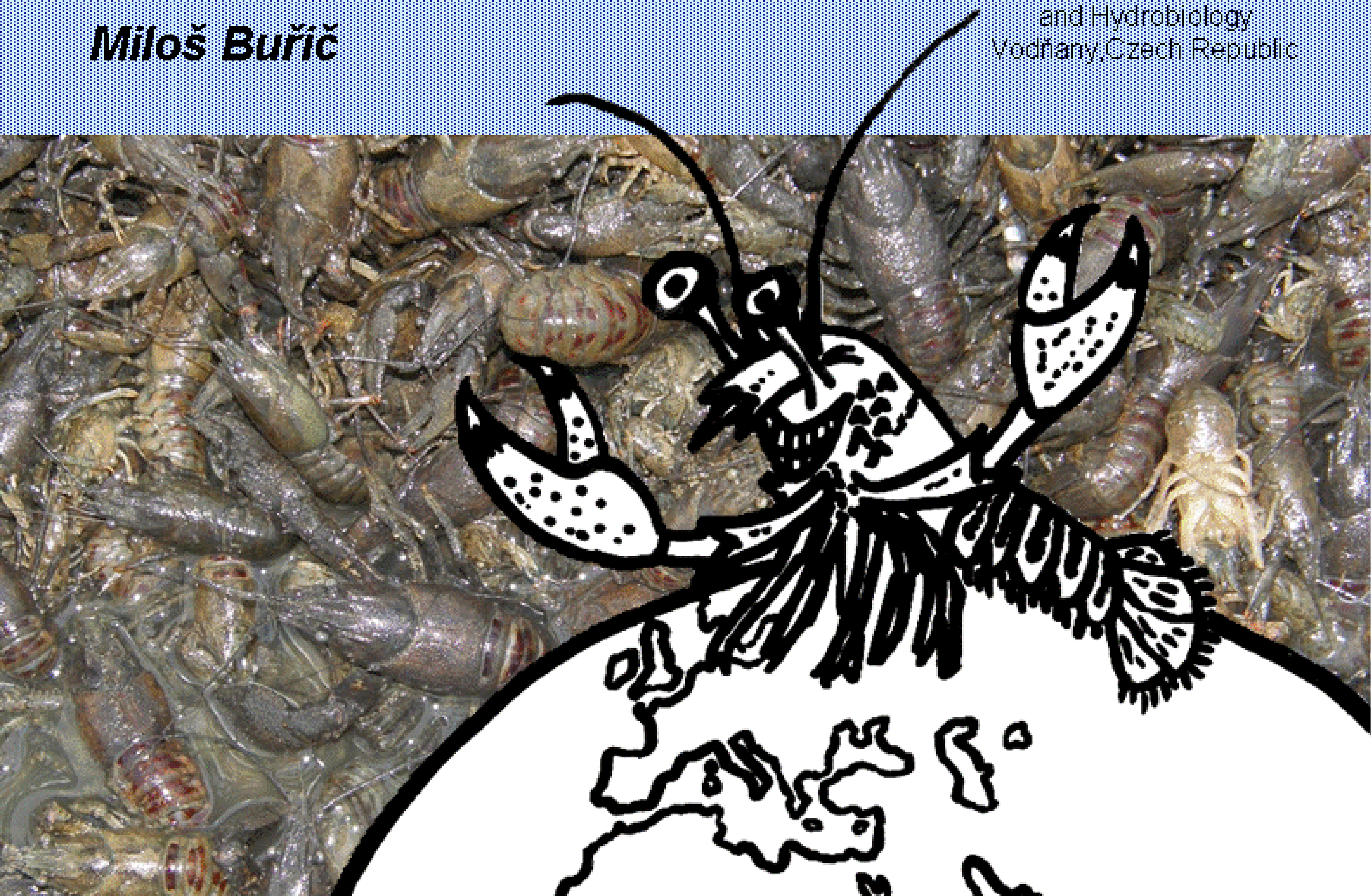
Biology of spiny-cheek crayfish (*Orconectes limosus*, Rafinesque, 1817) under conditions of the Czech Republic and the study of factors influencing its invasive spreading

Biologie raka pruhovaného (*Orconectes limosus*, Rafinesque, 1817) v podmínkách ČR a studium faktorů ovlivňujících jeho invazní šíření

University of South Bohemia
České Budějovice
Research Institute of Fish Culture
and Hydrobiology
Vodňany,Czech Republic

Miloš Buřič



Biology of spiny-cheek crayfish (*Orconectes limosus*, Rafinesque, 1817) under conditions of the Czech Republic and the study of factors influencing its invasive spreading

Biologie raka pruhovaného (*Orconectes limosus*, Rafinesque, 1817) v podmínkách ČR a studium faktorů ovlivňujících jeho invazní šíření

Miloš Buřič

Rector of University of South Bohemia České Budějovice (Prof. PhDr. Václav Bůžek, PhD.) and director of USB RIFH (Prof. Dipl.-Ing. Otomar Linhart, DSc.) will award Ph.D. in fisheries (24th September 2009, at 14:00 in USB RIFH) to the author of the thesis. From that time the author of the thesis can use the title Ph.D.

Supervisor:
Assoc. Prof. Dipl.-Ing. Pavel Kozák, Ph.D.
University of South Bohemia České Budějovice (USB)
Research Institute of Fish Culture and Hydrobiology (RIFCH)
Zátiší 728/II
389 25 Vodňany
Czech Republic

Head of Laboratory of Ethology and Nutrition of Fish and Crayfish:
Assoc. Prof. Dipl.-Ing. Pavel Kozák, Ph.D.

Director of USB RIFCH:
Prof. Dipl.-Ing. Otomar Linhart, DSc.

Board of doctorate study defense with referees:
Prof. Dipl.-Ing. Vlastimil Baruš, DSc. – head of the board
Prof. Dipl.-Ing. Petr Ráb, DSc. – board member
Prof. Zdeňka Svobodová, DVM, DSc. – board member
Assoc. Prof. Stanislav Navrátil, DVM, Ph.D. – board member
Assoc. Prof. M.Sc. Zdeněk Adámek, Ph.D. – board member
Assoc. Prof. M.Sc. Jana Pěknicová, Ph.D. – board member
Assoc. Prof. M.Sc. Josef Matěna, Ph.D. – board member
Assoc. Prof. M.Sc. Zdeněk Ďuriš, Ph.D. – referee
Prof. Francesca Gherardi, Ph.D. – international referee
Prof. Premek Hamr, Ph.D. – international referee

Date, and place of Ph.D. defense:
3rd September 2009, USB RIFCH, Vodňany

Name: Miloš Buřič

Title of thesis: Biology of spiny-cheek crayfish (*Orconectes limosus*, Rafinesque, 1817) under conditions of the Czech Republic and the study of factors influencing its invasive spreading

Ph.D. thesis, USB RIFCH Vodňany, 2009, 145 pages, with a summary in English and Czech.

ISBN: ??-?????-??-?

Contents

Chapter 1

General Introduction

1. Introduction

Human activities move many species out of their native ranges, where they may establish and have substantial ecological effects on native communities (Mack et al. 2000; Vitousek et al. 1996). A group of invaders with particularly important ecological effects are crayfish. These animals have often been introduced outside their native ranges by deliberate stocking (Hobbs et al. 1989). The large sphere of action for crayfish invaders are European inland waters, where native crayfish stock were depopulated in 19th century due to crayfish plague (Holdich et al. 1999). Apart from plague, other negative factors include pollution of surface waters, habitat alterations (especially unnecessary watercourse regulation resulting in shelter losses), competition with non-native species, or predation. In some regions, recent climate fluctuations also negatively influenced populations of native crayfish (Diéguez-Uribeondo 2006).

Non-indigenous crayfish (NIC) were introduced in Europe as crayfish plague resistant substitution for indigenous crayfish (IC), which should revive depleted bentic habitats. Other reasons for NIC introduction were: aquaculture, reduction of water vegetation and even reduction of animal pests (Henttonen and Huner 1999). Unfortunately, there were not assessed risks and threats associated with alien introductions into European freshwater ecosystems (Holdich et al. 1999). Once established, these animals may eliminate remaining IC and reduce populations of favoured food items such as gastropods, algae and macrophytes, which can have cascading trophic effects elsewhere in the ecosystem (Klocker and Strayer 2004; Nyström et al. 1996).

Another invisible threat was passed over. The crayfish from North America introduced to Europe (*Orconectes limosus,* Rafinesque 1817; *Pacifastacus leniusculus* Dana 1852; *Procambarus clarkii*, Girard 1852) often carry the crayfish plague pathogen (*Aphanomyces astaci* Schikora) and may transmit it to the IC populations (Vogt 1999). With the spread of NIC in recent decades, the crayfish plague outbreaks have regained intensity recently (Holdich 2003; Kozubíková et al. 2008). Furthermore, the IC can also be displaced through direct interactions or competition for resources with the non-native species (Holdich et al. 1999). Aggression and resource competition are important parts of crayfish interactions (Söderback 1991). Sympatric crayfish species compete for limited resources such as food, shelter and space, and larger crayfish routinely win competitive interactions with smaller individuals (Momot 1984). NIC are very well equipped for competition with IC due to early maturation, high fecundity, fast growth and high level of activity and aggressiveness (Lindqvist and Huner 1999).

NIC spreading causes recently the most important threat for IC stocks in Europe (Füreder et al. 2006), including Czech Republic (Petrusek et al. 2006). Hence, the knowledge about crayfish invaders (distribution, life cycle, ecology) is important to assess their invasive potential and develop an effective management to suppress their spreading. *Orconectes limosus* is actually the most distributed and actively spreading crayfish in Czech Republic, and the second most widespread NIC at European level.

2. Description and origin

2.1. Description

Orconectes limosus is a small-bodied species, with a total length (TL) reaching 5 to 8 cm in adult animals (Holdich et al. 2006; Holdich and Black 2007), rarely up to 12 cm (Pöckl et al. 2006). It can be distinguished by prominent lateral spines in front of the cervical groove and one pair of long post-orbital ridges with apical spine. The chelae are smooth and upper surface is covered with rows of pits. The color of the body is pale or dark brown or olive-green, tips of chelae are orange with black band visible on underside. A transverse brown-red band is present across each abdominal segment and on the pleura (Hamr 2002; Holdich et al. 2006; Pöckl et al. 2006).

2.2. Origin

Orconectes limosus, a temperate freshwater crayfish, is originally native to the east coast of the USA and Canada (Atlantic watershed) where it occurs in Quebec, New Brunswick, Vermont, Massachusetts, Rhode Island, New Jerey, New York, Connecticut, Delaware, District of Columbia, Maryland, Pennsylvania, Virginia and West Virginia (Hobbs 1974; Hamr 2002) and is a member of the family Cambaridae (Arthropoda: Decapoda). Within the cambarids, *Orconectes* is the 3rd most diverse genus, with 88 taxa (Taylor 2002).

3. Distribution in Europe and the Czech Republic

3.1. Europe

The first successful introduction into Europe took place in 1890 when some specimens were stocked into a fishpond in Barnowko (Poland). Further successful introductions in many European water-bodies, combined with natural dispersal, resulted in its presence in at least 20 European countries (Henttonen and Huner 1999; Holdich et al. 2006; Pöckl et al. 2006). *Orconectes limosus* is now present in Austria, Belarus, Belgium, Croatia, Czech Republic, England, France, Germany, Hungary, Italy, Lithuania, Luxembourg, Netherlands, Poland, Russia, Serbia, Slovakia, Switzerland (Pöckl et al. 2006), and probably also in Bulgaria, Romania, and Ukraine (Holdich et al. 2006).

2.2. The Czech Republic

Orconectes limosus is one of five crayfish species that are currently present in open waters of the Czech Republic. Although the first report of the presence of the spiny-cheek crayfish in the Czech territory was published at the end of the 1980's, the species seems to have been present in the Elbe river already in the 1960's. By the end of 20th century, it had spread widely in the Elbe watershed, due to its natural dispersal potential as well as a result of anthropogenic translocations (Kozák et al. 2004; Petrusek et al. 2006).

This species occurs in the Elbe and Vltava river systems and adjacent localities (ponds, sand pits etc.) in the Czech Republic (Petrusek et al. 2006), where it can obstruct re-establishment of IC (Holdich et al. 2006). The core of *O. limosus* distribution in the Czech Republic is the Elbe and Vltava rivers, with a high presence in the larger tributaries (e.g. Ohře, Jizera, Cidlina, Metuje, Úpa, Lužnice, Sázava) of these rivers. It is also found in smaller streams (i.e. those with a low stream order) which join the larger rivers. In the latter crayfish usually stay within a few hundred meters of the convergence with the major watercourse. The presence of the spiny-cheek crayfish close to the mouth of most small streams does not necessarily indicate a viable population, as the source population may be in the adjacent larger river or reservoir (Petrusek et al. 2006).

3. Habitat requirements

Orconectes limosus inhabits (in its natural range) soft-bottomed, silty, turbid waters such as large rivers, large streams and lakes with abundant aquatic vegetation (Hamr 2002). In

Europe it occurs in a variety of habitats such as rivers, lakes, and canals. It can occupy cooler, faster waters, but prefers calm, deep waters as ponds and lakes (Dehus et al. 1999). Although small brooks and streams are apparently not suitable habitats for *O. limosus* (Holdich et al. 2006), it was found in such habitats in Canada (McAlpine et al. 1991). *Orconectes limosus* shows tolerance to organic-rich waters and general pollutants (Holdich et al. 2006). It can burrow extensively (Holdich and Black 2007) and it is tolerant of its habitat drying out for a number of weeks (Holdich et al. 2006). In general it is very adaptable species able to withstand relatively unfavorable conditions.

4. Life history

4.1. Growth and maturation

American crayfish in Europe are usually characterized by high growth rates and early maturity (Lindqvist and Huner 1999). *Orconectes limosus* fully meet these characteristics. More molts, in contrast to molt increments, resulted in faster growth in crayfish (Lowery 1988). Hence the hatchlings of *O. limosus* grow faster than *Astacus astacus* (Linnaeus 1758) (Kozák et al 2007), when undergo 9 – 11 molts in their fist growing season (Andrews 1907; Price and Payne 1984). The high molt frequency could be a reason of high mortality rates in *O. limosus* during first year (Lindqvist and Huner 1999).

Orconectes limosus usually measure 18 – 30 mm carapace length (CL) in first winter (Holdich et al. 2006), which correspond with total length (TL) 22 – 60 mm (Andrews 1907) and 40 – 65 mm (Pieplow 1938) found after first growing season. Maturity is usually attained in the second summer at about 25 – 35 mm CL (Hamr 2002), but the mature and sexually active specimens were observed at the end of their first growing season (Kozák et al. 2007).

Crayfish decrease their growth after maturation (Reynolds 2002). Adult *O. limosus*, similarly as noble crayfish *Astacus astacus* (Skurdal and Taugbøl 2002), usually molt once (ovigerous females) or twice (males, females) per year (Holdich and Black 2007). In contrast Pieplow (1938) observed 3 molts per year for adult males and even females. Crayfish can reach 65 – 80 mm and 80 – 95 mm TL at the end of second and third year, respectively (Pieplow 1938). The average life span is 2 years, maximum life span is 4 years, and maximum size is 61 mm CL (Andrews 1907; Smith 1981; Van Den Brink 1988).

4.2. Form alternation

Cambarid males are known as cyclically dimorphic (Scudamore 1948; Hobbs 1989). Form I is first reached at the final juvenile molt (Suko 1953; Hobbs 2001). Form I (sexually active) and form II (sexually inactive) males occur in all known orconectid species (Hobbs, 1974). The change from the one form to another occurs among mature males during the semiyearly molts i.e. molting allows adult male crayfish to alternate from form I to form II and back to the reproductive stage (Hobbs 2001, Guiasu, 2002). It is generally believed that form II males revert to form I at the subsequent molt, as they normally undergo two molts per year (Capelli and Magnuson 1975; Smith 1981), but there is evidence that some form II males may go through two or more molts before reverting to the form I state (Payne 1978; Mazlum et al. 2007).

The breeding form is more aggressive (Bovbjerg 1956; Tierney et al. 2008) and can be distinguished by the sclerotization, amber-coloring, and lengthening of the terminal elements of the first pleopods (Andrews 1910; Pieplow 1938). In addition, the ischial hooks are more pronounced (Andrews 1910; Hobbs 2001), the first chelipeds are enlarged (Suko 1953; Eversole et al. 2006), and the sperm ducts are filled with recently formed spermatids

(Andrews 1910; Pieplow 1938). In form II males, the terminal elements of the first pleopod are not as well differentiated (and never corneous), and the ischial hooks become shorter and weaker (Pieplow 1938; Scudamore 1948).

In general it is assumed that adult females undergo only a single (summer) molt, do not undergo the form alternation, and grow only during this one summer molt (Boyd and Page 1978; Hamr and Berrilll 1985). That appears from females need, to delay their molt till release of dependent offspring (Scudamore 1948). But there is the evidence that orconectid females can undergo also the second (Scudamore 1948) or third molt (Pieplow 1938).

Wetzel (2002) found that females, like males, can undergo two molts each year from form I to form II in spring, and from form II back to form I in fall, and form I females have wider abdomens than form II conspecifics. It is not yet known how widespread this phenomenon is in other crayfish species or if males recognize and respond differently to the female forms (Tierney et al. 2008). But female form alternation need not occur in all population of *Orconectes* (Wetzel 2002). We could hypothesize, except abdomen width change (Wetzel 2002), that the changes in chelae length and width can occur, because of accompanying character in males form alternation (Scudamore 1948; Hobbs 2001). Naturally, the differences should be lower because females have smaller chelae than males (Huxley 1880; Rhodes and Holdich 1979). On the other hand, Stein (1976) resulted that chelae of females do not undergo the seasonal changes.

The reasons for form alternation could be to increase potential for growth and repair and to reach larger dimensions (Wetzel 2002). Why? Larger crayfish dominate smaller crayfish with smaller chelae (Bovbjerg 1956), can be more attractive for large males (Aquiloni and Gherardi 2008), are more resistant to predators (Stein 1976), and produce more offspring (Kozák et al. 2006; Larson and Magoulick 2008). On the other hand form alternation needs more molts that also involve costs as high vulnerability to predators during and after molting (Nyström 2002).

4.3. Mating, spawning and hatching

Mating takes place in autumn, as well as for IC (Van Den Brink 1988; Holdich et al. 2006), but females do not extrude their eggs after autumn mating period and waiting for spring season. Females store spermatophores in specialized cavity in the ventral side of the body, "*annulus ventralis*", during this time (Andrews 1907; Vogt 2002). The mating starts again in spring and after this period females spawn, usually during April and May (Hamr 2002; Holdich and Black 2007).

Orconectes limosus females can produce over 400 eggs (Holdich et al. 2006). The fecundity positively correlating with body size and it can range widely between 31 and 555 eggs (Pieplow 1938; Stucki 2002; Kozák et al. 2006). In general *O. limosus* females are able to produce large amounts of offspring in very small body size (Lindqvist and Huner 1999). The relatively small eggs are carried after spawning on the female pleopods till the hatching time (Reynolds 2002).

Egg incubation is very short, reaching about 45 days (Kozák et al. 2006). Compare to IC, which incubate their eggs during winter and the overall incubation time reaching about 8 months (Skurdal and Taugbøl 2002), *O. limosus* has an advantage because the spawning in spring and short incubation eliminates the potential egg losses during winter months and increases the possibility of successful reproduction. The newly hatched juveniles are dependent on their mother and very shortly molt into 2nd instar (2 – 4 days). Juveniles undergo the successive molt ca. one week later. The 3rd instar starts to be independent and feeding actively (Andrews 1907). The IC juveniles start to be independent sooner, in 2nd instar (Reynolds 2002) and have therefore shorter maternal care.

5. Ecology

5.1. Activity

Crayfish activity could be influenced by several factors as temperature (Barbaresi and Gherardi 2001; Bubb et al. 2004; Lozan 2000), light intensity (Bojsen et al. 1998; Gherardi 2002), shelter availability (Martin and Moore 2007; Barbaresi and Gherardi 2001), food availability (Gherardi 2002; Statzner et al. 2000), presence of predators (Jordan et al. 1996; Nyström 2002), and intra- or inter-specific interactions (Bubb et al. 2006; Gherardi, 2002).

Although most crayfish are classified as nocturnal animals (Gherardi 2002), spiny-cheek crayfish has been reported as a species with a high daily activity compared to IC (Lozan 2000; Stucki 2002). In general spiny-cheek crayfish is referred to as a highly active species (Lozan 2000). Despite the fact that *O. limosus* is a dangerous NIC, detailed data on activity or migrations linked to reproductive activities, temperature or year season are still lacking. There is a possible similarity with observed increased motional activity of *Procambarus clarkii* (Girard 1852) due to reproductive phase (Gherardi and Barbaresi 2000). Findings of Stucki (2002), who observed wandering *O. limosus* in early spring season and also checked daily activity, supported hypothesized similarity.

There can be expected high ability of *O. limosus* to move fast to expand to new localities. The motional activity could be lower than for *P. leniusculus* or *P. clarkii* with respect to *O. limosus* small size, because speed of movement is positively correlated to body size in crayfish (Gherardi et al. 2002).

5.2. Interactions

This species shows several characteristics such as rapid maturation, short-lifespan, high fecundity and second mating period, which facilitates its fast population growth, dispersal and invasive capabilities (Van Den Brink et al. 1988; Hamr 2002; Stucki 2002). If crayfish are classified into the two ecological groups referred to as r-selective and K-selected species, spiny-cheek crayfish will therefore be included in the first group. American invasive species are often more aggressive than IC (Lindqvist and Huner 1999) and could be stronger competitors for a native astacofauna even if its populations are free of crayfish plague (Schulz et al. 2006). In cases when the invading species is infected, the transfer of the disease resulting in a mass mortality of the native crayfish is likely to happen (Kozubíková et al. 2009).

Apart from crayfish plague transmission, aggression and resource competition are important parts of crayfish interactions (Söderback 1991). Sympatric crayfish species compete for limited resources such as food, shelter and space, and larger crayfish routinely win competitive interactions with smaller individuals (Momot 1984). Body size is a major determinant of dominance in crayfish (Butler and Stein 1985) and faster growing; hence, larger-sized crayfish are reported to outcompete smaller individuals of the same species, as well as smaller individuals of a different species (Momot 1984; Mather and Stein 1993). The size difference may provide a competitive advantage in conspecific interactions.

The predators were found to include perch, turbot, mink (Holdich et al. 2006), pike (Haertel et al. 2002), and birds (Holdich and Black 2007). Larger individuals of *O. limosus* are not much affected by fish predation, avoiding attacks by crossing the spinous chelipeds and keeping them locked in the shape of spinous ball, which is not easy to swallow. They also raise their chelipeds in defense (Holdich et al. 2006).

6. The role of O. limosus in European inland waters

Because of their size, population density, polytrophic links and importance as prey species, crayfish are important trophic components of freshwater ecosystems (Lodge and Hill 1994; Stenroth and Nyström 2003) and can be often considered as key species in both lentic and lotic habitats (Momot 1995, Nyström 2002). Elimination or introduction of crayfish can have substantial effects on the aquatic environment. Among other impacts, crayfish may alter macrophyte coverage (Matthews and Reynolds 1992; Nyström and Strand 1996), increase water turbidity (Angeler et al. 2001), with subsequent losses of diversity of various consumer tropic levels, including invertebrates, amphibians, and waterfowl (Rodriguez et al. 2005).

Indigenous crayfish (IC) species in Europe are endangered by several factors, such as fragmentation and alteration of habitats. One of the most significant threats is the continuous spread of NIC (Holdich and Gherardi 1999; Füreder et al. 2006), including fast spreading *O. limosus* (Henttonen and Huner 1999; Holdich and Black 2007). Invasive NIC may eliminate IC and reduce populations of favoured food items, which can have cascading trophic effects elsewhere in the ecosystem (Klocker and Strayer 2004; Nyström et al. 1996).

The North American crayfish introduced to Europe often carry the crayfish plague pathogen and may transmit it to the native European crayfish populations (Vogt 1999). *Orconectes limosus* is a major vector of the crayfish plague pathogen in Czech open waters (Kozubíková et al. 2006). With the spread of non-native species in recent decades, the crayfish plague outbreaks have regained intensity recently (Holdich 2003; Kozubíková 2008). Furthermore, the indigenous crayfish can also be displaced through direct interactions or competition for resources with the non-native species (Holdich et al. 1999).

Data regarding migratory ability and activity, seasonal migrations, habitat exploitation, and behavior of *O. limosus* are lacking. The wide geographical range and disease threat of *O. limosus*, combined with the lack of information through recent research, is an alarming situation for IC. Knowledge of behavior, habitat exploitation, and migratory ability and patterns may be crucial to assessing the invasive potential and ecology of *O. limosus* and its threat for IC.

7. How to obtain reliable data?

Orconectes limosus is a small-bodied species which complicates locating and re-sampling individuals in the field and requires careful choice of marking method. The visible implant alphanumeric tags (Isely and Stocket 2001) or visible implant elastomers (Clark and Kershner 2006) are useful tools for marking such small creatures. Mark and recapture methods are widely used in field (Abrahamsson 1973; Guan 1997; Bubb et al. 2002) and laboratory (Guan and Wiles 1996; Kuhlmann et al. 2008) experiments on crayfish. But, mark-recapture methods provide only a few answers such as abundance, growth and long term translocations.

However, laboratory studies can provide detailed data from defined conditions. Nevertheless, the findings can be different compared to natural habitat of crayfish. Hence the more descriptive method is required for observations in the field conditions. Relatively new technology such as radio-telemetry allows the study of movement and behavior patterns over extended time periods of even very small aquatic animals. Radio-telemetry has been utilized successfully with signal crayfish *P. leniusculus* (Bubb et al. 2004; 2006); red swamp crayfish, *P. clarkii* (Gherardi and Barbaresi 2000; Gherardi et al. 2002); noble crayfish, *A. astacus* (Bohl 1999; Schütze et al. 1999); and white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet 1858) (Robinson et al. 2000; Bubb et al. 2006). Radio-telemetry can also reveal whether *O. limosus* occupies a single shelter or alternates among a system of shelters within a home range, as some other decapods do (Vannini and Cannicci 1995).

IN THIS THESIS

The overall aim of present thesis was collect as much data as possible to evaluate probable reasons why *O. limosus* is such good invader, and to elucidate dark places in knowledge about this interesting freshwater crayfish species.

The specific objectives were to:

- Describe the fecundity and the time of embryonic development of the spiny-cheek crayfish
- Determine the growth and survival of young-of-the-year (YOY) of *A. astacus* and *O. limosus* in intra-specific and inter-specific competition treatments
- Evaluate marking methods for *O. limosus* (tag retention and readability, effects of tagging on survival and molt increments)
- Observe seasonal migrations, estimate annual changes in abundance, and determine the over-wintering location of *O. limosus* using radio-telemetry and mark-recapture method
- Study the activity patterns of *A. astacus* and *O. limosus* and to compare it in order to obtain an understanding of inter-specific relations between species
- Determine diurnal ranging patterns, seasonal migrations, shelter exploitation, habitat preferences, and home range of *O. limosus* using radio-telemetry
- Gain information on migratory activity and behavior influenced by reproductive activities - during and after spring mating period using radio-telemetry
- Elucidate patterns associated with male form alternation in the *O. limosus* (number of molts, their timing and a/synchrony; changes in body proportions; growth increments of successive molts; changes in copulatory stylet proportions)
- Provide evidence of form alternation in adult female *O. limosus* (changes associated with possible form alternation in experimental population of *O. limosus* throughout the year, directly aimed on events connected with yearly female cycle)

References

Abrahamsson S.A.A., 1973. The crayfish *Astacus astacus* in Sweden and the introduction of the American crayfish *Pacifastacus leniusculus*. Freshwater Crayfish 1: 27-39.

Andrews E.A., 1907. The young of the crayfishes *Astacus* and *Cambarus*. Smithson. Contrib. Knowl. Part of Volume XXXV, No. 1718. Smithsonian Institution. Washington. 79 pp.

Andrews E.A., 1910. Conjugation in the crayfish *Cambarus affinis*. J. Exp. Zool. 9: 235-364.

Angeler D.G., Sanchez-Carillo S., García G., Alvarez-Cobelas M., 2001. The influence of *Procambarus clarkii* (Decapoda: Cambaridae) on water quality and sediment characteristics in Spanish floodplain wetland. Hydrobiologia 464: 89-98

Aquiloni L., Gherardi F., 2008. Mutual mate choice in crayfish: large body size is selected by both sexes, virginity by males only. J. Zool. 274: 171-179.

Barbaresi S., Gherardi F., 2001. Daily activity of the white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet): a comparison between field and laboratory studies. J. Nat. Hist. 35 (12): 1861-1871.

Bohl E., 1999. Motion of individual noble crayfish *Astacus astacus* in different biological situations: in-situ studies using radio telemetry. Freshwater Crayfish 12: 677-687.

Bojsen B.H., Witthøfft H., Styrishave B., Andersen O., 1998. *In situ* studies on heart rate and locomotor activity in the freshwater crayfish, *Astacus astacus* (L.) in relation to natural fluctuations in temperature and light intensity. Freshwat. Biol. 39: 455-465.

Bovbjerg R.V., 1956. Some factors affecting aggressive behavior in crayfish. Physiol. Zool. 29: 127-136.

Boyd J.A., Page L.M., 1978. The Life History of the Crayfish *Orconectes kentuckiensis* in Big Creek, Illinois. Am. Midl. Nat. 99: 398-414.

Bubb D.H., Lucas M.C., Thom T.J., Rycroft P., 2002. The potential use of PIT telemetry for identifying and tracking crayfish in their natural environment. Hydrobiologia 483: 225-230.

Bubb D.H., Lucas M.C., Thom T.J., 2004. Movement and dispersal of the invasive signal crayfish *Pacifastacus leniusculus* in upland rivers. Freshwat. Biol. 49: 357-368.

Bubb D.H., Thom T.J., Lucas M.C., 2006. Movement, dispersal and refuge use of co-occuring introduced and native crayfish. Freshwat. Biol. 51: 1359-1368.

Butler M.J., Stein R.A., 1985. An analysis of mechanism governing species replacement in crayfish. Oceologia 66: 168-177.

Capelli G.M., Magnuson J.J., 1975. Reproduction, moulting, and distribution of *Orconectes propinquus* (Girard) in relation to temperature in a northern mesotrophic lake. Freshwater Crayfish 2: 415-427.

Clark J.M., Kershner M.W., 2006. Size-dependent effects of visible implant elastomer marking on crayfish (*Orconectes obscurus*) growth, mortality, and tag retention. Crustaceana 79 (3): 275-284.

Dehus P., Phillipson S., Bohl E., Oidtman B., Keller M., Lechleiter S., 1999. German conservation strategies for native crayfish species with regard to alien species. In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 149-153.

Eversole A.G., Mazlum Y., Spacil M.S., 2006. *Procambarus acutus acutus* (Girard) replacement of *P. clarkii* (Girard): a possible explanation. Freshwater Crayfish 15: 63-68.

Diéguez-Uribeondo J., 2006. The dispersion of the *Aphanomyces astaci*-carrier *Pacifastacus leniusculus* by humans represents the main cause of disappearance of the indigenous crayfish *Austropotamobius pallipes* in Navarra. Bull. Fr. Peche Pisci. 380-381: 1303-1312.

Füreder L., Edsman L., Holdich D.M., Kozák P., Machino Y., Pöckl M., Renai B., Reynolds J.D., Schulz H., Schulz R., Sint D., Taugbol T., Trouilhé M.C., 2006. Indigenous crayfish habitat and threats. In: Souty-Groset C., Holdich D.M., Noël P., Reynolds J.D., Haffner P. (eds.): Atlas of Crayfish in Europe. Publications Scientifiques du MNHN, Paris, pp. 25-48.

Gherardi F., 2002. Behaviour. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 258-290.

Gherardi F., Barbaresi S., 2000. Invasive crayfish: activity patterns of *Procambarus clarkii* in the rice fields of the lower Guadalquivir (Spain). Arch. Hydrobiol. 150: 153-168.

Gherardi F., Tricarico E., Ilhéu M., 2002. Movement patterns of an invasive crayfish, *Procambarus clarkii*, in temporary stream of southern Portugal. Ethol. Ecol. Evol. 14: 183-197.

Guan R.Z., 1997. An improved method for marking crayfish. Crustaceana 70: 641-652.

Guan R.Z., Wiles P.R., 1996. Growth, density and biomass of crayfish, *Pacifastacus leniusculus*, in a British lowland river. Aquat. Living Resour. 9 (3): 265-272.

Guiasu R.C., 2002. *Cambarus*. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 609-634.

Haertel S.S., Baade U., Eckmann R., 2002. No general percid dominance at mesotrophic lake conditions: insights from the quantification of predator-prey interactions. Limnologica 32: 1-13.

Hamr P., 2002. *Orconectes*. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 585-608.

Hamr P., Berrill M., 1985. The life histories of north-temperate populations of the crayfish *Cambarus robustus* and *Cambarus bartoni*. Can. J. Zool. 63: 2313-2322.

Henttonen P., Huner J.V., 1999. The introduction of alien species of crayfish in Europe: A historical introduction. In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 13-22.

Hobbs H.H., 1974. Synopsis of the Families and Genera of Crayfishes (Crustacea: Decapoda). Smithson. Contrib. Zool. 164: 33 pp.

Hobbs H.H. Jr., 1989. An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae). Smithson. Contrib. Zool. 480: 236 pp.

Hobbs H.H. III, 2001. Decapoda. In: Thorp J.H, Covich A.P. (eds.): Ecology and Classification of North American Freshwater Invertebrates, second edition. Academic press, London, pp. 955-1001.

Hobbs H.H., Jass J.P., Huner J.V., 1989. A review of global crayfish introductions with particular emphasis on two North American species (Decapoda: Cambaridae). Crustaceana 56: 299-316.

Holdich D.M., 2003. Crayfish in Europe – an overview of taxonomy, legislation, distribution, and crayfish plague outbreaks. In: Holdich D.M., Sibley P.J. (eds.): Management & Conservation of Crayfish, Proceedings of a conference held on 7th November 2002 at the Nottingham Forest Football Club, Nottingham, UK, Environment Agency, Bristol, UK., pp. 15-34.

Holdich D.M., Ackefors H., Gherardi F., 1999. Native and alien crayfish in Europe: Some conclusions. In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 281-292.

Holdich D.M., Gherardi F., 1999. Native and alien crayfish in Europe: An introduction. In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 3-9.

Holdich D.M., Haffner P., Noël P., Carral J., Füreder L., Gherardi F., Machino Y., Madec J., Pöckl M., Śmietana P., Taugbol T., Vigneux E., 2006. Species files. In: Souty-Groset C., Holdich D.M., Noël P., Reynolds J.D., Haffner P. (eds.): Atlas of Crayfish in Europe. Publications Scientifiques du MNHN, Paris, pp. 49-130.

Holdich D.M., Black J., 2007. The spiny-cheek crayfish, *Orconectes limosus* (Rafinesque, 1817) [Crustacea: Decapoda: Cambaridae], digs into the UK. Aquat. invasions 2: 1-16.

Huxley T.H., 1880. The crayfish. An introduction to study of Zoology. Kegan Paul & Co., London, 371 pp.

Isely J.J., Stockett P.E., 2001. Tag retention, growth, and survival of red swamp crayfish marked with a visible implant tag. N. Am. J. of Fish. Manage. 21: 422-424.

Jordan F., DeLeon C.J., McCreary A.C., 1996. Predation, habitat complexity, and distribution of the crayfish *Procambarus alleni* within a wetland habitat mosaic. Wetlands 16 (4): 452-457.

Klocker C.A., Strayer D.L., 2004. Interactions among an invasive crayfish (*Orconectes rusticus*), a native crayfish (*Orconectes limosus*), and native bivalves (Sphaeriidae and Unionidae). Northeast. Nat. 11: 167-178.

Kozák P., Policar T., Ďuriš Z., 2004. Migration ability of *Orconectes limosus* through a fishpass and notes on its occurrence in the Czech Republic. Bull. Fr. Peche Piscic. 372-373: 367-373.

Kozák P., Buřič M., Policar T., 2006. The fecundity, time of egg development and juveniles production in spiny-cheek crayfish (*Orconectes limosus*) under controlled conditions. Bull. Fr. Peche Piscic. 380-381: 1171-1182.

Kozák P., Buřič M., Policar T., Hamáčková J., Lepičová A., 2007. The effect of inter- and intra-specific competition on survival and growth rate of native juvenile noble crayfish *Astacus astacus* and alien spiny-cheek crayfish *Orconectes limosus*. Hydrobiologia 590: 85-94.

Kozubíková E., Petrusek A., Ďuriš Z., Kozák P., Geiger S., Hoffmann R., Oidtmann B., 2006. The crayfish plague in the Czech Republic – review of recent suspect cases and a pilot detection study. Bull. Fr. Peche Piscic. 380-381: 1313-1324.

