THE GENUS AUSTROPOTAMOBIUS IN THE AUSSERFERN REGION (TYROL, AUSTRIA) WITH AN OVERLAP IN THE DISTRIBUTION OF A. TORRENTIUM AND A. PALLIPES POPULATIONS

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ABSTRACT

Native populations of the stone crayfish Austropotamobius torrentium in Tyrol (Austria) are restricted to the region of Außerfern, where the lake “Haldensee” and the river “Archbach” are known to hold intact populations. Both records are of biogeographical importance because they are the southernmost located stock of the Bavarian-Tyrolean distribution area. In addition, the “Haldensee” is one of the highest locations holding stone crayfish in Austria. “Plansee”, a lake within the Archbach catchment contains a dense population of Austropotamobius pallipes that was introduced about 80 years ago. During recent field surveys a gradual immigration of A. pallipes into the habitat populated by A. torrentium was noticed. We characterised the native populations of A. torrentium in Haldensee, its immediate outflow and in the river Archbach by means of population parameters and crayfish morphometry. The potential influence of the non-indigenous A. pallipes as intruder in the A. torrentium population is discussed.

Key-words: freshwater crayfish, habitat condition, migration barrier, hybridisation.

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INTRODUCTION

The stone crayfish (*Austropotamobius torrentium*, Schrank, 1803) and the white-clawed crayfish (*Austropotamobius pallipes*, Lereboullet, 1858) are highly endangered throughout Europe due to various reasons (e.g. FÜREDER, OBERKOFLER and MACHINO, 2002). *A. pallipes* inhabits freshwaters mainly in south-western and western Europe from Italy to Spain and the British Islands, whereas *A. torrentium* is naturally found east of the distribution area of *A. pallipes* with only small overlapping regions in the Balkan (HOLDICH, 2002). In Austria, *A. torrentium* is widely distributed in all provinces but *A. pallipes* was only recorded from two small regions – one each in Carinthia and Tyrol. In the Carinthian valleys Gitschtal and Gaittal several populations of the latter species were found (ALBRECHT, 1981; MACHINO and FÜREDER, 1996), and two populations were known within the Tyrolean region Außerfern (FÜREDER and MACHINO, 1996; FÜREDER and MACHINO, 1998). The species overlap in Tyrol is manmade as the white-clawed crayfish was introduced into the area some decades ago (FÜREDER and MACHINO, 1995). Individuals of this population were observed to invade the downstream population of *A. torrentium* despite several potential migration barriers (FÜREDER and MACHINO, 1996). The *A. torrentium* populations in Archbach and Haldensee are of great interest as they form the southernmost border of the species’ Bavarian-Tyrolean distribution area and are the only records of stone crayfish in Tyrol (FÜREDER and HANEL, 2000).

Taking into account the still unclear species allocation of the *A. pallipes* complex and comparing the situation with that described in North America, where different species often are restricted to relatively small areas and show high potential for hybridisations when brought together (PERRY, FEDER and LODGE, 2001), this could also be possible within the genus *Austropotamobius* in Europe. Mixed populations of stone and noble crayfish (*Astacus astacus*, L.) are known from Bavaria (M. KELLER, pers. comm.), but other than within *Austropotamobius*, no hybridisation is expected between the different genera.

Because of the special situation of the co-occurrence of the two *Austropotamobius* species in a river in the region of Außerfern this study was carried out. Herein we characterise the populations from both species and investigate whether the actual situation poses threats to the native *A. torrentium*.

MATERIAL AND METHODS

Study-area

The region of Außerfern is situated in the north-western part of North Tyrol and is considered to originally been inhabited by the stone crayfish. Today crayfish populations occur in the following water bodies and sites:

