— 759 —

DISTRIBUTION AND REPRODUCTIVE BIOLOGY OF *AUSTROPOTAMOBIUS TORRENTIUM* IN BAVARIA AND DOCUMENTATION OF A CONTACT ZONE WITH THE ALIEN CRAYFISH *PACIFASTACUS LENIUSCULUS*

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ABSTRACT

In Bavarian freshwater systems, the stone crayfish *Austropotamobius torrentium* is still present in many headwaters of Danubian tributaries. Since the 1960s an alien species, the signal crayfish *Pacifastacus leniusculus*, was introduced several times independently in Germany. Compared to the previously introduced *Orconectes limosus*, this species also invaded higher reaches of freshwater streams, thereby becoming a direct competitor of *A. torrentium*. In addition, it carried the crayfish plague, *Aphanomyces astaci*, which is lethal to European species. So far, there are only few documented populations of *Pacifastacus leniusculus* from upper streams in Bavaria and to our knowledge no reports of coexistence with the stone crayfish, which may be outcompeted by this much larger species due to direct interactions or reproductive superiority. In this study, we document the distribution of the stone crayfish in Bavaria and give evidence for a contact zone of *Austropotamobius torrentium* and apparently uninfected *Pacifastacus leniusculus* in the western Bavarian Forest, with a limited range of overlap and a slow upward displacement of the native species in the time frame of three years. Comparative studies of reproductive biology in the field and in the laboratory showed higher fecundity, faster juvenile growth and earlier hatching of the introduced species. These new aspects of the biology of the two involved species suggest an ecological superiority of *P. leniusculus* and may explain the long-term displacement of the native species, even in the absence of the crayfish plague *Aphanomyces astaci*.

Key-words: Astacidae, invasive species, competition, reproduction, ecology.

DISTRIBUTION ET BIOLOGIE DE LA REPRODUCTION D'*AUSTROPOTAMOBIUS TORRENTIUM* EN BAVIÈRE ET DESCRIPTION D'UNE ZONE DE CONTACT AVEC L'ÉCREVISSE EXOTIQUE *PACIFASTACUS LENIUSCULUS*

RÉSUMÉ

Dans les écosystèmes d'eaux douces bavaroises, l'écrevisse des torrents *Austropotamobius torrentium* est encore présente au niveau des sources de nombreux affluents du Danube. Depuis les années 60, une espèce étrangère, l'écrevisse signal *Pacifastacus leniusculus*, a été introduite plusieurs fois indépendamment en Allemagne. Par comparaison avec *Orconectes limosus*, espèce introduite auparavant, *Pacifastacus leniusculus* a envahi une plus large étendue de cours d'eau, entrant de ce fait en compétition directe avec *Austropotamobius torrentium*. De plus, elle a amené avec elle la « peste » des

écrevisses, *Aphanomyces astaci*, qui est létal pour les espèces européennes. Jusqu'à maintenant, seules quelques populations de *Pacifastacus leniusculus* ont été documentées dans la partie supérieure des cours d'eau de Bavière et, à notre connaissance, aucune coexistence n'a été relevée avec l'écrevisse des torrents. Cette dernière pourrait être l'objet d'une exclusion compétitive par l'espèce étrangère, de taille beaucoup plus grande, à cause d'interactions directes ou d'une inégalité de succès reproductif. Dans cette étude, nous décrivons la distribution de l'écrevisse des torrents en Bavière et nous montrons l'existence d'une zone de contact entre *Austropotamobius torrentium* et *Pacifastacus leniusculus* apparemment non infectée dans la forêt bavaroise de l'ouest, avec une zone de recouvrement limitée et un déplacement lent de l'espèce native vers l'amont en l'espace de trois ans. Des études comparatives sur la biologie reproductive des deux espèces ont montré que l'espèce introduite présente une fécondité plus élevée, une croissance des juvéniles plus rapide et une éclosion plus précoce. Ces nouveaux aspects de la biologie de ces deux espèces suggèrent une supériorité écologique de *Pacifastacus leniusculus* et pourraient expliquer le déplacement à long terme de l'espèce native, même en l'absence de la « peste » des écrevisses *Aphanomyces astaci*.

Mots-clés : Astacidae, espèces invassives, compétition, reproduction, écologie.

INTRODUCTION

Previous to the introduction of alien species by humans, freshwater streams and lakes in Bavaria were exclusively inhabited by two autochthonous species of crayfish, the noble crayfish *Astacus astacus* (LINNAEUS, 1758) and the stone crayfish *Austropotamobius torrentium* (SCHRANK, 1803). While the larger species, *A. astacus,* used to be frequent in lakes and larger rivers all throughout Germany and eastern Europe including southern Scandinavia, the considerably smaller species, *A. torrentium* reaches its northern and western natural limit in southern Germany, its distribution approximately delimited by the course of the rivers Rhine in the west and Main in the north (ALBRECHT, 1983). The latter species nowadays is confined to headwaters and small water systems, adapted to water with turbulent flowthrough and, as the name stone crayfish suggests, rocky environments. In the Danube basin, BOHL (1989) and BOHL *et al*. (1999) characterise Bavarian crayfish stocks as being restricted to altitudes below 800 m, but the highest known population of *A. torrentium* has been reported to reach 1,024 m in Austria (MACHINO and FÜREDER, 1998). Due to the different size of the two endemic species and their habitat preferences, they have experienced different impacts by human exploitation and water pollution. In consequence, *Astacus astacus* has often been driven out of its preferred lowland habitats and have moved to higher grounds: 90% of noble crayfish stocks in Bavaria can be found at altitudes between 500-800 m (BOHL, 1987). Since *Austropotamobius torrentium* is the smallest of the European crayfish species (BOTT, 1950), it has never been of great economic interest and was probably never stocked (ALBRECHT, 1983; TROSCHEL and DEHUS, 1993). In addition, this species may have been impacted less by pollution of waters during the times of industrial development, which mostly affected the water quality of the larger rivers. Only locally confined deterioration of the water quality in smaller streams due to insecticides or eutrophication represent potential direct impact of human activities on this species. However, since approximately 110 years (OIDTMANN and HOFFMANN, 1999) there has been a much more severe threat as an indirect consequence of human activities: the stocking of American crayfishes in European waters, all of them potential carriers of the crayfish plague *Aphanomyces astaci*, which is lethal for European crayfishes. These introductions have limited drastically the distribution of all autochthonous species. Currently, three species of nonindigenous crayfish inhabit German freshwaters with moderate to high success. *Orconectes limosus* (RAFINESQUE, 1817) and the signal crayfish *Pacifastacus leniusculus* (DANA, 1852), both introduced for human consumption, are nowadays widespread. More recently, the red swamp crayfish

Procambarus clarkii GIRARD, 1852 has also been found in the wild, probably released as ornamental pet, but not in high enough numbers to cause a negative impact yet (BOHL *et al*., 1999). However, the first two alien crayfish species aided by the crayfish plague have completely displaced the native crayfish fauna from many of their natural habitats. The first species to be introduced, *O. limosus*, is normally not found in upper reaches of streams and therefore has not been a direct threat for the smaller *A. torrentium*. However, the introduction of *P. leniusculus* in the sixties of the last century, especially in smaller rivers and fishponds (BOHL, 1987), seems to have a far more severe effect on the stone crayfish population. Most unfortunately, this species has often been introduced by mistake, in the belief of stocking the similarly coloured noble crayfish *Astacus astacus*.