Kozubíková E., Petrusek A., Ďuriš Z., Martín M.P., Diéguez-Uribeondo J., Oidtmann B., 2008. The old menace is back: recent crayfish plague outbreaks in the Czech Republic. Aquaculture 274: 208-217.

Kozubíková E., Filipová L., Kozák P., Ďuriš Z., Martín M.P., Diéguez-Uribeondo J., Oidtmann B., Petrusek A., 2009. Prevalence of the crayfish plague pathogen *Aphanomyces astaci* in two American invasive crayfishes in the Czech Republic. Conservation Biology, *in press.*

Kuhlmann M.L., Badylak S.M., Carvin E.L., 2008. Testing the differential predation hypothesis for the invasion of rusty crayfish in a stream community: laboratory and field experiments. Freshwat. Biol. 53: 113-128.

Larson E.R., Magoulick D.D., 2008. Comparative life history of native (*Orconectes eupunctus*) and introduced (*Orconectes neglectus*) crayfishes in the Spring River Drainage of Arkansas and Missouri. Am. Midl. Nat. 160: 323-341.

Lindqvist O.V., Huner J.V., 1999. Life history characteristics of crayfish: what makes some of them good colonizers? In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 23-30.

Lodge D.M., Hill A.M., 1994. Factors governing species composition, population size and productivity of cool-water crayfishes. Nord. J. Freshwat. Res. 69: 111-136.

Lowery R.S., 1988. Growth, Moulting and Reproduction. In: Holdich D.M., Lowery R.S. (eds.): Freshwater Crayfish: Biology, Management and Exploitation, Croom Helm, London, pp. 83-113.

Lozan J.L., 2000. On the threat to the European crayfish: A contribution with the study of the activity behaviour of four crayfish species (Decapoda: Astacidae). Limnologica 30: 156-166.

Mack R.N., Simberloff D., Lonsdale W.M., Evans H., Clout M., Bazzaz F.A., 2000. Biotic invasions: Causes, epidemiology, global consequences, and control. Ecol. Appl. 10: 689-710.

Martin A.L., Moore P.A., 2007. Field observations of agonism in the crayfish, *Orconectes rusticus*: shelter use in a natural environment. Ethology 113 (12): 1192-1201.

Mather M.E., Stein R.A., 1993. Using growth/mortality trade-offs to explore a crayfish species replacement in stream riffles and pools. Can. J. Fish. Aquat. Sci. 50: 88-96.

Matthews M., Reynolds J.D., 1992. Ecological impact of crayfish plague in Ireland. Hydrobiologia 234: 1-6.

Mazlum Y., Can M.F., Eversole A.G., 2007. Morphometric relationship of length-weight and chelae length-width of eastern white river crayfish (*Procambarus acutus acutus*, Girard, 1852), under culture conditions. J. Appl. Ichthyol. 23: 616-620.

McAlpine D., Hogans W.E., Fletcher T.J., 1991. *Orconectes limosus* (Crustacea, Cambaridae) an addition to the crayfish fauna of New Brunswick. Can. Field-Nat. 105: 386-387.

Momot W.T., 1984. Crayfish production: a reflection of community energetics. J. Crust. Biol. 4: 35-54.

Momot W.T., 1995. Redefining the role of crayfish in aquatic ecosystems. Rev. Fish. Sci. 3: 33-63.

Nyström P., 2002. Ecology. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 192-235.

Nyström P., Brönmark C., Granéli W., 1996. Patterns in benthic food webs: a role for omnivorous crayfish? Freshwat. Biol. 36: 631-646.

Nyström P., Strand J.A., 1996. Grazing by a native and an exotic crayfish on aquatic macrophytes. Freshwat. Biol. 36: 673-682.

Payne J.F., 1978. Aspects of life histories of selected species of North American crayfishes. Fisheries 3: 5-8.

Petrusek A., Filipová L., Ďuriš Z., Horká I., Kozák P., Policar T., Štambergová M., Kučera Z., 2006. Distribution of the invasive spiny-cheek crayfish (*Orconectes limosus)* in the Czech Republic: past and present. Bull. Fr. Peche Piscic. 380–381: 903-917.

Pieplow U., 1938. Fischereiwissenschaftliche Monographie von *Cambarus affinis* Say. Zeitschr. Fisherei 36: 349-440.

Pöckl M., Holdich D.M., Pennerstorfer J., 2006. Identifying native and alien crayfish species in Europe. European Project CRAYNET, Université de Poitiers.

Price J.O., Payne J.F., 1984. Postembryonic to adult growth and development in the crayfish *Orconectes neglectus chaenodactylus* Williams, 1952 (Decapoda, Astacidea). Crustaceana 46 (2): 177-194.

Reynolds J.D., 2002. Growth and reproduction. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 152-191.

Rhodes C.P., Holdich D.M., 1979. On size and sexual dimorphism in *Austropotamobius pallipes* (Lereboullet), a step in assessing the commercial exploitation potential of the native British freshwater crayfish. Aquaculture 17(4): 345-358.

Robinson C.A., Thom T.J., Lucas M.C., 2000: Ranging behaviour of a large freshwater invertebrate, the white-clawed crayfish *Austropotamobius pallipes*. Freshwat. Biol. 44: 509-521.

Rodriguez C.F., Bécares E., Fernández-Aláez M., Fernández-Aláez C., 2005. Loss of diversity and degradation of wetlands as a result of introducing exotic crayfish. Biol. Invasions 7: 75-85.

Schulz H.K., Smietana P., Maiwald T., Oidtmann B., Schulz R., 2006. Case studies on the co-occurrence of *Astacus astacus* (L.) and *Orconectes limosus* (Raf.): snapshots of a slow displacement. Freshwater Crayfish 15: 212-219.

Schütze S., Stein H., Born O., 1999. Radio telemetry observations on migration and activity patterns of restocked noble crayfish *Astacus astacus* (L.) in the small river Sempt, North-East of Munich, Germany. Freshwater crayfish 12: 688-695.

Scudamore H.H., 1948. Factors influencing moulting and sexual cycles in the crayfish. The Biol. Bull. 95: 229-237.

Skurdal J., Taugbøl T., 2002. *Astacus*. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 467-510.

Smith D.G., 1981. Life history parameters of the crayfish *Orconectes limosus* in Southern New-England USA. Ohio J. Sci. 81: 169-172.

Söderback B., 1991. Interspecific dominance relationship and aggressive interactions in the freshwater crayfishes *Astacus astacus* (L.) and *Pacifastacus leniusculus* (Dana). Can. J. Zool. 69: 1321-1325.

Statzner B., Fièvet E., Champagne J.Y., Morel R., Herouin E., 2000. Crayfish as geomorphic agents and ecosystem engineers: Biological behavior affects sand and gravel erosion in experimental streams. Limnol. Oceanogr. 45 (5): 1030-1040.

Stein R.A., 1976. Sexual dimorphism in crayfish chelae: functional significance linked to reproductive activities. Can. J. Zool. 54: 220-227.

Stenroth P., Nyström P., 2003. Exotic crayfish in a brown water stream: effects on juvenile trout, invertebrates and algae. Freshwat. Biol. 48: 466-475.

Stucki T., 2002. Differences in live history of native and introduced crayfish species in Switzerland. Freshwater Crayfish 13: 463-476.

Suko T., 1953. Studies on the development of the crayfish. I. The development of secondary sex characters in appendages. Sci. Rep. Saitama Univ. 1: 77-96.

Taylor C.A., 2002. Taxonomy and conservation of native crayfish stocks. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 236-256.

Tierney A.J., Gunarante C., Jennison K., Monroy V., Donnelly L., 2008. Behavioral correlates and alternate male forms (form I and form II) in the crayfish *Orconectes rusticus*. J. Crust. Biol. 28: 596-600.

Van Den Brink F.W.B., Van Der Velde G., Geelen J.F.M., 1988. Life history parameters and temperature-related activity of an American crayfish, *Orconectes limosus* (Rafineque, 1817) (Crustacea, Decapoda), in the area of the major rivers in The Netherlands. Arch. Hydrobiol. 114: 275-289.

Vannini M., Cannicci S., 1995. Homing behaviour and possible cognitive maps in crustacean decapods. J. Exp. Mar. Biol. Ecol. 193: 67-91.

Vitousek P.M., D´Antonio C.M., Loope L.L., Westbrooks R., 1996. Biological invasions as global environmental change. Am. Sci. 84: 469-478.

Vogt G., 1999. Diseases of European freshwater crayfish, with particular emphasis on interspecific transmission of pathogens. In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 87-103.

Vogt G., 2002. Functional Anatomy. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 53-151.

Wetzel J.E., 2002. Form Alteration of Adult Female Crayfishes of the genus *Orconectes* (Decapoda: Cambaridae). Am. Midl. Nat. 147: 326-337.

Chapter 2

The fecundity, time of egg development and juveniles production in spiny-cheek crayfish (*Orconectes limosus*) under controlled conditions

Kozák P., Buřič M. and Policar T. 2006. Bulletin Français de la Pêche et de la Pisciculture, 380–381: 1171–1182.

The fecundity, time of egg development and juvenile production in spiny-cheek crayfish (*Orconectes limosus*) under controlled conditions

Pavel Kozák, Miloš Buřič, Tomáš Policar

University of South Bohemia in České Budějovice, Research Institute of Fish Culture and Hydrobiology, Zátiší 728/II, 389 25 Vodňany, Czech Republic

Abstract

We comprehensively describe the fecundity and time of embryonic development of the spiny-cheek crayfish (*Orconectes limosus*). Linear relationships between female size and ovarian fecundity, pleopodal fecundity, and production of juveniles at the 3rd fecundity stage, were confirmed. The ovarian fecundity was evaluated for the smallest as well as largest females in the sampled population (43-93 mm of body length); the number of oocytes (eggs) ranged widely, between 46 and 505. No significant difference was found between the ovarian and pleopodal fecundity observed just after egg laying. The value of the female gonadosomatic index just before laying was 4.2 ± 1.8% (0.8-7.7%). The mean diameter of eggs on female pleopods was 1.8 ± 0.2 mm (1.45-2.15 mm). A statistically significant difference was found between the numbers of eggs carried on individual pairs of pleopods. Mean time from laying to hatching was 46 ± 3.8 days (37-56 days), i.e. 647 ± 39.9 CTU (Celsius Temperature Units = degrees Celsius × days). Mean production of juveniles at the 3rd stage was found to be 135.7 ± 67.24 (15-243) juveniles. The early sexual maturation and also high number of juveniles at the 3rd stage per female gives this species a good predisposition for quick establishment in new localities.

Keywords: Crayfish, Orconectes limosus, lifecycle, Czech Republic, juveniles, egg development

1. Introduction

The spiny-cheek crayfish (*Orconectes limosus*) (Rafinesque) is originally native to the east coast of the USA and Canada (Hobbs 1974; Hamr 2002). This species was imported to Europe in 1891. Nowadays, it is widely distributed throughout most Western and Central European countries (Holdich et al. 1999). It is one of five crayfish species that are currently present in open waters of the Czech Republic. The noble crayfish *Astacus astacus* (Linnaeus), stone crayfish *Austropotamobius torrentium* (Schrank), narrow-clawed crayfish *Astacus leptodactylus* (Eschscholtz) and signal crayfish *Pacifastacus leniusculus* (Dana) are also present.

Although the first report of the presence of the spiny-cheek crayfish on the Czech territory was published at the end of the 1980's, the species seems to have been present in the river Elbe already in the 1960's. By the end of 20th century, it had spread widely in the Elbe watershed, due to its natural dispersal potential as well as a result of anthropogenic translocations (Kozák et al. 2004; Petrusek et al., *in review*). This species is a major vector of the crayfish plague pathogen, *Aphanomyces astaci*, in Czech open waters (Kozubíková et al., *in review*).

The spiny-cheek crayfish shows several characteristics such as rapid maturation, short-lifespan, high fecundity and second mating period, which facilitate its fast population growth, dispersal and invasive capabilities. In Québec populations, mating takes place in September-

October and again in March-April (Hamr 2002). According to Van Den Brink et al. (1988), the mating period for *O. limosus* occurs in autumn, just as it does for *A. astacus*, *A. leptodactylus* and *P. leniusculus*. Hamr (2002) states, however, that in American and European populations mating also takes place in spring, and eggs are carried from March to May. Van Den Brink et al. (1988) captured ovigerous females of *O. limosus* from mid-March to May. A similar period for egg development has been presented by Pieplow (1938), Smith (1981) and Orzechowski (1984). Smith (1981) and Van Den Brink et al. (1988) observed that larger and presumably older females of *O. limosus* extruded their eggs earlier in the season than smaller and younger ones. A temporal difference in the onset of the breeding period between younger and older females is probably a more common phenomenon in orconectid crayfish, as it is also known for *Orconectes immunis* (Tack 1941).

The potential reproductive capacity of crayfish is usually measured by ovarian egg counts, and realised reproductive capacity by pleopod egg counts. Pleopod egg counts are typically more variable and lower in total numbers than ovarian counts because of incomplete egg extrusion at spawning, eggs that fail to be fertilised, eggs that fail to attach after extrusion or because of losses of attached eggs during the incubation period for various reasons (Mason 1977; Savolainen et al. 1996). Pleopod egg counts are more informative when determining recruitment to a population and are therefore more useful when management of a population is involved (Lewis 2002). In *Austropotamobius pallipes*, the ovarian fecundity differs from pleopodal egg counts by 20-40% (Reynolds 2002). In White Lake (Ireland), the observed shortfall in *Austropotamobius pallipes* pleopodal egg number relative to ovarian fecundity was about 30%, but females immediately after spawning had retained at most 2% of their eggs in ovaries or oviducts. This suggests that the discrepancy between the two measures of fecundity is chiefly due to poor egg attachment, rather than to factors such as egg resorption or incomplete fertilisation (Savolainen et al. 1996; Reynolds 2002). Both ovarian and pleopodal fecundity correlated with female size (Stypińska 1973; Savolainen et al. 1996; Austin 1998*a*; Schultz and Śmietana 2001; Harlioğlu et al. 2004; Mazlum and Eversole 2004; Nakata and Goshima 2004; Huber and Schubart 2005; Maguire et al. 2005), but the relationship between the egg number and body size was strongly influenced by environmental conditions (Reynolds 2002). Different authors evaluated the pleopodal fecundity just after ovulation (Abrahamsson 1971; Austin 1998*a*,*b*; Leonard et al. 2001; Nakata and Goshima 2004; Celada et al. 2005*a*; Sáez-Royuela 2005), during incubation (Jones 1995; Lewis and Horton 1997; Mazlum and Eversole 2004; Celada et al. 2005*a*,*b*; Sáez-Royuela et al. 2005) or just before hatching (Söderbäck 1995; Savolainen et al. 1996; Celada et al. 2005*b*; Sáez-Royuela et al. 2005).

Stypińska (1973) recorded an average ovarian fecundity of spiny-cheek crayfish from 315 to 440 eggs related to size group (body length of females ranged from 75 to 104 mm). Kozák and Policar (in press) found a mean ovarian fecundity (females from 52-82 mm of body length) of 140 eggs, with a minimum of 76 and maximum of 290 eggs.

Pieplow (1938) observed pleopodal fecundity of 241-394 eggs in the spiny-cheek crayfish females (body length ranging from 66 to 92 mm) just two days after laying. Three weeks after the laying, however, the average pleopodal fecundity values were lower – 130 eggs with a minimum and maximum of 2 and 316 eggs, respectively. Holdich and Lowery (1988) presented a pleopodal fecundity of 400 eggs for a spiny-cheek crayfish female with a carapace length of 45 mm. Momot in Holdich and Lowery (1988), however, presented a much lower value of mean pleopodal fecundity – 163 eggs. Stucki (2002) found a mean average pleopodal fecundity of 139 eggs with minimum and maximum 31 and 555 eggs, respectively. Hamr (2002) collected data of several authors and presented pleopodal fecundity ranging between 57 and 440 eggs.

The aim of our study was to comprehensively describe the fecundity and the time of embryonic development of the spiny-cheek crayfish under Central European conditions.

2. Material and methods

Crayfish used in the experiment were collected on April 18 and April 26, 2004 in the Kořensko reservoir (Vltava River, South Bohemia, Czech Republic). We measured the body length, carapace length and weight of all crayfish.

2.1. Ovarian and pleopodal fecundity

Ovarian fecundity of 20 females was evaluated on April 18, just after catching. We selected individuals that represented both the smallest and largest size fractions of mature females in the sample. Total whole body wet weight of each individual was determined to the nearest 0.1 g on an electronic balance. Each crayfish was then dissected and its gonad was removed. The gonad was weighed on an electronic balance to the nearest 0.0001 g. The gonadosomatic index (GSI; ratio of wet weight of ovary to the whole body wet weight × 100) was calculated for each crayfish. The number of eggs in the ovaries was counted.

The laying of eggs took place at the end of April and beginning of May. Altogether, 16 females were kept in a special apparatus with shelters in a flow-through system throughout this period. Pleopodal fecundity was evaluated approximately 3 days after the laying. Eggs were removed and counted individually from each pleopod.

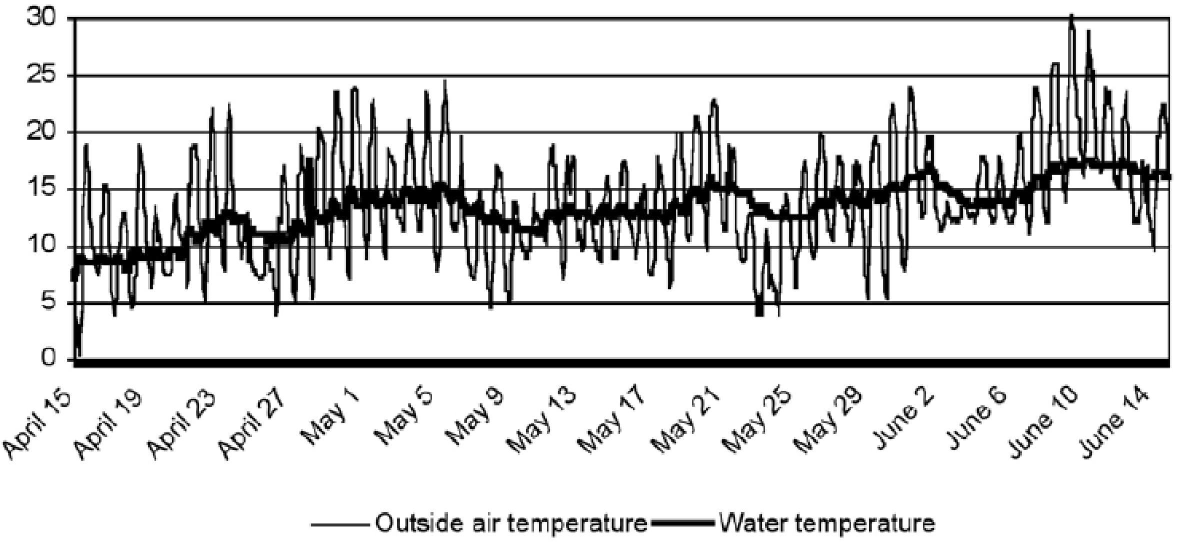


Figure 1. The course of water temperature and outside air temperature during experiments, measured every hour.

2.2. Number of juveniles at the 3rd stage

Altogether, 20 females without eggs were stocked individually on April 18 in the Rückel-Vacek hatching apparatus (originally used for hatching salmonid eggs) with a flow-through system. We observed the time of egg laying, hatching and the time of juvenile independence (3rd stage); observations were done only twice a week to minimize disturbance and manipulation. Just after reaching the 3rd stage, we counted the number of juveniles.

2.3. Time of embryonic development

Altogether, 80 females were kept in 4 special apparatuses (4 × 20 females) with shelters in a flow-through system. Females were individually marked with a glosspaint pen by writing their individual number on the carapace (see Sint and Füderer 2004). Females were checked daily to observe time of laying and subsequently time of juvenile hatching. This allowed us to record the specific time of laying and hatching, but unfortunately caused daily disturbance of females.

Water temperature and outside air temperature were measured automatically by temperature sensors RT-F52 (Qi Analytical Ltd. CZ) at one hour intervals during all experiments (Figure 1). The CTU (Celsius Temperature Units = degrees Celsius × days) was calculated.

2.4. Data analysis

Average values of the acquired parameters and SD were counted. Statistical significance was assessed using one-way analysis of variance (ANOVA, Statgraphics version 5), followed by Tukey HSD multiple range test comparisons. The difference between ovarian and pleopodal fecundity was tested by ANCOVA, using the female body length as a covariate. Linear regression was evaluated from the following parameters: ovarian and pleopodal fecundity, and number of juveniles at the 3rd stage, and assessed with Statgraphics version 5. Probability values < 0.01 were considered to be significant.

3. Results

3.1. Ovarian fecundity

The body length and weight of 20 mature females used in the experiment was 54.1 ± 12.64 mm (43-93 mm) and 5.08 ± 5.16 g (2.24-23.37 g), respectively. The mean ovarian fecundity was found to be 130.8 ± 107.6, ranging from 46 to 505 eggs. The ovarian fecundity showed a linear relationship ($r2 = 0.9042$, $P < 0.0000$ to the body length (Figure 2). The average size of eggs was found to be 1.2 ± 0.2 mm (0.9- 1.7 mm). We did not find any significant relationship between the size of eggs and their number. Respective to the size of females, however, the largest females had a higher number of the biggest eggs (1.6-1.7 mm), and had also the highest values of GSI (6.45- 7.74%). The average gonadosomatic index of females was found to be 4.2 ± 1.8% (0.83- 7.74%).

3.2. Pleopodal fecundity

The body length and weight of 16 females used in the experiment was 66.8 ± 1.68 mm (47-96 mm) and 10.86 ± 6.86 g (3.10-29.91 g), respectively. The mean pleopodal fecundity was found to be 217.8 ± 94.9 (95-492) eggs. The pleopodal fecundity again showed a linear relationship ($r2 = 0.8836$, $P < 0.0000$) to the body length (Figure 2). The estimated pleopodal fecundity was 10% lower than the estimated ovarian fecundity calculated by the resulting linear equations. However, we did not find a significant difference between ovarian and pleopodal fecundity when tested by ANCOVA using size as a covariate. The proportion of eggs placed on each pairs of pleopods and results of the pairwise comparison between different pairs are shown in the Table 1. We found a statistically significant difference ($P < 0.01$) between the egg numbers carried on individual pairs of pleopods. The highest numbers of eggs were placed on the 3rd and 4th pair of pleopods with 53.1 ± 23.1 (25-122) and 59.0 ±

24.1 (28-117) eggs, respectively. Practically no eggs were placed on the 1st pair of pleopods: 1.6 ± 1.5 (0-5) eggs. Altogether, 15.2 ± 7.8 (1-31) eggs were attached directly to the abdomen cuticle. The average size of eggs attached to the pleopods was 1.8 ± 0.21 mm (1.45-2.15 mm). We did not find any relationship between the size of a female and the size of eggs.

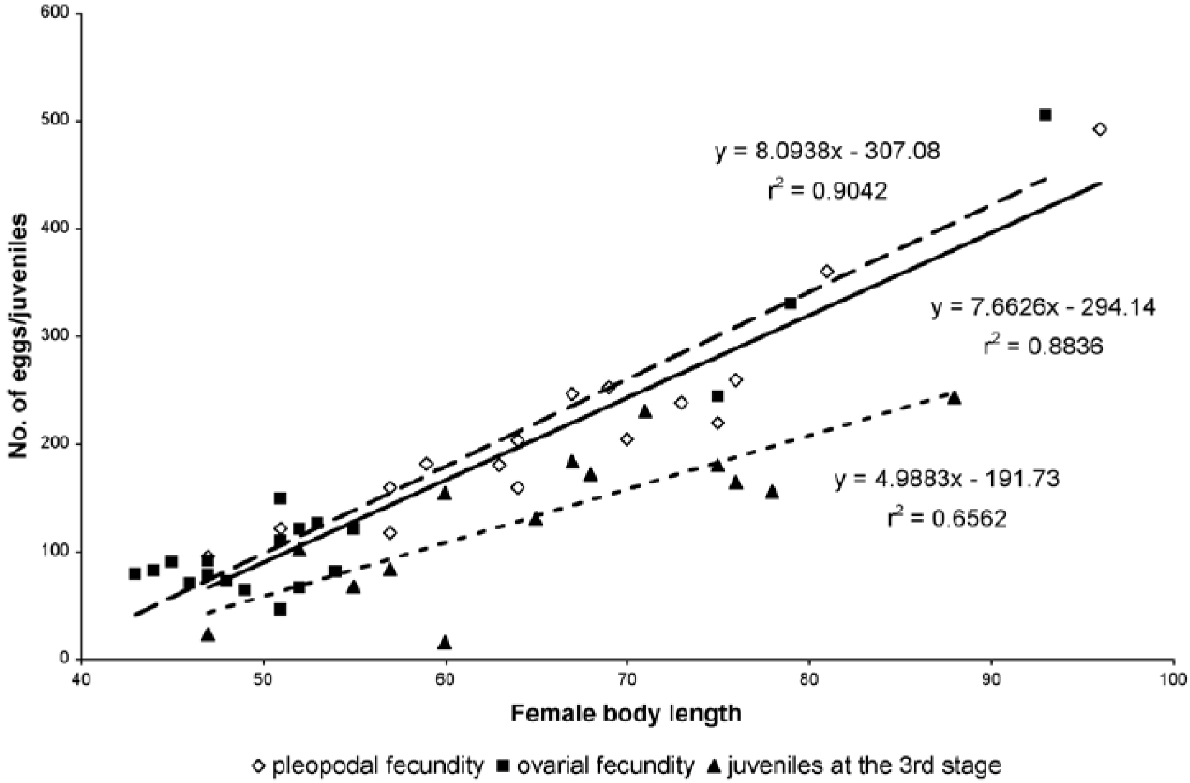


Figure 2. The relationship between the body length and ovarian fecundity, pleopodal fecundity, and production of juveniles at the 3rd stage in spiny-cheek crayfish females (*Orconectes limosus*).

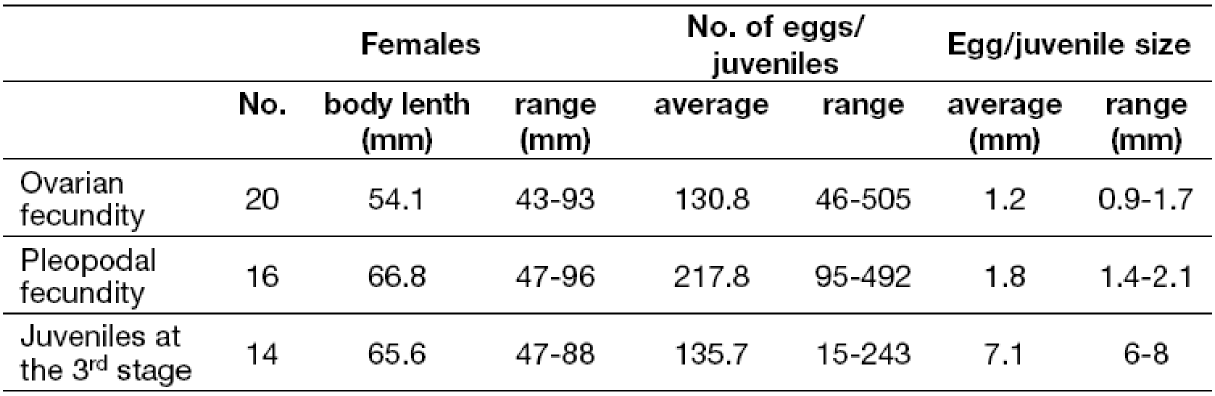
3.3. Juveniles at the 3rd stage

All 20 females laid eggs between April 22 and May 15. Six females lost eggs during the incubation. Juveniles hatched between June 1 and June 24, and moulted to the 3rd stage between June 22 and July 4. Total number of juveniles per female at the 3rd stage (calculated from the values of 14 females which had not lost eggs) was 135.7 ± 67.24. The body length and weight of the 14 females was 65.6 ± 10.92 mm (47-88 mm) and 9.14 ± 4.73 g (2.50-18.68 g), respectively. The number of juveniles showed a positive correlation with the female body length (r2 = 0.6562, P < 0.0004) (Figure 2).

Table 1. Percentage of eggs carried on individual pairs of pleopods (different alphabetic superscripts show significant differences in pairwise comparison; P < 0.01).

	1st pair	2nd pair	3rd pair	4th pair	5th pair	Others	Total no. of eggs
Average ± STD	1 ± 0,6^{e}	18 ± 2,7^{c}	25 ± 1,8^{ab}	27 ± 2,4^{a}	22 ± 3,2^{b}	7 ± 2,7^{d}	217,8 ± 94,93
Min-Max	0-2	14-24	21-27	23-32	16-27	1-11	95-492

Table 2. The size of females, fecundity and egg/juvenile size (average and range).



	Females			No. of eggs/ juveniles		Egg/juvenile size	
	No.	body lenth (mm)	range (mm)	average	range	average (mm)	range (mm)
Ovarian fecundity	20	54.1	43-93	130.8	46-505	1.2	0.9-1.7
Pleopodal fecundity	16	66.8	47-96	217.8	95-492	1.8	1.4-2.1
Juveniles at the 3rd stage	14	65.6	47-88	135.7	15-243	7.1	6-8

3.4. Time of embryonic development

The body length and weight of 80 mature females used in the experiment was 56.8 ± 10.06 mm (41-82 mm) and 5.91 ± 5.60 g (2.10-17.91 g), respectively. The time of laying was not dependent on the size of the female. We did not confirm that larger females extruded their eggs earlier. The disturbance also caused considerable losses of eggs during the incubation, especially if female was disturbed directly during the laying process. Only 43 females out of 80 females laid their eggs, and 26 of them reached the 1st juvenile stage. The laying of females in this experiment took place between April 18 and May 16. Hatching of juveniles occurred between June 12 and June 27. The mean time from laying to hatching of juveniles at the 1st stage was 46 ± 3.8 days (37-56 days), i.e. 647 ± 39.9 CTU (543-730 CTU). The water temperature slowly increased during the experiment from 8 to 17°C. Females laying the eggs later had a lower requirement of CTU for embryonic development.

4. Discussion

The higher fecundity of spiny-cheek crayfish in comparison with native European crayfish was confirmed. The ovarian fecundity of 130.8 ± 107.63 (46-505) oocytes per female is in agreement with our previous study (Kozák and Policar, in press). Stypińska (1973) presented an even higher average ovarian fecundity for the spiny-cheek crayfish, from 315 to 440 oocytes. Our results are comparable, however, if we take into account the size of females used in the experiments. The impact of spiny-cheek crayfish female size on fecundity has been confirmed by several studies, e.g. Pieplow (1938), Stypińska (1973), Schulz and Śmietana (2001). The literature data about average pleopodal fecundity of spiny-cheek crayfish are wide, ranging from 139 to 440 eggs per female, and there is always a large difference between minimum and maximum (altogether, the pleopodal fecundity ranges in the interval 35-555 eggs) (Pieplow 1938; Holdich and Lowery 1988; Stucki 2002; Hamr 2002). The pleopodal fecundity of 217.8 ± 94.93 (95-492) eggs recorded in our experiment is in agreement with the published data. We did not find a significant difference between ovarian fecundity and pleopodal fecundity observed just after laying when the results were corrected for female size. We can presume that the potential difference between ovarian fecundity and the number of pleopodal eggs at the end of embryonic development are caused mainly by losses of eggs during the incubation period. The magnitude of this difference may possibly reflect quality of environmental conditions. This phenomenon has already been observed in the spiny-cheek crayfish by Pieplow (1938), and by e.g. Celada et al. (2005*a*,*b*) and Sáez-Royuela et al. (2005) in other crayfish species. We also recorded large variability in the fecundity of females of a comparable size, similarly to Harioğlu et al. (2004).

The spiny-cheek crayfish has a higher average fecundity than native European crayfish, and comparable to non-native American species present in Europe. Savolainen et al. (1996) presented an average ovarian fecundity for the noble crayfish and signal crayfish of 166-264 oocytes with a minimum of 73 and maximum of 436 oocytes, and 377-456 (73-952) oocytes, respectively. They presented pleopodal fecundity of only 50-60% (max. 250 eggs) and 30-60% (max. 466 eggs) of ovarian fecundity for the noble and the signal crayfish, respectively. These observations, however, were carried out in spring rather than close to the egg laying time. For the signal crayfish, Celada et al. (2005a) presented an average pleopodal fecundity of up to 348 and 233 eggs at the beginning and at the end of embryonic development, respectively. Stypińska (1973) presented 130-325 and 210-345 oocytes for the noble crayfish and the narrow-clawed crayfish, respectively, dependent on the size of females. Schulz and Śmietana (2001) presented that fully-matured female spiny-cheek crayfish had a considerably higher number of eggs than narrow-clawed crayfish of the same body length. This difference amounted to approx. 200 eggs for females of 9 cm length and 300 eggs for females of 11 cm length. Lindquist and Louekari (1975) presented 248 oocytes for the noble crayfish. Stucki (2002) presented the following average pleopodal fecundities per female: noble crayfish 1 50, stone crayfish 60, narrow-clawed crayfish 1 80 and signal crayfish 11 4 eggs per one female. Maguire et al. (2005) founded a mean pleopodal fecundity of 62 eggs, ranging between 30 and 104 eggs, for the stone crayfish. The big eggs losses happen during the winter incubation among these crayfish species (Celada et al. 2005*a*,*b*), which is a significant advantage for the spiny-cheek crayfish, which carries eggs for one to two months only in spring (as described already by Andrews 1907).

Van Den Brink et al. (1988) recorded ovigerous females of the spiny-cheek crayfish in their studied catchments from March to May. In our experiment, females carried eggs in April and May, and the mean time from laying to hatching was 46 ± 3.8 days (37-56 days, i.e. 647 ± 39.9 CTU (543-730 CTU). This is much lower than for the noble crayfish, where the estimated requirement for hatching in nature is approximately 240 days (1,500 CTU), (Cukerzis 1973; Taugbøl and Skurdal 1990). Mason (1977), Hofmannn (1980), Winnicki et al. (2004), and Celada et al. (2005*a*,*b*) stated that 906-1,380 CTU are needed for embryonic development in the signal crayfish, which is still higher than values recorded in our experiment for the spiny-cheek crayfish. The requirement of higher CTU for egg development in European crayfish in comparison with the spiny-cheek crayfish is evident even if we take into account the artificially shortened incubation times under laboratory conditions (Hessen et al. 1987; Pérez et al. 1998; Carral et al. 2004; Policar et al. 2004).

The number of juveniles reaching the 3rd stage was also high (136 juveniles) in comparison with the noble crayfish and the signal crayfish at the 2nd stage (stage of independence). However, the number of juveniles in the 2nd or 3rd stage per female under laboratory conditions is mainly related to rearing conditions, so the differences of such values among various studies are difficult to interpret. In our previous study, under similar experimental conditions we obtained values of 47-97 and 133 juveniles at the 2nd stage for the noble crayfish (Policar and Kozák 2002, Policar et al. 2004) and the signal crayfish (Policar and Kozák 2002), respectively.

Acknowledgements

We are grateful to Adam Petrusek and two anonymous reviewers for all the comments and suggestions that have helped improving the manuscript. This investigation was financially supported by the Czech Ministry of Education (project USB RIFCH No. MSM6007665809) and the Czech Science Foundation (projects 206/03/0532 and 206/03/D064).

References

Abrahamsson S.A.A., 1971. Density, growth and reproduction in populations of *Astacus astacus* and *Pacifastacus leniusculus* in an isolated pond. Oikos 22: 373-380.

Andrews E.A., 1907. The young of the crayfishes *Astacus* and *Cambarus*. Smithson. Contrib. Knowl. Part of Volume XXXV, No. 1 718. Smithsonian Institution. Washington. 79 p.

Austin C.M., 1998*a*. A comparison of clutch and brood size in the Red Claw, *Cherax quadricarinatus* (von Martens) and the Yabby, *C. destructor* Clark (Decapoda: Parastacidae). Aquaculture 167: 135-145.

Austin C.M., 1998*b*. Intraspecific variation in clutch and brood size and rate of development in the yabby, *Cherax destructor* (Decapoda: Parastacidae). Aquaculture 167: 147-159.

Carral J.M., Pérez J.R., Celada J.D., Sáez-Royuela M., Melendre P.M., Aguilera A., 2004. Effects of dead egg removal frequency on stage 2 juvenile production in artificial incubation of *Austropotamobius pallipes* Lereboullet. Bull. Fr. Peche Piscic. 372-373: 425-430.

Celada J.D., Antolín J.I., Carral J.M., Sáez-Royuela M., Rodríguez R., 2005*a*. Successful sex ratio of 1M: 4F in the astacid crayfish *Pacifastacus leniusculus* Dana under captive breeding conditions. Aquaculture 244: 89-95.

Celada J.D., Antolín J.I., Carral J.M., Pérez M., Sáez-Royuela M., 2005*b*. Reproductive efficiency of the signal crayfish (*Pacifastacus leniusculus* Dana. Astacidae) at different densities under both culture and laboratory conditions. Aquaculture, in press.

