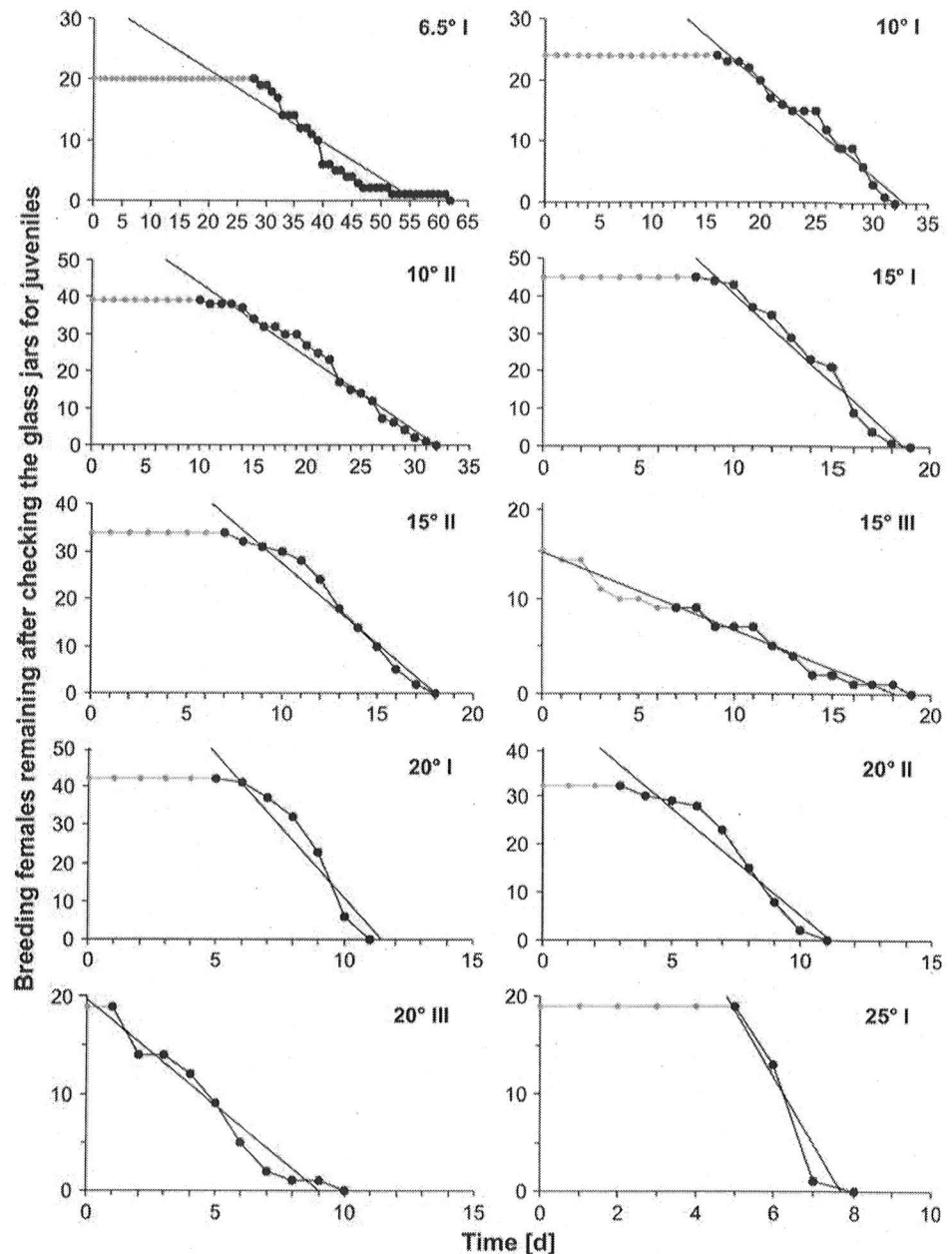
Clutch size and probability of survival

The brood size of the mysids caught in the field at the beginning of each experiment was 20 ± 4.5 larvae/female in run I, 18.2 ± 5.1 larvae/female in run II, and 6.2 ± 2.2 larvae/female in run III. The mean brood sizes (hatched juveniles) at all temperatures (Table 1) decreased as the development time increased. The probability of survival of both the mother ($R^2 = 0.62$) and the larvae ($R^2 = 0.83$) at each temperature tested is described with a nonlinear-regression curve (Fig. 3; Table 2). At 6.5 and 25°C, the probability of survival of the mothers (Fig. 3a) and of the larvae (Fig. 3b) was lower than at 10, 15, and 20°C. The optimal and maximum temperature for survival of both the mothers and the larvae was 15.9°C (Table 2).