Like the other native European crayfish species, *Astacus astacus*, *A. leptodactylus* (ESCHSCHOLTZ, 1823) and *Austropotamobius pallipes* (LEREBOULLET, 1858), the stone crayfish is considered to be a threatened species. It is listed in the German Red List (RL3, Appendix I) of endangered species (BINOT' *et al*., 1998) and also in Annex II (priority species) and in the Annex IV of European Community Directives for the Conservation of Natural habitats and wild Flora and Fauna (FFH 97/62/EU) as a species requiring special conservation measures.

The aim of this study was to map the distribution of crayfish species in the Bavarian Forest around Regensburg with special emphasis on the occurrence of alien crayfish and the documentation of displacement in zones of contact. In addition, we studied the reproductive biology of the Bavarian populations of the stone crayfish *Austropotamobius torrentium* and compared them with results from the literature and simultaneously reared signal crayfish *Pacifastacus leniusculus*.

MATERIAL AND METHODS

In the years 2002 to 2004, freshwater streams throughout Bavaria belonging to the Danube water catchment were sampled in order to map the distribution of crayfish species and the impact of alien species. Our sampling activities were centred in the surroundings of Regensburg (Oberpfalz) in the western Bavarian Forest. We used the map of E. BOHL "Bayerisches Landesamt für Wasserwirtschaft, Verbreitung der Krebsarten in Bayern, Stand: Nov. 2000" (web link: http:www.bayern.de/lfw/technik/ gewaesseroekologie/fischoeko/krebse/verbreitung.htm) as the current distribution status of native and introduced crayfish species in Bavaria.

Morphometric measurements were taken from all sampled *Austropotamobius torrentium* to estimate the population health status and to compare growth and sexual maturity. Signal crayfish *Pacifastacus leniusculus* from the Sulzbach (see below) were measured for morphometric comparisons of sexual maturity. From all specimens, three morphometric measurements were taken: carapace length including rostrum (CL), chelar propodus height (prh), dactylus length (dal). In addition, number and size of eggs from berried females were recorded. Total length (TL), was recorded from approximately 40 individuals (28 were used for a CL-TL linear regression), and was later considered to be too imprecise for correlating other measurements taken in the field.

In one freshwater stream in the vicinity of Regensburg, the Sulzbach (section near Bernhaldswald also known as Ellbach), we found a large population of the signal crayfish *Pacifastacus leniusculus* and a few kilometres upstream a healthy population of the stone crayfish *Austropotamobius torrentium*, syntopic with a few individuals of the noble crayfish *Astacus astacus*. In subsequent samplings, we followed the river downstream from the autochthonous populations until encountering the first individuals of the signal crayfish. This contact zone was observed on a regular basis during the following years, to determine the existence and the range of a zone of overlap and the possible upward shifting of the

contact zone. Exact geographic positions of this contact zone were recorded by a General Positioning System device (GPS acc. World Geodetic System of 1984 (WGS 84)).

Life animals, including ovigerous females, were carried back to the laboratory of the University of Regensburg and maintained in large aerated water tanks under constant 12 h-12 h light-dark conditions. Temperature was regulated between 4 to 20°C according to the season. In most cases, we used separate tanks for *Pacifastacus leniusculus* and *Austropotamobius torrentium* to study their reproductive biology. In one case, species were mixed for observation of direct interactions and to determine whether the studied population of signal crayfish was infected by *Aphanomyces astaci.*

For documentation of juvenile growth, three hatches of stone crayfish from Vils and Wertach tributaries were kept in the laboratory until spring 2003. Comparative data for juvenile development in the signal crayfish *Pacifastacus leniusculus* from Sulzbach were obtained one year before. Following the results of BOHL (1989) and GYDEMO (1989) that showed that survival rate of *Astacus astacus* juveniles was highest at a constant temperature of 20°C, both signal and stone crayfish hatchlings were kept under this condition with identical feeding regimes. Light – dark cycles were identical to the ones at which adult specimens were kept. Juveniles were removed in irregular intervals and preserved in 70% ethanol for measuring under a Leica MZ 75 stereomicroscope.

In newly discovered populations throughout Bavaria, the population age structure status was estimated according to a method suggested by BOHL (1989): In non-selective hand catches, crayfish individuals were classified in 5 mm total length (TL) intervals, starting at a minimum of 45 mm, which is considered the minimum size of adults and normally reached after 3 years. Because in crayfish total length is analogous to age, it is possible to estimate the demographic health of a population. According to the author, a sample size of 20 animals that fits into at least 6 size categories represents a stable population status. Since in some localities it proved very difficult to collect numbers of 20 or more, we changed this method to sample sizes of 15 using only 5 size categories. We used the carapace length (CL) as size/age marker and recalculated BOHL's (1989) intervals of 5 mm to CL intervals from 28 specimens total lengths. This had to be done for males and females separately, because STREISSL and HÖDL (2002a) showed that carapace growth in proportion to total growth differs between sexes. In six of the sampled populations we found enough crayfish to estimate the health status according to the described method.

RESULTS

Distribution and interaction of species

209 specimens of *Austropotamobius torrentium* (juveniles of unknown sex excluded) from 17 Bavarian populations belonging to the Danube water catchment were sampled from October 2002 to August 2003 (Figures 1-2). From 14 freshwater systems (Au, Bu, Da, Fo, Ga, Ho, Hö, Is, Ot, Pe, Sa, Su, Vi, We; see Table I) at least one adult specimen of *A. torrentium* was kept and conserved in 70% ethanol for type collection. These collections served to establish new records of the stone crayfish in several Bavarian localities. We found a sex ratio of 1:1.18 (female: male), including 47 juvenile specimens. The largest male measured 98 mm total length (48.75 mm CL), the largest female 90 mm (44.5 mm CL), which was carrying 68 eggs. The highest egg number of 110 was found in an other berried female 42.50 mm CL). This individual was, like two other ovigerous females (Table III), taken into the aquarium for studying juvenile growth. Undocumented populations of signal crayfish were found in three freshwater streams, two in the vicinity of Regensburg (Sulzbach, Spindelbach) and one in a tributary to the Rott in Niederbayern (Table II). Distribution records of native and alien crayfish species were deposited in the archives of the Oberpfalz county government (Bezirksregierung) and are shown in the maps in Figures 1-2.