Cukerzis J.M., 1973. Biologische Grundlagen der Methode der kunstlichen Aufzucht der Brut dese *Astacus astacus* L. Freshwater Crayfish 1: 187-202.

Hamr P., 2002. *Orconectes*. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 585-608.

Harlioğlu M.M., Barim Ö., Türkgülü I., Harlioğlu G.A., 2004. Potential fecundity of an introduced population, Keban Dam Lake, Elaziğ, Turkey, of freshwater crayfish, *Astacus leptodactylus leptodactylus* (Esch., 1852). Aquaculture 230: 189-195.

Hessen D.O., Taugbøl T., Fjeld E., Skurdal J., 1987. Egg development and lifecycle timing in the noble crayfish (*Astacus astacus*). Aquaculture 64: 77-82.

Hobbs H.H., 1974. Synopsis of the Families and Genera of Crayfishes (Crustacea: Decapoda). Smithson. Contrib. Zool. 164: 33p.

Hofman J., 1980. Die Flußkrebse/Freshwater Crayfish. 2. Auflage (von K.M. Strempel). Paul Parey, Hamburg, 110p.

Holdich D.M., Ackefors H., Gherardi F., 1999. Native and alien crayfish in Europe: Some conclusions. In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 281-292.

Holdich D.M., Lowery R.S., 1988. Freshwater Crayfish: Biology, Management and Exploitation, Croom Helm, London, pp. 498 p.

Huber M.G.J., Schubart C.D., 2005. Distribution and reproductive biology of *Austropotamobius torrentium* in Bavaria and documentation of a contact zone with the alien crayfish *Pacifastacus leniusculus*. Bull. Fr. Peche Piscic. 376-377: 759-776.

Jones C.M., 1995. Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae) I. Development of hatchery and nursery procedures. Aquaculture 138: 221-238.

Kozák P., Policar T., Annual course of gonad development in *Orconectes limosus*. Freshwater Crayfish 15, in press.

Kozák P., Policar T., Ďuriš Z., 2004. Migration ability of *Orconectes limosus* through a fishpass and notes on its occurrence in the Czech Republic. Bull. Fr. Peche Piscic. 372-373: 367-373.

Kozubíková E., Petrusek A., Ďuriš Z., Kozák P., Geiger S.H., Hoffmann R., Oidtmann B., 2006. The crayfish plague in the Czech Republic – Review of recent suspect cases and a pilot detection study. Bull. Fr. Peche Piscic. 380-381: 000-000.

Leonard B.V., Lennard W.A., Kildea D.G, 2001. A method for testing the effectiveness of artificial incubation of eggs vs. maternal brooding in the freshwater crayfish *Cherax destructor* (Decapoda: Parastacidae). Aquaculture 195: 299-309.

Lewis S.D., 2002. *Pacifastacus*. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 511-540.

Lewis S.D., Horton H.F., 1997. Life history and population dynamics of signal crayfish, *Pacifastacus leniusculus*, in Lake Billy Chinook, Oregon. Freshwater Crayfish 11: 34-53.

Lindqvist O.V., Louekari K., 1975. Muscle and hepatopancreas weight in *Astacus astacus* L. (*Crustacea, Astacidae*) in the trapping season in Finland. Ann. Zool. Fennici 12: 237-243.

Maguire I., Klobučar G.I.V., Erben R., 2005. The relationship between female size and egg size in the freshwater crayfish *Austropotamobius torrentium*. Bull. Fr. Peche Piscic. 376-377: 777-785.

Mason J.C., 1977. Artificial incubation of crayfish eggs (*Pacifastacus leniusculus* Dana). Freshwater Crayfish 3: 119-132.

Mazlum Y., Eversole A.G., 2004. Observations on the life cycle of *Procambarus acutus acutus* in South Carolina culture ponds. Aquaculture 238: 249-261.

Nakata K., Goshima S., 2004. Fecundity of the Japanese crayfish, *Cambaroides japonicus*: ovary formation, egg number and egg size. Aquaculture 242: 335-343.

Orzechowski B., 1984. Productivity of the freshwater crayfish *Orconectes limosus* Raf. (= *Cambarus affinis* Say) in Koronowo Basin. Acta Univ. Nicolai Copernici Prace Limnol. (in Polish) 14: 13-36.

Pérez J.R., Carral J.M., Celada J.D., Sáez-Royuela M., Romero M.P., 1998. Effects of different thermal treatments during embryonic development on the artificial incubation efficiency of crayfish (*Austropotamobius pallipes* Lereboullet) eggs. Control of the embryogenetic duration and implications for commercial production. Invertebr. Reprod. Dev. 34: 253-258.

Petrusek A., Filipová L., Ďuriš Z., Horká I., Kozák P., Policar T., Štambergová M., 2006. distribution of the invasive spiny-cheek crayfish (*Orconectes limosus)* in the czech republic – history and present. Bull. Fr. Peche Piscic. 380-381: 000-000.

Pieplow U., 1938. Fischereiwissenschaftliche Monographie von *Cambarus affinis* Say. Zeitschr. Fisherei 36: 349-440.

Policar T., Kozák P., 2002. Metody líhnutí a odchovu raků. (Methods of crayfish hatching and rearing) In: B. Vykusová (ed.): Proc. conf. Produkce násadového materiálu ryb a raků (Hatchery production of fish and crayfish stocks), Vodňany, pp. 63-71.

Policar T., Simon V., Kozák P., 2004. Egg incubation in the noble crafish (*Astacus astacus* L.): the effect of controlled laboratory and outdoor ambient condition on hatching success, growth and survival rate of juveniles. Bull. Fr. Peche Piscic. 372-373: 411-423.

Reynolds J.D., 2002. Growth and reproduction. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 152-191.

Sáez-Royuela M., Carral J.M., Celada J.D., Melendre P.M., Aguilera A., 2005. Comparison between individual and group mating of *Austropotamobius pallipes* under controlled conditions. Bull. Fr. Peche Piscic. 376-377: 699-704.

Savolainen R., Westman K., Pursiainen M., 1996. Fecundity of Finnish noble crayfish, *Astacus astacus* L., and signal crayfish, *Pacifastacus leniusculus*, in various natural habitats and in culture. Freshwater Crayfish 11: 319-338.

Schulz R., Śmietana P., 2001. Occurrence of native and introduced crayfish in northeastern Germany and northwestern Poland. Bull. Fr. Peche Piscic. 361: 629-641.

Sint D., Füderer L., 2004. Reintroduction of *Astacus astacus* L. in East Tyrol, Austria. Bull. Fr. Peche Piscic. 372-373: 301-314.

Smith D.G., 1981. Life history parameters of the crayfish *Orconectes limosus* in Southern New-England USA. Ohio J. Sci. 81: 169-172.

Söderbäck B., 1995. Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a Swedish lake: possible causes and mechanisms. Freshwat. Biol. 33: 291-304.

Stucki T.P., 2002. Differences in live history of native and introduced crayfish species in Switzerland. Freshwater crayfish 13: 463-476.

Stypińska M., 1973. The fecundity of three types of crayfish inhabiting Polish waters. Rocz. Nauk. Rol. 95 (1): 147-156 (in Polish).

Tack P.I., 1941. The life history and ecology of the crayfish *Cambaris immunis* Hagen. Am. Midl. Nat. 25: 420-466.

Taugbøl T., Skurdal J., 1990. Reproduction, moulting and mortality of female noble crayfish, *Astacus astacus* (L. 1858), from five Norwegian populations subjected to indoor culture conditions (Decapoda, Astacoidea). Crustaceana 58: 113-123.

Van Den Brink F.W.B., Velde G., Geelen J.F.M., 1988. Life history parameters and temperature related activity of an American crayfish, *Orconectes limosus* (Rafineque, 1817) (Crustacea, Decapoda), in the area of the major rivers in The Netherlands. Arch. Hydrobiol. 114 (2): 275-289.

Winnicki A. Pawlos D., Formicki K., Śmietana P., 2004. On selected morphomechanical characteristics of signal crayfish (*Pacifastacus leniusculus* Dana) eggs during incubation. Bull. Fr. Peche Piscic. 372-373: 431-438.

Chapter 3

The effect of inter- and intra-specific competition on survival and growth rate of native juvenile noble crayfish *Astacus astacus* and alien spiny-cheek crayfish *Orconectes limosus*

Kozák P., Buřič M., Policar T., Hamáčková J. and Lepičová A. 2007. Hydrobiologia 590, 85–94.

The effect of inter- and intra-specific competition on survival and growth rate of native juvenile noble crayfish *Astacus astacus* and alien spiny-cheek crayfish *Orconectes limosus*

Pavel Kozák., Miloš Buřič, Tomáš Policar, Jitka Hamáčková, Andrea Lepičová

University of South Bohemia in České Budějovice, Research Institute of Fish Culture and Hydrobiology in Vodňany, Zátiší 728/II, 389 25 Vodňany, Czech Republic

Abstract

Juvenile noble crayfish, *Astacus astacus* and spiny-cheek crayfish, *Orconectes limosus* were reared from the 2nd and the 3rd developmental stage (i.e. since the stage of independence), respectively, until the end of the growing season. The first period revealed very low survival rate of *O. limosus* in the communal stock (7±6.2%), probably caused by predation of *A. astacus*. Separately reared *O. limosus* proved significantly higher survival rate (49±9.2%) than those in the communal stock with *A. astacus*. The latter species also showed the highest survival rate in this case (90±7.1%). This was not found to differ from the survival rate of separately reared noble crayfish (86±3.1%). In general, both the significant effect of bigger size of *A. astacus* than that of *O. limosus* and higher intra-specific cannibalism of *O. limosus* were reflected during the first phase of rearing. Size and weight of both species were equivalent 11 weeks after hatching (8 weeks of rearing). The higher cumulative survival rate was reached in the intraspecific culture of *A. astacus* (90 %) while low cumulative survival of both species was found in communal stock (less than 50 %) in the second phase of rearing. At the end of the growing season, 19-week-old *O. limosus* attained a higher mean size and weight but were more variable in the group (29.3±5.17 mm and 616.4±412.53 mg) than *A. astacus* (21.2±1.82 mm and 229.9±59.45 mg). Single and communal stock did not differ. Growth of *O. limosus* was much faster than that of *A. astacus*.

Keywords: Orconectes limosus, Astacus astacus, competition, interaction, communal stock, cheliped injuries

1. Introduction

Human activities move many species outside of their native ranges, where they may establish and have substantial ecological effects on native communities (Mack et al. 2000; Vitousek et al. 1996). A group of invaders with particularly important ecological effects are the crayfish. These animals have often been introduced outside their native ranges by deliberate stocking (Hobbs et al. 1989). Once established, these animals may eliminate native crayfish species and reduce populations of favoured food items such as gastropods, algae and macrophytes, which can have cascading trophic effects elsewhere in the ecosystem (Klocker and Strayer 2004; Nyström et al. 1996). The crayfish from North America introduced to Europe (*Orconectes limosus* Rafinesque., *Pacifastacus leniusculus* Dana, *Procambarus clarkii* Girard) often carry the crayfish plague pathogen (*Aphanomyces astaci* Schikora) and may transmit it to the native European crayfish populations (Vogt 1999). With the spread of non-native species in recent decades, the crayfish plague outbreaks have regained intensity recently (Holdich 2003). Furthermore, the indigenous crayfish can also be displaced through direct interactions or competition for resources with the non-native species (Holdich et al.

1999). American crayfish are usually characterized by high growth rates, early maturity and large amounts of offspring, but also short life spans and high mortality rates. They are often aggressive and tolerate also deteriorated habitat conditions (Lindqvist and Huner 1999).

The most widespread non-native species in the Czech Republic is the spiny-cheek crayfish, *O. limosus* (Kozák et al. 2004; Petrusek et al. 2006). It was first intentionally introduced to Europe in 1890, when about 100 specimens from Pennsylvania were released to a fishpond in Barnowko (Berneuchen, Western Pommerania; currently in Poland close to the German border) (Kossakowski 1966; Hamr 2002). This crayfish has spread naturally or by secondary translocations to over 15 European countries, including the Czech Republic and its neighbours – Poland, Germany and Austria (Henttonen and Huner 1999; Holdich 2003). *O. limosus* occurs in the main Czech rivers and it is still being spread. It is a major vector of *A. astaci* in Czech open water (Kozubíková et al. 2006). There is no evidence of a long-term coexistence of *O. limosus* and the native noble crayfish *Astacus astacus* (Linnaeus) in running waters. The occurrence of *O. limosus* in a number of isolated still water bodies, especially flooded quarries or sandpits where several individuals of native species were also recorded (Petrusek et al. 2006), is certainly caused by frequent human-mediated translocations (especially by fishermen, recreational scuba divers etc.).

Apart from crayfish plague transmission, aggression and resource competition are important parts of crayfish interactions (Söderback 1991). This species shows several characteristics such as rapid maturation, short-lifespan, high fecundity and second mating period, which facilitates its fast population growth, dispersal and invasive capabilities (Van Den Brink et al. 1988; Holdich and Lowery 1988; Hamr 2002; Stucki 2002; Kozák and Policar, in press; Kozák et al. 2006). Sympatric crayfish species compete for limited resources such as food, shelter and space, and larger crayfish routinely win competitive interactions with smaller individuals (Momot 1984). Body size is a major determinant of dominance in crayfish (Butler and Stein 1985) and faster growing; hence, larger-sized, crayfish are reported to outcompete smaller individuals of the same species, as well as smaller individuals of a different species (Momot 1984; Mather and Stein 1993). The size difference may be providing a competitive advantage in conspecific interactions.

The specific objectives of this experiment were to determine the growth and survival of young-of-the-year (YOY) of *A. astacus* and *O. limosus* in intra-specific and inter-specific competition treatments.

2. Materials and Methods

2.1. Stocking material

At the end of October 2003, 20 egg-bearing females of *A. astacus* were collected from Světlohorská reservoir in Šumava Protected Landscape Area. Environmental conditions of the Světlohorská reservoir were described in detail by Policar and Kozák (2005). Twenty mature females of *O. limosus* (without pleopodal eggs) were collected on April 18, 2004 in the Kořensko reservoir (Vltava river, South Bohemia, Czech Republic). The females of the two species were placed in a separate fibreglass trough with 1.5 m^2 area with shelters, placed in the laboratory. Laying of eggs in *O. limosus* females took place between April 24 and May 10. Hatching of juveniles occurred between May 20 and June 19 in *A. astacus* and between June 11 and June 16 in *O. limosus*. Stage 1 hatchling are lecitrophic and immobile, remaining attached to the pleopods. The astacid stage 2 and cambarids stage 3 juveniles resemble adults and begin to be independent (Reynolds 2002; Muck et al. 2002; Andrews 1907). Moulting of *A. astacus* and *O. limosus* juveniles used in experiments to the second and third stage, respectively (which is the first stage of juvenile independence) occurred on June 28. Juvenile were stocked to the experiment on July 9, e.g. approximately one month old and 11 days after last moult. There was a statistical difference between sizes (and weights) of both species. Total length (TL) and weight of subsamples were measured to the nearest millimetre and 0.1 mg to estimate the starting size and weight of juveniles, respectively. The body size and weight of *A. astacus* at the second developmental stage were 11.1±0.60 mm (10-12 mm) and 44.37±3.201 mg (37.1-51.5 mg), respectively in comparison to the body size and weight of 7.1±0.73 mm (7-8 mm) and 8.83±1.816 mg (5.8-11.8 mg), respectively for the *O. limosus* at the third developmental stage.

2.2. Rearing conditions

All juveniles were stocked into nine 15 L aquaria (20x30 cm of bottom area). Two bricks (28.5 x 13.5 x 6.5 cm) with 40 holes (each of diameter 1x3 cm) were placed in each aquarium to provide refuges for the crayfish. Both species were reared separately at initial densities of 40 juveniles per aquarium (designated hereafter as single stocks), as well as in a communal stock in 1:1 ratio at densities of 20 juveniles per species and aquarium (333 individuals m^{-2} in all three treatments). The three treatments (single *A. astacus*, single *O. limosus*, mixed-species group) were carried out in triplicates. The rearing was divided into two eight-week experiments during which the crayfish were monitored at regular two-week intervals to check survival and growth rates and crayfish with cheliped injuries were monitored regularly. The crayfish were measured to the nearest 1 mm from the tip of the rostrum to the end of telson to give the total body length (TL). The animals were weighed to the nearest 0.1 mg. Prior to weighing, the animals were first put on filter paper to remove excess water trapped between the branchiostegites and appendages. The first experiment lasted until size and weight equilibration of juveniles of both species (altogether 4 two-week intervals = 8 weeks of rearing) to examine mainly the impact of size on predation risk. After every sampling, during this period, additionally crayfish were stocked to maintain the original density (40 crayfish per aquarium) because low survival of *O. limosus* in the communal stocks was observed. The second experiment again lasted 8 weeks (4 two-week periods) of rearing. The initial density of crayfish was half of the above (20 individuals per aquarium) because of larger size of crayfish and no crayfish were additionally stocked after the control catching to check the effect of inter-specific competition on cumulative survival and growth rate. This experiment was finished at the end of October. The crayfish were fed with frozen zooplankton twice a day at a ratio of 30 % of initial crayfish body mass adjusted at the beginning of each period. Mean (±SD) water temperature during the experiment was 20.6±1.2 °C. The oxygen level was measured daily and did not decrease below 7.5 $mg.l^{-1}$. Lights were set on 12h L: 12h D regime. Faeces and waste feed were siphoned out daily before feeding.

2.3. Statistical analysis

All data are presented as the mean ± SD. Statistical analyses were performed using Statistica software (6.1. version), StatSoft, Inc. Differences in survival and growth (TL, weight) were compared between groups by Anova, Tukey HSD and Kruskal-Wallis test. For all of these statistical tests, P values lower than or equal to 0.05 were considered indicative of a significant difference.

3. Results

3.1. Experiment 1: early phase growth

Period 1: In general, both the significant effect of larger size of *A. astacus* than that of the *O. limosus* and higher intra-specific cannibalism of *O. limosus* were reflected during the first phase of rearing. We found very low survival rate of *O. limosus* in the communal stock (7±6.2%), probably caused by predation by *A. astacus*. Separately reared *O. limosus* showed a significantly higher survival rate (49±9.2%) than those in the communal stock with *A. astacus*. *A. astacus* also shoved the highest survival rate in the communal stock with *O. limosus* (90±7.1%). It was not found to differ from the survival rate of separately reared *A. astacus* (86±3.1%) (Fig. 1).

The size and weight between both species still differed at the end of period 1. The *A. astacus* in communal stock reached higher weight than those in single treatment.

Period 2: High intra-specific cannibalism of *O. limosus* was reflected during the second period of rearing. We found the least survival (41±4.7%) in single *O. limosus*. The *O. limosus* in the communal stock had lower survival (72±10.2%) than *A. astacus* in both communal and monospecies rearing (93±6.2% and 93±3.5%). The size and weight between both species still differed at the end of the period 2 but without statistical difference between stocks of the same species. We recorded the change in spiny cheek crayfish behaviour as some of the individuals did not use shelters during the day and they staid out of them.

Period 3: We found a difference between the survival of *A. astacus* (87±6.12% and 88±6.2%) and *O. limosus* (53±6.2% and 53±12.0%). There was no inter-specific effect on survival. Size of both species was found equilibrated at the end of the period 3 (9 weeks after hatching). Only *O. limosus* in communal stock reached a lower weight than *A. astacus*. Statistical difference was found between the diffusion of size (and weight) of both species. In general, the size of *A. astacus* was relatively well balanced, ranging from 11 to 20 mm, with majority (more than 75 %) between 17 and 19 mm. Size of *O. limosus* substantially varied, ranging between 11 and 29 mm with majority between 14 and 20 mm.

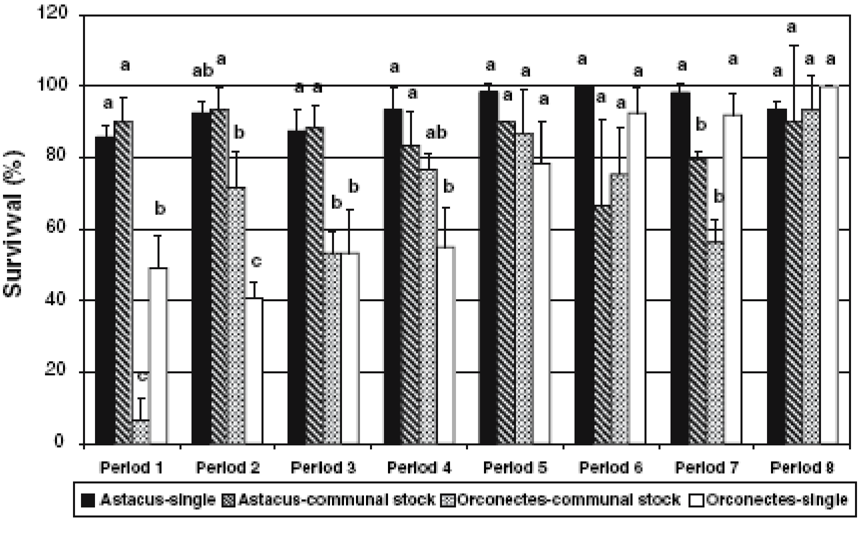


Figure 1. The survival of A. astacus and *O. limosus* in single and communal stocks in individual periods (different alphabetic superscripts show significant differences between stocks in each period; $P < 0.05$).

Period 4: The lowest survival was found in single *O. limosus* (55±10.8%) in comparison to high survival of *A. astacus* both alone and communally (93±6.2% and

83±9.4% respectively). The survival of *O. limosus* in the communal stock (77±4.7) did not differ from the others. The bigger disproportion in size inside the group of *O. limosus* persisted. While the mean size and weight of both species did not statistically differ, the variances of both samples were different (Fig. 2). The minimum and maximum size of *A. astacus* and *O. limosus* ranged between 15-23 mm and 12-36 mm, respectively. The *O. limosus* with both chelae in the communal stock was bigger than those ones without chelae.

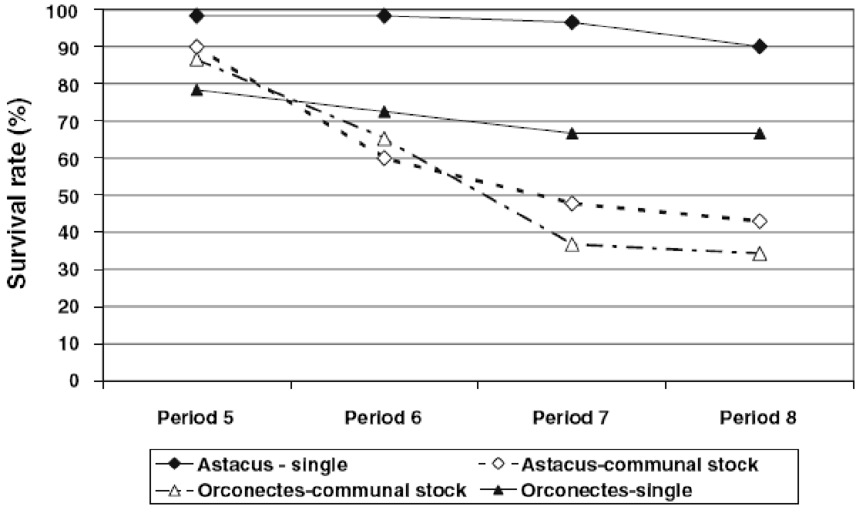


Figure 2. The cumulative survival rate of *A. astacus* and *O. limosus* in the single and communal stocks in the second experiment.

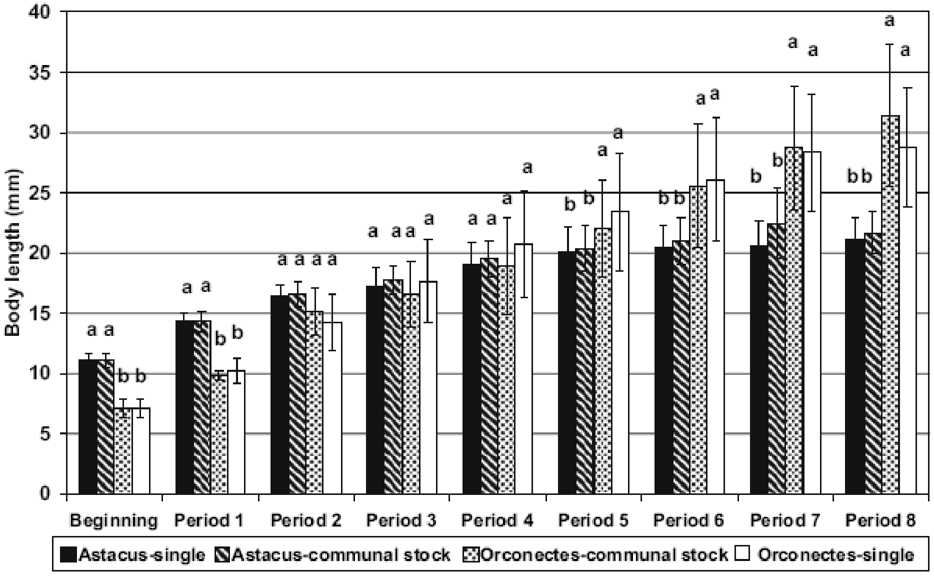


Figure 3. The mean body length of *A. astacus* and *O. limosus* in single and communal stock in individual periods (different alphabetic superscripts show significant differences between stocks in each period; P < 0.05).

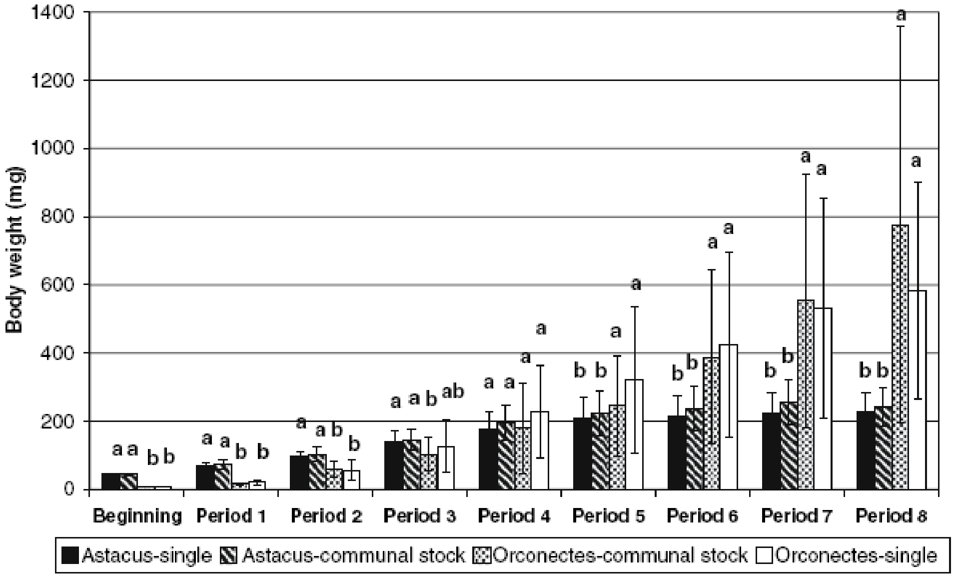


Figure 4. The mean body weight of *A. astacus* and *O. limosus* in the single and communal stock in individual periods (different alphabetic superscripts show significant differences between stocks in each period; P < 0.05).

3.2. Experiment 2: later growth

No significant difference was found in the survival during all periods in the second experiment except for period 7. There was higher survival in single populations (*A. astacus* 98±2.5% and *O. limosus* 92±6.0%) than in communal stock (*A. astacus* 65±2.1% and *O. limosus* 56±6.2%). The higher cumulative survival rate was reached by single *A. astacus* (90 %) while low cumulative survival of both species was found in communal stock (less than 50 %) (Fig. 3). At the end of period 5 the size and weight of *O. limosus* were bigger than those of *A. astacus* (Fig. 4, 5). Single and communal stocks did not differ. At the end of the growing season, 19-week-old *O. limosus* reached higher mean size and weight than *A. astacus* but they were highly variable in the group (29.3±5.17 mm and 616.4±412.53 mg) (Fig. 6, 7). The *A. astacus* gained mean size and weight of 21.2±1.82 mm and 229.9±59.45 mg, respectively. The sex ratio was either 1:1 or females were more abundant in all treatments. The biggest individuals of *O. limosus* in each aquarium were also females.

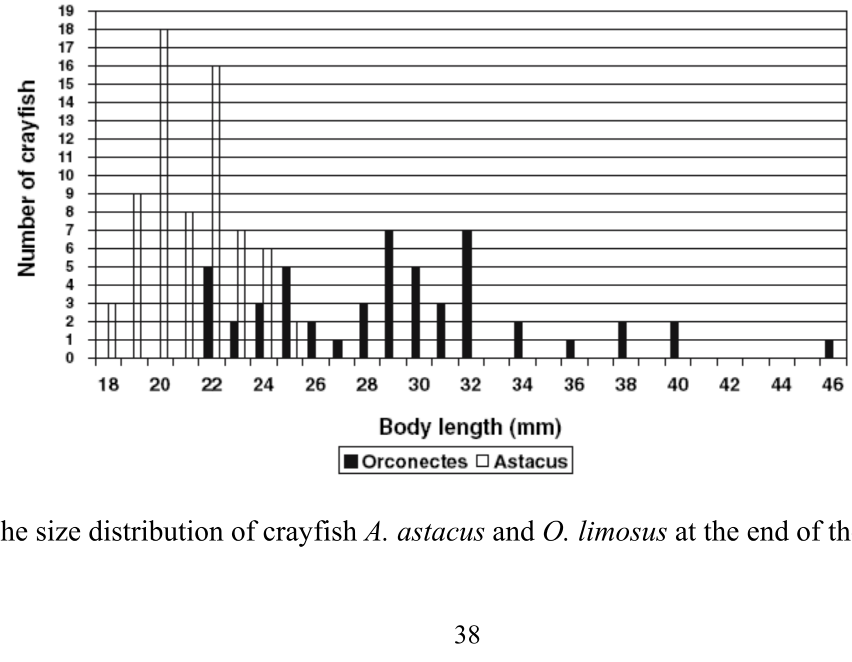


Figure 5. The size distribution of crayfish *A. astacus* and *O. limosus* at the end of the rearing period.

4. Discussion

The high mortality at the beginning of rearing caused mainly by cannibalism was probably due to lack of food. Gydemo and Westin (1993) reported that on average, 37% of the mortality of adult *A. astacus* was caused by cannibalism, but cannibalism increased to 94% when crayfish were being starved. The feeding rate of 30% would apper to be very low for the youngest stage of both species. The very low survival rate of *O. limosus* in the communal stock was certainly caused by predation by bigger *A. astacus*. Body size is a major determinant of dominance in crayfish (Butler and Stein 1985), larger crayfish routinely win competitive interactions with smaller individuals (Momot 1984). Mazlum and Eversole (2005) showed that *Procambarus acutus acutus* (Girard) YOY, which were larger than *P. clarkii* YOY at stocking, grew and survived at significantly greater rates. The body size advantage at hatching and through the faster growth of *P. a. acutus* was important in defining competitive interaction with *P. clarkii*. The bigger size of *A. astacus* at hatching and beginning of our experiment was advantage for them approximately half of the season because *O. limosus* grew faster even in communal stock. The *A. astacus* in communal stock reached higher weight than those in single treatment at the beginning of rearing. Ahvenharju et al. (2005) reported that larger individuals of *Pacifastacus leniusculus* benefited from the presence of smaller co-species. Higher mortality or inter-specific cannibalism showed *O. limosus* in comparison to *A. astacus* at this period. In contrast to the first period, single *O. limosus* had lower survival than those in communal stock which could be caused changes in their behaviour. They started to stay more outside of the shelters during the daytime which is typical for this species in contrast to the mainly nocturnal activity of *A. astacus* and they could compete better for the food at this time.

Although the average size and weight of both species was equivalent 9 weeks after hatching (8 weeks of rearing), the bigger disproportion in size inside the group of *O. limosus* was monitored and extended at the of rearing. The size of *A. astacus* was comparable with juveniles reared elsewhere under controlled conditions (Ackefors et al. 1995; Policar et al. 2004). We did not monitor the number and time of crayfish moulting in our experiment. Ackefors et al. (1995) and Ackefors (1999) found that under experimental conditions juveniles of *A. astacus* moult 6 times during the first year and reach stage 7. There were only small differences in the mean moult interval even with different feeding. For *O. limosus* there is a lack of published information regarding the number of developmental stages. Only very old information focused on moulting frequency of *O. limosus* was given by Andrews (1907). The *O. limosus* hatched in May 18 reached the stage 7 in July 17 with the 29 mm of body length. The body length in October ranged between 23 to 62 mm without mention of developmental stages. The high disproportion is in good relationship with our results. Muck et al. (2002) estimated that 63% of males and 55% of females of *Orconectes ozarkae* (Williams) matured during their first year of life and were capable of mating.

Ackefors et al. (1995) indicated that if males and females of *A. astacus* were reared individually there were probably no differences in growth rate between males and females during the juvenile stages but Gydemo and Westin (1989) found a tendency in males to attain larger sizes after 6 and 9 months of rearing. This indicates that juvenile males of *A. astacus* may be more aggressive than females, a behaviour which could favour the competition for food and optimal shelter sites. This is in contrary to *O. limosus* in our study where we found the biggest individuals in each aquarium to be females. Muck et al. (2002) showed that males and females of *O. ozarkae* grew at the same rate throughout the first growing season. From March until May males were larger than females. Adult males began to moult in March while females carried eggs and young until May, at which time they began to grow. Adult females began moulting after release of young and grew to the same size as males by July.

Chelae are important also in determining success in intra- and inter-specific crayfish interactions. Crayfish species with larger chelae win more fights than similar-sized species with smaller chelae (Gherardi 2002). Rutherford et al. (1995) reported that chelae length is an important indicator of fighting ability in crayfish. They found that in agonistic interactions between male *Orconectes rusticus* (Girard) the winner had significantly longer chelae than the loser. Higher density of crayfish increased the frequency of cheliped injuries (Savolainen et al. 2004). The *O. limosus* with both chelae in the communal stock were bigger than those ones without chelae at the end of the first experiment. Animals that had lost a cheliped were reported to be significantly smaller in comparison with juveniles with two chelipeds (Nyström 1994; Savolainen 2003). Smaller animals held together with larger ones had the highest number of injuries, probably because of aggressive behaviour of the dominant larger individuals. The smaller crayfish grew faster in the absence of large animals. However, smaller juveniles had more injuries than larger individuals even when reared separately (Ahvenharju et al. 2005). Chelae form a substatial proportion of crayfish total biomas. After a cheliped loss, crayfish are forced to regenerate the missing claw using a part of their energy reserves, which would otherwise be used in growth (Aiken and Wady 1992). Missing claws and the size of crayfish have a great effect on their overall success, behaviour and social status in the population. Mason (1979) reported that absence of chelae in *P. leniusculus* increased mortality due to cannibalism or damage by conspecifics with unbroken chelae.

Dominance hierarchies among crayfish, stabilize rapidly after fighting where one animal (the largest) was superdominant (Copp 1986). This could explain relatively high cumulative survival rate in both single stocks in the second phase of rearing. The low survival of both species in the communal stock indicated some inter-specific interaction which could affect social behaviour also inside the species.

Besides the tolerance to *A. astaci*, growth in *O. limosus* was much faster than in *A. astacus*. This is one trait in the life history of r-selected species which are adapted to unpredictable environments, exhibit rapid growth rate, short life spans and are able to take maximum advantage of abundant resources. Together with other behaviour and ecological properties, faster growth make *O. limosus* a successful invasive species in disturbed habitats and other areas that are subject to man-induced modifications. The only disadvantage was lower survival of *O. limosus* in both single and communal stocks which could be balanced by higher fecundity and early maturation of this species.

5. Conclusion

- *A. astacus* had higher survival than *O. limosus* in both treatments. The significant effect of bigger size of *A. astacus* than that of *O. limosus* on survival rate and higher intra-specific cannibalism of *O. limosus* were reflected during the first phase of rearing.
- Survival of both species was significantly higher in the intra-specific treatment than in inter-specific treatment in the second phase of rearing.
- The *O. limosus* grew much faster than *A. astacus*. Size and weight of both species was found equilibrated 11 weeks after hatching (8 weeks of rearing).
- The highest cumulative survival rate was attained by the single *A. astacus* (90 %) while low cumulative survival of both species was found in communal stock (less than 50 %) at the second phase of rearing. At the end of the growing season, 19-week-old *O. limosus* gained higher mean size and weight but they were highly variable in the group (29.3±5.17 mm and 616.4±412.53 mg) than *A. astacus* (21.2±1.82 mm and 229.9±59.45 mg).

Acknowledgements

This investigation was financially supported by the Czech Science Foundation project No. 206/03/D064, Ministry of Agriculture project No. QF3028 and by the USB RIFCH No. MSM6007665809.

References

Ackefors H., 1999. Observations on the yearly life cycle of *Astacus astacus* in a small lake in Sweden. Freshwater Crayfish 12: 413-429.