Discussion

Our experiments, which provide the first determination of the embryonic development time of a freshwater mysid, revealed a clear dependency of the development time of the brood of *L. benedeni* on the water temperature. The tested temperatures between 4

Fig. 1 Breeding females of *L. benedeni* remaining after checking the glass jars for juveniles every morning (see “Methods”) during the experiments. *Lettering* indicates the temperature (6.5–25°C) and the time (run I–III) of each experiment. Please pay attention to the different x axis scaling. Data represented by *black dots* were used in the linear regression



and 25°C represent the range observed in the littoral of Lake Constance (Stich and Brinker 2010; Hanselmann et al. 2011) and in most lakes in temperate zones in which *L. benedeni* is found (Wittmann and Ariani 2008; Audzijonyte et al. 2009).

At 4°C, eggs did not develop. The coldest temperature at which the larvae developed to juveniles was at 6.5°C, which is close to the temperature measured in the field at which the first breeding females were found in spring 2008 (6.6°C, Hanselmann et al. 2011). Because of the negative exponential relationship,

fluctuations at lower temperatures cause much higher changes in the development time than fluctuations at higher temperatures. Therefore, the speed at which water warms in spring and the higher water temperatures in winter that will probably be caused by climate warming (Straile et al. 2003) should have a greater influence on population growth, abundance, and the length of the reproductive period of the mysids than fluctuations in the maximum summer temperatures. The summer temperatures, on the other hand, influence the distribution and survival of *L. benedeni*.

Table 1 Results of the linear-regression model for the development time in each experiment (D_T), the percent of surviving females (\bar{q}), and the corresponding mean brood size (mean number of hatched juveniles per female \pm SD) of *L. benedeni*

Temperature (°C)	Run I					Run II					Run III				
	D_T (d)	R^2	P value	Brood size	\bar{q} (%)	D_T (d)	R^2	P value	Brood size	\bar{q} (%)	D_T (d)	R^2	P value	Brood size	\bar{q} (%)
6.5	—	—	—	—	—	56.16	0.86	<0.001	7.7 \pm 4.8	40	—	—	—	—	—
10	32.88	0.97	<0.001	18.1 \pm 5.0	48	31.19	0.97	<0.001	12.5 \pm 6.4	78	—	—	—	—	—
15	18.66	0.97	<0.001	11.9 \pm 3.9	90	18.18	0.96	<0.001	18.1 \pm 6.0	68	18.17	0.97	<0.001	6.0 \pm 2.8	43
20	11.45	0.91	0.008	17.9 \pm 4.8	84	11.17	0.92	<0.001	11.9 \pm 4.2	64	9.02	0.94	<0.001	12.2 \pm 1.9	48
25	7.70	0.92	0.04	12.2 \pm 4.0	38	—	—	—	—	—	—	—	—	—	—

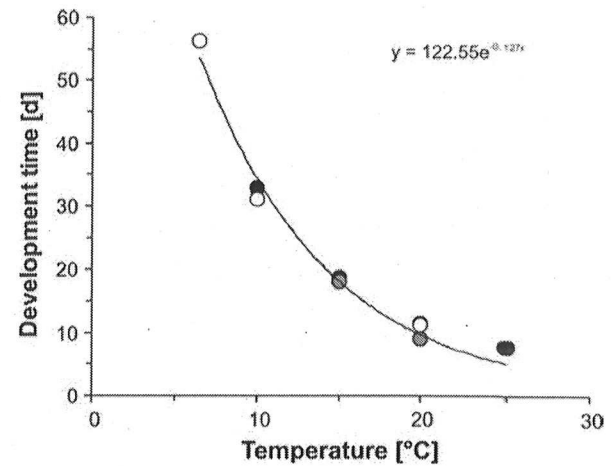


Fig. 2 Development time of *L. benedeni* at the indicated temperatures and time. Results from Fig. 1 were combined in an exponential curve (black dots run I, white dots run II, grey dots run III; see text for dates of each set of experiments)