Studies were intensified in the Sulzbach-Otterbach river system. The first of these two rivers harbours stone crayfish in its upper reaches and a dense population of signal crayfish all the way down to the confluence with the Otterbach, whereas the latter was
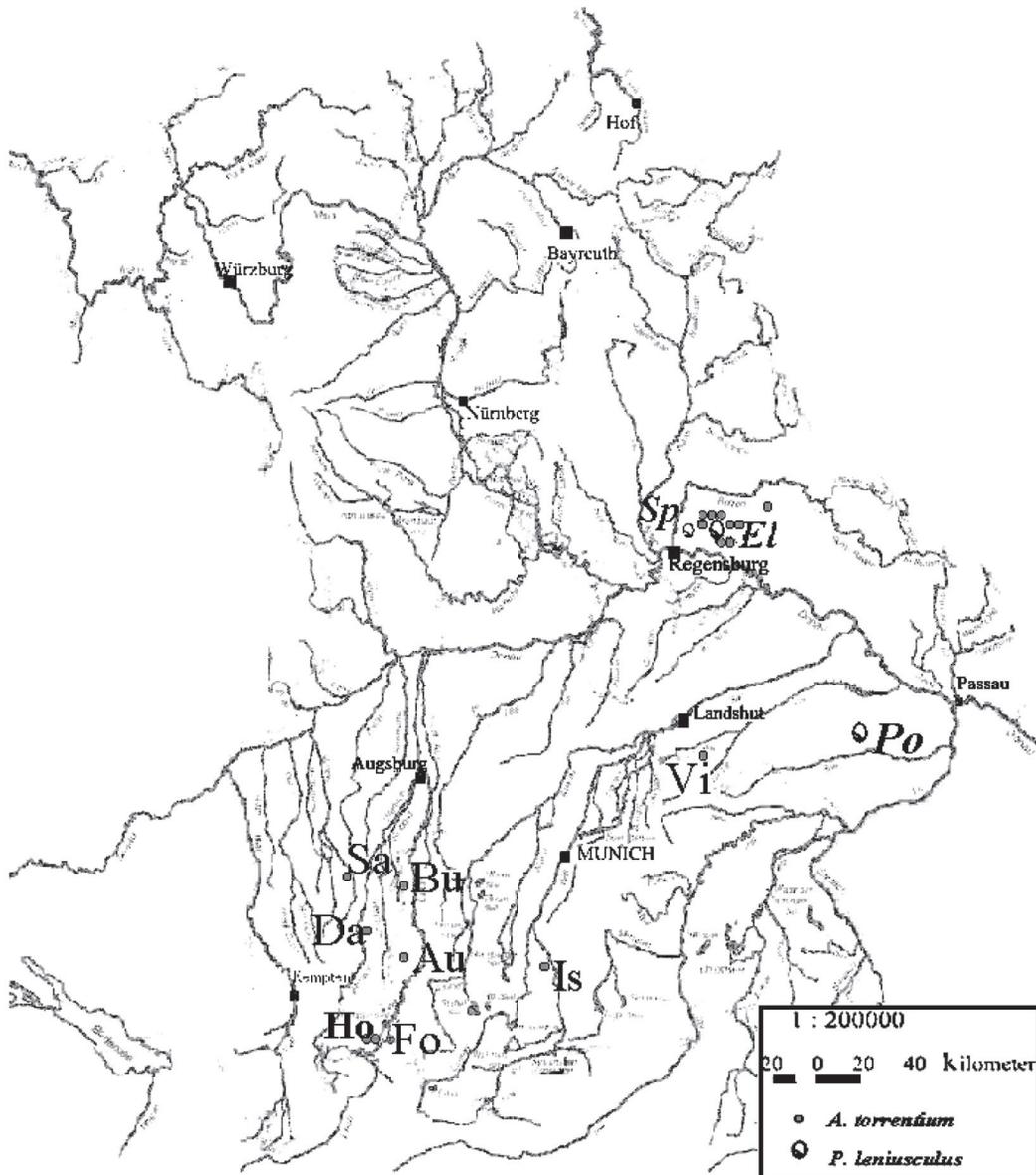

Figure 1
   Map of surveyed Bavarian populations, from 2002-2003 with 3 new occurrences of signal crayfish (in italics); abbreviations for localities are explained in Table I; local populations in the vicinity of Regensburg are not named.

Figure 1
   Carte de suivi des populations bavaroises, de 2002 à 2003 avec 3 nouvelles occurrences de l'écrevisse signal (en italique) ; les abréviations des localités sont expliquées dans le Tableau I ; les populations locales situées à proximité de Regensburg ne sont pas nommées.

Table I
    Localities in alphabetical order and number of sampled *Austropotamobius torrentium* from 17 populations and three new findings of *Pacifastacus leniusculus* in Bavaria.

Tableau I
    Localités classées par ordre alphabétique et nombre d'*Austropotamobius torrentium* échantillonnés dans 17 populations et trois nouveaux sites de *Pacifastacus leniusculus* en Bavière.
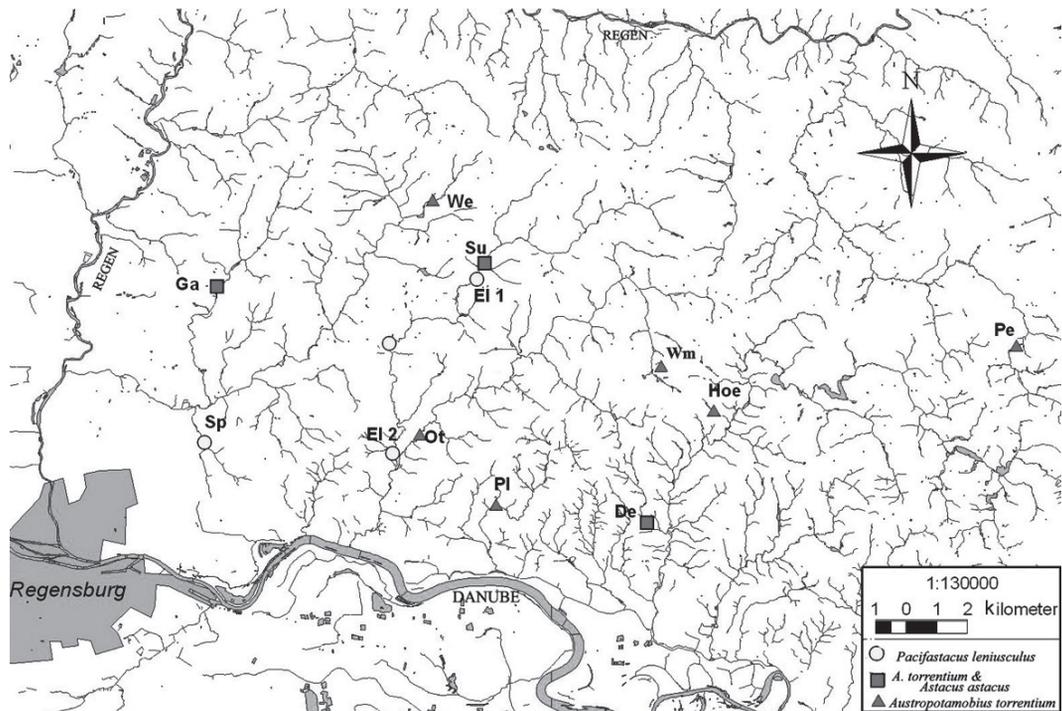

Figure 2
    Map of the area under survey in the surroundings of Regensburg (near the Bavarian Forest region) from 2002-2004, with special emphasis on a contact zone of signal crayfish and native species; abbreviations for localities are explained in Table I.