Ackefors H., Gydemo R., Keyser P., 1995. Growth and moulting in confined juvenile noble crayfish *Astacus astacus* (L.) (Decapoda, Astacidae). Freshwater Crayfish 10: 396-409.

Ahvenharju T., Savolainen R., Tulonen J., Ruohonen K., 2005. Effects of size grading on growth, survival and cheliped injuries of signal crayfish (*Pacifastacus leniusculus* Dana) summerlings (age 0+). Aquacult. Res. 36: 857-867.

Aiken D.E., Waddy S.L., 1992. The growth process in crayfish. Rev. Aquat. Sci. 6: 335-381.

Andrews E.A., 1907. The young of the crayfishes *Astacus* and *Cambarus*. Smithson. Contrib. Knowl. Part of Volume XXXV, No. 1718. Smithsonian Institution. Washington. 79 pp.

Butler M.J., Stein R.A., 1985. An analysis of mechanism governing species replacement in crayfish. Oceologia 66: 168-177.

Copp N.H., 1986. Dominance hierarchies in the crayfish *Procambarus clarkii* (Girard, 1852) and the question of learned individual recognition (Decapoda, Astacidea). Crustaceana 51: 9-24.

Gherardi F., 2002. Behaviour. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 152-191.

Gydemo R., Westin L., 1989. Growth and survival of juvenile Astacus astacus L. in optimized water temperature. In: De Pauw N., Jaspers E., Ackefors H., Wilkins N. (eds.): Aquaculture – a biotechnology in progress. European Aquaculture Society, Bredene, Belgium, pp. 383-391.

Gydemo R., Westin L., 1993. Effects of starvation, constant light and partial dactylotomy on survival of noble crayfish, *Astacus astacus* (L.), under high density laboratory conditions. Freshwater Crayfish 9: 79-83.

Hamr P., 2002. *Orconectes*. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 585-608.

Henttonen P., Huner J.V., 1999. The introduction of alien species of crayfish in Europe: A historical introduction. In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 13-22.

Hobbs H.H., Jass J.P., Huner J.V., 1989. A review of global crayfish introductions with particular emphasis on two North American species (Decapoda, Cambaridae). Crustaceana 56: 299-316.

Holdich D.M., 2003. Crayfish in Europe – an overview of taxonomy, legislation, distribution, and crayfish plague outbreaks. In: Holdich D.M., Sibley P.J. (eds.): Management & Conservation of Crayfish, Proceedings of a conference held on 7th November 2002 at the Nottingham Forest Football Club, Nottingham, UK, Environment Agency, Bristol, UK., pp. 15-34.

Holdich D.M., Lowery R.S., 1988. Freshwater Crayfish: Biology, Management and Exploitation, Croom Helm, London, 498 pp.

Holdich D.M., Ackefors H., Gherardi F., 1999. Native and alien crayfish in Europe: Some conclusions. In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 281-292.

Klocker C.A., Strayer D.L., 2004. Interactions among an invasive crayfish (*Orconectes rusticus*), a native crayfish (*Orconectes limosus*), and native bivalves (Sphaeriidae and Unionidae). Northeast. Nat. 11: 167-178.

Kossakowski J., 1966. Raki. [Crayfish.] Panstwowe wydawnictwo rolnicke i lesne, Warszawa (in Polish).

Kozák P., Policar T. Annual course of gonad development in *Orconectes limosus*. Freshwater Crayfish 15, *in press*.

Kozák P., Buřič M., Policar T., 2006. The fecundity and juveniles production in spiny-cheek crayfish (*Orconectes limosus*). Bull. Fr. Peche Piscic. 380-381: *in press*.

Kozák P., Policar T., Ďuriš Z., 2004. Migration ability of *Orconectes limosus* through a fishpass and notes on its occurrence in the Czech Republic. Bull. Fr. Peche Piscic. 372-373: 367-373.

Kozubíková E., Petrusek A., Ďuriš Z., Kozák P., Geiger S., Hoffmann R., Oidtmann B., 2006. The crayfish plague in the Czech Republic – review of recent suspect cases and a pilot detection study. Bull. Fr. Peche Piscic. 380-381, *in press*.

Lindqvist O.V., Huner J.V., 1999. Life history characteristics of crayfish: what makes some of them good colonizers? In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 23-30.

Mack R.N., Simberloff D., Lonsdale W.M., Evans H., Clout M., Bazzaz F.A., 2000. Biotic invasions: Causes, epidemiology, global consequences, and control. Ecol. Appl. 10: 689-710.

Mason J.C., 1979. Effects of temperature, photoperiod, substreate and shelter on survival, growth and biomass accumulation of juvenile *Pacifastacus leniusculus* culture. Freshwater Crayfish 4: 73-82.

Mather M.E., Stein R.A., 1993. Using growth/mortality trade-offs to explore a crayfish species replacement in stream riffles and pools. Can. J. Fish. Aquat. Sci. 50: 88-96.

Mazlum Y., Eversole A.G., 2005. Growth and survival of *Procambarus acutus acutus* (Girard, 1852) and *P. clarkii* (Girard, 1852) in competitive settings. Aquacult. Res. 36: 537-545.

Momot W.T., 1984. Crayfish production: a reflection of community energetics. J. Crust. Biol. 4: 35-54.

Muck J.A., Rabeni C.F., DiStefano R.J., 2002. Life-history characteristics of the crayfish *Orconectes ozarkae* in a Missouri Ozark stream. Freshwater Crayfish 13: 359-370.

Nyström P., 1994. Survival of juvenile signal crayfish (*Pacifastacus leniusculus*) in relation to light intensity and density. Nord. J. Freshwat. Res. 69: 162-166.

Nyström P., Brönmark C., Granéli W., 1996. Patterns in benthic food webs: a role for omnivorous crayfish? Freshwat. Biol. 36: 631-646.

Petrusek A., Filipová L., Ďuriš Z., Horká I., Kozák P., Policar T., Štambergová M., 2006. Distribution of the invasive spiny-cheek crayfish (*Orconectes limosus)* in the Czech Republic: history and present. Bull. Fr. Peche Piscic. 380-381, *in press*.

Policar T., Simon V., Kozák P., 2004. Egg incubation in the noble crafish (*Astacus astacus* L.): the effect of controlled laboratory and outdoor ambient condition on hatching success, growth and survival rate of juveniles. Bull. Fr. Peche Piscic. 372-373: 411-423.

Policar T., Kozák P., 2005. Comparison of trap and baited stick catch efficiency for noble crayfish (*Astacus astacus* L.) in the course of the growing season. Bull. Fr. Peche Piscic. 376-377, 675 - 686.

Reynolds J.D., 2002. Growth and reproduction. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 152-191.

Rutherford P.L., Dunham D.W., Allison V., 1995. Winning agonistic encounters by male crayfish *Orconectes rusticus* (Girard) (*Decapoda, Cambaridae*): chela size matters but chela symmetry does not. Crustaceana 68: 526-529.

Savolainen R., Ruohonen K., Tulonen J., 2003. Effects of bottom substrate and presence of shelter in experimental tanks on growth and survival of signal crayfish, *Pacifastacus leniusculus* (Dana) juveniles. Aquacult. Res. 34: 289-297.

Savolainen R., Ruohonen K., Railo E., 2004. Effect of stocking density on growth, survival and cheliped injuries of stage 2 juvenile signal crayfish *Pacifastacus leniusculus* Dana. Aquaculture 231: 237-248.

Söderback B., 1991. Interspecific dominance relationship and aggressive interactions in the freshwater crayfishes *Astacus astacus* (L.) and *Pacifastacus leniusculus* (Dana). Can. J. Zool. 69: 1321-1325.

Stucki T., 2002. Differences in live history of native and introduced crayfish species in Switzerland. Freshwater crayfish 13: 463-476.

Van Den Brink F.W.B., Velde G., Geelen J.F.M., 1988. Life history parameters and temperature-related activity of an American crayfish, *Orconectes limosus* (Rafineque, 1817) (Crustacea, Decapoda), in the area of the major rivers in The Netherlands. Arch. Hydrobiol. 114: 275-289.

Vitousek P.M., D´Antonio C.M., Loope L.L., Westbrooks R., 1996. Biological invasions as global environmental change. Am. Sci. 84: 469-478.

Vogt G., 1999. Diseases of European freshwater crayfish, with particular emphasis on interspecific transmission of pathogens. In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 87-103.

Chapter 4

Evaluation of different marking methods for spiny-cheek crayfish (*Orconectes limosus*)

Buřič M., Kozák P., and Vích P. 2008. Knowledge and Management about Aquatic Ecosystems, 389 (02): 1–8.

Evaluation of different marking methods for spiny-cheek crayfish (*Orconectes limosus*)

Miloš Buřič, Pavel Kozák, Petr Vích

University of South Bohemia in České Budějovice, Research Institute of Fish Culture and Hydrobiology, Zátiší 728/II, 389 25 Vodňany, Czech Republic

Abstract

Different methods of marking were evaluated for *Orconectes limosus* (CL = carapace length 12 – 37 mm) under laboratory conditions: the use of passive integrated transponders, visible implant elastomers, visible implant alphanumeric tags, and cauterization. High survival (>90%) was obtained with all methods tested. Tag retention and readability were evaluated immediately after marking, before and after ecdysis, with moult increments and number of moults been recorded. Marking using cauterization negatively affects the percent moult increment of crayfish. The average PMI of 15 ± 0.88 % did not differ between males (15 ± 1.41 mm) and females (15 ± 1.12 mm). It was found that PMI gradually decreased with an increase in CL. Sixty-one moults were observed, with 12 crayfish (6 juveniles and 6 adults) presenting two moults. Time between moults varied from 3 to 7 weeks. CL was increased with over 28 ± 2.68% due to two moults. Three females moulted twice after successful hatching and carrying of juveniles.

Keywords: Crayfish, Orconectes limosus, marking, moult increment.

1. Introduction

Orconectes limosus (Rafinesque, 1817), a temperate-water crayfish native to north eastern USA and south-eastern Canada, shows tolerance to organic-rich waters and general pollutants. Although it occurs in rivers, its preferred habitats are ponds and lakes (Hamr 2002). The first successful introduction into Europe took place in 1890 when some specimens were stocked into a fishpond in Barnowko (Poland). Further introductions in France and successful secondary introductions in many European water-bodies, combined with natural dispersal, has resulted in its presence in several European countries (Henttonen and Huner 1999; Holdich et al. 2006), including Czech Republic (Petrusek et al. 2006).

O. limosus is a fast spreading, crayfish plague carrying, invasive species, which poses a serious threat to indigenous crayfish (Henttonen and Huner 1999; Holdich and Black 2007). Early maturation (Holdich et al. 2006), high fecundity (Stucki 2002), fast growth (Kozák et al. 2007), and a short life span (Holdich et al. 2006), suggest that *O. limosus* is a r-selected species (Henttonen and Huner 1999). More moults, in contrast to moult increments, resulted in faster growth in crayfish (Lowery 1988). However, similar to noble crayfish *Astacus astacus* (Skurdal and Taugbøl 2002), adult *O. limosus* usually moult once (ovigerous females) or twice (males, females) per year (Holdich and Black 2007).

Despite the fact that *O. limosus* is one of the largest macroinvertebrates in several American and European freshwater water-bodies, and its wide distribution and invasiveness, the ecology of this species was less studied. In order to obtain information on population dynamics a suitable observation method, which does not influence survival, growth and behaviour, is crucial. Mark and recapture methods are widely used in field (Abrahamsson

1973; Guan 1997; Bubb et al. 2002) and laboratory (Guan and Wiles 1996; Barki et al. 1997; Kuhlmann et al. 2008) experiments on crayfish.

Objectives of the present study were: 1) to evaluate four different marking methods for *O. limosus* (tag retention and readability, effects of tagging on survival), 2) to evaluate the effect of tagging on moult increments, and to observe the relationship with number of moults.

2. Material and methods

2.1. Crayfish

O. limosus specimens were captured (in overall 155 pcs.) in the brook 'Pšovka' in central Czech Republic during the beginning of April 2007. Crayfish were acclimated to laboratory conditions for 14 days before tagging. Total length (TL; from tip of the rostrum to the posterior median edge of the telson) and carapace length (CL; from the tip of the rostrum to the posterior median edge of the cephalothorax) were measured to the nearest 1 mm with vernier calipers, and wet body weight of crayfish to the nearest 0.01 g with an electronic balance (Kern & Sohn GmbH, Balingen, Germany). Average TL and CL of crayfish used were 53.5 ± 13.83 mm and 24.9 ± 0.69 mm, respectively, with an average body weight of 6.2 ± 0.4 g. Crayfish were partitioned into five groups (20 crayfish in each group with sex ratio 1:1) according to treatment. There was not significant difference between groups in size.

2.2. Marking

Passive integrated transponders (PITs). Crayfish were tagged with PIT tags of 12 x 2.1 mm in size and 0.1 in weight. Animals were held around the cephalothorax with the ventral surface upwards, and an incision of ca. 3 mm wide and deep was made through the cuticle and abdominal musculature of the third abdominal segment with the tip of a sterile large (diameter 2.5 mm) hypodermic needle. The tag was inserted through the incision by gently pushing the tag anteriorally to fit between the edge of abdomen and hindgut (Figure 1A). After tagging the number of the PIT was read on a portable reader (Datamars S.A., Lugano, Switzerland).

Visible implant alphanumeric tags (VI Alpha tags). VI Alpha tags of 2.5 x 1 mm, made from soft plastic (Northwest Marine Technology^{TM}, Shaw Island, USA), were used. Tagging at the third abdominal segment was carried out similarly as for PITs using the tip of the injector. The tag was inserted through the incision by gently pushing it with the rod of the injector (Figure 1B), whereafter the injector was slowly withdrawn.

Visible implant elastomers (elastomers). After thorough mixing of the two elastomer components in equal parts it was loaded into an injection syringe (Northwest Marine Technology^{TM}, Shaw Island, USA). Tagging was done as described above with a syringe needle inserted into the marking location (Figure 1C). When a long narrow mark was created the injector was slowly withdrawn. It is important that the tag created is fully complete on the target tissue; extrusion of the material from the needle must cease before the needle is withdrawn so that material does not project through the needle wound; this is likely to cause rapid loss of the tag.

Cauterization. Crayfish were marked with a portable gas soldering iron (Ferm, Zwolle, Netherlands) by touching the carapace for a few seconds to create red-orange spots on the cuticle, which were visible after moulting as white spots. A modified system for numerical marking of crayfish (Abrahamsson 1973), with the use of five number marks (Figure 1D), was applied.

2.3. Experimental conditions

The study was conducted between 19 April and 28 June 2007 over a 70 day period in Vodňany. Groups of marked crayfish were kept in 5 circular tanks with a volume of 0.12 m^3 and diameter of 0.6 m supplied with shelters. Tag retention, readability, survival and number of moults were determined on a weekly basis, and moult increments were measured with vernier calliper. Tanks were regularly cleaned, with water temperature similar to outdoor water temperature. Water temperature was measured every 3 hours using data loggers (RT-F53, Qi Analytical, Prague, Czech Republic), and dissolved oxygen level twice daily. Crayfish were fed on frozen chironomid larvae and fish meat three times per week.

2.4. Statistical analysis

T-tests were used to compare moult increments between males and females, and between successive (1^{st} and 2^{nd}) moults. Data for survival and increments in particular groups were analysed by the non-parametric Kruskal-Wallis test. Spearman rank correlation was used to determine the relationship between percent moult increment (PMI) and CL. The null hypothesis was rejected at $\alpha = 0.05$. Data are presented as average ± standard error of mean. For data analysis was used program Statistica 7.0 (StatSoft, Inc.).

3. Results

3.1. Evaluation of marking methods

Most of the injuries on the cuticle caused by tagging were cured within a few days. All methods evaluated were found suitable for tagging of *O. limosus*. Very small crayfish (13 – 14 mm CL) were successfully marked by VI Alpha tags and elastomers. Survival did not differ ($H = 5.699$; $p = 0.223$) among marking methods (Figure 2). Mortality mainly occurred during the time when crayfish moulted. All groups showed a high survival rate.

High retention and readability of all type of tags were found (Table I). The spots after cauterization appeared well grown over after moult, which led to more difficult readability. Although VI Alpha tags before and after moult presented high readability, several tags were lost in this group. The slow setting and smudging of elastomers caused the impaired readability, but moulting did not affect further changes.

Table 1. Percentage of readable marks before and after moulting and tag retention as influenced by different marking methods.

Marking method	Readability (%)		Tag retention
	Before moult	After moult	(%)
PITs	100	100	100
Cauterization	100	80	100
VI Alpha tags	100	100	85
VI elastomers	80	80	100

3.2. Moult increments and number of moults

The average total moult increment and percentage moult increment (PMI) of CL was found as 3 ± 0.15 mm and 15 ± 0.88 %, respectively. Average total increments did not differ ($t = 1.325$; $p = 0.191$) between males (3.2 ± 0.21 mm) and females (2.8 ± 0.19 mm). This was also illustrated (t = -0.01; p = 0.994) in PMI, with values of 15 ± 1.41 and 15 ± 1.12 mm for males and females, respectively. With an increase in CL the PMI gradually decreased (Spearman rank correlation, $r_s = -0.617$, $p < 0.05$). This trend was also observed within sex (Spearman rank correlation, males, $r_s = -0.683$, $p < 0.05$; females, $r_s = -0.551$, $p < 0.05$). PMI values of 13.9 ± 1.73%, 17.7 ± 1.74% and 14.9 ± 0.99% were obtained for PITs, VI Alpha tag, and elastomer treatments, respectively, with a lower (H = 10.3; p = 0.016) value (9.7 ± 1.26%) derived for the group marked by cauterization. However, CL of moulted crayfish did not differ (H = 3.01; p = 0.221) among groups.

Sixty-one moults occurred during the experiment. Twelve crayfish (5 males, 7 females) moulted twice, with double moults observed in 6 juveniles (3 males, 3 females) and 6 adults (2 males, 4 females). Time between moults varied from 3 to 7 weeks. CL increased by over 28 ± 2.68% due to two moults. Of four adult females three presented two successive moults in 3 to 4 weeks after successful hatching and carrying of juveniles. Three adult crayfish underwent two moults during a 4 - 6 week period. A significant difference (t = 3.01; p = 0.011) between PMI in the 1st (17.3 ± 2.17%) and 2nd (9.2 ± 1.36%) moults was found.

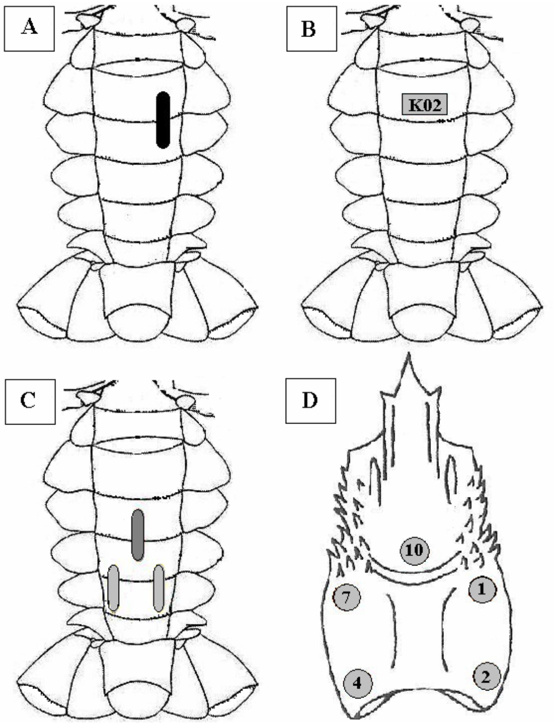


Figure 1. Position of tags. A – Passive integrated transponder inserted in abdominal musculature. B – Position of visible implant alphanumeric tag under transparent ventral abdominal cuticle. C – Location of individual marks made by visible implant elastomers under transparent ventral abdominal cuticle. D – System for numerical marking of crayfish using cauterization.

4. Discussion

All methods evaluated were found to be suitable for successful marking of O. *limosus*. Application of the different methods was simple and fast, fulfilling the requirement of creating minimum stress. Elastomers and VI Alpha tags were clearly visible through the ventral somites, were retained after moulting, and did not interfere with survival and growth. The above methods are preferred to other marking methods for crustaceans, because they allow long-term individual identification and the tagging of small size-class animals. Other tags such as external marks are often lost during moults (Gherardi et al. 2000), whereas use of internal transponders (Bubb et al. 2002) and cauterization are hampered by the size of the animal. In addition, with cauterization spots are visible for only ca. 2 moults (Abrahamsson 1973). Other workers have found that cauterization spots can be seen for more than 2 moults. Marking by PITs is possible only for big adults (> 25 mm CL). On the other hand passive integrated transponders allowed fast and exact determining of marked crayfish. Marking of crayfish can negatively affect growth, e.g. external tags (Graaf 2007) or punching holes in uropods and telson (Guan 1997). Although the values of PMI in all experimental groups were high, the crayfish marked using cauterization had a significantly lower PMI than other groups, hence cauterization could not be recommended for study of growth in crayfish. VI Alpha tags are recommended as an effective inexpensive method to identify individual crayfish for long-term studies. On the other hand, several tags could be lost during moulting.

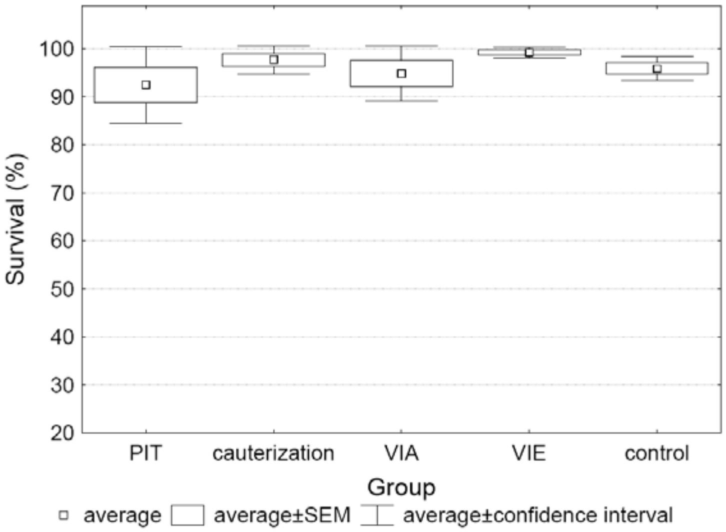


Figure 2. Survival of crayfish in particular experimental groups (Kruskal-Wallis test; H = 5.699; p = 0.223).

The observed PMI of *O. limosus* is comparable to PMI of juvenile *A. astacus* in their first growing season (Ackefors et al. 1995), and higher than for juvenile *Austropotamobius pallipes* and *Procambarus clarkii* (5 – 10%) (Paglianti and Gherardi 2004). The present study presents evidence that three females which successfully hatched and carried juveniles, together with three other adults, were able to moult twice during one month. This is in contrast to the finding of Holdich and Black (2007) that adults of *O. limosus* usually moult once (ovigerous females) or twice (males) per year, but in agreement to results obtained for *Orconectes neglectus chaenodactylus* (Price and Payne 1978). The short intermoult period probably lead to the lower PMI at second moult. Faster growth in *O. limosus* is attributed to a high PMI and moult frequency, because growth is accomplished as a product of moult increment and moult frequency (Reynolds 2002). Fast growth contribute to the invasive success of *O. limosus*. A trend of a decrease in PMI with an increase in CL was found, in accordance to results obtained with *P. leniusculus* (Guan and Wiles 1996; Buřič et al. 2007).

Acknowledgements

This investigation was financially supported by the Czech Ministry of Education (project USB RIFCH No. MSM6007665809) and the Czech Science Foundation (project GAAV IAA601870701).

References

Abrahamsson S.A.A., 1973. The crayfish *Astacus astacus* in Sweden and the introduction of the American crayfish *Pacifastacus leniusculus*. Freshwater crayfish 1: 27-39.

Ackefors H., Gydemo R., Keyser P., 1995. Growth and moulting in confined juvenile noble crayfish *Astacus astacus* (L.) (Decapoda, Astacidae). Freshwater crayfish 10: 396-409.

Barki A., Levi T., Hulata G., Karplus I., 1997. Annual cycle of spawning and molting in the red-claw crayfish, *Cherax quadricarinatus*, under laboratory conditions. Aquaculture 157: 239-249.

Bubb D.H., Lucas M.C., Thom T.J., Rycroft P., 2002. The potential use of PIT telemetry for identifying and tracking crayfish in their natural environment. Hydrobiologia 483: 225-230.

Buřič M., Kozák P., Kanta J., Kouba A., Policar T., 2007. The effect of water temperature on number of moults and growth of juvenile signal crayfish (*Pacifastacus leniusculus* D.). Bulletin VÚRH Vodňany 43 (1): 16-26.

Gherardi F., Barbaresi S., Salvi G., 2000. Spatial and temporal patterns in the Movement of *Procambarus clarkii*, an invasive crayfish. Aquat. Sci. 62: 179-193.

Graaf M., 2007. Tag retention, survival and growth of marron *Cherax tenuimanus* (Crustacea: Decapoda) marked with coded micro wire tags. Mar. Freshwat. Res. 58: 1044-1047.

Guan R.Z., Wiles P.R., 1996. Growth, density and biomass of crayfish, *Pacifastacus leniusculus*, in a British lowland river. Aquat. Living Res. 9 (3): 265-272.

Guan R.Z., 1997. An improved method for marking crayfish. Crustaceana 70: 641-652.

Guan R., Wiles R.P., 1998. Feeding ecology of the signal crayfish *Pacifastacus leniusculus* in the British lowland river. Aquaculture 169: 177-193.

Hamr P., 2002. *Orconectes*. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 585-608.

Henttonen P., Huner J.V., 1999. The introduction of alien species of crayfish in Europe: A historical introduction. In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 13-22.

Holdich D.M., Haffner P., Noël P., Carral J., Füderer L., Gherardi F., Machino Y., Madec J., Pöckl M., Śmietana P., Taugbol T., Vigneux E., 2006. Species files. In: Souty-Groset C., Holdich D.M., Noël P., Reynolds J.D., Haffner P. (eds.): Atlas of Crayfish in Europe. Publications Scientifiques du MNHN, Paris, pp. 49-130.

Holdich D.M., Black J., 2007. The spiny-cheek crayfish, *Orconectes limosus* (Rafinesque, 1817) [Crustacea: Decapoda: Cambaridae], digs into the UK. Aquat. invasions 2, 1-16.

Kozák P., Buřič M., Policar T., Hamáčková J., Lepičová A., 2007. The effect of inter- and intra-specific competition on survival and growth rate of native juvenile noble crayfish *Astacus astacus* and alien spiny-cheek crayfish *Orconectes limosus*. Hydrobiologia 590: 85-94.

Kuhlmann M.L., Badylak S.M., Carvin E.L., 2008. Testing the differential predation hypothesis for the invasion of rusty crayfish in a stream community: laboratory and field experiments. Freshwat. Biol. 53: 113-128.

Lowery R.S., 1988. Growth, Moulting and Reproduction. In: Holdich D.M., Lowery R.S. (eds.): Freshwater Crayfish: Biology, Management and Exploitation, Croom Helm, London, pp. 83-113.

Paglianti A., Gherardi F., 2004. Combined effecs of temperature and diet on growth and survival of young-of-year crayfish: A comparison between indigenous and invasive species. J. Crust. Biol. 24: 140-148.

Petrusek A., Filipová L., Ďuriš Z., Horká I., Kozák P., Policar T., Štambergová M., Kučera Z., 2006. Distribution of the invasive spiny-cheek crayfish (*Orconectes limosus*) in the Czech Republic: past and present. Bull. Fr. Peche Piscic. 380-381: 903-917.

Price J.O., Payne J.F., 1978. Multiple summer molts in adult *Orconectes neglectus chaenodactylus* Wiliams. Freshwater Crayfish 4: 93-104.

Reynolds J.D., 2002. Growth and reproduction. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 152-191.

Skurdal J., Taugbøl T., 2002. *Astacus*. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 467-510.

Stucki T.P., 2002. Differences in live history of native and introduced crayfish species in Switzerland. Freshwater crayfish 13: 463-476.

Chapter 5

Seasonal migrations of *Orconectes limosus* (Rafinesque, 1817) to a small tributary of large river

Buřič M., Kouba A., and Kozák P. 2009. Freshwater crayfish 17 (submitted)

Seasonal migrations of *Orconectes limosus* (Rafinesque, 1817) to a small tributary of large river

Miloš Buřič, Antonín Kouba, Pavel Kozák

University of South Bohemia in České Budějovice, Research Institute of Fish Culture and Hydrobiology, Zátiši 728/II, CZ 389 25 Vodňany, Czech Republic

Abstract

Seasonal migrations of *Orconectes limosus* between brook and reservoir has been observed in the small tributary of a large reservoir, identified as suitable habitat for indigenous crayfish. Sixteen captures (between April 2007 and October 2008) and one radio-telemetry study (from October to November 2007) have been conducted. A significant trend of downstream migration to the reservoir was found in autumn. *O. limosus* showed a high ability for rapid downstream movements (max. 139m per day). Seasonal fluctuations in crayfish density were observed and suggested that the occurrence of crayfish in the brook goes through periodic temporal changes. A lower water temperature in the brook throughout the year, and possible fish predation pressure in reservoir, could contribute to this phenomenon. The occurrence of *O. limosus* in reservoirs of big rivers, and their ability to penetrate small tributaries accentuate their serious threat to indigenous crayfish.

Keywords: Abundance, invasive crayfish, radio-telemetry, small stream, temporal migrations.

1. Introduction

The continuous spread of non-indigenous species (NIC), with their capacity to transmit crayfish plaque, is one of the most significant threats for indigenous crayfish (IC) species in Europe (Holdich and Gherardi 1999; Füderer et al. 2006). *Orconectes limosus* (Rafinesque) is now widespread in at least 20 European countries (Pöckl et al. 2006). This species also occurs in the Elbe and Vltava river systems and adjacent localities in the Czech Republic (Petrusek et al. 2006), where it can obstruct re-establishment of IC (Holdich et al. 2006).

The core of *O. limosus* distribution in Czech Republic is the Elbe and Vltava rivers, with a high presence in the larger tributaries (of 6th or higher stream order) of these rivers. It is also found in smaller streams (i.e. those with a low stream order) which join the larger rivers. In the latter crayfish usually stay within a few hundred meters of the convergence with the major watercourse (Petrusek et al. 2006). Although small brooks and streams are apparently not suitable habitats for *O. limosus* (Holdich et al. 2006), it was found in such habitats in Canada (McAlpine et al. 1991). The occurrence of *O. limosus* close to the mouth of most small streams suggests that it may enter the small watercourses only temporarily (Petrusek et al. 2006).

O. limosus is a small-bodied species, with a total length (TL) reaching 5 to 8 cm in adult animals (Holdich and Black 2007). Such a small size complicates locating and re-sampling of individuals under field conditions. Knowledge about migratory ability and activity or seasonal migrations of *O. limosus* is therefore lacking.

The objective of the present study was (1) to estimate annual changes in abundance, (2) to observe seasonal migrations, and (3) to determine the location (brook or reservoir) of *O. limosus* during winter.

2. Material and methods

2.1. Study site

The Velký brook is a small tributary of a large reservoir (Orlík Reservoir) of the Vltava river in southwest Czech Republic, found to be a suitable habitat for *Astacus astacus* (Linnaeus), a species of European IC (Skurdal and Taugbøl 2002). During former times *A. astacus* also occurred in the upper parts of the brook. The brook is situated at an altitude of 360 m above sea level at 49°20'N, 14°18'E. Its width and depth vary from 0.6 to 2 m, and 0.1 to 0.6 m, respectively. The substratum is variable, from sand and silt to large boulders, mostly covered by coarse gravel. Most of the study area is wooded. The abundant established crayfish population, consisting of only *O. limosus*, was probably introduced into the reservoir by fishermen. Only small fish, mainly *Barbatula barbatula* (Linnaeus), are found in the brook. In this study the lowest (ca 200 m) part of the brook was divided into 23 sections, each 5 to 10 m long, according to the presence of pools, large boulders, or other distinct markers in order to improve identification of shelters and movements. During the study water temperature in both the reservoir and brook was continuously measured every 3 h using data loggers (RT-F53, Qi Analytical, Prague, Czech Republic).

2.2. Captures

In total 16 captures (between April 2007 and October 2008) have been conducted. Crayfish were caught by hand searching of all potential shelters (2 persons for 2 hours). In all crayfish carapace length (CL) were measured to the nearest 0.01 mm using a digital caliper (Schut Geometrical Metrology, Groningen, The Netherlands), and weighted to the nearest 0.01 g by means of an electronic balance (Kern & Sohn GmbH, Balingen, Germany). All crayfish caught were released into locations near to the capture area (<0.25 m) after measurements.

2.3. Radio-telemetry

A radio-telemetry study has been conducted in autumn (from October to November 2007). Radio-transmitters (Biotrack Ltd., Wareham, U.K.), 14 x 6 x 3 mm and 0.5 g in weight, were glued to the carapace of suitable individual crayfish. Tag weight represented 6.9 ± 1.77 % of wet body weight, and was similar to those in other radio-telemetry studies with crayfish (Gherardi and Barbaresi 2000, Robinson et al. 2000, Bubb et al. 2002, 2004, 2006). Tagged crayfish were released close to the location found (<0.25 m) within 10 min after capturing, and tracked with a Sika receiver and flexible three-element Yagi antenna (Biotrack Ltd., Wareham, U.K.). The position of crayfish and distance moved, and daytime shelter location, were recorded once a day over a period of 5-6 days per week.

2.4. Calculations and statistical analysis

Non-parametric Mann-Whitney U-tests were used for comparison of distances moved, with Spearman rank correlations calculated to evaluate the relationship between temperature and abundance. The null hypothesis was rejected at a significance level of $\alpha = 0.05$. Data are presented as average ± S.D, and data analysis was performed with the software STATISTICA 8.0 (StatSoft, Inc.).

3. Results

3.1. Captures

The number of crayfish caught positively correlated with water temperature (Spearman rank correlation, $r_S = 0.868$, $P < 0.05$), as illustrated in Fig. 1. Data presented in Table 1 indicated that the transition time to colonization of the brook occurred between April and June. An opposite trend was found from October to November. During summer the population in the brook increased, and successful colonization of all suitable parts of the brook was recorded. Males inhabited the brook at an earlier stage than females.

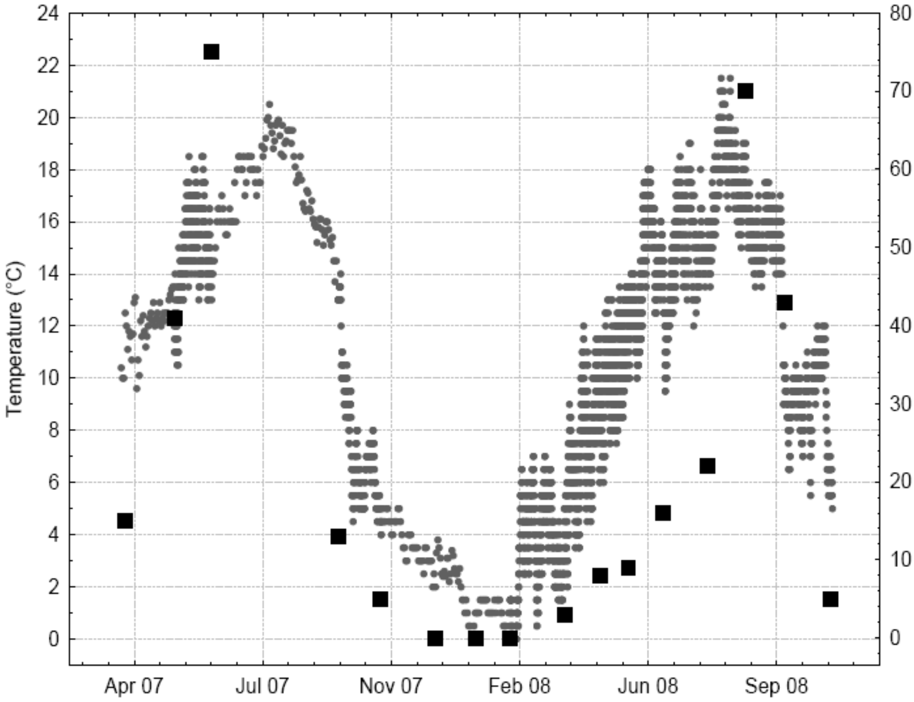


Figure 1. Influence of water temperature on abundance of *O. limosus*. Black squares are presenting crayfish abundance in the brook, with grey dots indicating water temperature.

3.2. Radio-telemetry

During the experiment a gradual decrease from 13 °C to 5 °C in water temperature was recorded in the brook. In the reservoir the decrease was substantially slower, and initial (October 3) and final (November 5) temperatures were higher (16 °C and 11 °C, respectively). From 19 to 27 October migratory activity (percentage of active crayfish) reached 77.3 ± 5.25 %. During this period, 76.5 % of movements longer than 10 m, and 91.7 % movements longer than 25 m were observed. These movements were initiated by a gradual temperature decrease in the brook to 5 °C. However, the influence of water temperature on movements was not significant. Distances moved per day (5.9 ± 11.95 m and 0.7 ± 0.59 m for downstream and upstream movements, respectively) differed ($U = 199.5$, $P = 0.028$), with a mean total distance of 6.7 ± 11.99 m per day. Maximum values for upstream and downstream movements were 12 and 139 m per day, respectively. The majority of crayfish migrated downstream to the reservoir during this observation period, with only a single tagged crayfish remaining in the brook.