*Haldensee (HAL) and its immediate outflow*

Haldensee lies in the Tannheimertal at an altitude of 1,124 m a.s.l. and holds one of the highest elevated crayfish populations in Tyrol (MACHINO and FÜREDER, 1998). It has a surface area of 72.7 ha and a maximum depth of 22 meter (TIROLER LANDESREGIERUNG, 2005). The first section of the Berger Ache, which is the only outlet in the north-western side of the lake, is populated by *Austropotamobius torrentium*. Along the northern side, the lake is bordered by a road, whereas the eastern shore is shallower and surrounded by meadows. Parts of the western shoreline are highly frequented by swimmers in summer. There only at a few spots, reed is providing for a more heterogeneous habitat. A forest reaches to the southern lakeshore, which in most areas is steeper and the bottom consists of rocks of varying size. Thus, shoreline and bottom substrate offer rich shelter for crayfish. This, together with the reed stands, is the only part of the lake where stone crayfish are found in the lake littoral. Scuba divers reported their presence also in deeper waters. In this study the animals from the lake as well as from the outlet are included.
Plansee (PLS)

Plansee is a lake near the town of Reutte at 976 m a.s.l., it has a maximum depth of 78 m and is connected to the Heiterwanger See through a narrow channel. Both lakes have a combined surface area of 2,900 ha (TIROLER LANDESREGIERUNG, 2005) and are used to generate electricity from hydrodynamic power. FÜREDER (2002) recorded dense populations of *Austropotamobius pallipes* along the northern and eastern lakeshore of Plansee, whereas Heiterwanger See is inhabited by the same crayfish species, but at lower densities. These populations were founded through introduction, which probably took place around 1920 (FÜREDER and MACHINO, 1995).

Archbach (ABU)

The river Archbach is the outlet of Plansee and drains into the river Lech. The river continuum is interrupted several times, not only by the natural Stuiben Waterfalls, but also by retaining walls and power stations for hydropower generation. Even though considerable water-level fluctuations of up to about 1.5 m per day occur, the river is populated with *A. torrentium* near the village of Pfisch (840 m) and several other river sections (FÜREDER L., unpubl. data). Above and below a hydropower dam crayfish were observed walking around in very shallow to almost no water during the night. Recently, this reach of the Archbach was modified by flood control measures, but habitat availability for crayfish was considered, and measures were defined to include effective shelter between rocks (FÜREDER L. unpubl. data).

Hüttenmühlsee (HMS)

Hüttenmühlsee is an impoundment within the river Archbach, formed by a retaining wall and lying upstream of the above mentioned stretch near Pfisch. Some sections of the bank are affected by measures of flood protection, and like the areas further downstream, they offer a suitable habitat for crayfish. Some areas of the bottom are covered with *Chara* sp., which offers shelter for crayfish too. Daily water level fluctuations are generally less than 0.5 m in this area. Recently, an increasing number of *A. pallipes* was found within the native *A. torrentium* population in Hüttenmühlsee, especially in the upper parts of the water storage area (Table I).

<table>
<thead>
<tr>
<th>date</th>
<th>location</th>
<th>AUT</th>
<th>AUP</th>
<th>AUT / AUP</th>
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</thead>
<tbody>
<tr>
<td>20.09.2004</td>
<td>HMS</td>
<td>56</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>14.10.2004</td>
<td>HMS</td>
<td>29</td>
<td>13</td>
<td>2.23</td>
</tr>
<tr>
<td>15.10.2004</td>
<td>HMS</td>
<td>8</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>21.09.2004</td>
<td>ABU</td>
<td>33</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>14.10.2004</td>
<td>ABU</td>
<td>11</td>
<td>2</td>
<td>5.5</td>
</tr>
</tbody>
</table>
Crayfish were caught during night hours by hand with the help of powerful spotlights. In addition baited traps were exposed overnight whenever it was possible. In Archbach and Hüttenmühlsee, both species *A. torrentium* and *A. pallipes* have been observed walking side by side and were found in one trap. This leads to the assumption that here both species share the same habitat.