Figure 2
    Carte de la région sous surveillance aux alentours de Regensburg (près de la région forestière bavaroise) de 2002 à 2004, avec une considération particulière pour la zone de contact entre l'écrevisse signal et les espèces natives ; les abréviations des localités sont expliquées dans le Tableau I.

completely devoid of alien crayfish species (Figure 2). The length of the Sulzbach river section inhabited by signal crayfish was 8.9 km, reaching densities of approximately 2-4 individuals per square metre. In the search for the contact zone in the Sulzbach we found one single male stone crayfish near the bridge of the road between Hauzendorf and Altenthann (49°06.413'N; 12°17.896'E) in early August of 2002, and only one signal crayfish was found directly downstream of the bridge. The contact zone shifted upwards between 2002 and 2003 by 350 m. That year in september, 14 signal crayfish were found all the way up from the bridge to the vicinity of a farm with fishponds. Near the farm,

Localities Abbreviation	N	Locality	Nearby village	Drainage system rivers → Danube	Date of sampling	County
A. torrentium						
Au	**22**	Auerberg	Loxhub	Kronbach → Lech	9/6/03	Schwaben
Bu (with *A. astacus*)	**23**	Buchloe	Hausen	Schanzgraben (fish pond) → Gennach → Wertach → Lech	4/6/03	Schwaben
Da	**21**	Dachssee	Bernbach	Hühnerbach → Wertach → Lech	3/17/03	Schwaben
De (with *A. astacus*)	**3**	Deßgraben	Pangerlhof	Deßgraben → Wildbach	3/30/03	Oberpfalz
Fo	**6**	Forggensee	Rieden	Schleichbach → Lech	5/31/03	Schwaben
Ga (with *A. astacus*)	**23**	Gambach	Hauzenstein	Gambach → Wenzenbach → Regen	8/31/03	Oberpfalz
Wm	**6**	Weißmühlbach	Brennberg	Weißmühlbach → Otterbach	5/6/03	Oberpfalz
Ho	**22**	Hopfensee	Enzensberg	small tributary to lake → Füssener Achen	5/31/03	Schwaben
Hö	**1**	Höllbach	Brennberg	Höllbach → Wildbach	5/6/03	Oberpfalz
Is	**12**	Isar	Königsdorf	Rottach → Isar	6/9/03	Oberbayern
Ot	**8**	Otterbach	Unterlichtenwald	upstream before Sulzbach drains	12/7/03	Oberpfalz
Pe	**7**	Perlbach	Kothmühl	Mietnach → Perlbach → Regen	4/30/03	Oberpfalz
Pl		Perlenbach	Wiesent	Perlenbach (1 individual, not measured)	4/30/03	Oberpfalz
Sa	**5**	Salgen	Mattsies	small forest creek → Flossach → Mindel	10/4/02	Schwaben
Su (with *A. astacus*)	**17**	Sulzbach	Hornismühle	Sulzbach → Otterbach	5/3/03	Oberpfalz
Vi	**17**	Vilsbiburg	Kollmansberg	fish ponds → Vils‡ Inn	11/30/03	Niederbayern
We	**16**	Wenzenbach	Zuchmühl	Wenzenbach → Regen	7/4/03	Oberpfalz
Total	**209**	**113 males**	**96 females**			
P. leniusculus						
El	x	Ellbach	Bernhardswald	lower part of Sulzbach → Otterbach	2002-2004	Oberpfalz
Sp	x	Spindelbach	Irlbach	Spindelbach → Wenzenbach → Regen	2003	Oberpfalz
Po	x	Poldinger Bach	Polding	Poldinger Bach → Gambach → Rott	2003	Niederbayern

three stone crayfish (the lowest 49°06.627'N; 12°17.962'E) were found syntopic with the farthest upstream individuals of signal crayfish. In 2004, the contact zone remained more or less constant (49°06.665'N; 12°18.088'E) and was intensely surveyed, revealing a zone of overlap of the two species of approximately 120 m length. One single small male of *Astacus astacus* was also found (49°06.638'N, 12°18.078'E). The contact zone of 2002 could not be sampled in 2004, because the river section was flooded due to a beaver dam. Crayfish of the two species found in the 2004 overlap zone showed that only small individuals of stone crayfish were present and in part injured. Only a mid-size female (25,86 CL) with several appendages missing -including both claws- and otherwise no large size stone crayfish, could be found in the contact zone. Several specimens (smaller invasive signal crayfish and stone crayfish) sampled from the contact zone were kept together in the aquarium since September 2004. Direct interactions indicating interspecific aggression, could not be observed in a period of 10 weeks.

Native species and signal crayfish from the Sulzbach were kept in the same aquarium for over 3 months in 2003, without any contagious symptoms of crayfish plague. Furthermore, a large male of *Astacus astacus* found in the upper Sulzbach in May 2002, was kept in a mixed aquarium with changing *Pacifastacus leniusculus* individuals from student-excursions to the Ellbach River section of Sulzbach, stayed uninfected for 1.5 years and died after 2 successful moults (CL 49.52 mm, TL 96.4 mm) because of the burn spot disease (fungus *Ramularia astaci*). For that reason, and because we have never seen signs of crayfish plague disease in the field, we assume that *Aphanomyces astaci* is probably absent from the Sulzbach signal crayfish population.

Estimates of population structure

Six Bavarian populations (Au, Bu, Da, Ho, Su, Vi; abbreviations according to Table I) of the stone crayfish *Austropotamobius torrentium*, of which at least 15 animals had a CL bigger than 22.5 mm, showed a sufficient age structure according to the method described above (modified method of BOHL, 1989). According to our calibration of CL from TL based on 28 individuals we calculated a ratio of CL/TL = 0.489 ± 0.018 for 16 females and 0.496 ± 0.016 for 12 males. Therefore our size categories were redefined as intervals of 2.5 mm carapace lengths starting at a minimum of 22.5 mm CL length, as opposed to the intervals of 5 mm TL starting with 45 mm TL animals as used by BOHL (1989). To give an overview on the frequency of sampled sizes, an histogram including 47 individuals with a CL < 22.5 mm, and excluding stages without sex differentiation CL < 10 mm, is shown in Figure 4.

Reproductive biology

The comparison of egg size in the field (Table II) showed that the average diameter remained more or less constant during the cold period from 30 November 2002 until the beginning of May (5/3/2003) in 18 apparently healthy stone crayfish with presumable no preceding egg loss. The data obtained by measuring five egg diameters in each of 14-berried females during that time period resulted in an average size of 2.47 ± 0.15 mm egg diameter for *Austropotamobius torrentium* in cold regime upstream waters. The average egg diameter increased drastically in 4 measured individuals (6/9/2003) in the waters near Auerberg (Au) shortly before spawning: 2.91 ± 0.15. The same day and in the same river one female already carried the first juveniles. In this study over the whole collecting time an average egg number of 62.9 ± 19.4 was found in 25-berried females (Table II). Egg size diameter did not show any significant correlation, neither positive to maternal female size (CL), nor negative to total amount of eggs. The stone crayfish with the highest reproductive capacity of 3.45 eggs per mm CL corresponding to 108 eggs and a carapace length of 31.30 mm was found already late in the gestation period on 5 April 2003 (Table II). Overall, there was only a slight tendency that larger females carried more eggs. The corresponding

positive correlation between egg number and CL could only be tested in March 2003 (Pearson rank sum test N = 8, p = 0.005), which may be explained by small sample sizes in the other months and a large variation of egg numbers depending on the condition of the females. In March 2003, an average egg number of 68 ± 21 in eight females between 26.9 and 41.5 mm CL was counted, which corresponds to a normalized number of eggs of 2.06 ± 0.41 per mm CL (Table II).

Table II

Ovigerous females of *Austropotamobius torrentium* from Bavarian Danube tributaries, in the years 2002-2003 with mean number and size of eggs in comparison to fecundity of *Pacifastacus leniusculus*.