Mating was observed during autumn. Crayfish mating or staying outside shelters during diurnal hours were often recorded, confirming daytime activity during this season.

4. Discussion

In the present study movements of crayfish over greater distances were sporadic. Crayfish remained in one area for several days, with occasional movements to new locations, similarly as observed by Bubb et al. (2002, 2004) in *Pacifastacus leniusculus* (Dana). Maximum distances moved showed that the relatively small *O. limosus* has the ability to perform rapid long movements, as expected. Greater distances moved per day have been observed in *A. astacus* (Schütze et al. 1999). This contradiction to present results might be attributed to 1) the small size of *O. limosus*, with speed of movement positively correlated to body size in crayfish (Gherardi et al. 2002), and 2) the release of crayfish to a familiar environment (Bohl 1999). A relatively high number of crayfish was observed to be active during diurnal hours, similar to results obtained for *O. limosus* by Lozan (2000) and Stucki (2002). However, the majority of freshwater crayfish (such as *A. astacus*) display nocturnal activity (Gherardi 2002).

The found mean distance moved per day is comparable to those observed for *P. leniusculus*, but higher than in *Austropotamobius pallipes* (Lereboullet) (Bubb et al. 2006). Differences were found in distances moved downstream or upstream, with a significant tendency to move downstream in autumn. Increased migratory activity could probably be related to a temperature decrease to less than 5 °C. This fact supports the close relationship between abundance of crayfish and water temperature in the brook. However, the influence of water temperature on movements observed by radio-telemetry was not significant.

From telemetry and capture data, we can assume that the brook is probably inhabited by *O. limosus* only during the growing season. The hypothesis that *O. limosus* migrate periodically from the brook to over-wintering sites in the reservoir is a possible explanation for the increasing crayfish density in spring, subsequent decrease in autumn, and the trend to autumn downstream migration. This might explain the occurrence of *O. limosus* exclusively in the lower parts of tributaries of large rivers (Petrusek et al. 2006).

The crayfish might use the brook for safe molting and for protection of themselves and their offspring against predation by predatory fish, *e.g.* perch (*Perca fluviatilis*, Linnaeus), pike (*Esox lucius*, Linnaeus) and eel (*Anquilla anquilla*, Linnaeus) (Haertel et al. 2002, 2005), fish species that are abundant in the reservoir. In addition, a lower temperature may be a limiting factor in the spread of *O. limosus* and in their permanent settlement upstream. The fact that the species does not usually penetrate far upstream in small watercourses supports this suggestion, and may protect some populations of IC from the transmission of crayfish plaque by direct contact with carriers. On the other hand, *O. limosus* is able to rapidly penetrate the brook, which is the habitat of IC, inhabit it in the short term, and enable the transmission of this disease.

In general, the Velký brook is an example of a site suitable for establishment of the native *A. astacus* (Skurdal and Taugbøl 2002), but is threatened by the vector of crayfish plaque. In addition, *O. limosus* in the Orlík reservoir and its tributaries are infected with crayfish plaque (Kozubíková et al. 2006). Despite the fact that *O. limosus* have not been shown to prefer this type of water body (Holdich et al. 2006) it is able to inhabit it at least during the growing season. During former times *A. astacus* occurred in upper parts of the brook, but its prospective spread or re-establishment is prevented by the presence of the invading *O. limosus*.

Table 1. Total number of crayfish caught, carapace length (CL, mm), wet weight (g), and male:female ratio of crayfish caught during specific captures. The same specification of juvenile and adult crayfish apart was described below. Data are presented as mean ± SD. None crayfish was caught during December, January and February.

date	**Apr 07**	**May 07**	**Jun 07**	**Oct 07**	**Nov 07**	**Mar 08**	**Apr 08**	**May 08**	**Jun 08**	**Jul 08**	**Aug 08**	**Sep 08**	**Oct 08**
n	15	41	75	13	5	3	8	9	16*	22*	70	43	5
CL (mm)	25.4±7.04	21.7±4.65	24.1±3.61	27.8±4.74	29.8±5.30	32.3±2.03	32.8±3.02	29.1±6.05	27.0±8.58	26.3±3.91	28.2±7.67	24.2±8.29	33.9±3.27
W (g)	5.5±5.26	3.4±4.65	4.3±2.22	6.7±3.30	8.5±3.70	9.7±1.60	11.3±2.84	8.6±4.86	7.6±6.21	4.9±2.53	7.3±4.60	5.2±5.04	9.9±2.19
♂:♀	11:4	23:18	32:43	7:6	2:3	2:1	8:0	7:2	11:5	4:18	35:35	16:27	1:4
juvenile - n	7	26	28	1	1	0	0	3	7*	3*	16	22	0
CL (mm)	20.1±1.73	19.1±2.78	20.9±1.74	18.2	20.9	x	x	21.3±0.78	18.1±1.97	20.9±0.54	16.0±3.66	16.8±1.94	x
W (g)	2.1±0.64	1,9±0.85	2.48±0.73	1.45	2.45	x	x	2.6±0.46	1.5±0.55	2.1±0.37	1.1±0.72	1.2±0.37	x
♂:♀	6:1	11:15	10:18	1:0	0:1	x	x	1:2	5:2	0:3	8:8	9:13	x
Adult – n	8	15	47	12	4	3	8	6	9*	19*	54	21	5
CL (mm)	30.1±6.13	26.3±3.32	26.0±3.02	28.6±3.75	32.0±0.82	32.3±2.03	32.8±3.02	33.1±1.55	33.9±3.22	27.1±3.40	31.8±3.70	31.9±4.49	33.9±3.27
W (g)	8.5±5.37	6.1±2.33	5,4±2.05	7.2±2.89	9.9±1.56	9.7±1.60	11.3±2.84	11.6±2.21	12.8±3.51	5.5±2.38	9.1±3.47	9.5±4.04	9.9±2.19
♂:♀	5:3	12:3	22:25	6:6	2:2	2:1	8:0	6:0	6:3	4:15	27:27	7:14	1:4

*In June and July a considerable part (50 and 30% of study area, respectively) of the brook was flooded by a high reservoir water level.

Acknowledgements

This investigation was financially supported by the Czech Ministry of Education (project USB RIFCH No. MSM6007665809) and the Czech Science Foundation (projects GAAV IAA601870701).

References

Bohl E., 1999. Motion of individual noble crayfish *Astacus astacus* in different biological situations: in-situ studies using radio telemetry. Freshwater crayfish 12: 677-687.

Bubb D.H., Lucas M.C., Thom T.J., 2002. Winter movements and activity of signal crayfish *Pacifastacus leniusculus* in an upland river, determined by radio telemetry. Hydrobiologia 483: 111-119.

Bubb D.H., Lucas M.C., Thom T.J., 2004. Movement and dispersal of the invasive signal crayfish *Pacifastacus leniusculus* in upland rivers. Freshwat. Biol. 49: 357-368.

Bubb D.H., Thom T.J., Lucas M.C., 2006. Movement, dispersal and refuge use of co-occuring introduced and native crayfish. Freshwat. Biol. 51: 1359-1368.

Füderer L., Edsman L., Holdich D.M., Kozák P., Machino Y., Pöckl M., Renai B., Reynolds J.D., Schulz H., Schulz R., Sint D., Taugbol T., Trouilhé M.C., 2006. Indigenous crayfish habitat and threats. In: Souty-Groset C., Holdich D.M., Noël P., Reynolds J.D., Haffner P. (eds.): Atlas of Crayfish in Europe. Publications Scientifiques du MNHN, Paris, pp. 25-48.

Gherardi F., 2002. Behaviour. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 258-290.

Gherardi F., Barbaresi S., 2000. Invasive crayfish: activity patterns of *Procambarus clarkii* in the rice fields of the lower Guadalquivir (Spain). Arch. Hydrobiol. 150: 153-168.

Gherardi F., Tricarico E., Ilhéu M., 2002. Movement patterns of an invasive crayfish, *Procambarus clarkii*, in temporary stream of southern Portugal. Ethol. Ecol. Evol. 14: 183-197.

Haertel S.S., Baade U., Eckmann R., 2002. No general percid dominance at mesotrophic lake conditions: insights from the quantification of predator-prey interactions. Limnologica 32: 1-13.

Haertel-Borer S.S., Zak D., Eckmann R., Baade U., Holker F., 2005. Population density of the crayfish, *Orconectes limosus*, in relation to fish and macroinvertebrate densities in a small mesotrophic lake – Implications for the lake's food web. Int. Rev. Hydrobiol. 90: 523-533.

Holdich D.M., Gherardi F., 1999. Native and alien crayfish in Europe: An introduction. In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 3–9.

Holdich D.M., Haffner P., Noël P., Carral J., Füderer L., Gherardi F., Machino Y., Madec J., Pöckl M., Śmietana P., Taugbol T., Vigneux E., 2006. Species files. In: Souty-Groset C., Holdich D.M., Noël P., Reynolds J.D., Haffner P. (eds.): Atlas of Crayfish in Europe. Publications Scientifiques du MNHN, Paris, pp. 49-130.

Holdich D.M., Black J., 2007. The spiny-cheek crayfish, *Orconectes limosus* (Rafinesque, 1817)[Crustacea: Decapoda: Cambaridae], digs into the UK. Aquat. invasions 2: 1-16.

Kozubíková E., Petrusek A., Ďuriš Z., Kozák P., Geiger S., Hoffmann R., Oidtmann B., 2006. The crayfish plague in the Czech Republic - Review of recent suspect cases and a pilot detection study. Bull. Fr. Peche Piscic. 380–381: 1313-1323.

Lozan J.L., 2000. On the threat to the European crayfish: A contribution with the study of the activity behaviour of four crayfish species (Decapoda: Astacidae). Limnologica 30: 156-166.

McAlpine D., Hogans W.E., Fletcher T.J., 1991. *Orconectes limosus* (Crustacea, Cambaridae) an addition to the crayfish fauna of New Brunswick. Can. Field-Nat. 105: 386-387.

Petrusek A., Filipová L., Ďuriš Z., Horká I., Kozák P., Policar T., Štambergová M., Kučera Z., 2006. Distribution of the invasive spiny-cheek crayfish (*Orconectes limosus)* in the Czech Republic: past and present. Bull. Fr. Peche Piscic. 380–381: 903-917.

Pöckl M., Holdich D.M., Pennerstorfer J., 2006. Identifying native and alien crayfish species in Europe. European Project CRAYNET.

Robinson C.A., Thom T.J., Lucas M.C., 2000. Ranging behaviour of a large freshwater invertebrate, the white-clawed crayfish *Austropotamobius pallipes*. Freshwat. Biol. 44: 509-521.

Schütze S., Stein H., Born O., 1999. Radio telemetry observations on migration and activity patterns of restocked noble crayfish *Astacus astacus* (L.) in the small river Sempt, North-East of Munich, Germany. Freshwater crayfish 12: 688-695.

Skurdal J., Taugbøl T., 2002. *Astacus*. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 467-510.

Stucki T.P., 2002. Differences in live history of native and introduced crayfish species in Switzerland. Freshwater crayfish 13: 463-476.

Chapter 6

Comparison of day and night activity between noble (*Astacus astacus*) and spiny-cheek crayfish (*Orconectes limosus*)

Musil M., Buřič M., Policar T., Kouba A., and Kozák P. 2009. Freshwater crayfish, 17 (submitted).

Comparison of day and night activity between noble (*Astacus astacus*) and spiny-cheek crayfish (*Orconectes limosus*)

Martin Musil, Miloš Buřič, Tomáš Policar, Antonín Kouba, Pavel Kozák

University of South Bohemia in České Budějovice, Research Institute of Fish Culture and Hydrobiology, Zátiši 728/II, CZ 389 25 Vodňany, Czech Republic

Abstract

Several studies have shown that American crayfish species are more active during the day compared to European species. Spiny-cheek crayfish (*Orconectes limosus* Raf.) is known as an aggressive nocturnal species with higher daytime activity than noble crayfish (*Astacus astacus* L.). The aim of the present study was to compare day and night activity of these two species under laboratory conditions with the use of video techniques. Three groups were included: noble crayfish only, spiny-cheek crayfish only, and a mixture of both species. Substantial differences were found between species. Higher ($P < 0.05$) day and night activity was found for spiny-cheek crayfish than for noble crayfish, with more spiny-cheek females than males active, and higher ($P < 0.05$) night than day activity in noble crayfish, when kept as individual species. However, the above activities were modified when both species were kept together, with an increased day activity found in noble crayfish, and night activity similar between species. A higher activity during the day might influence the vulnerability of noble crayfish to day time predators.

Key words: Astacus astacus, Orconectes limosus, shelter use, interaction, video-tracking.

1. Introduction

Indigenous crayfish (IC) species in Europe, including noble crayfish (*Astacus astacus*, Linnaeus), are endangered by several factors. One of the most significant threats is the continuous spread of non-indigenous species (NIC) and their function as crayfish plaque vectors (Holdich and Gherardi 1999; Füderer et al. 2006).

The spiny-cheek crayfish (*Orconectes limosus*, Rafinesque), a small-bodied species with a total length (TL) reaching 5 to 8 cm in adult animals (Holdich et al. 2006; Holdich and Black 2007), was introduced into Europe in 1890, and is now widespread in at least 20 European countries (Pöckl et al. 2006). With its life cycle, activity, aggressiveness, and carrier of crayfish plaque (Kozubíková et al. 2008), it presents a serious danger to IC (Hamr 2002, Henttonen and Huner 1999, Holdich and Black 2007, Kozák et al. 2006). Spiny-cheek crayfish is referred to as a highly active species. Although most crayfish are classified as nocturnal animals (Gherardi 2002), spiny-cheek crayfish has been reported as a species with a high daily activity compared to IC (Lozan 2000; Stucki 2002).

Noble crayfish is the most widespread IC in Europe, and can attain a TL of more than 15 cm (Holdich et al. 2006). However, the effect of its bigger size could be eliminated by its slower life-cycle, lower fecundity and low aggressiveness (Gydemo 1989; Holdich et al. 2006; Lindquist and Huner 1999; Policar et al. 2004). If crayfish would be classified into the two ecological groups referred to as r-selective and K-selected species, spiny-cheek crayfish will be included in the first group and noble crayfish in the second (Linquist and Huner 1999).

Noble crayfish is considered, unlike spiny-cheek crayfish, as a typical nocturnal animal (Westman et al. 2000; Skurdal and Taugbol 2002).

With the most common characteristic evaluated in behavioral studies as activity, the above two species are suitable representatives of NIC and IC for studies of inter-specific interactions. Crayfish activity could be influenced by temperature (Barbaresi and Gherardi 2001; Bubb et al. 2004; Lozan 2000), light intensity (Bojsen et al. 1998; Gherardi 2002), shelter availability (Martin and Moore 2007; Barbaresi and Gherardi 2001), food availability (Gherardi 2002; Statzner et al. 2000), presence of predators (Jordan et al. 1996; Nyström 2002), and inter-specific interactions (Bubb et al. 2006; Gherardi, 2002).

In the present study the activity patterns of European (noble crayfish) and American (spiny-cheek crayfish) crayfish species were studied and compared in order to obtain an understanding of inter-specific relations between species. The main objectives were to assess overall, day and night, and sex depended activity for both crayfish species kept either as individual species or in a mixed species group.

2. Material and methods

2.1. Animals

Spiny-cheek crayfish used in the experiment were collected from the Kořensko water reservoir (Vltava River, South Bohemia, Czech Republic), and noble crayfish from the Kramata pond (South Bohemia, Czech Republic), in 2004. Total body length (from tip of the rostrum to the posterior median edge of the telson) and carapace length (CL; from tip of the rostrum to the posterior edge of cephalothorax) were measured using a vernier caliper, with wet body weight determined with an electronic balance (Kern & Sohn GmbH, Balingen, Germany). Each animal was marked with a specific visible number with the use of white nail varnish.

Crayfish were subjected to three different groups (each with 3 replicates): noble crayfish (16) only, spiny-cheek crayfish (16) only, and a mixed group of 8 noble crayfish and 8 spiny-cheek crayfish. Sex ratio in each group was 1:1. There was not significant difference between groups in size inside the species. Average TL and CL of spiny-cheek crayfish were 71.1 ± 7.79 and 34.5 ± 3.76 mm, respectively, with an average body weight of 10.8 ± 4.77 g. Corresponding length values for noble crayfish were 95.0 ± 11.71 and 49.2 ± 6.86 mm, respectively, with an average body weight of 32.4 ± 14.24 g.

2.2. Experimental conditions

The study was conducted in the experimental facilities of the RIFCH Vodňany (South Bohemia, Czech Republic). Squared experimental tank (1x1 m) was filled with 10 cm of tap water, and water was kept stagnant during the experiment. Tank bottom was covered with a 1 – 2 cm layer of sand and gravel, and stone lines were provided as hiding-places on the opposite sides of tank. Water temperature (13.0 ± 2.25 °C) was continuously monitored using a data logger (RT-F53, Qi Analytical, Prague, Czech Republic), and dissolved oxygen (10.2 ± 2.52 $mg.l^{-1}$) was measured twice daily using an Oximeter (Oxi 315i, WTW GmbH, Weilheim, Germany). Crayfish were fed on spinach leaves, frozen chironomid larvae and fish meat in excess 12 hours before observations to prevent the effect of starvation. Animals were acclimatized for 7 days in experimental tank before initiation experiments

A day time regime (7:00 – 19:00) was created with the use of two fluorescent tubes (36 W each, Royal Philips Electronics, Eindhoven, Netherlands), whereas a 60 W red light-bulb (Royal Philips Electronics, Eindhoven, Netherlands) provided a night treatment (19:00 –

7:00). Changeover between regimes was done with a clock timer. A gradual change of light intensity for the simulation of dawn and dusk was followed.

2.3. Activity measurements

Movement of animals was recorded continuously over a 72-hour period on a video recorder (Grundig VS 170, Grundig Intermedia GmbH, Nürnberg, Germany) mounted above the experimental tank. A super slow program (EP) was used for recording, and the play back using accelerated program on videotape reproducer and visualized by Easy Grab 5.6 for Picolo (Euresys, Liege, Belgium). Time spent outside shelters (TSOS; %) during the day and night periods were calculated from video tapes for individual crayfish.

2.4. Data analysis

Data was analysed according to ANOVAS with the statistical package Statistica 8.0 (StatSoft, Inc.), with LSD-tests used to identify differences among treatments. Relationships between noble and spiny-cheek crayfish for TSOS in the mixed species groups were evaluated with the use of Spearman rank correlation coefficients. The null hypothesis was rejected at $\alpha = 0.05$. Data are presented as average ± standard deviation.

3. Results

3.1. Overall time spent outside the shelter

No significant intra-specific differences in TSOS were detected between crayfish kept as individual species or in a mixed group for both spiny-cheek crayfish and noble crayfish. However, inter-specific differences were significant. Spiny-cheek crayfish spent significantly more time outside the shelter than noble crayfish in groups consisting of individual species and as mixed species. Some females in the spiny-cheek crayfish only group never used shelters, whereas other individuals, independent of group, never left the shelters.

Table 1. Time spent outside shelters (TSOS; mean ± SD) for noble crayfish and spiny-cheek crayfish in individual and mixed species groups. Data with different superscripts in the same row with different superscripts differ ($P < 0.05$).

	Individual species		Mixed species	
TSOS	*Astacus astacus*	*Orconectes limosus*	*Astacus astacus*	*Orconectes limosus*
Day	7.8 ± 5.19^b	49.5 ± 4.15^a	13.5 ± 8.60^b	53.0 ± 17.10^a
Night	22.4 ± 6.87^b	53.3 ± 7.98^a	18.1 ± 8.87^b	62.5 ± 12.79^a
Overall	15.1 ± 9.51^b	51.4 ± 6.64^a	15.8 ± 9.04^b	57.7 ± 15.82^a

3.2. Day vs. night

As expected, noble crayfish in the group containing only this species were significantly more outside shelters during nighttime than daytime hours, with no difference ($P > 0.05$) found for spiny-cheek crayfish. Surprisingly, there were no intra-specific differences ($P > 0.05$) in TSOS between day and night hours for both species in the mixed group.

Although non-significant, noble crayfish in the mixed group showed higher and lower TSOS in day and night hours, respectively, compared to the group of noble crayfish only.

The values of day and night TSOS for spiny-cheek crayfish were higher ($P < 0.05$) than for noble crayfish in both the groups consisting of individual or mixed species. Both species reached a peak in activity peak soon after the start of the nighttime hours (Fig. 1); between 8 and 9pm in noble crayfish, and 8 and 10pm in spiny-cheek crayfish. A significant Spearman rank correlation ($r_S = 0.399$) presented evidence that a decrease in TSOS in one species was related to an increase in the other species in the mixed species group. The behavior of both species was influenced by communal stock.

3.3. Males vs. females

TSOS in noble crayfish was not significantly different between sexes in groups either as individual or mixed species. Although this was also found for spiny-cheek crayfish kept in the mixed species group, females in the spiny-cheek crayfish only group had a higher ($P < 0.05$) TSOS value than males (Fig. 2, Fig. 3).

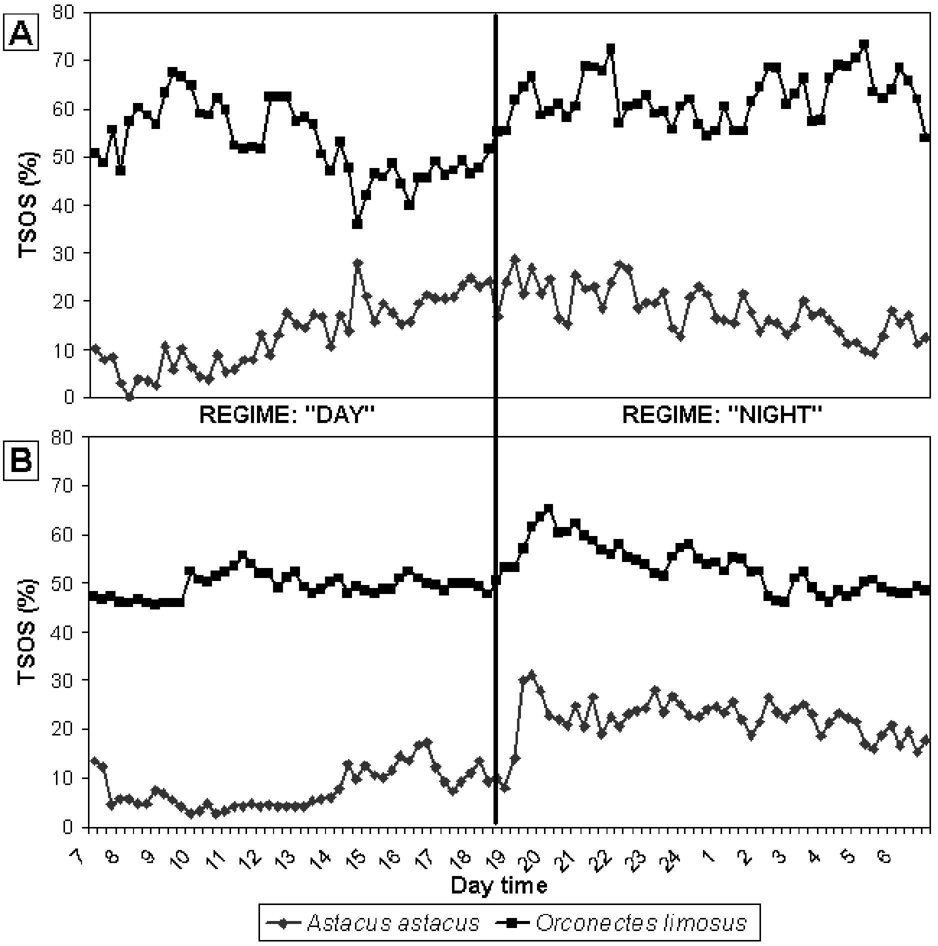


Figure 1. Time spent outside shelters (TSOS) for noble crayfish and spiny-cheek crayfish over a 24 hour-period in mixed species (A) and individual (B) groups. Black line in middle presents the change from day to night hours.

Figure 2. Time spent outside shelters (TSOS) for noble crayfish and spiny-cheek crayfish for males and females in individual and mixed species groups. Data with different superscripts differ ($P < 0.05$). Data are presented as mean (squares), standard error of mean (boxes) and confidential interval (whiskers).

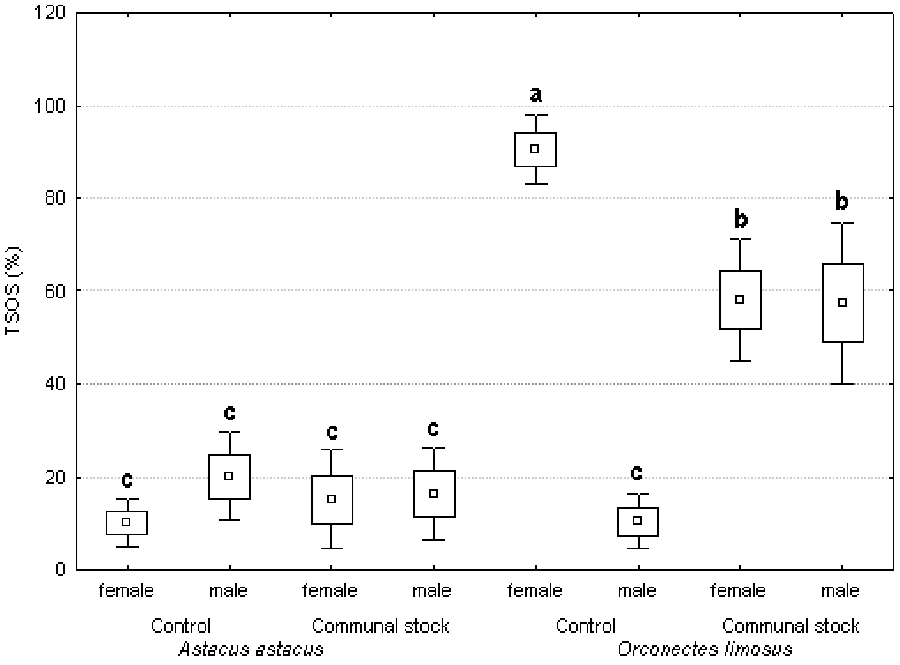
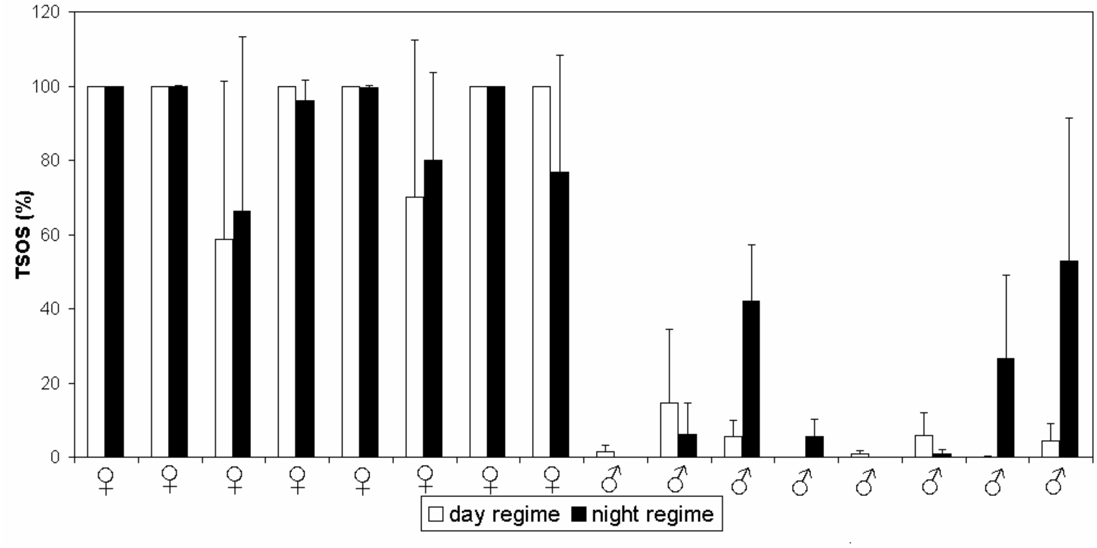


Figure 3. Time spent outside shelters (TSOS) for noble crayfish and spiny-cheek crayfish for males and females in individual species groups during day and night hours. Data are presented as mean ± standard deviation.



4. Discussion

Present results showed that noble crayfish is more active, although not limited, during night than day time hours. Both day and night TSOS was higher for spiny-cheek than noble crayfish. Signal crayfish (*Pacifastacus leniusculus* Dana), another representative of NIC, was found to be more active (about 13%) during the day than noble crayfish (Lozan 2000), but values were less than those found for spiny-cheek crayfish in the present study. TSOS was similar for day and nighttime hours in spiny-cheek crayfish in the current study. A high activity was described in spiny-cheek crayfish by Lozan (2000), Stucki (2002) and Pieplow (1938). However, values presented by Lozan (2000) and Söderback (1995) during the day in spiny-cheek crayfish 12% and 22%, respectively, were lower than found in the present study. Lozan (2000) also reported lower (about 38%) values in nighttime for noble crayfish. These differences could probably be attributed to differences in methodology.

In accordance to results obtained in nature with noble (Cukerzis 1988; Hamrin 1987) and other (e.g. white-clawed crayfish, *Austropotamobius pallipes* Lereboullet) crayfish species (Robinson et al. 2000; Gherardi 2002), activity peaked for both species in the present study soon after the start of night time hours. Higher night TSOS of noble crayfish in groups consisting of only this species is in accordance with the general opinion that this species is a typical night active creature (Abrahamsson 1983; Bojsen et al. 1998; Stucki 2002; Westman 2000). The observed changes in daytime behavior of noble crayfish in the mixed species group (increased day TSOS to reach similar values than night TSOS, modified 24h activity) was probably due to the presence of spiny-cheek crayfish. This postulation is confirmed by a significant correlation between TSOS of both species during a 24-hour period in the mixed species group.

Activity in noble crayfish was not influenced by sex, as have been found for signal crayfish (Bubb et al. 2004), white-clawed crayfish (Robinson et al. 2000), and rusty crayfish (*Orconectes rusticus*, Girard) (Byron and Wilson 2001). However, females in a group consisting of only spiny-cheek crayfish showed an increased TSOS compared to males, although some females often took a position outside the shelter and were not really active. Similarly, Mainwald et al. (2006) observed a decreased use of shelters by spiny-cheek crayfish independent of sex. The above has not been observed when spiny-cheek crayfish were accommodated together with noble crayfish, presenting evidence of the influence of a different species onto activity.

Aggressive dominance between these two species could have an influence on behavior when they are kept together. In signal crayfish aggressive dominance during inter-specific interactions when together with noble crayfish was found (Söderback 1991). However, spiny-cheek crayfish has a smaller body size compared to adult noble crayfish. Further research is needed to evaluate aggressive interactions between spiny-cheek and noble crayfish.

The influence of spiny-cheek crayfish presence on noble crayfish stock could be indirect. In general, refuges can be a critical resource for crayfish survival (Gherardi 2002) and their use leads to a decrease of predation risk and protection from environmental extremes (Lodge and Hill 1994). One mechanism of displacement of native crayfish species by invading crayfish could be caused by diurnal displacement of the native species from refuge (Bubb et al. 2006), which could lead to higher vulnerability to predation (Lodge and Hill 1994). In the present study noble crayfish spent more time outside shelters in the mixed species groups, which is in accordance to the above.

Our results suggest that there are inter-specific interactions between studied species, which could contribute to noble crayfish displacement. However, there is probably a complexity of mechanisms that influenced the chances of IC, which necessitates future studies for identification.

Acknowledgement

This investigation was financially supported by the Czech Ministry of Education (project USB RIFCH No. MSM6007665809 and ME 855) and the Czech Science Foundation (projects GAAV IAA601870701).

References

Abrahamsson S.A.A., 1983. Trappability, locomotion and diel pattern of activity of the crayfish *Astacus astacus* and *Pacifastacus leniusculus* Dana. Freshwater Crayfish 5: 239-253.

Barbaresi S., Gherardi F., 2001. Daily activity of the white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet): a comparison between field and laboratory studies. J. Nat. Hist. 35 (12): 1861-1871.

Bojsen B.H., Witthøfft H., Styrishave B., Andersen O., 1998. *In situ* studies on heart rate and locomotor activity in the freshwater crayfish, *Astacus astacus* (L.) in relation to natural fluctuations in temperature and light intensity. Freshwat. Biol. 39: 455-465.

Bubb D.H., Lucas M.C., Thom T.J., 2004. Movement and dispersal of the invasive signal crayfish *Pacifastacus leniusculus* in upland rivers. Freshwat. Biol. 49: 357-368.

Bubb D.H., Thom T.J., Lucas M.C., 2006. Movement, dispersal and refuge use of co-occuring introduced and native crayfish. Freshwat. Biol. 51: 1359-1368.

Byron C.J., Wilson K.A., 2001. Rusty crayfish (*Orconectes rusticus*) movement within and between habitats in Trout Lake, Vilas County, Wisconsin. J. N. Am. Benthol. Soc. 20: 606-614.

Cukerzis J.M., 1988. *Astacus astacus* in Europe. In: Holdich D.M., Lowery R.S. (eds.): Freshwater Crayfish: Biology, Management and Exploitation, Croom Helm, London, pp. 309-340.

Füderer L., Edsman L., Holdich D.M., Kozák P., Machino Y., Pöckl M., Renai B., Reynolds J.D., Schulz H., Schulz R., Sint D., Taugbol T., Trouilhé M.C., 2006. Indigenous crayfish habitat and threats. In: Souty-Groset C., Holdich D.M., Noël P., Reynolds J.D., Haffner P. (eds.): Atlas of Crayfish in Europe. Publications Scientifiques du MNHN, Paris, pp. 25-48.

Gherardi F., 2002. Behaviour. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 258-290.

Gydemo R., 1989. Studies on reproduction and growth in the noble crayfish, *Astacus astacus* L. PhD dissertation. University of Stockholm, Stockholm, Sweden.

Hamr P., 2002. *Orconectes*. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 585- 608.

Hamrin S., 1987. Seasonal crayfish activity as influenced by fluctuating water levels and presence of fish predators. Holarctic Ecol. 10: 45-51.

Henttonen P., Huner J.V., 1999. The introduction of alien species of crayfish in Europe: A historical introduction. In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 13- 22.

Holdich D.M., Gherardi F., 1999. Native and alien crayfish in Europe: An introduction. In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 3-9.

Holdich D.M., Haffner P., Noël P., Carral J., Füderer L., Gherardi F., Machino Y., Madec J., Pöckl M., Śmietana P., Taugbol T., Vigneux E., 2006. Species files. In: Souty-Groset C., Holdich D.M., Noël P., Reynolds J.D., Haffner P. (eds.): Atlas of Crayfish in Europe. Publications Scientifiques du MNHN, Paris, pp. 49-130. ¨

Holdich D.M., Black J., 2007. The spiny-cheek crayfish, *Orconectes limosus* (Rafinesque, 1817)[Crustacea: Decapoda: Cambaridae], digs into the UK. Aquat. invasions 2: 1-16.

Jordan F., DeLeon C.J., McCreary A.C., 1996. Predation, habitat complexity, and distribution of the crayfish *Procambarus alleni* within a wetland habitat mosaic. Wetlands 16 (4): 452-457.

Kozák P., Buřič M., Policar T., 2006. The fecundity, time of egg development and juveniles production in spiny-cheek crayfish (*Orconectes limosus*) under controlled conditions. Bull. Fr. Peche Piscic. 380-381: 1171-1182.

Kozubíková E., Petrusek A., Ďuriš Z., Martin M.P., Dieguez-Uribeondo J., Oidtmann B., 2008. The old menace is back: Recent crayfish plague outbreaks in the Czech Republic. Aquaculture 274 (2-4): 208-217.

Lindquist O., Huner J.V., 1999. Life history characteristcs of crayfish: What makes some of them good colonizers? In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 23-28.

Lodge D.M., Hill A.M., 1994. Factors governing species composition, population size and productivity of cool-water crayfishes. Nord. J. Freshwat. Res. 69: 111-136.

Lozan J.L., 2000. On the threat to the European crayfish: A contribution with the study of the activity behaviour of four crayfish species (Decapoda: Astacidae). Limnologica 30 (2): 156-166.

Mainwald T., Schulz H.K., Smietana P., Schulz R., 2006. Aggressive interactions and interspecific competition between the indigenous crayfish *Astacus astacus* (Linne) and the non-indigenous *Orconectus limosus* (Rafinesque). Freshwater Crayfish 15: 203-211.

Martin A.L., Moore P.A., 2007. Field observations of agonism in the crayfish, *Orconectes rusticus*: shelter use in a natural environment. Ethology 113 (12): 1192-1201.