**Morphometry**

A total number of 142 crayfish (37 specimens of *A. torrentium* from HAL, 30 *A. pallipes* from PLS, 33 *A. torrentium* and 1 *A. pallipes* from ABU, 33 *A. torrentium* and 8 *A. pallipes* from HMS) was investigated for this study. For all individuals the following morphological characters were measured with a calliper to the nearest 0.1 mm: Claw length (CLL), claw width (CLW) and claw height (CLH), length of the claw palm (CPL), length of the claw finger (CFL), rostrum length (ROL) and rostrum width (ROW), head length (HEL) and head width (HEW), areola length (ARL) and areola width (ARW), abdomen length (ABL), abdomen width (ABW, at the second abdominal segment) and abdomen height (ABH, at the second abdominal segment), telson length (TEL) and telson width (TEW), carapace width (CPW), width at the cervical groove (CGW), width of the carapace at the hind edges (CEW), and carapace height (CPH, between the second and third pereopod) (Figure 1 A and B). Total length (TL) was measured to the nearest 1.0 mm by placing the crayfish in a stretched position on its back on scale paper. All bilateral parameters were measured on the right side except when injuries or regenerations were observed which chiefly affected the claws. In this case measurements were taken on the left side. Weight was recorded to the nearest 0.1 g. Post orbital length (POL) was calculated as HEL + ARL, carapace length (CPL) as ROL + HEL + ARL. Beside these measurements, the sex and the number of injuries, counted as missing or injured claws, legs, antennae, carapace and abdomen, were recorded.

To avoid misleading comparisons between adults and juveniles, crayfish with TL less than 60 mm and 50 mm for *A. pallipes* and *A. torrentium*, respectively, were excluded from the analysis. These size limits were chosen based on observations in the field and on findings by GRANDJEAN *et al.* (1997) and STREISSL and HÖDL (2002). Males and females were analysed separately, as they are known to show sexual dimorphism (GRANDJEAN *et al.*, 1997; GRANDJEAN and SOUTY-GROSSET, 2000; STREISSL and HÖDL, 2002).

Beside general population examinations, a stepwise multivariate discriminant analysis was performed. The size normalization procedure and selection of the relevant morphological characters was done as described in SINT, DALLA VIA and FÜREDER (2005). In addition, several ratios (e.g. claw length to claw width, CLL/CLW) were calculated and included in the analysis. The populations of Plansee (PLS) and Haldensee (HAL) were used to create a model for discriminating *A. torrentium* and *A. pallipes* which was then applied to the animals of the mixed populations of Archbach (ABU) and Hüttenmühlsee (HMS).

All the analyses were performed using Microsoft® Excel 2002, SP-2 and SPSS for Windows 11.0.1.

**RESULTS**

A total number of 148 crayfish (78 males and 70 females) was caught in autumn 2004. They consisted of 109 individuals of *A. torrentium* from three populations and 39 individuals of *A. pallipes* from 2 populations. Six specimens of *A. torrentium* from HAL had to be excluded due to a TL below 50 mm, thus a valid number of 142 crayfish was used for the analyses.

The percentage of injured individuals was higher for females than for males and higher for *A. torrentium* than for *A. pallipes*. Within the females of *A. pallipes* 85.7% were
injured in HMS and 76.5% in PLS, for males the rate of injured individuals was 54.5% in HMS and 66.1% in PLS. For *A. torrentium* the number of injured crayfish was highest in HMS (females: 73.3%, males: 50.0%) followed by HAL (females: 60.0%, males: 38.1%) and ABU (females: 40.0%, males: 33.3%).

Within the species, the females were not only more often injured but had also a higher number of injuries per individual, which was significant for *A. torrentium* (*p* = 0.001),
but not for *A. pallipes* (p = 0.466). Between the species the mean number of injuries was higher for *A. pallipes* than for *A. torrentium*, but no significant difference was found, either for males (p = 0.071), or for females (p = 0.181) (Table II).

The ratio of *A. torrentium* to *A. pallipes* in the total catch clearly decreased from September to October for both locations with mixed populations (Table I).

The ratio of males to females showed only small differences between the crayfish populations. For stone crayfish it was between 0.95 in HAL and 1.20 in HMS and ABU. For white-clawed crayfish it was 1.14 in PLS and 1.67 in HMS (Table II).

Both sexes of *A. pallipes* showed higher TL, CLL and weight in HMS than in PLS, but only for the females the difference was significant (p < 0.001). Within the males of *A. torrentium* the individuals from HAL were significantly smaller (TL, CLL) and weighed less than those from HMS (p ≤ 0.001). The females from HAL differed in all three characters significantly from those from HMS (p < 0.001) and ABU (p < 0.05).