Because of decreasing productivity over 20°C, as observed by the lower survival at 25°C, the establishment, density, and continuing dispersion of the mysids could be limited by increasing summer temperatures (Adrian et al. 2009). *L. benedeni* is well adapted to the temporal conditions in Lake Constance, as indicated by the maximal survival of larvae and adults at 15.9°C. The mean temperature in the field during the reproduction period in 2008 was $15.6 \pm 5.7^\circ\text{C}$, with a minimum of 6.6°C at the beginning on April 2 and a maximum of 22.9°C in midsummer on July 30 (Hanselmann et al. 2011). This could also explain the rapid increase in *L. benedeni* after its introduction in Lake Constance (Hanselmann, unpublished observations).

One precondition for this type of experiment is continuous reproduction. Because we started the experiments early in the year at the beginning of the reproduction period, there was a plateau, i.e., no hatching, at the beginning of runs I and II. Because of this pattern, we conclude that at the beginning of the spring experiments, the clutch age distribution was skewed toward younger clutches, whereas in the summer samples (run III), clutch ages were more evenly distributed, i.e., unlike the spring samples, the summer samples contained relatively old clutches that hatched immediately in the first day after the start of the experiment. Nevertheless, we assumed for all experiments that the clutch that hatched last was fertilized just before the females were caught. As the

Table 2 Results of the nonlinear regression model for the probability of survival of females and larvae of *L. benedeni*, with calculations of the optimal temperature (maximum) and of the temperature dependent development time

	Parameter	Estimated value	SE	T value	P value	R ²	Maximum (°C)
Optimal temperature						Formula f	$ax^2 + bx + d$
Females	a	0.443	0.140	3.159	0.013	0.6196	15.94
	b	14.108	4.079	3.459	0.009		
	d	402.950	26.823	1.502	0.171		
Juveniles	a	0.556	0.103	5.421	<0.001	0.8247	15.87
	b	17.635	2.985	5.909	<0.001		
	d	51.841	19.627	2.641	0.030		
Temperature dependent development time						Formula f	$a \times e^{bx}$
	a	122.549	8.27	14.81	<0.001	0.985	
	b	0.127	0.006	19.58	<0.001		

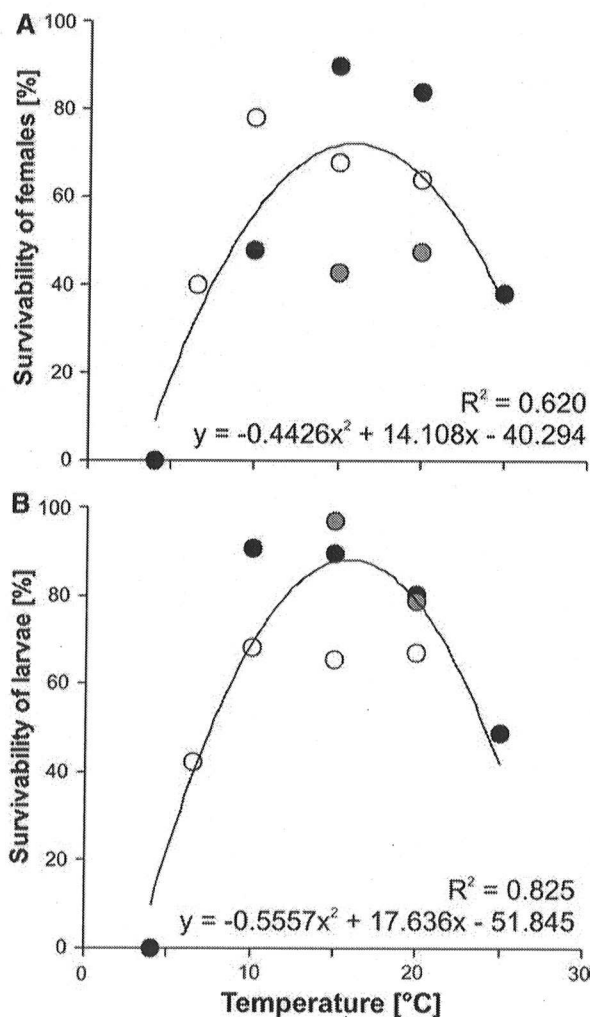


Fig. 3 Probability of survival of females **a** and larvae **b** at the temperatures indicated in three experiments (filled circle run I, open circle run II, filled gray circle run III; see text for dates of each set of experiments)

decline of breeding females, respectively the hatching of the juveniles during our experiments, is well described by a straight line (Fig. 1), we concluded that the reproduction was continuous and that our sampling size of 50 females was large enough to include a newly breeding female.