Nyström P., 2002. Ecology. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 192-235.

Pieplow U., 1938. Fischereiwissenschaftliche Monographie von *Cambarus affinis* SAY. Zeitschr. für Ficherei 36: 349-440.

Pöckl M., Holdich D.M., Pennerstorfer J., 2006. Identifying native and alien crayfish species in Europe. European Project CRAYNET.

Policar T., Simon V., Kozák P., 2004. Egg incubation in the noble crayfish (*Astacus astacus* L.): The effect of controlled laboratory and outdoor ambient condition on hatching success, growth and survival rate of juveniles. Bull. Fr. Peche Piscic. 372-373: 411-423.

Robinson C.A., Thom T.J., Lucas M.C., 2000. Ranging behaviour of a large freshwater invertebrate, the white-clawed crayfish *Austropotamobius pallipes*. Freshwat. Biol. 44: 509-521.

Skurdal J., Taugbøl T., 2002. *Astacus*. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 467-510.

Söderback B., 1991. Interspecific dominace relationship and aggressive interactions in the freshwater crayfish *Astacus astacus L.* and *Pacifastacus leniusculus* DANA. Can. J. Zool. 69: 1321-1325.

Söderback B., 1995. Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a Swedish lake: Possible causes and mechanisms. Freshwat. Biol. 33: 291-304.

Statzner B., Fièvet E., Champagne J.Y., Morel R., Herouin E., 2000. Crayfish as geomorphic agents and ecosystem engineers: Biological behavior affects sand and gravel erosion in experimental streams. Limnol. Oceanogr. 45(5): 1030-1040.

Stucki T.P., 2002. Differences in live history of native and introduced crayfish species in Switzerland. Freshwater crayfish 13: 463-476.

Westman K., 2000. Comparison of the crayfish *Pacifastacus leniusculus* Dana, a species introduced into Finland, with the native species, *Astacus astacus* L., in allopatry and sympatry. PhD Dissertation. University of Helsinky, Helsinky, Finland.

Chapter 7

Movement patterns and ranging behavior of the invasive spiny-cheek crayfish in a small reservoir tributary

Buřič M., Kozák P., and Kouba A. 2009. Fundamental and applied limnology (in press).

Movement patterns and ranging behavior of the invasive spiny-cheek crayfish in a small reservoir tributary

Miloš Buřič, Pavel Kozák, Antonín Kouba

University of South Bohemia in České Budějovice, Research Institute of Fish Culture and Hydrobiology, Zátiši 728/II, CZ 389 25 Vodňany, Czech Republic

Abstract

Nineteen adult *O. limosus* (carapace length 26.3–35.7 mm) were radio-tagged and tracked in a small tributary to a reservoir during two time periods, from May to June and from October to November 2007. High nocturnal activity (33.3%) and relatively high diurnal activity (18.2%) were observed, with a positive effect of cloudy weather on nocturnal movements. Tracked crayfish showed a high ability for rapid upstream (62 m) and downstream (69 m) movements during short (3 h) time periods. The average movement distances per day were one order of magnitude lower (7.2 and 6.7 m in spring and autumn, respectively). The maximum daily movement was 139 m. A significant trend to downstream migration to the reservoir was observed in autumn. Fifteen specimens displayed homing behavior, with the use of a system of shelters. A preference for soft bottomed, deeper areas of the brook, and for slow flow areas, such as pools, with the presence of organic matter was observed. Fluctuations in crayfish density, together with downstream migration in autumn, suggested that crayfish inhabit the brook only during the growing season, over-wintering in the reservoir. This study provides information on the spatial and temporal behavior of invasive species in small tributaries suitable for indigenous crayfish and illustrates their serious threat for it.

Keywords: Orconectes limosus, telemetry, migration, homing behavior, non-indigenous species.

1. Introduction

Although indigenous crayfish (IC) species in Europe are endangered by several changes, such as fragmentation and alterations of habitat, one of the most significant threats is the continued spread of non-indigenous species (NIC) with their capacity to transmit crayfish plaque (Holdich and Gherardi 1999; Füderer et al. 2006). The spiny-cheek crayfish (*Orconectes limosus* Rafinesque 1817), a temperate-water crayfish native to the north-eastern USA and south-eastern Canada, was introduced into Europe in 1890, and is now widespread in at least 20 European countries (Pöckl et al. 2006). The species occurs in the Elbe and Vltava river systems and adjacent localities in the Czech Republic (Petrusek et al. 2006), where it hampers re-establishment of indigenous crayfish (IC) (Holdich et al. 2006).

Orconectes limosus is present mainly in the western part of the Czech Republic (Elbe watershed). The core of its distribution is the River Elbe and River Vltava, with a presence in larger tributaries (of 6th or higher stream order) of these rivers. It is also found in smaller streams (i.e. those with a low stream order), which join larger rivers. In these cases the crayfish usually stay within a few hundred meters of the confluence with the major watercourse and do not penetrate far upstream into the tributary (Petrusek et al. 2006). Small brooks and rivulets are apparently not suitable habitats for *O. limosus* (Holdich et al. 2006). However, such habitats are used in Canada (McAlpine et al. 1991). The presence of the spiny-cheek crayfish close to the mouth of most small streams does not necessarily indicate a viable population, as the source population may be in the adjacent larger river or reservoir, and crayfish may enter the small watercourse only temporarily (Petrusek et al. 2006).

Orconectes limosus is a small-bodied species, with a total length (TL) reaching 5 to 8 cm in adult animals (Holdich et al. 2006; Holdich and Black 2007). Such a small size complicates locating and re-sampling individuals in the field. Data regarding migratory ability and activity, seasonal migrations, habitat exploitation, and behavior of *O. limosus* are therefore lacking. The wide geographical range and disease threat of *O. limosus*, combined with the lack of information through recent research, is of concern with respect to IC. Knowledge of behavior, habitat exploitation, and migratory ability and patterns may be crucial to assessing the invasive potential and ecology of *O. limosus* and its threat for IC.

In former times, the indigenous *Astacus astacus* abundantly occurred throughout the Czech Republic, including the river Vltava watershed (Kozák and Policar 2006; Štěpán 1933). In the study site the last specimen of this species was caught in the summer 2002, when *O. limosus* established in the lower part of the brook (Kozák, pers. comm.). The presence of invading *O. limosus* was linked to crayfish plague infection, and 25-100 % of *O. limosus* specimens were tested positive using the PCR-based detection (Kozubíková et al. 2006).

New technology such as radio-telemetry allows the study of movement and behavior patterns over extended time periods of even very small aquatic animals. Radio-telemetry has been utilized successfully with signal crayfish *Pacifastacus leniusculus* (Dana 1852) (Bubb et al. 2002; 2004; 2006); red swamp crayfish, *Procambarus clarkii* (Girard 1852) (Gherardi and Barbaresi 2000; Gherardi et al. 2002; Barbaresi et al. 2004; Aquiloni et al. 2005); noble crayfish, *Astacus astacus* (Linnaeus 1758) (Bohl 1999; Schütze et al. 1999); and white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet 1858) (Robinson et al. 2000; Bubb et al. 2006). Radio-telemetry can also reveal whether *O. limosus* occupies a single shelter or alternates among a system of shelters within a home range, as do some other decapods (Vannini and Cannicci 1995).

In order to gain information on habitat requirements, patterns of resource utilization, and the potential for inter-specific interaction of *O. limosus*, studies were conducted to: 1) determine diurnal patterns, seasonal migrations, shelter exploitation, and home range; 2) examine differences in population densities among habitat types; 3) estimate annual changes in abundance; and 4) to determine the location (brook or reservoir) of crayfish during winter. The study site was chosen as a suitable habitat for *A. astacus*, a species of European IC.

2. Material and methods

2.1. Study site

The Velký brook is a small tributary of a large reservoir, Orlík Reservoir, of the river Vltava in the southwestern Czech Republic. It is situated at an altitude of 360 m above sea level at 49°20' N, 14°18' E. Its width and depth vary (in different parts of the brook) from 0.6 to 2 m, and 0.05 to 0.6 m, respectively. The substratum is variable, from sand and silt to large boulders, most covered by coarse gravel. Most of the study area is wooded. In general, this brook presents a suitable habitat type for the native *A. astacus* (Skurdal and Taugbøl, 2002).

The crayfish population, consisting of only *O. limosus*, was probably introduced into the reservoir by fishermen. The brook is well populated with small benthic invertebrates (Annelida, Gastropoda, Amphipoda, Ephemeroptera larvae, Trichoptera, and Diptera), and with organic matter, which is also a good food source for crayfish. Only small fish, mainly *Barbatula barbatula* and *Pseudorasbora parva*, are found in the brook. Both crayfish and fish are common prey of mink (*Mustela vison*) at the study site.

We divided the lowest ca 200 m portion of the brook into 23 sections, each 5 to 10 m long, according to the presence of pools, large boulders or other distinct markers (for better specification of shelters and movements).

2.2. Crayfish capture and radio-tagging

The study was conducted in two phases, late spring (29 May-27 June; 9 crayfish) and late autumn (3 October-5 November; 10 crayfish). Crayfish were caught by hand by searching in all potential shelters at the beginning and end of experiments. Captures were carried out in each section of the brook mentioned above. Total length (TL; from tip of the rostrum to the posterior median edge of the telson) and carapace length (CL; from the tip of the rostrum to the posterior median edge of the cephalothorax) were measured to the nearest 0.1 mm with vernier calipers. Wet body weight of crayfish to the nearest 0.1 g was determined with an electronic balance (Kern & Sohn GmbH, Balingen, Germany). Average TL and CL of spiny-cheek crayfish used in experiments were 61.2 ± 5.86 and 29.4 ± 2.99 mm, respectively, with an average body weight of 7.8 ± 2.39 g. All locations where crayfish were found were charted. Males and females were identified using distinctive characters on the ventral side of the crayfish i.e. by male copulatory stylets (modified pleopods) and female annulus ventralis.

Radio-transmitters, type Pip, powered by Ag379 batteries (Biotrack Ltd., Wareham, U.K.), 14 x 6 x 3 mm and 0.5 g weight, with a lifespan of about one month, were glued to the carapace of suitable individual crayfish. Tag to animal-size ratios were similar to those in other radio-telemetry studies with crayfish (Gherardi and Barbaresi 2000; Robinson et al. 2000; Bubb et al. 2002; 2004; 2006), tag length 23.1 ± 2.11% of TL, with tag weight representing 6.9 ± 1.77% of wet body weight. Tagged crayfish were released close to the capture location (<0.25 m) within 10 min of capture.

2.3. Radio-telemetry data collection

Tagged crayfish were tracked with a Sika receiver and flexible three-element Yagi antenna (Biotrack Ltd., Wareham, U.K.). Data collections in late spring (study 1) were made over seven time periods during diurnal (06:00-10:00, 10:00-14:00, 14:00-18:00, 18:00-21:00) and nocturnal hours (21:00-00:00, 00:00-03:00, 03:00-06:00). The nocturnal observations included the time of nigh-fall (after 21:00), late night and also the daybreak (before 6:00). The position of all crayfish and the distance moved during each time period was recorded. Crayfish that changed position were termed as active in a particular time period. Observations were made three times per week in the period more than one month long. The late autumn study (study 2), looking at long-term migration patterns, comprised one observation per day, recording the daytime shelter location, 5-6 days per week for more than one month. Positions were described on a scaled map of the study site, and the distance from the previous shelter was calculated.

2.4. Habitat measurements

During the studies, water temperature in both the reservoir and the brook was measured every 3 h using data loggers (RT-F53, Qi Analytical, Prague, Czech Republic). Weather was classified as cloud coverage of less than 30 % (clear sky), 30-60 % (somewhat cloudy) and coverage of more than 60 % (cloudy).

2.5. Calculations and statistical analysis

For each radio-tracked crayfish, total and mean distance moved, distance moved during day and night, during cloudy and sunny weather, and upstream and downstream movements were calculated. Number of active crayfish during the defined time periods was included in study 1. Home range was determined to be the space in which a crayfish used a system of re-visited shelters (Vannini and Cannicci 1995).

Students t-tests or non-parametric Mann-Whitney U-test (when data showed non-normal distribution) were used for comparison of carapace length and nocturnal and diurnal activity among groups. Data for distances moved were analyzed according to analysis of variance (ANOVA) techniques. Observed and expected values for habitat preference were evaluated using χ^2 tests with Yates' correction. The level of significance at which the null hypothesis was rejected was $\alpha = 0.05$. Data are presented as average ± S.E. with figures showing average ± S.E. and confidence intervals.

3. Results

3.1. Late spring

The number of active crayfish during nocturnal hours (33.3 ± 3.73%) was higher (U = 231.5; P = 0.004) than during diurnal hours (18.2 ± 2.53%). The number of active crayfish increased from 6.3 ± 1.63%, to 21.2 ± 4.86%, to 39.1 ± 7.31% in the periods 6:00-10:00, 18:00-21-00 and 3:00-6:00, respectively (Fig 1A). The rapid decrease of active crayfish was observed between periods 3:00-6:00 and 6:00-10:00.

The average distances moved at night were significantly higher than in daylight (F = 17.61; P = 0.0001). The mean distance moved of 0.1 ± 0.14 m, 2.1 ± 0.77 m, 0.7 ± 0.21 m, 1.6 ± 0.44 m, 12.2 ± 5.73 m, 7.3 ± 2.14 m and 9.8 ± 5.12 m was observed in consecutive time periods 06:00-10:00, 10:00-14:00, 14:00-18:00, 18:00-21:00, 21:00-00:00, 00:00-03:00 and 03:00-06:00, respectively. Movement during observation periods in the day or at night did not differ (Fig. 1B). The average distance moved during a cloudy night (16.5 ± 6.67 m) was significantly higher (F = 4.05; P = 0.0492) than in nights with a clear sky (6.9 ± 2.29 m).

The maximum values during a single observation period (3 h) for upstream and downstream movements were 62 and 69 m, respectively, observed during nocturnal hours. Maximum distance moved in a single day was 74 m, with a mean distance of 7.2 ± 1.82 m per day. Mean distance of movement downstream (4.4 ± 1.66 m) and upstream (2 ± 0.43 m) did not differ significantly (U = 931.0; P = 0.9384). The frequency of upstream and downstream movements was similar ($\chi^2 = 0.65$; P = 0.4213).

The observed crayfish often moved from their shelters and later returned. The spatial behavior of crayfish was designated as homing behavior if movements were undertaken to reach a known area repeatedly. We defined a minimum of at least 4 returns to the shelters in home area. Eight crayfish displayed a homing behavior, repeatedly using 3-8 shelters within a home area (Fig. 2A). Two and three home areas were recorded for crayfish A and D, respectively. The place where crayfish were hidden during observations was named as shelter. We calculated the time spent in particular shelters in the home area - the most exploited shelter was marked as the hub shelter. It was often centrally located as a hub for movements within the home area. Exploitation of a hub shelter reached a mean of 48.9 ± 4.49% of time spent in the home area. The mean number of shelters in a home area was 5.3 ± 0.56, with the mean distance between shelters being 1.1 ± 0.13 m. The mean greatest distance between two shelters within a home area was 3.1 ± 0.46 m. Crayfish D resided at three home areas. It spent two days in the first area, then moved 22 m downstream to a second area, where a relatively short time was spent. Subsequently it left this area and moved 40m downstream to a third area. After 14 days it left this third area and returned more than 60 m to the first home area, where it used the same shelter as before. We did not observe more than one tracked specimens in one shelter and any aggressive interaction over shelters.

A 65.6 ± 12.45% ($\chi^2 = 9.69$; $P = 0.0018$) occurrence close to sources of organic matter was found, with a preference (74.2 ± 12.13%; $H = 15.07$; $P = 0.0046$) for a sand substratum with a silt layer compared to hard-bottomed sections. There was also a preference for brook sections deeper than 0.2 m ($\chi^2 = 52.64$; $P < 0.0000$) and pools (86.3 ± 6.76%; $H = 16.11$; $P = 0.0003$). The slow- and fast-flowing parts of the brook were used only for migration, not for settlement. Crayfish were observed to take refuge under rocks or relatively small stones. In study 1, D-shaped burrows characteristic of *O. limosus* were found in the steep clay bank of pools in the brook. These burrows were occupied only in spring.

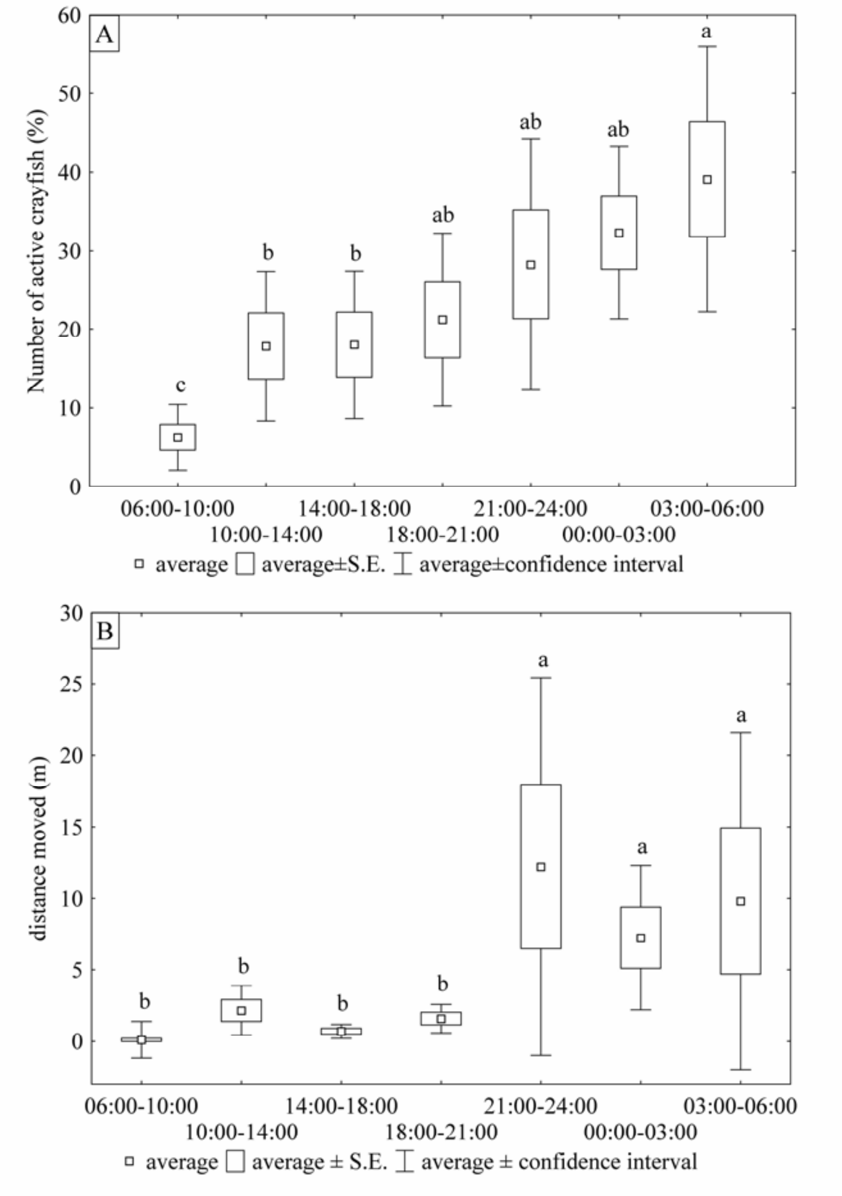


Figure 1. A - The number of active crayfish (%) during particular monitoring periods in late spring ($H = 27.88$; $P = 0.009$). **B -** Mean distances ($F=17.61$; $P = 0.0001$) moved during particular monitoring periods in late spring. Different letters indicate significant differences between groups after a post-hoc test.

Figure 2. Routes followed by the radio-tracked crayfish in study 1 [A] and study 2 [B]. Points represent shelters, squares the main shelter, and circles indicate home areas.

3.2. Late autumn

From 19 to 27 October migratory activity reached 77.3 ± 5.25%. During this period, 76.5% of movements greater than 10 m, and 91.7% greater than 25 m were observed (Fig. 3). Although the total number of downstream and upstream movements (51 downstream and 41 upstream) did not differ ($\chi^2 = 1.09$; $P = 0.2971$), distances per day (5.9 ± 2.39 m and 0.7 ± 0.12 m for downstream and upstream movements, respectively) differed ($H = 14.35$; $P = 0.0008$), with a mean total distance of 6.7 ± 2.4 m per day. The maximum values for upstream and downstream movements were 12 and 139 m per day, respectively. During the experiment, supernormal flow was recorded in the brook, with a gradual decrease of temperature from 13 °C to 5 °C. However, no significant relationship between site conditions and movements was found.

Throughout study 2, the autumn period of mating was observed. Crayfish mating or standing outside the shelter during diurnal hours were often recorded, confirming daytime activity in this season. The majority of the crayfish migrated downstream to the reservoir during this observation period, with only a single tagged crayfish remaining in the brook.

Homing behavior was seen in seven crayfish in study 2 (Fig. 2B). Exploitation of a hub shelter reached a mean value of 37.9 ± 3.53% of time spent in the home area. The mean number of shelters in the home area was 5.4 ± 0.37. The maximum distance between shelters in the home area was 2.6 ± 0.5 m, with an average value of 1.3 ± 0.19 m. Again, we did not observe more than one tracked specimens in one shelter and any aggressive interaction over shelters. There were no significant differences between homing behavior data in studies 1 and 2.

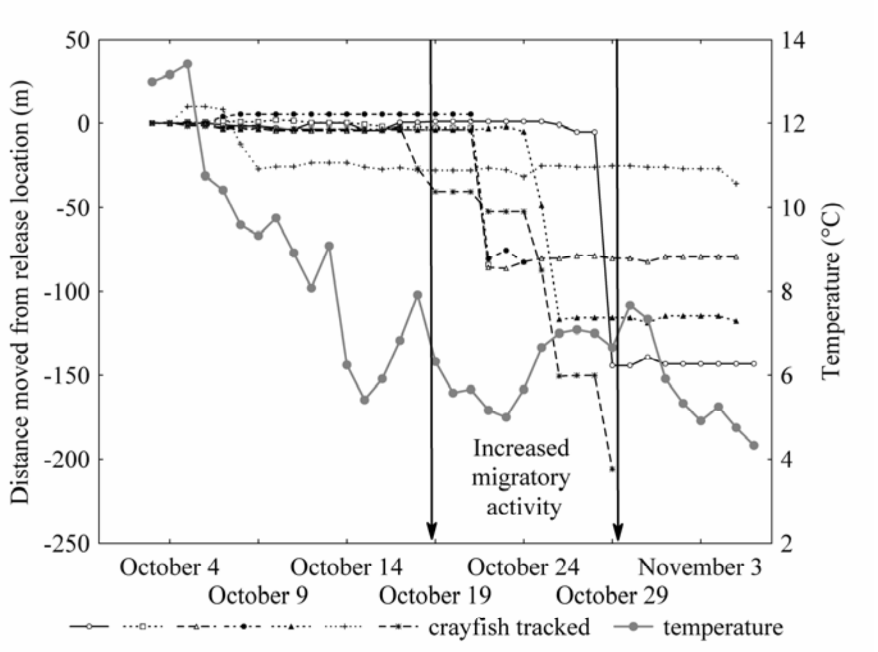


Figure 3. Example plot of movements of 7 radio-tracked crayfish. Positive values refer to locations upstream and negative values to locations downstream from release location.

3.3. Changes in crayfish abundance

Crayfish abundance in the study site is recorded in Table 1. The mean length of males and females differed only during the second capture (t = -2.83; P = 0.0059). Juvenile specimens predominated during the 1^{st} and 2^{nd} captures. The average CL in the brook population increased during the year to a maximum in November (F = 11.47; P < 0.0000).

May is apparently the transition time to colonization of the brook, where an important role is played by the large pool near the influx. A month later, the population in the brook had increased, and successful colonization of all suitable parts of the brook was recorded. A reverse trend was observed in autumn. In October a residual population inhabited the brook, with only five crayfish remaining the in the upper part in November.

In spring the mean water temperature in the brook (14.9 ± 1.58 °C) was significantly (t = -40.61; P < 0.0000) lower than that in the reservoir (21.7 ± 1.9 °C). This trend was also observed in late autumn (t = -22.19; P < 0.0000), with average temperatures of 7.8 ± 2.3 °C and 13 ± 2.08 °C detected in the brook and reservoir, respectively.

Table 1. Crayfish caught in particular captures, average carapace length (CL) ± standard error of mean, number of males and females. Means of CL in the same column with different superscripts differ ($P < 0.05$).

Date	Crayfish captured (n)	CL (mm)	Males		Females	
			n	CL (mm)	n	CL (mm)
29 May	41	21.7 ± 0.73^d	23	22.5 ± 0.79	18	20.7 ± 1.3
27 June	75	24.1 ± 0.42^c	32	25.4 ± 0.72	43	23.1 ± 0.45
3 October	12	27.8 ± 1.31^b	6	29.1 ± 2.57	6	27.2 ± 1.19
5 November	5	29.8 ± 2.36^a	2	33.7 ± 0.7	3	27.2 ± 3.15

4. Discussion

Radio-tags did not hinder movement; crayfish were able to take refuge under stones and mate. This is in accordance with studies of other crayfish species (e.g. Bubb et al. 2002; Gherardi et al. 2002, Robinson et al. 2000). No apparent post-release 'fright response' in radio-tagged crayfish, as reported Robinson et al. (2000) for *A. pallipes*, was found in the present study.

Higher nocturnal activity was observed, as expected, but without differences in particular nocturnal monitoring periods, as described by Robinson et al. (2000) for *A. pallipes*. The majority of freshwater crayfish display nocturnal activity (Gherardi 2002). However, a relatively high number of crayfish were active during diurnal hours in the present study, confirming the results of Lozan (2000) and Stucki (2002) found with *O. limosus*. In contrast to findings with *P. leniusculus* (Bubb et al. 2002), water temperature was not correlated to activity in *O. limosus*.

As seen in *P. leniusculus* (Bubb et al. 2002; 2004), movement over greater distances was sporadic, with crayfish remaining in an area for several days, with occasional movements to new locations. Average distances moved during night periods were significantly higher than during day periods. This is in agreement with results obtained with *P. clarkii* (Gherardi and Barbaresi 2000; Aquiloni et al. 2005) and *A. pallipes* (Robinson et al. 2000). The night activity increased in cloudy weather i.e. there was strong moonlight avoidance. The likely explanation of this pattern is that moonlight provides better conditions for night predation, and cloudy weather can be a factor that minimizes the risk of being preyed on. The mammal predators occurring in study site, mink (*Mustela vison*) and otter (*Lutra lutra*), are mainly active at night (Green and Green, 1981; Bowman *et al*., 2007).

Maximum values for upstream and downstream movement showed the ability of the relatively small *O. limosus* to perform rapid long distance movements, as expected. Greater distances moved per day have been observed in *A. astacus* (Bohl 1999; Schütze et al. 1999). This may be explained by the small size of *O. limosus*, since speed of movement is positively correlated to body size in crayfish (Gherardi et al. 2002). A high ability to move upstream allows *O. limosus* to become more widespread, As a consequence, *Orconectes limosus* is able to rapidly penetrate the upper parts of the brook, resulting in a threat to native crayfish species.

The mean distance that *O. limosus* moved per day was comparable to that seen in the invasive crayfish *P. leniusculus*, but higher than in *A. pallipes* (Bubb et al. 2006). The migratory ability of *P. clarkii* during the wandering phase is dramatically greater; this species can reach up to 17 km over 4 days (Gherardi and Barbaresi 2000).

The total number of movements downstream and upstream did not differ, as found for *P. leniusculus* by Bubb et al. (2002; 2004). A difference was found in the distance moved, with a significant tendency to move downstream in autumn. This migratory activity could probably be related to a temperature decrease to less than 7 °C. However, the influence of water temperature on activity or distance moved was not significant. Temperature decrease is probably responsible only for initiation of downstream migration. Supernormal flow recorded in the brook indicated that *O. limosus* could move under conditions of high flow rates, including moving upstream. This ability has been described by Kozák et al. (2004), and no effect of high flows on mortality or apparent displacement of crayfish downstream was observed by Bubb et al. (2002).

Orconectes limosus were more abundant in sandy/silt substrata and pools, as also described by Hamr (2002), Holdich et al. (2006) and Henttonen and Huner (1999), and in deeper parts, but always in the presence of a sufficient number of shelters such as big stones, rocks or driftwood. Shelters provide refuges from predation and cannibalism (Capelli and Magnuson 1983). In the absence of natural shelters, *O. limosus* is able to construct burrows (Holdich and Black 2007). In the present study, two D-shaped burrows were observed to be occupied by crayfish in the spring. Their use was probably associated with the reproductive season, when the females carry eggs (Holdich and Black 2007). The preference for home areas with an abundance of organic matter suggested that the crayfish choose places with high food availability. Organic matter is, in itself, an adequate food source for crayfish, and also provides a habitat for smaller invertebrates, also the prey of crayfish (Guan and Wiles 1998; Nyström 2002).

Orconectes limosus display homing behavior, and occupy a simple system of shelters. The most exploited shelter was often centrally located as a hub for movements within the home area. One crayfish resided at three home areas and returned to the 1st home area to use the original shelter. This differs from results of Robinson et al., (2000), who did not observe a return of displaced animals to home sites. We can conclude that *O. limosus* is capable of homing like some other decapods (Vannini and Cannicci 1995).

From telemetry data, and capture data, we can hypothesize that the brook is probably inhabited by *O. limosus* only during its growing season. The hypothesis that *O. limosus* migrate periodically from the brook to over-wintering sites in the reservoir is a possible explanation for the increasing crayfish density in spring, subsequent decrease in autumn, the trend to autumn downstream migration, and the abundance of crayfish near the stream mouth. This might explain the occurrence of *O. limosus* exclusively in the lower reaches of tributaries of large rivers (Petrusek et al. 2006). The crayfish might use the brook for safe molting and for protection against predation by predatory fish, *e.g.* perch (*Perca fluviatilis*), pike (*Esox lucius*) and eel (*Anquilla anquilla*) (Haertel et al. 2002; 2005). These fish are abundant in the reservoir. In addition, the lower temperature may be a limiting factor in the spread of *O. limosus* and in their permanent settlement upstream. The fact that the species does not usually penetrate far upstream in small watercourses may protect some populations of IC (*A. astacus* and *Austropotamobius torrentium*) from the transmission of crayfish plaque by direct contact with its carriers (Petrusek et al. 2006). On the other hand, *O. limosus* is able to rapidly move upstream, so can penetrate the habitat of IC in the short term and enable the transmission of this disease.

In general, the Velký brook is an example of a site suitable for establishment of native noble crayfish (Skurdal and Taugbøl 2002), but threatened by the vector of crayfish plaque. Indeed, *O. limosus* in the Orlík reservoir and its tributaries are infected with crayfish plaque (Kozubíková et al. 2006). Despite the fact that *O. limosus* have not been shown to prefer this type of water body (Holdich et al. 2006) it is able to inhabit the brook, at least during the growing season, when the water temperature increased. The more favorable conditions provide warmer water in reservoir, especially during winter. Nevertheless, the prospective spread or re-establishment of *A. astacus* in the upper parts of the brook is prevented by the presence of invading, *O. limosus*.

Acknowledgements

This investigation was financially supported by the Czech Ministry of Education (project USB RIFCH No. MSM6007665809), the Czech Science Foundation (projects GAAV IAA601870701) and the South Bohemian University Foundation (project 88/2006/P-VÚRH). We also thank Dr. James Sales for his help during correcting the manuscript, and deeply appreciate Dr. Alan Pike and Ms. Kathleen Hills of the contribution of the Lucidus Consultancy for correcting this manuscript.

References

Aquiloni L., Ilhéu M., Gherardi F., 2005. Habitat use and dispersal of the invasive crayfish *Procambarus clarkii* in ephemeral water bodies of Portugal. Mar. Freshwat. Behav. Physiol. 38: 225-236.

Barbaresi S., Santini G., Tricarico E., Gherardi F., 2004. Ranging behaviour of the invasive crayfish, *Procambarus clarkii* (Girard). J. Nat. Hist. 38: 2821-2832.

Bohl E., 1999. Motion of individual noble crayfish *Astacus astacus* in different biological situations: in-situ studies using radio telemetry. Freshwater crayfish 12: 677-687.

Bowman J., Kidd A., Gorman R., Schulte-Hostedde A., 2007. Assessing the potential for impacts by feral mink on wild mink in Canada. Biol. Conserv. 139: 12-18.

Bubb D.H., Lucas M.C., Thom T.J., 2002. Winter movements and activity of signal crayfish *Pacifastacus leniusculus* in an upland river, determined by radio telemetry. Hydrobiologia 483: 111-119.

Bubb D.H., Lucas M.C., Thom T.J., 2004. Movement and dispersal of the invasive signal crayfish *Pacifastacus leniusculus* in upland rivers. Freshwat. Biol. 49: 357-368.

Bubb D.H., Thom T.J., Lucas M.C., 2006. Movement, dispersal and refuge use of co-occuring introduced and native crayfish. Freshwat. Biol. 51: 1359-1368.

Capelli G.M., Magnuson J.J., 1983. Morphoedaphic and biogeographic analysis of crayfish distribution in northern Wisconsin. J. Crust. Biol. 3: 548-564.

Füderer L., Edsman L., Holdich D.M., Kozák P., Machino Y., Pöckl M., Renai B., Reynolds J.D., Schulz H., Schulz R., Sint D., Taugbol T., Trouilhé M.C., 2006. Indigenous crayfish habitat and threats. In: Souty-Groset C., Holdich D.M., Noël P., Reynolds J.D., Haffner P. (eds.): Atlas of Crayfish in Europe. Publications Scientifiques du MNHN, Paris, pp. 25-48.

Gherardi F., 2002. Behaviour. In: Holdich, D.M. (ed.): Biology of Freshwater Crayfish. Blackwell Science Ltd., London, pp. 258-290.

Gherardi F., Barbaresi S., 2000. Invasive crayfish: activity patterns of *Procambarus clarkii* in the rice fields of the lower Guadalquivir (Spain). Arch. Hydrobiol. 150: 153-168.

Gherardi F., Tricarico E., Ilhéu M., 2002. Movement patterns of an invasive crayfish, *Procambarus clarkii*, in temporary stream of southern Portugal. Ethol. Ecol. Evol. 14: 183-197.

Green J., Green R., 1981. The otter (*Lutra lutra* L.) in Western France. Mamm. Rev. 11: 181-187.

Guan R., Wiles R.P., 1998. Feeding ecology of the signal crayfish *Pacifastacus leniusculus* in the British lowland river. Aquaculture 169: 177-193.

Haertel S.S., Baade U., Eckmann R., 2002. No general percid dominance at mesotrophic lake conditions: insights from the quantification of predator-prey interactions. Limnologica 32: 1-13.

Haertel-Borer S.S., Zak D., Eckmann R., Baade U., Holker F., 2005. Population density of the crayfish, *Orconectes limosus*, in relation to fish and macroinvertebrate densities in a small mesotrophic lake – Implications for the lake's food web. Int. Rev. Hydrobiol. 90: 523-533.

Hamr P., 2002. *Orconectes*. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish. Blackwell Science Ltd., London, pp. 585-608.

Henttonen P., Huner J.V., 1999. The introduction of alien species of crayfish in Europe: A historical introduction. In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 13-22.

Holdich D.M., Gherardi F., 1999. Native and alien crayfish in Europe: An introduction. In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 3-9.

Holdich D.M., Haffner P., Noël P., Carral J., Füderer L., Gherardi F., Machino Y., Madec J., Pöckl M., Śmietana P., Taugbol T., Vigneux E., 2006. Species files. In: Souty-Grosset C., Holdich D.M., Noël P., Reynolds J.D., Haffner P. (eds.): Atlas of Crayfish in Europe. Publications Scientifiques du MNHN, Paris, pp. 49-130.

Holdich D.M., Black J., 2007. The spiny-cheek crayfish, *Orconectes limosus* (Rafinesque, 1817)[Crustacea: Decapoda: Cambaridae], digs into the UK. Aquat. invasions 2: 1-16.

Kozák P., Policar T., 2006. The present status of the astacofauna in the Czech Republic. In: 23rd World Congress of Czechoslovak Society of Arts and Sciences. *Book of abstracts*, České Budějovice, June 25-July 2, 2006, pp. 89.

Kozák P., Policar T., Ďuriš Z., 2004. Migration ability of *Orconectes limosus* through a fishpass and notes on its occurrence in the Czech Republic. Bull. Fr. Peche Piscic. 372-373: 367-373.

Kozubíková E., Petrusek A., Ďuriš Z., Kozák P., Geiger S., Hoffmann R., Oidtmann B., 2006. The crayfish plague in the Czech Republic - Review of recent suspect cases and a pilot detection study. Bull. Fr. Peche Piscic. 380–381: 1313-1323.

Lozan J.L., 2000. On the threat to the European crayfish: A contribution with the study of the activity behaviour of four crayfish species (Decapoda: Astacidae). Limnologica 30: 156-166.