In the mixed populations, in all cases the means of TL, CLL and weight were higher for *A. pallipes* than for *A. torrentium*, but a statistically significant difference was only found for females (p < 0.001).

Within all populations of both species the highest average TL was found for individuals of *A. pallipes* from HMS (for males: 91.4 mm; females: 88.7 mm), and the

### Table II

<table>
<thead>
<tr>
<th>Population</th>
<th>Species</th>
<th>Sexes</th>
<th>m / f</th>
<th>CLL (mm)</th>
<th>TL (mm)</th>
<th>Weight (g)</th>
<th>Injuries</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLS AUP</td>
<td>males</td>
<td>16</td>
<td>1.14</td>
<td>37.3 (8.7)</td>
<td>78.9 (10.1)</td>
<td>20.8 (9.1)</td>
<td>0.9 (0.2)</td>
</tr>
<tr>
<td>HMS AUP</td>
<td>males</td>
<td>5</td>
<td>1.67</td>
<td>42.0 (8.9)</td>
<td>91.4 (9.7)</td>
<td>30.8 (10.7)</td>
<td>0.8 (0.4)</td>
</tr>
<tr>
<td>ABU AUP</td>
<td>males</td>
<td>18</td>
<td>1.20</td>
<td>36.5 (8.3)</td>
<td>78.4 (10.6)</td>
<td>21.9 (10.1)</td>
<td>0.5 (0.2)</td>
</tr>
<tr>
<td>HAL AUP</td>
<td>males</td>
<td>18</td>
<td>1.20</td>
<td>30.6 (3.4)</td>
<td>72.8 (6.8)</td>
<td>15.9 (2.7)</td>
<td>0.7 (0.2)</td>
</tr>
<tr>
<td>PLS AUP</td>
<td>females</td>
<td>14</td>
<td>1.14</td>
<td>19.6 (1.6)</td>
<td>66.5 (4.6)</td>
<td>8.0 (1.7)</td>
<td>2.2 (0.4)</td>
</tr>
<tr>
<td>HMS AUP</td>
<td>females</td>
<td>3</td>
<td>1.67</td>
<td>29.2 (2.9)</td>
<td>88.7 (4.9)</td>
<td>19.9 (2.6)</td>
<td>1.3 (0.9)</td>
</tr>
<tr>
<td>ABU AUP</td>
<td>females</td>
<td>15</td>
<td>1.20</td>
<td>23.1 (2.5)</td>
<td>68.7 (5.3)</td>
<td>10.3 (2.7)</td>
<td>0.8 (0.3)</td>
</tr>
<tr>
<td>HAL AUP</td>
<td>females</td>
<td>15</td>
<td>1.20</td>
<td>21.5 (1.9)</td>
<td>64.9 (4.3)</td>
<td>8.6 (1.8)</td>
<td>0.9 (0.2)</td>
</tr>
<tr>
<td>PLS AUT</td>
<td>females</td>
<td>22</td>
<td>0.95</td>
<td>18.2 (1.9)</td>
<td>58.1 (5.2)</td>
<td>6.6 (1.7)</td>
<td>0.8 (0.3)</td>
</tr>
</tbody>
</table>

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smallest in specimens from HAL (males: 64.7 mm, females: 58.1 mm). The highest mean CLL was also found in the *A. pallipes* population from HMS for males (42.0 mm) and females (29.2 mm), and the smallest in specimens of *A. pallipes* from HAL (males: 25.8 mm, females: 18.2 mm; Table II, Figure 2A).

The weight was strongly correlated to TL and CLL ($r^2 > 0.9$, $p < 0.01$). As a consequence, for white-clawed crayfish it was highest in HMS and lowest in HAL.

The ratio of CPL: CPH was significantly higher for both sexes in *A. torrentium* than in *A. pallipes* ($p < 0.001$) (Figure 2B).

Multivariate discriminant analysis was well suited to distinguish *A. pallipes* from *A. torrentium*. 100% of the original and of the cross-validated cases from PLS and HAL were correctly classified and the eigenvalues were high (27.5 for males, 37.9 for females). The derived model was based on different morphological characters for males and females, these were CLL, ABB, CLL/CLW, CLL/CFL, ARL/ARW for males and CFL/CPL, ROL/ROW, ARL/ARW, CPL/CPH, TEL/TEW and TL/CPL for females. Details on the loadings are given in Table III.