Tableau II

Femelles ovigères d'*Austropotamobius torrentium* dans les affluents du Danube bavarois, dans les années 2002-2003 avec le nombre moyen et la taille des œufs comparés à la fécondité de *Pacifastacus leniusculus*.

Month	Number and range of carapace lengths [mm]	Mean egg number ± standard deviation	Mean egg number per mm carapace length [mm]$^{-1}$	Mean egg diameter [mm]
November 2002	4/22.0-35.6	56.8 ± 16.5	2.35 ± 0,29	2.45 ± 0.17
March 2003	8/26.9-41.5	67.6 ± 21.2	2.06 ± 0.41	5/2.49 ± 0.16
April 2003	5/26.6-37.4	77.6 ± 18.9	2.44 ± 0.67	No data
May 2003	4/26.3-31.1	48.8 ± 17.4	1.65 ± 0,53	2.48 ± 0.12
June 2003	4/35.4-44.5	55.3 ± 9.7	1.39 ± 0.11	2.91 ± 0.15
P. leniusculus Sulzbach, May 2002	3/40.5-45.2	137.7 ± 26.46	3.21 ± 0.34	2.65 ± 0.14

Three signal crayfish *Pacifastacus leniusculus* caught in May 2002 had overall more eggs, normally more than hundred, also if normalized per mm CL: 3.21 ± 0.34 (Table II). Despite the small sample sizes in the comparison of the two species groups, a Mann-Whitney-U-test showed a significant difference (p = 0.002) in the size-relative fecundity. However, a smaller average egg size (2.65 ± 0.14) was determined in the signal crayfish (Table II). Two weeks later (5/27/2002), first stage juveniles hatched from the signal crayfish females.

In two ovigerous stone crayfish from the Wertach drainage kept in the laboratory in since 17 March 2003 at a constant temperature of approximately 16°C, the time of hatching was almost simultaneous: 31st of May and 1st of June. One maternal crayfish from the Vils kept during the winter at temperatures stepwise increasing from 4°C in November to 16°C in April lost all the eggs until 27 March 2003 (Table III).

Signal and stone crayfish were equal in carapace length of first juvenile stages and showed no significant differences on day 2 after birth (CL 3.40 ± 0.24 versus 3.40 ± 0,20 mm, respectively). In contrast, eight *P. leniusculus* juveniles from two hatches were significantly larger in carapace length on postnatal days 23-24 than four juveniles of *A. torrentium* on days 24-25 (Mann-Whitney-U-test p = 0.011), although only stadium 2 juveniles had been reached in both species (Figure 3). This difference was more obvious in stadium 3 and highly significant (p < 0.001) on postnatal day 44, when almost all signal crayfish juveniles had reached stadium 4, whereas stone crayfish remained in stadium 3 and survival rate diminished rapidly. The last juvenile of *A. torrentium* died after 72 days while moulting into stadium 4. The corresponding time growth increment of juvenile development in the laboratory for both species is shown in Figure 3.
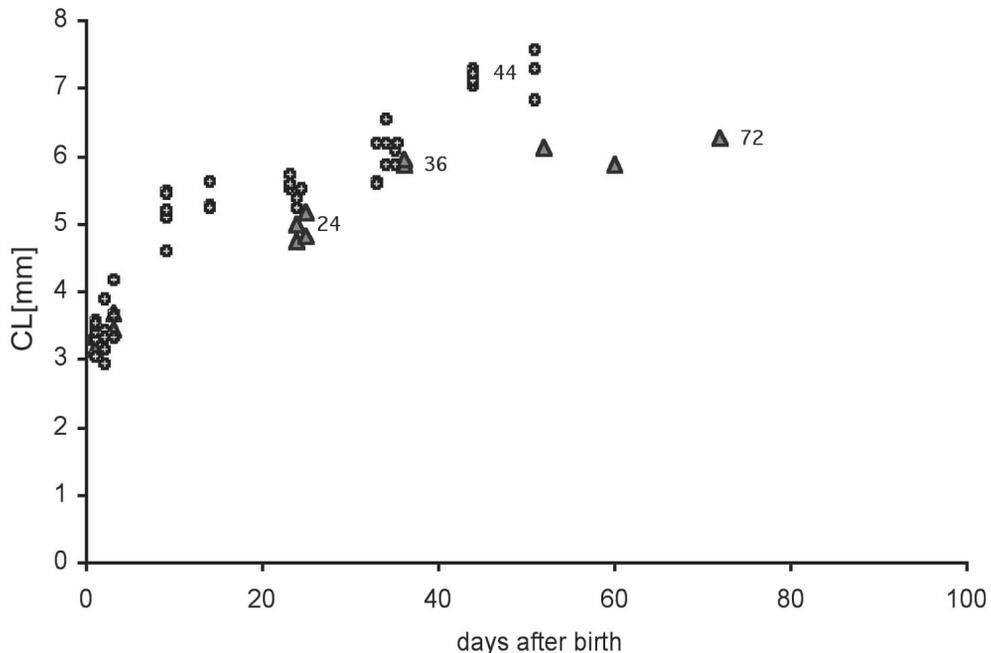
Figure 3
   Juvenile growth of carapace length (CL) in *Austropotamobius torrentium* (triangles) from May-July 2003 and *Pacifastacus leniusculus* (circles) from May-July 2002 in the laboratory under constant environmental conditions (20°C); carapace lengths are expressed as the distance from the tip of the rostrum the posterior carapace fold.

Figure 3
   Croissance de la longueur de la carapace des juvéniles (CL) chez *Austopotamobius torrentium* (triangles) de mai à juin 2003 et de *Pacifastacus leniusculus* (cercles) de mai à juin 2002 en laboratoire sous conditions environnementales constantes ; Les longueurs des carapaces sont exprimées comme la distance du bout du rostre au pli postérieur de la carapace.

DISCUSSION

Austropotamobius torrentium is still present in many tributaries to the Danube River in Bavaria, showing a patchy distribution in some areas. The overall sex ratio of 1:1.18 (female: male) was balanced considering that adult female crayfish have a characteristic cycle of activity over the seasons and hide in their shelters after mating from October until juvenile hatching in spring (BOHL, 1989). In crayfish, maternal territoriality is often used as an offspring defence strategy (FIGLER *et al.*, 2001).