McAlpine D., Hogans W.E., Fletcher T.J., 1991. *Orconectes limosus* (Crustacea, Cambaridae) an addition to the crayfish fauna of New Brunswick. Can. Field-Nat. 105: 386-387.

Nyström P., 2002. Ecology. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish. Blackwell Science Ltd., London, pp. 192-235.

Petrusek A., Filipová L., Ďuriš Z., Horká I., Kozák P., Policar T., Štambergová M., Kučera Z., 2006. Distribution of the invasive spiny-cheek crayfish (*Orconectes limosus)* in the Czech Republic: past and present. Bull. Fr. Peche Piscic. 380-381: 903-917.

Pöckl M., Holdich D.M., Pennerstorfer J., 2006. Identifying native and alien crayfish species in Europe. European Project CRAYNET.

Robinson C.A., Thom T.J., Lucas M.C., 2000: Ranging behaviour of a large freshwater invertebrate, the white-clawed crayfish *Austropotamobius pallipes*. Freshwat. Biol. 44: 509-521.

Schütze S., Stein H., Born O., 1999. Radio telemetry observations on migration and activity patterns of restocked noble crayfish *Astacus astacus* (L.) in the small river Sempt, North-East of Munich, Germany. Freshwater crayfish 12: 688-695.

Skurdal J., Taugbøl T., 2002. *Astacus*. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish. Blackwell Science Ltd., London, pp. 467-510.

Stucki T.P., 2002. Differences in live history of native and introduced crayfish species in Switzerland. Freshwater crayfish 13: 463-476.

Štěpán V., 1933. Soudobý stav rakařství v Čechách (The current status of astacology in Bohemia) (in Czech), Vodňany, Nakl. A. Dvořák.

Vannini M., Cannicci S., 1995. Homing behaviour and possible cognitive maps in crustacean decapods. J. Exp. Mar. Biol. Ecol. 193: 67-91.

Chapter 8

Spring mating period in *Orconectes limosus*: The reason for movement!

Buřič M., Kouba A., and Kozák P. 2009. Aquatic Sciences (submitted).

Spring mating period in *Orconectes limosus*: The reason for movement!

Miloš Buřič., Antonín Kouba, Pavel Kozák

University of South Bohemia in České Budějovice, Research Institute of Fish Culture and Hydrobiology, Zátiši 728/II, CZ 389 25 Vodňany, Czech Republic

Abstract

Adult *Orconectes limosus* males (carapace length 32.5 ± 2.48 mm; weight 10.8 ± 2.63 g) were radio-tagged and tracked in a small tributary to a reservoir during and after spring mating period, from April to June. Higher average movement distances per day were observed, when mating occurred (14.9 ± 21.4 m) and correlation between distance moved and water temperature was not seen. Tracked crayfish showed a high ability for rapid upstream and downstream movement, maximum 118 m and 95 m per day, respectively. Crayfish were observed active even during day hours. When water temperature increased up to 10 °C, crayfish spring migration suddenly collapsed. That was affected by the end of mating and the start of spawning. The low values then slowly increased with increasing water temperature. *Orconectes limosus* was found able to move rapidly upstream in small watercourses in the short term, which can signify a threat of penetration of native crayfish habitats and enable the transmission of crayfish plaque. The threat is very high especially in mating season, due to increased motional activity.

Key words: invasive species, radio-telemetry, reproduction, migration.

1. Introduction

Orconectes limosus (Rafinesque 1817), a temperate-water crayfish native to the north-eastern USA and south-eastern Canada, is now widespread in at least 20 European countries (Pöckl et al. 2006). It is present mainly in big rivers and their larger tributaries (of 6th or higher stream order). It can be found also in smaller streams (i.e. those with a low stream order), which join larger rivers (Petrusek et al. 2006). Small brooks and rivulets are apparently not suitable habitats for *O. limosus* (Holdich et al. 2006). However, such habitats are used by *O. limosus* in Canada (McAlpine et al. 1991). Crayfish may enter the small watercourse only temporarily (Petrusek et al. 2006; Buřič et al. 2009).

Orconectes limosus is a small-bodied species, with a total length (TL) reaching 5 to 8 cm in adult animals (Holdich et al. 2006; Holdich and Black 2007). The small size complicates locating and re-sampling individuals in the field and requires careful choice of marking method (Buřič et al. 2008). However, radio-telemetry allows the study of movement and behaviour patterns over extended time periods of even very small aquatic animals. Radio-telemetry has been utilized successfully in crayfish (e.g. Robinson et al. 2000; Gherardi et al. 2000; Bubb et al. 2004) including one study in *O. limosus* (Buřič et al. 2009).

For *O. limosus* two mating periods are described, in autumn and then in spring (Hamr 2002; Holdich and Black 2007; Kozák et al. 2007). Detailed data on migratory activity linked to reproductive activities of *O. limosus* are still lacking. There is a possible similarity with observed increased motional activity of *Procambarus clarkii* (Girard 1852) due to reproductive phase (Gherardi and Barbaresi 2000). Findings of Stucki (2002), who observed wandering *O. limosus* in early spring season and also checked daily activity, supported hypothesized similarity.

In order to gain information on migratory activity influenced by reproductive activities, the study was conducted to observe crayfish behaviour and movements during and after spring mating period.

2. Material and methods

2.1. Study site

The Velký brook is a small tributary of a large reservoir, Orlík Reservoir, of the Vltava river in the south-western Czech Republic. It is situated at an altitude of 360 m above sea level at 49°20' N, 14°18' E. Its width and depth ranged from 0.6 to 2 m, and 0.05 to 0.6 m, respectively. The substratum is variable, from sand and silt to large boulders, mostly covered by coarse gravel. Most of the study area is wooded. During the study, water temperature in both the reservoir and the brook was measured every 3 h using data loggers (RT-F53, Qi Analytical, Prague, Czech Republic). The crayfish population, consisting of only *O. limosus*, was probably introduced into the reservoir by fishermen.

2.2. Crayfish capture and radio-tagging

The study was conducted between April 17 and June 10. Crayfish were caught by hand searching at the beginning of experiment. Carapace length (CL; from the tip of the rostrum to the posterior median edge of the cephalothorax) was measured to the nearest 0.1 mm with digital callipers (Schut Geometrical Metrology, Groningen, The Netherlands). Wet body weight of crayfish to the nearest 0.1 g was determined with an electronic balance (Kern & Sohn GmbH, Balingen, Germany). Average CL of spiny-cheek crayfish used in experiments was 32.5 ± 2.48 mm, with an average body weight of 10.8 ± 2.63 g. All locations, where crayfish were found, were charted.

Radio-transmitters, type Pip, powered by Ag379 batteries (Biotrack Ltd., Wareham, U.K.), 14 x 6 x 3 mm and 0.5 g weight, were glued to the carapace of suitable individual crayfish. Tag to animal-size ratio was similar to those in other radio-telemetry studies with crayfish (Gherardi and Barbaresi 2000; Robinson et al. 2000; Bubb et al. 2002; 2004; 2006), tag weight representing 4.9 ± 1.17 % of wet body weight. Tagged crayfish were released close to the capture location (<0.25 m) within 10 min of capture.

2.3. Radio-telemetry data collection

Thirteen radio-tagged males were tracked with a Sika receiver and flexible three-element Yagi antenna (Biotrack Ltd., Wareham, U.K.). Data collections comprised one observation per day, recording the daytime shelter location, 5-6 days per week for two months. Positions were described on a scaled map of the study site, and the distance from the previous shelter was calculated. The presence of crayfish standing out of the shelter, moving or mating was observed during day time hours.

2.4. Calculations and statistical analysis

For each radio-tracked crayfish, total and mean distance moved as well as upstream and downstream movements were calculated.

Wilcoxon test was used for comparison of movements during and after mating period together with upstream and downstream movements. Spearman rank correlation was used for estimation of water temperature to migration activity correlations. The differences in frequency of upstream and downstream movements were evaluated using χ^2 tests with Yates' correction. The level of significance at which the null hypothesis was rejected was $\alpha = 0.05$. Data are presented as average ± standard deviation.

3. Results

The maximum values for upstream and downstream movements were 118 and 95 m per day, respectively. The mean distance of 7.4 ± 15.6 m per day was observed between April 17 and June 10. Mean distance of movement downstream (7.3 ± 15.4 m) and upstream (7.5 ± 15.7 m) did not differ significantly ($Z = 0.59$; $P > 0.05$). The frequency of upstream and downstream movements was similar ($\chi^2 = 0.90$; $P > 0.05$). The course of movements in time with day temperatures is shown in Fig. 1.

Table 1. Mean distances moved per day during and after mating period and maximal distances moved in upstream and downstream direction. Different superscripts in the same row differ significantly at $\alpha = 0.05$ (Wilcoxon test, Z).

	Mean distance per crayfish and day [m]				Maximal distance per day [m]
	During mating	After mating	Z	P	
Upstream	14.6 ± 20.9^a	2.3 ± 5.6^b	6.92	$> 10^{-6}$	118
Downstream	15.2 ± 21.7^a	2.1 ± 5.3^b	6.35	$> 10^{-6}$	95
Altogether	14.9 ± 21.4^a	2.2 ± 5.5^b	9.43	$> 10^{-6}$	-
Position change (%)	87.8 ± 13.2^a	38.9 ± 19.4^b	4.87	10^{-6}	-

3.1. Effect of mating period

Females were often observed outside their shelters and fully exposed to predators during day hours in mating period, including mating pairs. Mating took place till the time when females have spawned (spawning finished in May 12), once females were hidden they stopped their exposure. Three egg-carrying females were observed occupying simple D-shaped burrows in clay-loam banks.

During mating period males exhibit very high motional activity expressed by mean value 14.9 ± 21.4 m per day. There was no difference between upstream and downstream movement values ($Z = -0.02$; $P > 0.05$) and frequency of upstream and downstream movements was also similar ($\chi^2 = 1.24$; $P > 0.05$). Crayfish showed the opposite pattern when mating was finished. The mean moved distance reached 2.2 ± 5.5 m per day and was significantly lower than in mating period. There was no difference between upstream and downstream movement values ($Z = 0.12$; $P > 0.05$) and frequency of upstream and downstream movements was also similar ($\chi^2 = 0.02$; $P > 0.05$). The comparison of these two observed periods is shown in Table 1.

3.2. Effect of temperature

When temperature increased up to 10 °C motional activity suddenly decreased, which was reflected by negative correlation of distance moved per day and water temperature (Spearman rank correlation, $r_S = -0.622$, $P < 0.05$). However, the distance moved per day and water temperature did not correlated during mating period (Spearman rank correlation, $r_S = -$

0.086, $P > 0.05$) and positively correlated after mating period (Spearman rank correlation, r_S = 0.477, $P < 0.05$).

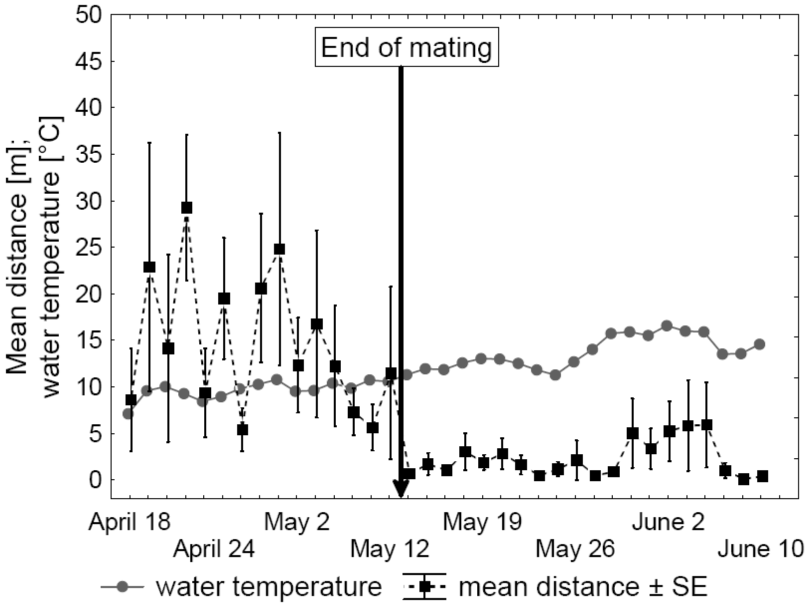


Figure 1. Course of migration activity during and after mating period. Mean distance travelled ± SE and average day water temperature are shown.

4. Discussion

As seen in *Pacifastacus leniusculus* (Dana 1852) (Bubb et al. 2002; 2004), *O. limosus* (Buřič et al. 2009) or *P. clarkii* (Aquiloni et al. 2005; Gherardi et al. 2002), movement over greater distances was sporadic, with crayfish remaining in an area for several days, with occasional movements to new locations. That is valid with exception of observed explosive spring mating season, when most crayfish changed their position every day. No apparent post-release 'fright response' in radio-tagged crayfish, as reported Robinson et al. (2000) for *Austropotamobius pallipes* (Lereboullet 1858), was found in the present study.

The mean distance that *O. limosus* moved per day during and after mating period was higher and lower, respectively, than that seen in the invasive crayfish *P. leniusculus* and European native *A. pallipes* (Bubb et al. 2006). However, the greater migratory ability has been observed in *Astacus astacus* (Linnaeus 1758) (Bohl 1999; Schütze et al. 1999) and also *P. clarkii* reached during the wandering phase dramatically higher values; this species can reach up to 17 km over 4 days (Gherardi and Barbaresi 2000; Gherardi et al., 2000). This difference could be explained by smaller size of *O. limosus*, since speed of movement is positively correlated to body size in crayfish (Gherardi et al. 2002), and also by the smaller home range of *O. limosus* (Buřič et al. 2009) than for *P. clarkii* (Barbaresi et al. 2004). Maximum values for upstream and downstream movement showed the ability of the relatively small *O. limosus* to perform rapid long distance movements, as expected. Two times higher maximal upstream movement and similar maximal downstream movement was observed in present study, compared to *O. limosus* observations of Buřič et al. (2009). A high ability to move upstream and downstream allows *O. limosus* to become more widespread.

The total number of movements and distance moved downstream and upstream did not differ in present study, as found also for *P. leniusculus* by Bubb et al. (2002; 2004). Therefore spring migrations probably do not have a final destination, but only mating with more females as a target. On the other hand, *O. limosus* displayed significant trend of downstream migration in autumn (Buřič et al. 2009).

In general, crayfish are nocturnal creatures hidden in shelters during day light (Gherardi 2002). But, Lozan (2000) and Stucki (2002) found increased day activity in *O. limosus,* compared to European native crayfish. In present study crayfish were often observed waiting near their shelter, moving or mating during diurnal hours, confirming also the results of Musil et al. (2009). The mammal predators occurring in study site, mink (*Mustela vison*, Schreber 1777) and otter (*Lutra lutra*, Linnaeus 1758), are otherwise active mainly at night (Green and Green 1981; Bowman et al. 2007), nevertheless crayfish are exposed to high predatory risk during reproductive season. Shelters provide refuges from predation and cannibalism (Capelli and Magnuson 1983) and especially egg-carrying females need suitable shelters to shield their broods (Gherardi 2002). In the absence of natural shelters, *O. limosus* is able to construct burrows (Holdich and Black 2007). In present study, three egg-carrying females were observed using burrows. Burrows were observed to be occupied by *O. limosus* only in spring and their use is thus associated with the reproductive season after spawning (Holdich and Black 2007; Buřič et al. 2009).

Bubb et al. (2002) described positive effect of water temperature on *P. leniusculus* movements as well as Lozan (2000) or Van Der Brink et al. (1988) in laboratory studies in several crayfish including *O. limosus*. In contrast to those findings, water temperature was observed negatively correlating with *O. limosus* movements during study. However, this was concluded as false and misleading finding. The calculated correlation was not caused by influence of temperature, but influenced by the end of mating period. Hence, the separation into mating and non mating period was necessary to reach an adequate result. Then the hectic searching of suitable mate was found as a stronger factor than an influence of water temperature. That specifies results of Van Der Brink et al. (1988), who found the water temperature as a major factor in the regulation of *O. limosus* activity. When reproductive activities have been finished, the migrations significantly dropped, but the movements were positively affected by increasing water temperature.

Orconectes limosus is able to move rapidly upstream, so it can penetrate the habitat of indigenous crayfish (IC) in small watercourses in the short term. The mating season, reflected as increased migratory activity, multiplies the threat of penetration into upper parts of brooks potentially inhabited by IC. Even short inhabiting of IC habitats by *O. limosus* could enable the transmission of crayfish plaque (Petrusek et al. 2006), because it is a vector of this dangerous disease (Holdich et al. 2006).

Acknowledgement

This investigation was supported financially by the Czech Ministry of Education (project USB RIFCH No. MSM6007665809), and the Czech Science Foundation (projects GAAV IAA601870701).

References

Aquiloni L., Ilhéu M., Gherardi F., 2005. Habitat use and dispersal of the invasive crayfish *Procambarus clarkii* in ephemeral water bodies of Portugal. Mar. Freshwat. Behav. Physiol. 38: 225-236.

Barbaresi S., Santini G., Tricarico E., Gherardi F., 2004. Ranging behaviour of the invasive crayfish, *Procambarus clarkii* (Girard). J. Nat. Hist. 38: 2821-2832.

Bohl E., 1999. Motion of individual noble crayfish *Astacus astacus* in different biological situations: in-situ studies using radio telemetry. Freshwater crayfish 12: 677-687.

Bowman J., Kidd A., Gorman R., Schulte-Hostedde A., 2007. Assessing the potential for impacts by feral mink on wild mink in Canada. Biol. Conserv. 139: 12-18.

Bubb D.H., Lucas M.C., Thom T.J., 2002. Winter movements and activity of signal crayfish *Pacifastacus leniusculus* in an upland river, determined by radio telemetry. Hydrobiologia 483: 111-119.

Bubb D.H., Lucas M.C., Thom T.J., 2004. Movement and dispersal of the invasive signal crayfish *Pacifastacus leniusculus* in upland rivers. Freshwat. Biol. 49: 357-368.

Bubb D.H., Thom T.J., Lucas M.C., 2006. Movement, dispersal and refuge use of co-occuring introduced and native crayfish. Freshwat. Biol. 51: 1359-1368.

Capelli G.M., Magnuson J.J., 1983. Morphoedaphic and biogeographic analysis of crayfish distribution in northern Wisconsin. J. Crust. Biol. 3: 548-564.

Buřič M., Kozák P., Vích P., 2008. Evaluation of different marking methods for spiny-cheek crayfish (*Orconectes limosus*). Knowl. Manage. Aquat. Ecosyst. 389 (02): 1-8.

Buřič M., Kozák P., Kouba A., 2009. Movement patterns and ranging behaviour of the invasive spiny-cheek crayfish in a small reservoir tributary in the Czech Republic. Fundam. Appl. Limnol., *in press*.

Gherardi F., 2002. Behaviour. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 258-290.

Gherardi F., Barbaresi S., 2000. Invasive crayfish: activity patterns of *Procambarus clarkii* in the rice fields of the lower Guadalquivir (Spain). Arch. Hydrobiol. 150: 153-168.

Gherardi F., Barbaresi S., Salvi G., 2000. Spatial and temporal patterns in the movement of *Procambarus clarkii*, an invasive crayfish. Aquat. Sci. 62: 179-193.

Gherardi F., Tricarico E., Ilhéu M., 2002. Movement patterns of an invasive crayfish, *Procambarus clarkii*, in temporary stream of southern Portugal. Ethol. Ecol. Evol. 14: 183-197.

Green J., Green R., 1981. The otter (*Lutra lutra* L.) in Western France. Mamm. Rev. 11: 181-187.

Hamr P., 2002. *Orconectes*. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish, Blackwell Science Ltd., London, UK, pp. 585-608.

Holdich D.M., Haffner P., Noël P., Carral J., Füderer L., Gherardi F., Machino Y., Madec J., Pöckl M., Śmietana P., Taugbol T., Vigneux E., 2006. Species files. In: Souty-Groset C., Holdich D.M., Noël P., Reynolds J.D., Haffner P. (eds.): Atlas of Crayfish in Europe. Publications Scientifiques du MNHN, Paris, pp. 49-130.

Holdich D.M., Black J., 2007. The spiny-cheek crayfish, *Orconectes limosus* (Rafinesque, 1817)[Crustacea: Decapoda: Cambaridae], digs into the UK. Aquat. invasions 2: 1-16.

Kozák P., Buřič M., Policar T., Hamáčková J., Lepičová A., 2007. The effect of inter- and intra-specific competition on survival and growth rate of native juvenile noble crayfish *Astacus astacus* and alien spiny-cheek crayfish *Orconectes limosus*. Hydrobiologia 590: 85-94.

Lozan J.L., 2000. On the threat to the European crayfish: A contribution with the study of the activity behaviour of four crayfish species (Decapoda: Astacidae). Limnologica 30: 156-166.

McAlpine D., Hogans W.E., Fletcher T.J., 1991. *Orconectes limosus* (Crustacea, Cambaridae) an addition to the crayfish fauna of New Brunswick. Can. Field-Nat. 105: 386-387.

Musil M., Buřič M., Policar T., Kouba A., Kozák P., 2009. Comparison of day and night activity between noble (*Astacus astacus*) and spiny-cheek crayfish (*Orconectes limosus*). Freshwater Crayfish 17, *in review*.

Petrusek A., Filipová L., Ďuriš Z., Horká I., Kozák P., Policar T., Štambergová M., Kučera Z., 2006. Distribution of the invasive spiny-cheek crayfish (*Orconectes limosus)* in the Czech Republic: past and present. Bull. Fr. Peche Piscic. 380-381: 903-917.

Pöckl M., Holdich D.M., Pennerstorfer J., 2006. Identifying native and alien crayfish species in Europe. European Project CRAYNET.

Robinson C.A., Thom T.J., Lucas M.C., 2000: Ranging behaviour of a large freshwater invertebrate, the white-clawed crayfish *Austropotamobius pallipes*. Freshwat. Biol. 44: 509-521.

Schütze S., Stein H., Born O., 1999. Radio telemetry observations on migration and activity patterns of restocked noble crayfish *Astacus astacus* (L.) in the small river Sempt, North-East of Munich, Germany. Freshwater crayfish 12: 688-695.

Stucki T.P., 2002. Differences in live history of native and introduced crayfish species in Switzerland. Freshwater crayfish 13: 463-476.

Van Den Brink F.W.B., Van Der Velde G., Geelen J.F.M., 1988. Life history parameters and temperature-related activity of an American crayfish, *Orconectes limosus* (Rafineque, 1817) (Crustacea, Decapoda), in the area of the major rivers in The Netherlands. Arch. Hydrobiol. 114: 275-289.

Chapter 9

Moulting and growth in relation to form alternation of male spiny-cheek crayfish, *Orconectes limosus*

Buřič M., Kouba A., and Kozák P.2009. Zoological Studies (submitted).

.

Moulting and growth in relation to form alternation of male spiny-cheek crayfish, *Orconectes limosus*

Miloš Buřič, Antonín Kouba, Pavel Kozák

University of South Bohemia in České Budějovice, Research Institute of Fish Culture and Hydrobiology, Zátiši 728/II, 389 25 Vodňany, Czech Republic

Abstract

Morphological changes associated with form alternation in male *Orconectes limosus* are described with new findings and explanations of existing equivocalness. Most males (84.4%) underwent 2 molts, with a one month interval between molts. A small proportion (8.9%) (higher initial size than twice molting) molted once, without form alternation. A smaller proportion (6.7%) (higher initial size than twice and once molted) did not molt at all. Molting began after water temperatures remained up to 16 °C for 2 months. Form I gonopods were longer, wider and more robust than form II gonopods, which were similar to those of juveniles, apart from possessing wider protopodites and shorter endopodites. Chelae of form I males were longer and wider than in form II. Juveniles had shorter and narrower chelae. The chelae and abdomen of form I were more robust than those of form II and juveniles, which did not differ. Change of body ratios did not occur in crayfish molted once without form alternation. Negative allometric growth of chela length (ChL) and width (ChW) and abdomen width (AbW) was observed after the molt to form II. Crayfish body size increased more at the 1st moult (to form II), and, at the molt back to form I. The ChL, ChW and weight (W) increments were 3 (ChL) and 4 (ChW, W) times that of body size increments and dramatically higher than at the previous molt. Form alternation seems to have a function in utilizing resources effectively to increase size as well as attractive appearance for females.

Keywords: invasive species, crayfish, intra-sex dimorphism, growth.

1. Introduction

Orconectes limosus (Rafinesque 1817) is a member of the family Cambaridae (Arthropoda: Decapoda) native to North America and eastern Asia. *Orconectes limosus* has become a widespread invasive species in many European countries and is also spreading in some provinces of Canada (Hamr 2002; Holdich et al. 2006). Its life history is different from that of crayfish of the native family Astacidae (Van Den Brink et al. 1988; Chybowski 2007). Cambarid males are dimorphic, alternating between a reproductively active condition (form I), and a reproductively inactive state (form II) (Scudamore 1948; Hobbs 1989). These forms occur in all known *Orconectes* species (Hobbs 1974). The changes in characteristics associated with form alternation are the form of the hooks on the ischia of the 3rd pereiopods, body proportions, and conformation of the gonopods (Scudamore 1948; Hobbs 2001).

Form alternation occurs during semi-yearly molts (Hobbs 2001; Guiasu 2002). Form I is first reached at the final juvenile molt (Suko 1953; Hobbs 2001). This breeding form is more aggressive (Bovbjerg 1956; Tierney et al. 2008) and can be distinguished by the sclerotization, amber-coloring, and lengthening of the terminal elements of the first pleopods (Andrews 1910; Hobbs 1989). In addition, the ischial hooks are more pronounced (Andrews 1910; Hobbs 2001), the first chelipeds are enlarged (Lutz and Wolter 1990; Eversole et al. 2006), and the sperm ducts are filled with recently formed spermatids (Pieplow 1938; Hobbs

2001). In form II males, the terminal elements of the first pleopod are not as well differentiated (and never corneous), and the ischial hooks become shorter and weaker (Scudamore 1948; Payne 1978).

Typically, all cambarid adult males in a population molt at approximately the same time, but in some species molting is asynchronous, and adults of both forms can be found in the same population (Hamr and Berrilll 1985; Hobbs 2001). Transformation to the non-reproductive state usually takes place in midsummer (Berrill and Arsenault 1984; Hamr and Berrilll 1985). It is generally believed that form II males revert to form I at the subsequent molt, as they normally undergo two molts per year (Capelli and Magnuson 1975; Smith 1981), but there is evidence that some form II males may go through two or more molts before reverting to the form I state (Payne 1978; Mazlum et al. 2007).

Although there are many life history studies that refer to form alternation in cambarid crayfishes, most are based on samples from different sites. Few cambarid crayfish have been studied continuously for extended periods (Hobbs 2001). Data are lacking on molting, growth, and also on the number and significance of changes in body and gonopod proportions between forms in crayfish maintained in controlled conditions. These data are essential to a better understanding of the complex cycle in cambarid crayfish.

The objective of this study was to elucidate patterns associated with male form alternation in the *O. limosus* life history. Form alternation was investigated in an experimental population of *O. limosus* over a seasonal cycle. The aims were, 1) to record the number of molts, and their timing and a/synchrony; 2) to describe the changes in body proportions; 3) to compare growth increments of successive molts; and 4) to describe the changes in copulatory stylet proportions.

2. Materials and methods

2.1. Animals

Specimens of *O. limosus* (both sexes) were captured (n = 1157) in the Černovický brook (South Bohemia, Czech Republic) in August 2007. They were acclimated to laboratory conditions during autumn and winter and placed in experimental tanks in February 2008. Each crayfish was identified as to sex and reproductive state by external appearance. Males were classified as reproductively active (form I) or inactive (form II) using the criteria of Pieplow (1938) and Hobbs (1989). All males were in form I at the start of the experiment. The crayfish were held in mixed sex confinement (females:males 2:1) in which visual, chemical and tactile contact, including mating behavior was possible. Individual crayfish were marked with VI Alpha tags (Northwest Marine Technology^{TM}, Shaw Island, USA) following Isely and Stockett (2001) and Buřič et al. (2008).

2.2. Experimental conditions

Crayfish were stocked circular tanks (0.6 m diameter, volume 0.18 m^3) and supplied with ~ 3 shelters per crayfish. Photoperiod and water temperature was natural ambient, from natural daylight and a flow through water supply. Tanks were cleaned regularly, and dissolved oxygen was measured twice daily using an oximeter (Oxi 315i, WTW GmbH, Weilheim, Germany). Water temperature was measured every 3 h using data loggers (RT-F53, Qi Analytical, Prague, Czech Republic), the pH was measured daily (pH 315i, WTW GmbH, Weilheim, Germany). Water temperature, dissolved oxygen level, and pH are shown in Fig. 1. Crayfish were fed in excess on fish pellets, frozen chironomid larvae, and carrot two to five times per week (depending on season and amount of uneaten feed).

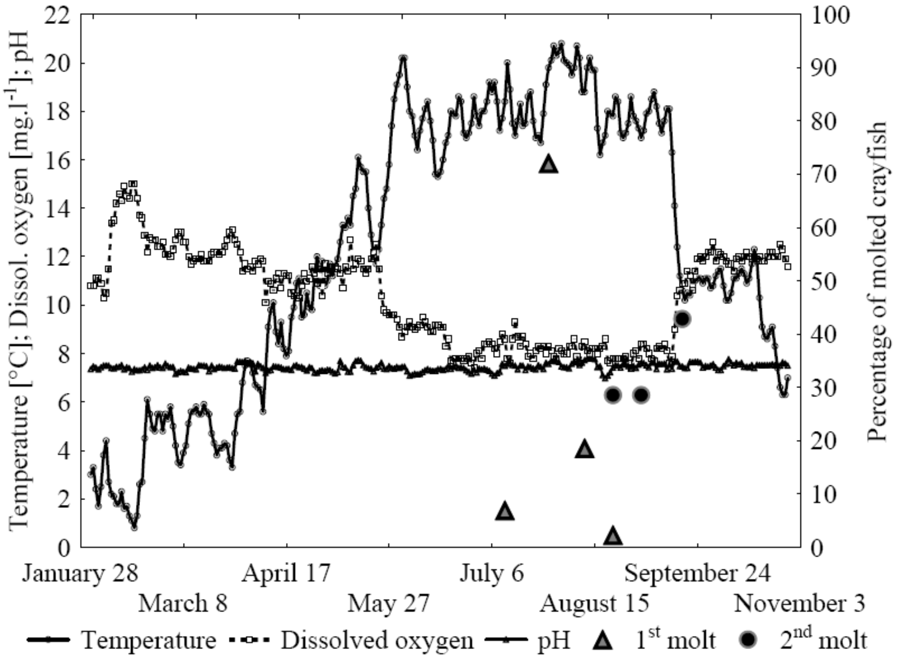


Figure 1. Temperature (°C), dissolved oxygen (mg l^{-1}) and pH over the course of the experiment. Triangles represent the percent of *Orconectes limosus* (from all molted) that underwent 1st molt at a given time, and the circles the percent of *Orconectes limosus* that underwent 2nd molt at given time.

2.3. Data collection

Crayfish were measured before and after molting (when the exoskeleton was fully hardened). Carapace length (CL, from tip of the rostrum to the posterior edge of cephalothorax), postorbital carapace length (POCL, from the edge of eye socket to the posterior edge of the cephalothorax), chela length (ChL, from the tip of propodus to carpal joint), chela width (ChW, at the widest part), and abdomen width (AbW, the width at the 2nd pleonite) were measured to the nearest 0.01 mm with digital callipers (Schut Geometrical Metrology, Groningen, The Netherlands) (Fig. 2*a*). The dimensions of the right chelae were used for analysis, with damaged or regenerated specimens excluded. Wet body weight was measured to the nearest 0.01 g with an electronic balance (Kern & Sohn GmbH, Balingen, Germany). Molting and form alternation were recorded. Growth increment was calculated for each molted crayfish, with the equation (Brewis and Bowler 1982):

$$L_i = (L_a - L_b) \times 100 / L_b \quad [\%]$$

where L_i = length increment, L_a = length after molt, and L_b = length before molt.

There was a need to determine the body dimensions for calculating ratios of body proportions. Total length, measured from the tip of the rostrum to the posterior edge of the telson, is an often used parameter to describe crayfish size (e.g., Pieplow 1938; Olsson et al. 2008). But, since the abdomen and telson are mobile, errors can result from curling or curving of the abdomen. Measurement of carapace length is more reliable, since it is a rigid immovable structure, but rostrum length may vary among individuals and also can be damaged. Therefore the POCL was designated as the most reliable parameter for body size ratios calculated in this study. The ChL/POCL, ChW/POCL, ChW/ChL, and AbW/POCL ratios were calculated.

Samples of 30 crayfish were euthanized in clove oil for copulatory stylet measurement. Form I and juveniles (~ one year old) were taken at the beginning of experiment, and form II taken on July 22. The stylets were dissected, fixed in 96% ethanol, labelled, and stored for later processing. The mesial and lateral surfaces of fixed stylets were photographed under stereomicroscope (Olympus, Lens, France) coupled by adapter with digital camera (Olympus, Lens, France). Measurements of the stylet were made to the nearest 0.001 mm with the QuickPHOTO CAMERA 2.2 program (Olympus, Hamburg, Germany). These comprised total length of the gonopodium (GL, from base to tip of the copulatory stylet), base width (BW, width of the gonopod base), maximum width of the gonopodium (GW), endopodite length (EL) and width (EW), and protopodite width (PW) (Fig. 2*b*). The ratios, GL/POCL, GW/POCL, BW/GL, GW/GL, EL/GL, and PW/GL were calculated.

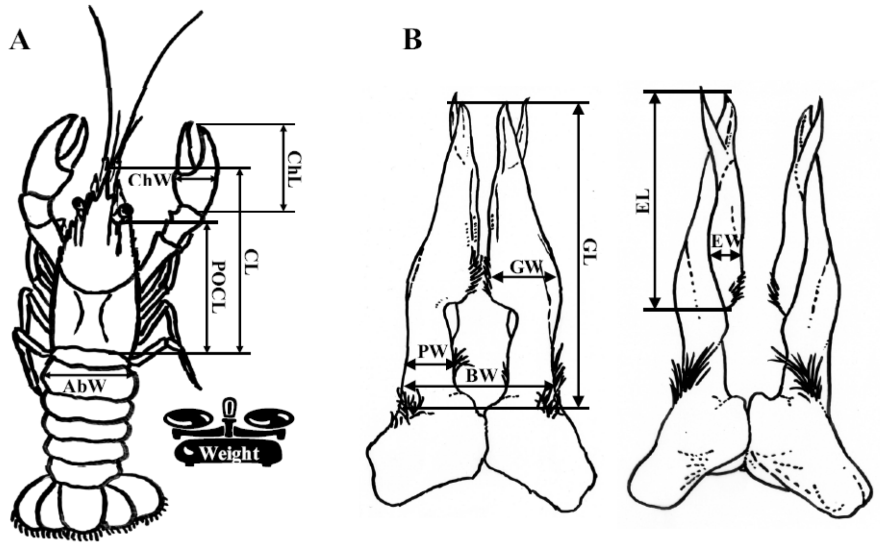


Figure 2. Designation of measurements of *Orconectes limosus* (a) body dimensions and (b) copulatory stylet dimensions (lateral and mesial view) CL – carapace length, POCL – postorbital carapace length, ChL – chela length, ChW – chela width, AbW – Abdomen width, GL – total length of gonopodium, BW – base width, GW – maximal width of gonopodium, EL – endopodite length, EW – endopodite width, and PW – protopodite width.

2.4. Data analysis

The data were analyzed with Statistica 8.0 (StatSoft., Inc.). All values were examined for normal distribution (Kolmogorov-Smirnov test) and homoscedaticity (Levene test). The *t* test was used for comparison of molting time and molt increments; the paired *t* test for comparison of body proportions in individual crayfish before and after molting; one way ANOVA with Tukey's post hoc test for comparison of body proportions and copulatory stylet proportions of form I, form II, and juveniles; and Spearman rank correlation for testing AbW/POCL to body size correlation. The null hypothesis was rejected at $\alpha = 0.05$. Data are presented as averages ± standard deviation.

3. Results

3.1. Number of molts and time of molting

The majority of males (84.4%) molted twice, from form I to form II and subsequently back to the form I. A small proportion of crayfish (8.9%) molted just once during the year but remained as reproductively active form I. A smaller proportion (6.7%) did not molt and remained as form I. The initial CL and POCL of twice molted (CL = 31.4 ± 2.92 mm; POCL = 23.0 ± 2.21 mm), once molted (CL = 34.3 ± 1.47 mm; POCL = 25.4 ± 1.10 mm), and non-molted crayfish (CL = 38.2 ± 1.53 mm; POCL = 28.8 ±1.19 mm) differed significantly ($F_{CL} = 20.05$, $p_{CL} < 10^{-5}$; $F_{POCL} = 21.98$, $p_{POCL} < 10^{-6}$). There were no form II males at the conclusion of the experiment. Males began molting 2 months after water temperature had increased to 16 °C. Crayfish that molted from form I to form II did so between July 11 and August 22; and the highest proportions of newly molted crayfish were seen on July 28 (72.1%) and August 11 (18.6%). The second molt, back to form I, occurred between August 22 and September 18. All of the males that molted only once did so between August 11 and August 22. The time between the end of mating (when females oviposited) and molting to form II was 65.8 ± 7.10 days or 1171.8 ± 135.89 degree days (d°). The time between the 1st molt and the 2nd molt back to form I was 32.7 ± 6.34 days or 590.2 ± 99.82 d°. Males undergoing a single molt did so a significantly longer time ($t_{days} = 3.93$, $p_{days} = 0.0003$; $t_{degree\ days} = 4.01$, $p_{degree\ days} = 0.0002$) after the end of mating (82.7 ± 5.19 days and 1499.8 ± 94.42 d°).