When the frequency of discriminant values was plotted for all four populations, two well-separated groups corresponding to the two species became visible around the centroids (males: 4.376 and -5.835, females: 5.929 and -5.929), with only a few crayfish in-between for both sexes (Figure 3).

Figure 2
Box plots (median [line], quartiles [box], 10th and 90th percentiles [whiskers]) of the total length (A) and the ratio of carapace length to carapace height (CPL/CPH) (B) of male crayfish individuals in Plansee (PLS), Hüttenmühlsee (HMS), Haldensee (HAL) and Archbach (ABU).
Table III
Discriminant Analysis: Standardized canonical discriminant function coefficients for males and females for the morphological parameters (see Figure 2A for location). Eigenvalue, and canonical correlation coefficient (ccc) are given.

<table>
<thead>
<tr>
<th>morphol. parameter</th>
<th>discriminant function</th>
<th>morphol. parameter</th>
<th>discriminant function</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL</td>
<td>1.036</td>
<td>CFL / CPL</td>
<td>-0.509</td>
</tr>
<tr>
<td>ABW</td>
<td>0.921</td>
<td>ROL / ROW</td>
<td>1.076</td>
</tr>
<tr>
<td>CLL / CLW</td>
<td>1.114</td>
<td>ARL / ARW</td>
<td>-0.768</td>
</tr>
<tr>
<td>CLL / CFL</td>
<td>0.832</td>
<td>CPL / CPH</td>
<td>-0.890</td>
</tr>
<tr>
<td>ARL / ARW</td>
<td>-0.939</td>
<td>TEL / TEW</td>
<td>1.401</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TL / CPL</td>
<td>0.727</td>
</tr>
<tr>
<td>eigenvalue</td>
<td>27.502</td>
<td>eigenvalue</td>
<td>37.855</td>
</tr>
<tr>
<td>ccc</td>
<td>0.982</td>
<td>ccc</td>
<td>0.987</td>
</tr>
</tbody>
</table>

DISCUSSION

It is well known that differences in crayfish morphology may occur not only on the level of species but also between distinct populations, caused by adaptations to local environmental conditions (GRANDJEAN and SOUTY-GROSSET, 2000; SINT, DALLA VIA and FÜREDER, 2005).

In this study, both surveyed species *Austropotamobius pallipes* and *A. torrentium* had smaller individuals at higher elevation (PLS and HAL). This may be attributed to the cooler water temperatures resulting in a shorter active phase and therefore also shorter period of growth during the summer months. This becomes apparent in Haldensee, where the water temperature was about 1.5 to 2°C lower than in Archbach and Hüttenmühlsee at every measurement. This hypothesis is also supported by the fact that we found very small egg-bearing females of *A. torrentium* in June (TL min 46 mm; mean 58 mm), while STREISSL and HÖDL (2002) reported the onset of maturity for stone crayfish females in the size class of 59-65 mm. Intraspecific size differences could be disregarded because all data were normalized for size and small animals were excluded from statistical analysis in order to assure comparisons only between mature crayfish. The observed size differences between *A. torrentium* and *A. pallipes* were expected, because it is well known that *A. pallipes* can reach a TL of up to 12 cm (MACHINO et al., 2004; HAGER, 1996) while *A. torrentium* reaches only about 10 cm (HAGER, 1996).

The sex-ratio was found to be close to 1 in all populations, which corresponds well to former findings (STREISSL and HÖDL, 2002; GRANDJEAN and SOUTY-GROSSET, 2000).