The deviation at 20°C in run III (summer) from runs I and II (spring) could possibly be caused by the lower number of females. At the end of July, when we started the experiment, only few breeding females were found. At this time, the abundance of the population of *L. benedeni* in Lake Constance was lower probably because of size-selective predation by juvenile perch on the large-length classes of the mysid (Hanselmann et al. 2011). Because of reduced adults available in the field, it could be that in our sampling no female was included with a clutch fertilized the night before. So the last clutch could be fertilized some days before catching, which would lead to an underestimation of the development time. The described equation (Fig. 2) could therefore, underestimate but never overestimate the egg development time.

Compared to previous methods, such as storing the eggs out of the marsupium in petri dishes (Manton 1928; Modlin 1979; Wittmann 1981a; Johnston et al. 1997; Fockede et al. 2006; Gheki et al. 2007), our experimental method avoided the stress of removing the larvae from the marsupium and handling them during water exchange. We reduced negative effects resulting from possible oxygen deficits by allowing the female to care for the brood. Therefore, we introduced a facile method for working in vivo with the larvae of *L. benedeni*, which can be used to answer ecotoxicological questions (Verslycke et al. 2007).

In our future studies on population processes of *L. benedeni*, we intend to quantify the changes in population size over time, and finally develop a model based on the mysids characteristics (inter alia rates in relation to environmental factors such as the embryonic development time, growth rates, and birth rates) to calculate and predict population dynamics and mortality rates.

Acknowledgments We thank Bettina Hodapp and Philipp Menzel for their help in the laboratory and Karen A. Brune for editing the English language.