The demographic survey revealed a healthy status in terms of a well balanced age structuring with five or more size categories in all populations of *A. torrentium* for which sufficient (15 or more) adult animals could be collected. The pooled data show that the most frequent size class of adult animals is between 27.5 and 30 mm CL (Figure 4). The demography in the Sulzbach was tested approximately 500 metres upstream of a contact zone with *P. leniusculus*. Out of 17 animals, four females carrying 32-72 eggs were found at sizes beyond 50 mm TL, which supports the impression of a healthy population at this point. Ongoing spot checks in the years 2003 and 2004 were equal to this picture. Another stone crayfish population that may be close to a potential contact zone with signal crayfish
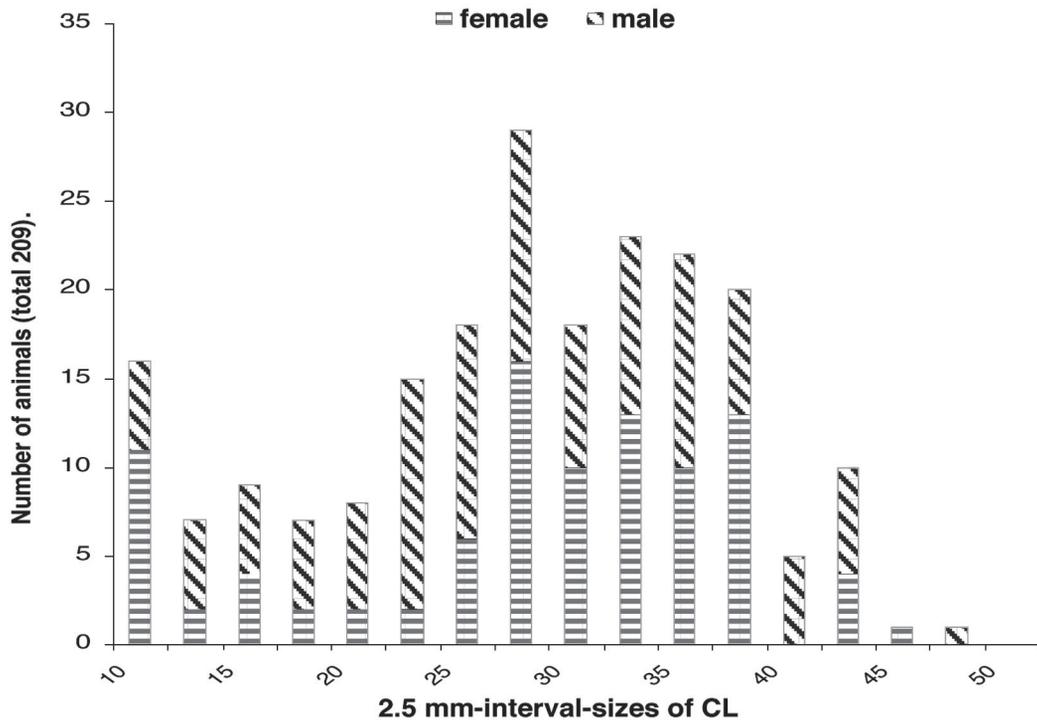
Figure 4

   Size and sex frequency distribution in *Austropotamobius torrentium* from Bavaria. The histogram pools data from sampled localities 2002/2003 (relevant adult size > 22.5 mm CL) collected individuals (Table I).

Figure 4

   Distribution des fréquences de la taille et du sexe d'*Austropotamobius torrentium* en Bavière. L'histogramme regroupe des échantillons de différents sites prélevés entre 2002 et 2003.

is the population from the Gambach. This little stream merges with the Wenzenbach near the village Irlbach a few hundred metres upstream of the Spindelbach, where a newly recorded *P. leniusculus* population has been found (Figure 2). The Gambach population could not be classified as healthy, based on our preliminary results of eight adult individuals and 15 juveniles (CL 10.35-12.70 mm) from August 2003. Additional sampling will be needed to determine the demography of this population and the distribution of *P. leniusculus* in this area, in order to study the location and the effect of the contact zone. Interestingly, in both areas where we newly recorded the presence of alien signal crayfish in the Bavarian Forest (Sulzbach and Spindelbach), nearby crayfish populations are comprised of both native species *Austropotamobius torrentium* and *Astacus astacus* in a syntopic assemblage. This makes these two areas ideal study areas for continuing observations to determine the impact of *P. leniusculus* on both native species. Another record in the literature of syntopic occurrence of natural populations of *A. torrentium* and *A. astacus* is the one by MACHINO (1997) from Slovenia.

   The Bavarian county government Oberpfalz is recording increasing numbers of alien crayfish species *Pacifastacus leniusculus* and *Orconectes limosus* (T. RING, Fischereifachberatung Oberpfalz and E. BOHL, Institute of Water Research, 2004; pers. comm.) that have been released repeatedly into fishponds. Especially stocking

of fishponds with signal crayfish, has happened until recently, sometimes under the impression of dealing with the noble crayfish. The documentation of an extended and dense signal crayfish population in the Bavarian Forest, which covers approximately 9 km length of the Sulzbach, indicates the high invasive potential of this species. LIGHT (2003) examined native populations of *P. leniusculus* in 33 North American larger rivers over a time period of 5 years. In his study, signal crayfish reached yearly travel distances of up to 270 meters (with a maximum of 120 meters daily travel for single individuals), and female crayfish had an upstream tendency in spring before juveniles hatched, probably to counteract downstream drifting of the small juveniles in spring, which is typical for many freshwater stream organisms (LIGHT, 2003).

In a study of introduced *P. leniusculus* populations in Great Britain, tremendously fast expansion rates of up to 1,000 meter a year were detected (HOLDICH, 2000). Many studies proved that stream obstructions of only a few meters are not very effective in stopping an upstream invasion and were easily avoided by bypassing them on land (PEKNY, 1995; PÖCKL, 2002). The case Sulzbach in the Bavarian Forest shows, that habitat preferences of *P. leniusculus* are less specific than known from literature (VORBURGER and RIBI, 1999b; PÖCKL, 2002; BOHL, 1987, 1989), because here it is found in a fast flowing water regime with steep and rocky stretches that did not seem to limit its density. In contrast to the high mobility of *P. leniusculus*, *A. torrentium* shows a strong local fidelity to a specific shelter as emphasized by several authors (BOHL, 1989; RENZ and BREITHAUPT, 2000; LAURENT, 1988; PEKNY, 1995; STREISSL and HÖDL, 2002b). There is only a very limited average range of about 3.6 m per week (RENZ and BREITHAUPT, 2000) with the exception of male ranging behaviour during the mating season in October (BOHL, 1987, 1989). Single individuals were found to move distances from 44 m RENZ and BREITHAUPT (2000) to 50 m STREISSL and HÖDL (2002b), but this was interpreted as a stress response to preceding capture and marking.

Due to these different degrees of invasive potential, it becomes evident that *P. leniusculus*, once established a breeding population, must invade into nearby habitats of *A. torrentium* and *A. astacus* at several localities in Bavaria. The question remains, what mechanisms prevail in the displacement of the native species and what approximate time ranges it takes the signal crayfish to completely exclude them. We recorded previously not cited breeding populations of signal crayfish and in one case in the Bavarian Forest (Sulzbach in the Falkensteiner Vorwald) observed the contact zone with local crayfish over three consecutive years. So far we can only state that coexistence is possible over a few hundred metres, for at least one year, even though the stone crayfishes were underrepresented and did not look healthy. The contact zone was shifted upstream by 350 m within two years. Additional and more extensive observations are necessary in this population, as well as in the one from the Gambach (see above) to obtain a larger long-term dataset, from which more definite conclusions can be drawn.