Although individuals of *A. pallipes* were larger than those of *A. torrentium*, they had more non-lethal injuries than the latter in the mixed populations from ABU and HMS.
(Table II). This could indicate the occurrence of altered inter- or intraspecific competition (STUCKI and ROMER, 2001). Different crayfish species are known to have different aggression potentials (WESTMAN and SAVOLAINEN, 2001; VORBURGER and RIBI, 1999; FURRER, CANTIENI and DUVOISIN, 1999; SÖDERBÄCK, 1995), but up to now no comparative investigations dealing with the aggression potentials of *A. pallipes* and *A. torrentium* living in the same habitat have been performed. According to the findings of VORBURGER and RIBI (1999), *A. torrentium* is able to exclude even larger specimens of the signal crayfish (*Pacifastacus leniusculus*, (Dana, 1852)) from shelter and may show a generally more aggressive behaviour than the latter. Thus, the greater number of injuries observed on white-clawed crayfish in HMS could well be due to interspecific competition with stone crayfish. This relatively higher aggression of *A. torrentium* is also supported by observations during field work where they showed much more aggressive behaviour towards the researchers’ hands during handling than did white-clawed or noble crayfish.

However, the increasing numbers of *A. pallipes* in Hüttenmühlsee – as has been found recently - argue against such an interspecific dominance of *A. torrentium*. This points to the possibility that in this particular case *A. pallipes* may have an advantage,
probably due to its larger size, since dominance is strongly connected to total length and aggressive interactions are mainly found between equal sized crayfish (VORBURGER and RIBI, 1999).

The results of the multivariate discriminant analysis allow a very good distinction between *A. pallipes* and *A. torrentium* as the variation between the two species is higher than the within species variation needed for distinction (BÜHL and ZÖFEL, 2002; HAIR et al., 1998). This was also found for the differentiation between *A. pallipes* and *A. astacus* (SINT, DALLA VIA and FÜREDER, 2005). However, there is also a small number of individuals with a discriminant value around zero for both sexes (Figure 3) and they could not be allocated to one of the two species with absolute certainty. This is confirmed by the fact that in some cases it was not easy to determine the exact species during fieldwork. According to CESARONI, ALLEGRUCCI and SBORDONI (1992) as well as PERRY, FEDER and LODGE (2001) hybrids are known to show morphological characters of both parental species, but no information is available about hybridisation between *A. torrentium* and *A. pallipes*. Interbreedings have already been reported for the genera Astacus (FURRER, CANTIENI and DUVOISIN, 1999), Orconectes (PERRY, FEDER and LODGE, 2001; PERRY et al., 2001) and Procambarus (CESARONI, ALLEGRUCCI and SBORDONI, 1992) although in some cases the outcome of the mating between two different species is not clear. While FURRER, CANTIENI and DUVOISIN (1999) reported hybrids between *A. leptodactylus* females and *A. astacus* males with a slightly altered chela shape, they did not find any surviving offspring when *A. astacus* females were paired with *A. leptodactylus* males. *Astacus astacus* was also observed to mate with *Pacifastacus leniusculus*, but this did not result in viable offspring (SÖDERBÄCK, 1995).

Hybridisation is considered to be one of the main threats to endangered species - the more, the closer related the parental species are (PERRY, LODGE and FEDER, 2002; RHYMER and SIMBERLOFF, 1996). So in North America, where the rusty crayfish (*Orconectes rusticus* (Girard, 1852) was originally restricted to the tributaries of the Ohio River; today it is displacing *O. propinquus* (Girard, 1852) and *O. virilis* (Hagen, 1870) due to hybridisation and competition, wherever it was introduced by man (PERRY, LODGE and FEDER, 2002; PERRY et al., 2001). So the invasion of potential competitors may pose an additional threat to the autochthonous stone crayfish.

Thus, it is of fundamental importance to know whether these two species can coexist and whether hybrids are generated, e.g. in Archbach, because only few populations of *A. torrentium* still exist in Tyrol (FÜREDER and MACHINO, 1996; FÜREDER and MACHINO, 1999). Hydropower development, habitat degradation and river engineering have substantially contributed to the decline of this endangered crayfish species in the region. Conservation measures that have recently been implemented, may improve the situation (FÜREDER L. unpubl. data). In the necessary flood protection measures at several river sections activities to extend and improve the crayfish habitat were included. A larger area with side channels offering heterogeneous aquatic habitats were considered in the river management plan. How the situation further develops highly depends on the close collaboration between water district managers, river engineers and nature conservation authorities.

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