References

- Adrian R, O'Reilly CM, Zagarese H, Baines SB, Hessen DO, Keller W, Livingstone DM, Sommaruga R, Straile D, Van Donk E, Weyhenmeyer GA, Winder M (2009) Lakes as sentinels of climate change. *Limnol Oceanogr* 54:2283–2297
- Aßmann C, Von Elert E, Gergs R (2009) Effects of leaf litter and its fungal colonization on the diet of *Limnomysis benedeni* (Crustacea: Mysida). *Hydrobiologia* 636:439–447
- Audzijonyte A, Wittmann KJ, Ovcarenko I, Väinölä R (2009) Invasion phylogeography of the Ponto Caspian crustacean *Limnomysis benedeni* dispersing across Europe. *Divers Distrib* 15:346–355
- Bij de Vaate A, Jazdzewski K, Ketelaars HAM, Gollasch S, Van der Velde G (2002) Geographical patterns in range extension of Ponto Caspian macroinvertebrate species in Europe. *Can J Fish Aquat Sci* 59:1159–1174
- Davis CC (1966) A study of the hatching process in aquatic invertebrates, XXII. Multiple membrane shedding in *Myxidium columbiae* (Zimmer) (Crustacea: Mysidacea). *Bull Mar Sci* 16:124–131
- Dediu II (1966) Répartition et caractéristique écologique des Mysides des bassins des rivières Dniestr et Pruth. *Rev Roum Biol/Ser Zool* 11:233–239
- Focke N, Ghekiere A, Bruwier S, Janssen CR, Vinex M (2006) Effect of salinity and temperature on the intramarsupial development of the brackish water mysid *Neomysis integer* (Crustacea: Mysidacea). *Mar Biol* 148:1339–1356
- Fritz B, Nisch A, Wittkugel C, Mörtl M (2006) First record of *Limnomysis benedeni* Czerniavsky in Lake Constance (Crustacea: Mysidacea). *Lauterbornia* 58:157–160
- Gergs R, Hanselmann AJ, Eisele I, Rothhaupt KO (2008) Autecology of *Limnomysis benedeni* Czerniavsky, 1882 (Crustacea: Mysida) in Lake Constance, southwestern Germany. *Limnologica* 38:139–146
- Ghekiere A, Focke N, Verslycke T, Vincx M, Janssen CR (2007) Marsupial development in the mysid *Neomysis integer* (Crustacea: Mysidacea) to evaluate the effects of endocrine disrupting chemicals. *Ecotox Environ Saf* 66:9–15
- Hanselmann AJ, Gergs R, Rothhaupt KO (2011) Seasonal shifts in the life cycle of the Ponto Caspian invader *Limnomysis benedeni* (Crustacea: Mysida): a physiological adaptation. *Hydrobiologia* 673:193–204
- Ishikawa M, Oshima Y (1951) On the life history of a mysid crustacean *Neomysis japonica* Nakazawa. *Bull Jpn Soc Sci Fish* 16:461–472
- Johnston NM, Ritz DA, Fenton GE (1997) Larval development in the Tasmanian mysids *Anisomysis mixta australis*, *Tenagomysis tasmaniae* and *Paramesopodopsis rufa* (Crustacea: Mysidacea). *Mar Biol* 130:93–99
- Leuven RSEW, van der Velde G, Baijens I, Snijders J, van der Zwart C, Lenders HJR, bij de Vaate A (2009) The river Rhine: a global highway for dispersal of aquatic invasive species. *Biol Invasions* 11:1989–2008
- Manton SM (1928) On the embryology of a mysid Crustacean, *Hemimysis Lamornae*. *Philos Trans R Soc Lond B Biol Sci* 216:363–463
- Mauchline J (1973) The broods of British Mysidacea (Crustacea). *J Biol Assoc UK* 53:801–817
- Mauchline J (1980) The biology of mysids and euphausiids. *Adv Mar Biol* 18:1–681
- Modlin RF (1979) Development of *Mysis stenolepis* (Crustacea: Mysidacea). *Am Midl Nat* 101:250–254
- Morgan MD (1980) Life history characteristics of two introduced populations of *Mysis relicta*. *Ecology* 61:551–561
- Murano M (1964) Fisheries biology of a marine relict mysid *Neomysis intermedia* Czerniavsky. IV. Life cycle, with special reference to growth. *Aquiculture (Jpn)* 12:109–117
- Porter ML, Meland K, Price W (2008) Global diversity of mysids (Crustacea: Mysida) in freshwater. *Hydrobiologia* 595:213–218
- R Development Core Team (2008) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Sala OE, Chapin FS III, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. *Science* 287:1770–1774
- Stich HB, Brinker A (2010) Oligotrophication outweighs effects of global warming in a large, deep, stratified lake ecosystem. *Glob Chang Biol* 16:877–888
- Straile D, Jöhnk K, Roszknecht H (2003) Complex effects of winter warming on the physicochemical characteristics of a deep lake. *Limnol Oceanogr* 48:1432–1438
- Vermonden K, Leuven RSEW, Van der Velde G (2010) Environmental factors determining invasibility of urban waters for exotic macroinvertebrates. *Divers Distrib* 16:1009–1021
- Verslycke T, Ghekiere A, Raimondo S, Janssen C (2007) Mysid crustaceans as standard models for the screening and testing of endocrine disrupting chemicals. *Ecotoxicology* 16:205–219
- Westheide W, Rieger R (1996) Spezielle Zoologie, Erster Teil: Einzeller und Wirbellose Tiere. Gustav Fischer Verlag, Stuttgart
- Wittmann KJ (1978) Adoption, replacement, and identification of young in marine Mysidacea (Crustacea). *J Exp Mar Biol Ecol* 32:259–274
- Wittmann KJ (1981a) Comparative biology and morphology of marsupial development in *Leptomysis* and other

- mediterranean Mysidacea (Crustacea). J Exp Mar Biol Ecol 52:243 270
- Wittmann KJ (1981b) On the breeding biology and physiology of marsupial development in mediterranean *Leptomysis* (Mysidacea: Crustacea) with special reference to the effects of temperature and egg size. J Exp Mar Biol Ecol 53:261 279
- Wittmann KJ (1984) Ecophysiology of marsupial development and reproduction in Mysidacea (Crustacea). Oceanogr Mar Biol Annu Rev 22:393 428
- Wittmann KJ, Ariani AP (2008) Reappraisal and range extension of non indigenous Mysidae (Crustacea, Mysida) in continental and coastal waters of eastern France. Biol Invasions 11:401 407