Our field sampling showed that the occurrence of stone crayfish is not only limited by competing crayfish species. There are long stretches in the Sulzbach above the syntopic noble and stone crayfish populations, where no crayfish could be found, which resembles the patchy distribution described in the literature (TROSCHEL and DEHUS, 1993; BOHL, 1989). In one case a large population of *A. torrentium* could be recorded (eight individuals measured): the Otterbach river in the Bavarian forest showed a continuous stretch with stone crayfish to a length of almost 6 kilometres and might reflect a preserved natural habitat of this species. It may be advisable to intensify studies in this river, e.g. test-fishing for population size (ABRAHAMSSON, 1965), to learn more about population structure and life history in *A. torrentium*. As the German name Otterbach suggests, an otter (*Lutra lutra*) could be observed in 2002, and tracks were found in the following years. This gives further evidence for a healthy crayfish population, because otters are important crayfish predators. Creeks with ambivalent habitat and water structures, like the Höllbach, were not studied

in detail, but probably could show similar patterns. The high risk for the population in the Otterbach would be the potential contact with the signal crayfish in the adjacent Sulzbach (Ellbach-section between Bernhardswald and Unterlichtenwald). In the more rural upper sections of the Otterbach above the village of Forstmühle (F in Figure 2) no more crayfish could be found all the way up to the upper creek regions to the northeast (Weißmühlbach, Wm), where *A. torrentium* individuals were again recorded. A similar situation could be found in the Sulzbach, where stone crayfish were only remarkably abundant in forest region stretches. Exact causes were unsure, but increasing settlement and agricultural activity with only scattered trees may be a reason for no or very sparse incidence of stone crayfish, explaining its patchy distribution.

The question what mechanisms are most important in the displacement of the native species by the signal crayfish in the absence of the crayfish plague was also addressed in the laboratory. In this study we could show that the invasive species has not only the higher invasive potential due to the larger dispersing range of adults (see above), but also shows several reproductive advantages in terms of fecundity and juvenile growth. Female signal crayfish carry more and smaller eggs than female stone crayfish. This is not only due to their overall larger size, but also to a denser egg mass, expressed as number of eggs per millimetre carapace length (Table II). Despite the differences in the size of the eggs, first stage juveniles are of the same size when hatching (Figure 3). *Austropotamobius torrentium*, as the smallest of the European crayfish species, shows the lowest fecundity with 50-100 eggs, but hardly more than 60 (BOHL, 1989). An example from the literature concerning this low egg production, is given by STUCKI and ROMER (2001): in Swiss Lake Aegeri females carried an average of 48 eggs with a diameter of 2.64 mm. Interestingly, in our study a higher average reproductive capacity of approximately 63 (± 19) eggs was found in 25 berried females. GRANDJEAN *et al*. (2000) pointed out a great egg lost in a population of *Austropotamobius pallipes* suffering of over-density: from 55 eggs (December) to 30 eggs in May. This was obviously not the case in the wild populations of *A. torrentium*, but happened to the berried females in the aquarium (Table III).

In the laboratory, juveniles of *P. leniusculus* showed significant faster growth than those of *A. torrentium*. On the 44th day of juvenile development, many individuals had moulted to third stage juveniles, whereas juveniles of *A. torrentium* reached this stage much later, after 72 days at ambient conditions (Figure 3).

Table III
   Ovigerous females of *Austropotamobius torrentium* during the winter 2002-2003, with number and growth of eggs during maintenance in laboratory culture.

Tableau III
   Femelles ovigères d'*Austropotamobius torrentium* durant l'hiver 2002-2003, avec le nombre et la croissance des œufs cultivés en laboratoire.

Female/locality	Date of collection	Carapace length [mm]	Weight [g] incl. eggs	Egg count	Egg diameter growth [mm] (N = 10)
Vi6/Vilsbiburg	30 Nov. 2002	31.85	9.7	70	2.38 - 2.9 in 142 days (last N = 3)
Da6/Dachsee	17 March 2003	42.50	19.9	110	2.73 - 2.81 in 23 days
Da17/Dachsee	17 March 2003	31.65	9.5	60	2.85 - 2.91 in 17 days

In addition to the higher fecundity and the faster juvenile growth of *Pacifastacus leniusculus*, a third factor in the early life history of these species, that may increase competitive advantage of the signal crayfish was observed in the wild : the earlier hatching of the juveniles of the signal crayfish (late May) as compared to the stone crayfish (mid June). This means that, in coexistence, juvenile signal crayfish would be already 2-3 weeks old and considerably larger than juvenile stone crayfish at their birth and would be the better competitors for shelter and even could predate on them. Juvenile mortality in the laboratory washigh due to predation (intra- and interspecific).

STREISSL and HÖDL (2002a) reported that both male and female stone crayfish in the Austrian Biberbach reached the age of sexual maturity, depending on water temperature and food supplies, after 3-4 years. The same authors estimated the relevant sexual size, according to increase of allometric growth in male chelae, which starts in males with a total length (TL) exceeding 50 mm. Female size of sexual maturity was estimated by catchments of berried females with lengths ranging from 59-65 mm. Male chelar size in crayfish in general is an important sexual trait, as also shown within the genus *Austropotamobius* by VILLANELLI and GHERARDI, 1998. The reason for the increase of chelar size is that they are closely linked to social behaviour, e.g. used during copulation and in competing for mates – *A. pallipes* female chooses the mate on the basis of his size and his potential dominance status – (VILLANELLI and GHERARDI, 1998).

Recalculating the total length from a linear regression with data of 28 *A. torrentium* individuals confirmed the results of STREISSL and HÖDL (2002a), that the relation TL to CL is sex-specific. The equations were only used for the age structure method (modified from BOHL, 1989), and to facilitate a comparison with literature data. In both species measured (*A. torrentium* and *P. leniusculus*) we found male individuals with proportionally large claws. Absolutely and relatively to CL signal crayfish claws were much bigger, which could be an important dominance factor in habitat displacement, as LEVENBACH and HAZLETT (1996) already stated. According to our data on the divergence in propodus height (prh) in different sexes, *A. torrentium* reaches the puberty moult at a carapace length of about 26 mm, while in *Pacifastacus leniusculus* the propodus height increased at a size of 36 mm CL (Figure 5). However, the smallest ovigerous stone crayfish female in this study measured only 22.5 mm CL, (45 mm TL, 63 eggs). Also GRANDJEAN *et al*. (2000) stated, that females of the sister species Austropotamobius *pallipes* reach maturity at a smaller size (CL 22-24 mm) than males (CL 26-29 mm). The possible disadvantage of larger size to reach sexual maturity in the signal crayfish is probably compensated by faster growth, as was already shown for juvenile individuals (Figure 3). It remains to be studied how long it takes signal crayfish to reach puberty in comparison to the 3-4 years that were determined for the stone crayfish by STREISSL and HÖDL (2002a).

Since *A. torrentium* is distinctly smaller than *P. leniusculus* and aggressive dominance is strongly size-dependent (STUCKI and ROMER, 2001; VORBUGER and RIBI, 1999a), it can be expected that the latter is also the dominant species as adults, even though *A. torrentium* did not show an increased frequency of limb loss living syntopically with larger species like *Astacus leptodactylus* or *Astacus astacus* (see STUCKI and ROMER, 2001). Direct interactions could also play an important role in species displacement (SÖDERBÄCK, 1995; STUCKI and ROMER, 2001; VORBURGER and RIBI, 1999a). In agonistic fight experiments, adult *Austropotamobius torrentium* had a higher preference for experimental shelters and often defended these even against larger *P. leniusculus* (VORBURGER and RIBI, 1999a), which may stress the importance of competitive advantages during the juvenile phase as stated above that will cause competitive displacement and can explain the ongoing invasion. Currently, juvenile individuals of both species are raised together in the laboratory to determine the starting point of possible interactions, the establishment of aggressive dominance and the long-term growth rates under artificial aquarium conditions.
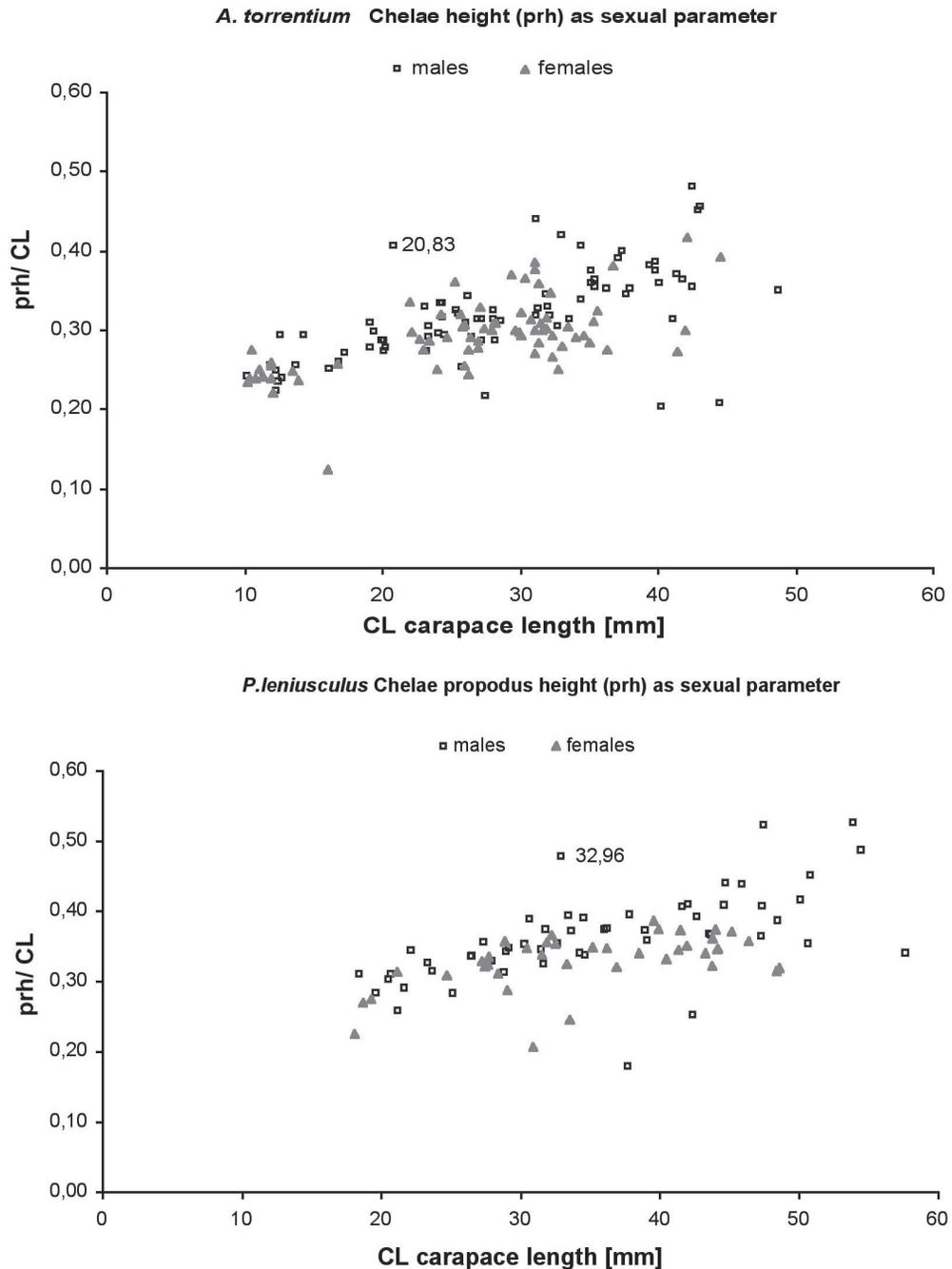
A. torrentium   Chelae height (prh) as sexual parameter

□ males   ▲ females

prh/ CL

□ 20,83

CL carapace length [mm]

P.leniusculus Chelae propodus height (prh) as sexual parameter

□ males   ▲ females

prh/ CL

□ 32,96

CL carapace length [mm]

Figure 5

Size relationships between chelar propodus height (prh) and carapace length (CL) in a) *Austropotamobius torrentium* **(various populations in Bavaria: m N = 78, f N = 71) and b)** *Pacifastacus leniusculus* **(from Sulzbach m N = 58 f N = 44) separated by sexes for optical determination of sexual maturity.**

Figure 5

Rapport entre la taille de chelar propodus (prh) et la longueur de la carapace (CL) chez a) *Austropotamobius torrentium* **(diverses populations en Bavière : m N = 78, f N = 71) et b)** *Pacifastacus leniusculus* **(de Sulzbach m N = 58 f N = 44) séparés par sexe pour une détermination optique de la maturité sexuelle.**

Finally, several concepts for management and conservation for some populations of *A. torrentium*, especially in the surroundings of Regensburg shall be proposed. In view of the great risk of importing the crayfish plague fungus together with carrier animals from outside (HOLDICH, 2000; OIDTMANN *et al*., 2002), all measures suitable to secure already established populations of fish and crayfish should have clear superiority over artificial stocking. In some cases, like the Wenzenbach tributaries, it would be necessary to prevent signal crayfish extending into the main river (if not already happened) and at the same time start controlled restocking with autochthonous individuals from adjacent rivers. No matter if stone or noble crayfish, viable populations adapted to the particular local conditions of climate and waters with their genetic pools are always indispensable for securing long-term survival of species. This study has shown that there has to be a stronger emphasis in protecting upper rivers stretches and in the importance of maintaining of the natural habitat structures, avoiding straightening, channeling in pipes or other water obstructions.

Another important factor is public awareness and could be strengthened with information politics in order to increase the knowledge about the different crayfish species in the public and especially in local fishermen and farmers before restocking ponds. In cooperation with public and governmental institutions a final aim could be to intensify fishing of alien crayfish, to enhance stocking with native crayfish in midstream waters and to preserve the genetic diversity in isolated native crayfish population, often restricted to upper courses of the drainage systems. We are currently in progress to carry out a genetic comparison of stone crayfish throughout German populations to determine the amount of gene flow and genetic similarity between them. Following the advises of a larger European scale mapping for conservation guidelines (HOLDICH, 2002), a complete mapping of Bavarian freshwaters crayfish could serve as a good basis for future goals and immediate conservation measures.

CONCLUSION

In this study we report several undocumented populations of the autochthonous stone crayfish *Austropotamobius torrentium* and noble crayfish *Astacus astacus* as well as three new populations of the introduced signal crayfish *Pacifastacus leniusculus* from Bavarian waters. In one case, we have determined a zone of overlap in the contact zone of native and alien crayfish species. A direct effect of the introduced species is evidenced by an upstream shift of the contact zone in the time range of three years. Furthermore, field and laboratory studies suggest ecological competitive advantages of *Pacifastacus leniusculus* due to higher fecundity and faster growth of juveniles. This could play an important role in species replacement in the surveyed Sulzbach. During field studies in 2003 it could be observed that reproduction cycle starts earlier in the invasive species (end of May) than in the stone crayfish (second week in June). That may also render an additional advantage to the older and larger signal crayfish juveniles in interspecific competition in the contact zone when competing for shelter and food. Several concepts for better management and conservation for populations of *A. torrentium*, especially in Bavaria are proposed.

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