Table 1. Ratios of body proportions of form I, form II, and juvenile male *Orconectes limosus*. Data are presented as mean ± standard deviation. Values in the same row with different superscripts differ significantly (α = 0.05). CL, carapace length; POCL, postorbital carapace length; ChL, chela length; ChW, chela width; AbW, abdomen width.

	Form I n = 184	Form II n = 129	Juvenile n = 30	F	*p*
POCL/CL	0.73 ± 0.016^{a}	0.74 ± 0.015^{a}	0.72 ± 0.015^{b}	15.38	$<10^{-5}$
ChL/POCL	1.07 ± 0.060^{a}	0.98 ± 0.067^{b}	0.79 ± 0.049^{c}	269.13	$<10^{-6}$
ChW/POCL	0.38 ± 0.024^{a}	0.33 ± 0.025^{b}	0.27 ± 0.014^{c}	320.94	$<10^{-6}$
ChW/ChL	0.36 ± 0.015^{a}	0.34 ± 0.015^{b}	0.34 ± 0.019^{b}	41.05	$<10^{-6}$
AbW/POCL	0.62 ± 0.020^{a}	0.60 ± 0.015^{b}	0.60 ± 0.019^{b}	27.20	$<10^{-6}$
CL (mm)	32.5 ± 3.69^{a}	33.1 ± 2.94^{a}	19.0 ± 2.04^{b}	209.33	$<10^{-6}$
POCL (mm)	23.8 ± 2.93^{a}	24.4 ± 2.20^{a}	13.6 ± 1.49^{b}	193.77	$<10^{-6}$
Weight (g)	10.5 ± 3.72^{a}	9.2 ± 2.65^{a}	1.7 ± 0.59^{b}	88.44	$<10^{-6}$

3.2. Changes in body proportions

The summary of ratios of body proportions in form I, form II and juveniles are given in Table 1. It is evident that form I had longer and wider chelae than form II and juveniles, and form II had longer and wider chelae than juveniles. In addition, form I had broader abdomens and a higher ChW/ChL ratio than form II and juveniles. Detailed information on changes in ratios of body proportions in observed individuals after successive molts is given in Table 2. Changes were not observed in those few crayfish that molted without form alternation (form I → form I), with the exception of the AbW/POCL ratio, which decreased.

Table 2. Changes in ratios of body proportions in *Orconectes limosus* before and after moulting: A) from form I to form II; B) from form II back to form I; C) from form I without form alternation. Data are presented as mean ± standard deviation. Values in the same row with different superscripts differ significantly ($\alpha = 0.05$). CL, carapace length; POCL, postorbital carapace length; ChL, chela length; ChW, chela width; AbW, abdomen width.

A 1st molt	Form I (before molt)	Form II (after molt)	residual	*t*	*p*
POCL/CL	0.73 ± 0.013^{b}	$0.74\pm0.01.48^{a}$	0.01±0.013	-4.13	0.0002
ChL/POCL	1.04 ± 0.071^{a}	0.98 ± 0.069^{b}	-0.06±0.017	18.55	$<10^{-6}$
ChW/POCL	0.37 ± 0.029^{a}	0.33 ± 0.025^{b}	-0.04±0.009	27.08	$<10^{-6}$
ChW/ChL	0.35 ± 0.016^{a}	0.33 ± 0.017^{b}	-0.02±0.009	13.17	$<10^{-6}$
AbW/POCL	0.63 ± 0.015^{a}	0.60 ± 0.015^{b}	-0.3±0.011	14.69	$<10^{-6}$

B 2nd molt	Form II (before molt)	Form I (after molt)	residual	*t*	*p*
POCL/CL	0.75 ± 0.018^{a}	0.75 ± 0.012^{a}	0.001±0.017	-0.08	0.9354
ChL/POCL	0.93 ± 0.071^{b}	1.03 ± 0.073^{a}	0.09±0.011	-20.49	$<10^{-5}$
ChW/POCL	0.31 ± 0.034^{b}	0.36 ± 0.031^{a}	0.04±0.010	-11.35	$<10^{-4}$
ChW/ChL	0.33 ± 0.017^{a}	0.34 ± 0.013^{a}	0.01±0.013	-2.02	0.0996
AbW/POCL	0.60 ± 0.017^{a}	0.61 ± 0.022^{a}	0.004±0.013	-0.81	0.4469

C Single molt	Form I (before molt)	Form I (after molt)	residual	*t*	*p*
POCL/CL	0.74 ± 0.006^{a}	0.74 ± 0.010^{a}	-0.002±0.016	0.20	0.8553
ChL/POCL	1.10 ± 0.061^{a}	1.09 ± 0.063^{a}	-0.01±0.018	1.08	0.3581
ChW/POCL	0.39 ± 0.015^{a}	0.37 ± 0.024^{a}	-0.02±0.019	2.40	0.0959
ChW/ChL	0.35 ± 0.024^{a}	0.34 ± 0.026^{a}	-0.01±0.026	0.59	0.5945
AbW/POCL	0.63 ± 0.012^{a}	0.60 ± 0.012^{b}	-0.03±0.007	8.70	0.0032

3.3. Growth increments in successive molts

The 1st molt (to form II) can be characterized by a low increment of weight increase, AbW, and ChL, and by reduction in ChW. On the other hand, a greater growth increment was seen in CL and POCL than was observed in the 2nd molt (back to form I). With this exception, the 2nd molt was characterized by higher growth of observed parts of the body. The AbW increment was higher than in the 1st molt and similar to CL and POCL increments in 2nd molt. The increments of ChL, ChW and weight were significantly higher than those observed in 1st molt and higher than CL, POCL and AbW growth increments in 2nd molt. The AbW/POCL decreased with increasing body size (Spearman rank correlation, $r_S = -0.5873$, $p < 0.05$). Data are summarized in Table 3.

Table 3. Percent molt increments in particular molts in *Orconectes limosus*; after 1st (to the form II) and 2nd molt (back to the form I). Data are presented as mean ± standard deviation. Values in the same row with different superscripts differ (α = 0.05). CL, carapace length; POCL, postorbital carapace length; ChL, chela length; ChW, chela width; AbW, abdomen width.

Increments (%)	Molt to Form II	Molt back to Form I	*t*	*p*
CL	6.6 ± 1.74^{a}	4.9 ± 1.93^{b}	2.39	0.0216
POCL	6.8 ± 2.04^{a}	5.0 ± 1.77^{b}	2.23	0.0314
ChL	1.2 ± 1.70^{b}	15.3 ± 2.62^{a}	-17.35	$<10^{-6}$
ChW	-4.9 ± 2.58^{b}	19.5 ± 5.17^{a}	-19.01	$<10^{-6}$
AbW	2.3 ± 2.40^{b}	5.7 ± 2.61^{a}	-3.31	0.0019
Weight	1.4 ± 6.09^{b}	19.3 ± 4.10^{a}	-7.42	$<10^{-6}$

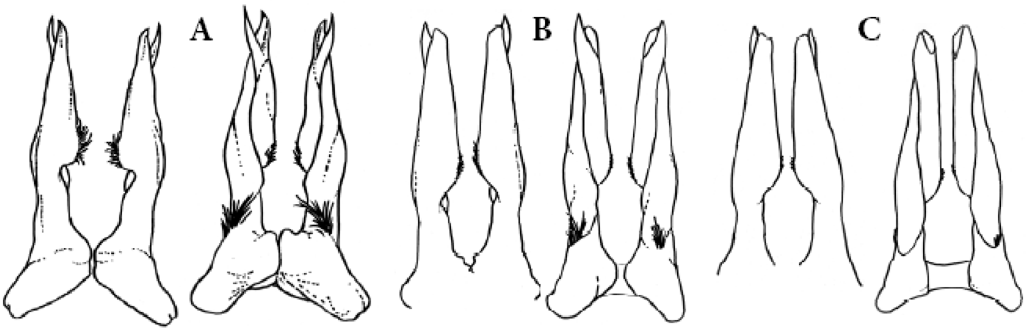
Table 4. Ratios of copulatory stylet proportions in form I, form II and juvenile *O. limosus*. Data are presented as mean ± standard deviation. Values in the same row with different superscripts differ (α = 0.05). CL, carapace length; POCL, postorbital carapace length; GL, total length of the gonopodium; GW, maximum width of the gonopodium; BW, base width; PW, protopodite width; EL, endopodite length.

	Form I n = 30	Form II n = 30	Juveniles n = 30	F	*p*
GL/POCL	0.37 ± 0.034^{a}	0.35 ± 0.024^{b}	0.35 ± 0.019^{b}	8.56	0.0004
GW/POCL	0.08 ± 0.008^{a}	0.07 ± 0.005^{b}	0.07 ± 0.005^{b}	48.36	$<10^{-6}$
GW/GL	0.22 ± 0.019^{a}	0.20 ± 0.009^{b}	0.20 ± 0.013^{b}	25.55	$<10^{-6}$
BW/GL	0.54 ± 0.031^{a}	0.54 ± 0.019^{ab}	0.52 ± 0.034^{b}	3.59	0.0321
PW/GL	0.14 ± 0.008^{a}	0.14 ± 0.008^{a}	0.13 ± 0.010^{b}	8.73	0.0003
EL/GL	0.68 ± 0.036^{a}	0.63 ± 0.026^{b}	0.68 ± 0.031^{a}	20.13	$<10^{-6}$
CL (mm)	31.5 ± 3.36^{a}	32.9 ± 2.63^{a}	19.0 ± 2.04^{b}	252.84	$<10^{-6}$
POCL (mm)	22.3 ± 2.60^{a}	24.3 ± 2.05^{a}	13.6 ± 1.49^{b}	253.75	$<10^{-6}$
Weight (g)	9.1 ± 3.36^{a}	9.1 ± 2.18^{a}	1.7 ± 0.59^{b}	250.02	$<10^{-6}$

3.4. Changes in morphology of copulatory stylets

In general, copulatory stylets in form I crayfish are larger than those of form II and juveniles. Form II gonopods showed proportions similar to those of juveniles, with three differences: form II had wider protopodites, shorter endopodites (Table 4), and gonopodia possessed more differentiated parts (Fig. 3).

Figure 3. Lateral and mesial views of the 1st pleopod of male *Orconectes limosus* (a) form I, (b) form II, (c) juvenile.



4. Discussion

The life history of *O. limosus* has been studied in its original area of distribution (Smith 1981; Momot 1988) as well as in areas of introduction and secondary expansion in Europe (Van Den Brink et al. 1988; Ďuriš et al. 2006). Comprehensive data (based on long term observation of individual crayfish) describing molting and growth patterns associated with form alternation are still lacking. Most existing data was gathered from samples rather continuous observation.

The typical life cycle of male *Orconectes*, reported by Hobbs (2001) in North America, is periodic form alternation throughout life, associated with two molts per year, in spring (May/June) and summer (August). Different molting strategies were found in the present study. In most cases males underwent a cycle through form II, consisting of 2 molts. The 1st molt took place between mid-July and mid-August, later than is usually reported (Van Den Brink et al. 1988; Larson and Magoulick 2008), and the 2nd molt between mid-August and mid-September. The time between successive molts was therefore shorter than the 8 weeks reported by Stein (1976) or Hobbs (2001). Muck et al. (2002) reported the 1st molting in water temperatures up to 9 °C (April/May) and the 2nd molting at temperatures to 8 °C (August/September). In the present study crayfish first molted following 2 months of water temperatures up to 16 °C. The last crayfish underwent their 2nd molt in temperatures exceeding 10 °C. It can be assumed higher temperature requirements of *O. limosus*, which is documented in degree days for the first time in the present study. This surprising finding offers questions about differences between North American and European *O. limosus* habitats, which could alter its requirements. However, the typical *O. limosus* habitats described by Hamr (2002) are similar to those in Europe (Holdich et al. 2006; Holdich and Black 2007).

The general assumption that form II males revert to the form I state with the subsequent molt (Stein et al. 1977; Smith 1981) was confirmed. However, there were two exceptions to the common life history model. A small proportion of males molted only once and did not change form. Suko (1960) also reported a small number of *Procambarus clarkii* (Girard 1852) males molting only once. Suggested reasons have been low pH conditions that may prevent normal mineralization during molting (Hobbs 2001), water temperature (Mundahl and Benton 1990), or the influence of food quality and availability (Reynolds 2002; Olsson et al. 2008). This does not appear to apply to the present study, where crayfish were held under identical conditions, consisting of optimal pH, natural water temperature (without high divergences from the seasonal normal values), high levels of dissolved oxygen, an adequate number of shelters, and sufficient food. Those not changing form underwent the molt later and showed a higher initial CL and POCL than males that molted twice. Belated molting of once molted males, could explain the assumption of Pieplow (1938), based on gastrolith presence, that *O. limosus* molts three times per year.

The second exception was a small proportion of males that did not molt throughout the year. The initial CL and POCL of these non-molting males were higher than in twice molted and once molted males. These two exceptions could point to an explanation for observation of form I in a population year round (Hobbs 1981; Hamr and Berrill 1985). The reason for this is unclear, but could be explained by the size reached, since the CL and POCL of non-molted males were close to the maximum values in the source population. This suggests decreasing growth, as a function of number of molts, with increasing size and age. This strategy can positively influence the survival of large males, since molting crayfish are more vulnerable to cannibalism and predation (Nyström 2002). The number of molts had no influence on the function of form I copulatory stylets, even non-molted crayfish successfully mated.

The twice molting crayfish molted in relative synchrony, limiting the time for the possibility of encounters between form I and form II males (except with non-molting and crayfish molting once). Molting in synchrony and form alternation was also reported by Hamr and Berrill (1985) and Hobbs (2001).

Juvenile, form I, and form II cambarid crayfish naturally differ in body conformation (Suko 1953; Craig and Wolters 1988). In general, form I have more pronounced ischial spines and enlarged chelae. In form II, the ischial hooks are shorter and weaker, and the chelae are less robust (Stein et al. 1977; Hobbs 2001). Those patterns have been confirmed for *O. limosus* (Pieplow 1938; Chybowski 2007). Pieplow (1938) described these differences as large and visible, while Chybowski (2007) reported them as very small. The differences can be explained by varying environmental conditions. Morphometric traits may be affected by feeding behavior, foraging efficiency, and the availability and quality of food resources (Lindquist and Lahti 1983; France 1985). Hence we measured water temperature, dissolved oxygen level, and pH level and fed crayfish to excess in our experiment to record important environmental variables.

The most significant aspect of form alternation, apart from morphology of the copulatory stylets, is the change in chela dimensions relative to body size. Form I males have longer and wider chelae than do form II. Juveniles had shorter and narrower chelae than both, from I and form II. The ChW/ChL and AbW/POCL ratios in form I were also higher (i.e., chelae and abdomen are more robust) than those of form II and juveniles, which did not differ from each other. Chelae have important functions, including prey capture and manipulation, defense against predators, inter- and intra-specific interactions, and for reproduction (Stein 1976; Holdich 2002). Chela dimorphism may be connected to their use. The form I can be characterized by higher inter-male aggression (Bovbjerg 1956; Tierney et al. 2008), competition for females (Hobbs 2001), and higher exposure of males to potential predation (Berrill and Arsenault 1984). Large chelae are advantageous in competition for limited resources and in aggressive behavior (Söderbäck 1991; Nakata and Goshima 2003). Males with large chelae are more successful in copulating with females and often overpower females much larger than themselves (Mason 1970; Stein 1976). Because large females produce more eggs than small females (Kozák et al. 2006; Larson and Magoulick 2008), males that copulate successfully with large females can increase their contribution to the gene pool of the population. This may explain the high investment in building large chelae in form I.

Following the breeding season, form I males molt to form II in which there is no breeding behavior (Payne 1978). Form II males spent significantly more time in shelters, displayed fewer agonistic acts, and spent less time fighting than did form I males (Tierney et al. 2008). Form II males therefore do not require large chelae because they face minimal predatory risk, are sexually inactive, and are less often exposed to agonistic encounters. Synchrony of molting leads to lower exposure to agonistic interactions, which can protect form II from encounters with aggressive form I. Form II usually occurs during summer (Hobbs 2001; Guiasu 2002), when water temperatures are high and food availability should be maximal (Stein 1976; Nyström 2002). Large chelae do not appear to be necessary for prey capture and manipulation, and may even be disadvantageous for this purpose (Stein 1976).

Negative allometric growth (relative to body size) of ChL, ChW and AbW was observed when crayfish molted to form II. Chela width decreased. Minimal (but not negative) growth increments of ChL and ChW were also reported by Pieplow (1938) and Stein (1976). Conversely, CL and POCL increments were higher, suggesting that the priority of the 1st molt was to increase body size while conserving energy. The minimal chela growth during molting to form II therefore makes ecological sense. Less energy is expended in growing, as well as carrying, large chelae which would have no practical function. If they not necessary for prey capture and manipulation or for predator defense (Stein 1976), there is no reason to grow massive chelae or a wider abdomen.

There is a need to increase body size before the 2nd molt. First because body size is the most important attribute for success in aggressive interactions, including more frequent initiation of, and successful, aggressive interactions, and mating success, including more frequent copulation and interruption of copulating pairs (Berrill and Arsenault 1984; Aquiloni and Gherardi *2008b*). Second, a larger size entering the 2nd molt means more resources will be available for growing longer and wider chelae. Cambarid crayfish must increase their size through only two molts annually (Stein 1976; Reynolds 2002), and must use resources effectively. Hence they invest more in growth at the molt to form II.

The 2nd molt, reverting to form I, was characterized by higher growth of ChL and ChW, with increments three (ChL) and four (ChW) times higher that seen in POCL. Larger ChL increments in the reproductive molt were also reported by Pieplow (1938). The growth increment of AbW was similar to that of POCL after the 2nd molt, the annual increment is therefore lower than the POCL increment. This resulted in a slow decrease of the AbW/POCL ratio with increasing body size, possibly due to the low importance of AbW. On the other hand, much higher annual increments of ChL and ChW than of POCL were observed. This resulted in positive allometric growth of ChL and ChW (relative to body size), as has been reported in other species (Boyd and Page 1978; Acosta and Perry 2000). The changes in body proportions occurred in form alternation seem to have a function of utilizing resources effectively to increase size as well as attractive appearance for females, because females prefer bigger males with large chelae (Aquiloni and Gherardi 2008a).

The 1st pair of pleopods, known as copulatory stylets or gonopods, in form I are longer, more rigid, and more sharply pointed than those of form II (Pieplow 1938; Hobbs 2001). The gonopod consists of three poorly differentiated parts: a protopodite bearing a mesial endopodite, and a lateral exopodite (Suko 1953; Hobbs 2001). The present study confirmed the often cited observation of longer gonopods in form I due to lengthening of the terminal elements. One of these terminal elements is soft and flexible, whereas the second is rigid and ends in a deep and convoluted groove. Form I gonopods were also observed to be wider and more robust (higher GW/GL ratio) than those in form II. Form II gonopods show a different structure, with both terminal elements being shorter, sclerotised, calcified, and rounded. In general, form II gonopods are similar to juvenile gonopods, but have a wider protopodite and shorter endopodite. The wider protopodite may be necessary as support for the enlarged gonopods of form I.

This study provides new findings as well as complementary data clarifying misunderstood parts of famous cycle of cambarid crayfishes, which contribute to the better understanding of life history of widely distributed crayfish in North American and European inland waters, and probably also other species of genus *Orconectes*.

Acknowledgements

This investigation was financially supported by the Czech Ministry of Education (project USB RIFCH No. MSM6007665809), and the Czech Science Foundation (projects GAAV IAA601870701). We also deeply appreciate the assistance of the Lucidus Consultancy for correcting this manuscript.

References

Acosta C.A., Perry S.A., 2000. Differential growth of crayfish *Procambarus alleni* in relation to hydrological conditions in the marl prairie wetlands of Everglade National Park, USA. Aquat. Ecol. 34: 389-395.

Andrews E.A., 1910. Conjugation in the crayfish *Cambarus affinis*. J. Exp. Zool. 9: 235-364.

Aquiloni L., Gherardi F., 2008*a*. Mutual mate choice in crayfish: large body size is selected by both sexes, virginity by males only. J. Zool. 274: 171-179.

Aquiloni L., Gherardi F., 2008*b*. Evidence of female cryptic choice in crayfish. Biol. Lett. 4: 163-165.

Berrill M., Arsenault M., 1984. The breeding behaviour of a northern temperate orconectid crayfish, *Orconectes rusticus*. Anim. Behav. 32: 333-339.

Bovbjerg R.V., 1956. Some factors affecting aggressive behavior in crayfish. Physiol. Zool. 29: 127-136.

Boyd J.A., Page L.M., 1978. The Life History of the Crayfish *Orconectes kentuckiensis* in Big Creek, Illinois. Am. Midl. Nat. 99: 398-414.

Brewis J.M., Bowler K., 1982. Growth of the freshwater crayfish *Austropotamobius pallipes* in Northumbria. Freshw. Biol. 12: 187-200.

Buřič M., Kozák P., Vích P., 2008. Evaluation of different marking methods for spiny-cheek crayfish *(Orconectes limosus)* [online]. Knowl. Manage. Aquat. Ecosyst. 389: 1-8.

Capelli G.M., Magnuson J.J., 1975. Reproduction, moulting, and distribution of *Orconectes propinquus* (Girard) in relation to temperature in a northern mesotrophic lake. Freshw. Crayfish 2: 415-427.

Chybowski L., 2007. Morphometrics, fecundity, density, and feeding intensity of the spiny-cheek crayfish, *Orconectes limosus* (Raf.) in natural conditions. Arch. Pol. Fish. 15: 175-241.

Craig R.J., Wolters W.R., 1988. Sources of variation in body size traits, dressout percentage and their correlations in crayfish, *Procambarus clarkii*. Aquaculture 72: 49-58.

Ďuriš Z., Drozd P., Horká I., Kozák P., Policar T., 2006. Biometry and demography of the invasive crayfish *Orconectes limosus* in terminal and residential localities in the Czech Republic. Bull. Fr. Peche Piscic. 380-381: 1215-1228.

Eversole A.G., Mazlum Y., Spacil M.S., 2006. *Procambarus acutus acutus* (Girard) replacement of *P. clarkii* (Girard): a possible explanation. Fresh. Crayfish 15: 63-68.

France R.L., 1985. Relationship of crayfish (*Orconectes virilis*) growth to population abundance and system productivity in small oligotrophic lakes in the Experimental Lakes Area, Northwestern Ontario. Can. J. Fish. Aquat. Sci. 42: 1096-1102.

Guiasu R.C., 2002. *Cambarus*. In: Holdich D.M. (ed.): Biology of freshwater crayfish. Blackwell Science Ltd., London, UK, pp. 609-634.

Hamr P., 2002. *Orconectes*. In: Holdich D.M. (ed.): Biology of freshwater crayfish. Blackwell Science Ltd., London, UK, pp. 585-608.

Hamr P., Berrill M., 1985. The life histories of north-temperate populations of the crayfish *Cambarus robustus* and *Cambarus bartoni*. Can. J. Zool. 63: 2313-2322.

Hobbs H.H. Jr., 1974. Synopsis of the families and genera of crayfishes (Crustacea: Decapoda). Smithson. Contrib. Zool. 164: 32pp.

Hobbs H.H. Jr., 1981. The crayfishes of Georgia. Smithson. Contrib. Zool. 318: 549pp.

Hobbs H.H. Jr., 1989. An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae). Smithson. Contrib. Zool. 480: 236pp.

Hobbs H.H. III., 2001. Decapoda. In: Thorp J.H., Covich A.P. (eds.): Ecology and Classification of North American Freshwater Invertebrates, second edition. Academic press, London, UK, pp. 955-1001.

Holdich D.M., 2002. Background and Functional Morphology. In: Holdich D.M. (ed.): Biology of freshwater crayfish. Blackwell Science Ltd., London, UK, pp. 3-29.

Holdich D.M., Black J., 2007. The spiny-cheek crayfish, *Orconectes limosus* (Rafinesque, 1817)[Crustacea: Decapoda: Cambaridae], digs into the UK. Aquat. invasions 2: 1-16.

Holdich D.M., Haffner P., Noël P., Carral J., Füderer L., Gherardi F., Machino Y., Madec J., Pöckl M., Śmietana P., Taugbol T., Vigneux E., 2006. Species files. In: Souty-Groset C., Holdich D.M., Noël P., Reynolds J.D., Haffner P. (eds.): Atlas of Crayfish in Europe. Publications Scientifiques du MNHN, Paris, pp. 49-130.

Isely J.J., Stockett P.E., 2001. Tag retention, growth, and survival of red swamp crayfish marked with a visible implant tag. N. Am. J. of Fish. Manage. 21: 422-424.

Kozák P., Buřič M., Policar T., 2006. The fecundity, time of egg development and juveniles production in spiny-cheek crayfish (*Orconectes limosus*) under controlled conditions. Bull. Fr. Peche Piscic. 380-381: 1171-1182.

Larson E.R., Magoulick D.D., 2008. Comparative life history of native (*Orconectes eupunctus*) and introduced (*Orconectes neglectus*) crayfishes in the Spring River Drainage of Arkansas and Missouri. Am. Midl. Nat. 160: 323-341.

Lindquist O.V., Lahti E., 1983. On the sexual dimorphism and condition index in the crayfish, *Astacus astacus* L., in Finland. Freshw. Crayfish 5: 3-11.

Lutz C.G., Wolter W.R. 1990. Multivariate morphological variation in a population of red swamp crayfish, *Procambarus clarkii* (Girard). Freshw. Crayfish 8: 56-67.

Mason J.C., 1970. Copulatory behavior of the crayfish, *Pacifastacus trowbridgii* (Stimpson). Can. J. Zool. 48: 969-976.

Mazlum Y., Can M.F., Eversole A.G., 2007. Morphometric relationship of length-weight and chelae length-width of eastern white river crayfish (*Procambarus acutus acutus*, Girard, 1852), under culture conditions. J. Appl. Ichthyol. 23: 616-620.

Momot W.T., 1988. *Orconectes* in North America and elsewthere. In: Holdich D.M., Lowery R.S. (eds.): Freshwater crayfish: Biology, Management and Exploitation. Chapman & Hall, London, UK, pp. 262-282.

Muck J.A., Rabeni C.F., DiStefano R.J., 2002. Life-history characteristics of the crayfish *Orconectes ozarkae* in a Missouri Ozark stream. Freshw. Crayfish 13: 359-370.

Mundahl N.D., Benton M.J., 1990. Aspects of the thermal ecology of the rusty crayfish *Orconectes rusticus* (Girard). Oecologia 82: 210-216.

Nakata K., Goshima S., 2003. Competition for shelter of preferred sizes between the native crayfish species *Cambaroides japonicus* and the alien crayfish species *Pacifastacus leniusculus* in Japan in relation to prior residence, sex difference, and body size. J. Crust. Biol. 23: 897-907.

Nyström P., 2002. Ecology. In: Holdich D.M. (ed.): Biology of freshwater crayfish. Blackwell Science Ltd., London, UK, pp. 192-235.

Olsson K., Nyström P., Stenroth P., Nilsson E., Svensson M., Granéli W., 2008. The influence of food quality and availability on trophic position, carbon signature, and growth rate of an omnivorous crayfish. Can. J. Fish. Aquat. Sci. 65: 2293-2304.

Payne J.F., 1978. Aspects of life histories of selected species of North American crayfishes. Fisheries 3: 5-8.

Pieplow U., 1938. Fischereiwissenschaftliche Monografie von *Cambarus affinis* Say. Zeitschrift für Ficherei und deren Hilfswissenschaften 36: 349-440.

Reynolds J.D., 2002. Growth and reproduction. In: Holdich D.M. (ed.): Biology of freshwater crayfish. Blackwell Science Ltd., London, UK, pp. 152-191.

Scudamore H.H., 1948. Factors influencing moulting and sexual cycles in the crayfish. The Biol. Bull. 95: 229-237.

Smith D.G., 1981. Life history parameters of the crayfish *Orconectes limosus* (Raf.) in southern New England. Ohio J. Sci. 81: 169-172.

Söderbäck B., 1991. Interspecific dominance relationship and aggressive interactions in the freshwater crayfishes *Astacus astacus* (L.) and *Pacifastacus leniusculus* (Dana). Can. J. Zool. 69: 1321-1325.

Stein R.A., 1976. Sexual dimorphism in crayfish chelae: functional significance linked to reproductive activities. Can. J. Zool. 54: 220-227.

Stein R.A., Murphy M.L., Magnuson J.J., 1977. External morphological changes associated with sexual maturity in the crayfish (*Orconectes propinquus*). Am. Midl. Nat. 97: 495-502.

Suko T., 1953. Studies on the development of the crayfish. I. The development of secondary sex characters in appendages. Sci. Rep. Saitama Univ. 1: 77-96.

Suko T., 1960. Studies on the development of the crayfish. VI. The reproductive cycle. Sci. Rep. Saitama Univ. 3B: 79-91.

Tierney A.J., Gunarante C., Jennison K., Monroy V., Donnelly L., 2008. Behavioral correlates and alternate male forms (form I and form II) in the crayfish *Orconectes rusticus*. J. Crust. Biol. 28: 596-600.

Van Den Brink F.W.B., Van Der Velde G., Geelen J.F.M., 1988. Life history parameters and temperature-related activity of an American crayfish, *Orconectes limosus* (Rafineque, 1817) (Crustacea, Decapoda), in the area of the major rivers in The Netherlands. Arch. Hydrobiol. 114: 275-289.

Chapter 10

Intra-sex dimorphism in female *Orconectes limosus*

Buřič M., Kouba A. and Kozák P. 2009. Journal of Limnology (submitted).

Intra-sex dimorphism in female *Orconectes limosus*

Miloš Buřič, Antonín Kouba, Pavel Kozák

University of South Bohemia in České Budějovice, Research Institute of Fish Culture and Hydrobiology, Laboratory: Ethology, nutrition of fish and crayfish, 389 25 Vodňany, Czech Republic.

Abstract

The unique cycle of female form alternation has been described in an experimental population of *Orconectes limosus* during a year long observation. Significant cyclic changes observed in chela length, width, and robustness, as well as in abdomen width, demonstrated form alternation similar to that in conspecific males. Females alternate between sexually active and sexually inactive forms with a short time interval between successive molts as well as different growth patterns of some body parts. Form alternation enables an economical reaching of larger chelae, abdomen and body dimensions. Larger females, once molting, do not have to alter between forms and are sexually active. They grow slowly and lose chela robustness. Form alternation may facilitate utilization of resources effectively to increase size of body parts important to survival and reproduction.

Keywords: crayfish, form alternation, invasive species, growth.

1. Introduction

Orconectes limosus (Rafinesque 1817) is a representative of the Family Cambaridae (Arthropoda: Decapoda) and is native to the Atlantic watershed of North America. It is invasive in many European countries and is also spreading in some provinces of Canada (Hamr 2002; Holdich et al. 2006).

Cambarid males are cyclically dimorphic (Hobbs 1989). Form I (sexually active) and form II (sexually inactive) males occur in all known orconectid species of genus *Orconectes* (Hobbs 1974), with associated alterations in body proportions, such as longer and wider chelae (Hobbs 2001). The change in form occurs in mature males during the semi-annual molts, allowing adult males to alternate from form I to form II and back during the reproductive season (Guiasu 2002).

In general, it has been assumed that adult females do not exhibit form alternation but undergo one (summer) molt and growth occurs during this molt (Van Deventer 1937). This may be explained by the need to delay molting until release of dependent offspring (Scudamore 1948). However, there is evidence that some females of genus *Orconectes* undergo two molts (Scudamore 1948; Wetzel 2002), and in *O. limosus* were found even three molts per year (Pieplow 1938). However, the assumption of a single molt per year is the most widespread. This implies that adult females should either be considerably smaller than similar age males or have a larger growth increment per molt than males.

Wetzel (2002) investigated some *Orconectes* species and found that females, like males, can undergo two annual molts, from form I to form II in the spring, and from form II back to form I in the fall. Female form I had noticeably wider abdomens than form II conspecifics. It is not yet known how widespread this phenomenon is in other crayfish species or if males recognize and respond differently to the female forms (Tierney et al. 2008), but female form alternation may not occur in all *Orconectes* species (Wetzel 2002). Suko (1960) observed that when juveniles separate from the maternal appendages of *Procambarus clarkii*, the female undergoes a molt changing from the reproducing form I to non-reproducing form II. He reported many adults molt twice a year and a few once but did not document distinctive physical characteristics of the two forms, if any occurred.

The main objective of the study was to investigate changes associated with possible form alternation related to the annual reproductive cycle in an experimental population of female *O. limosus*. Specific aims were to 1) record the number of molts and timing of molting, 2) describe morphological differences in body proportions between hypothetical female forms I and II, and 3) quantify growth increase increments with successive molts.

2. Material and methods

2.1. Animals

Specimens of *O. limosus* of both sexes (n = 1157) were captured in the Černovický brook (South Bohemia, Czech Republic) in August 2007. They were acclimated to laboratory conditions for one month and placed in experimental tanks in September 2008. In April 2008, 160 oviposited females were chosen as the sexually active hypothetical form I and placed in experimental tanks. They were held in mixed sex confinement (female:male 2:1) in which visual, chemical, and tactile contact, including mating behavior, was possible. Individual crayfish were marked with VI Alpha tags (Northwest Marine Technology^{TM}, Shaw Island, USA), following Buřič et al. (2008).

2.2. Experimental conditions

Crayfish were placed in circular tanks (0.6 m diameter, volume 0.18 m^3) supplied with ~ 3 shelters per crayfish. Photoperiod and water temperature was natural ambient, provided by natural daylight and a flow-through water supply. Tanks were cleaned regularly, and dissolved oxygen was measured twice daily using an oximeter (Oxi 315i, WTW GmbH, Weilheim, Germany). Water temperature was measured every 3 h using data loggers (RT-F53, Qi Analytical, Prague, Czech Republic).The pH was measured daily (pH 315i, WTW GmbH, Weilheim, Germany). Crayfish were fed to satiation on fish pellets, frozen chironomid larvae, and carrot two to five times per week (depending on season and the amount of food left uneaten).

2.3. Data collection

Crayfish were measured before and after molting (when the exoskeleton was fully hardened). Carapace length (CL, from tip of the rostrum to the posterior edge of cephalothorax), postorbital carapace length (POCL, from the edge of the eye socket to the posterior edge of the cephalothorax), chela length (ChL, from the tip of the propodus to carpal joint), chela width (ChW, at the widest part), and abdomen width (AbW, the width at the 2nd pleonite) were measured to the nearest 0.01 mm with digital calipers (Schut Geometrical Metrology, Groningen, The Netherlands). The dimensions of the right chela were used for analysis, with damaged or regenerated specimens excluded. Wet body weight was measured to the nearest 0.01 g with an electronic balance (Kern & Sohn GmbH, Balingen, Germany). Molting, glair gland development, and mating were recorded. Percent molt increment (PCMI) was calculated for each molted crayfish, with the equation (after Brewis and Bowler 1982):

$$PCMI = (L_a - L_b) \times 100 / L_b \quad [\%]$$

where $PCMI$ = percent molt increment, L_a = length after molt, and L_b = length before molt. Similarly, percent annual growth increment (PCYI) was calculated for twice molted crayfish.

Total length (from the tip of the rostrum to the posterior edge of telson) is a commonly used parameter to describe crayfish size, but, as the abdomen and telson are mobile, measurement errors can result from curling or curving of the abdomen. Carapace length is more reliable, as it is a rigid immovable structure, although rostrum length may vary among individuals and can also be damaged. The POCL was selected as the most reliable parameter for body size ratios calculated in this study. The ChL/POCL, ChW/POCL, ChW/ChL, and AbW/POCL ratios were calculated.

2.4. Data analysis

Data were analyzed with the program Statistica 8.0 (StatSoft., Inc.). All values were examined for normal distribution (Kolmogorov-Smirnov test) and homoscedaticity (Levene test). The *t* test was used for comparison of molting time; the paired *t* test for comparison of body proportions in individual crayfish before and after molting; one way ANOVA with Tukey's post hoc test for comparison of body proportions of form I (twice molted), form I (once molted), and form II females; ANCOVA with Tukey's post hoc test for comparison of PCMI of form I (once molted), form I (twice molted), and form II females using POCL as covariate; and regression analysis for testing the relationship of body size to body ratios and to weight. The null hypothesis was rejected at $\alpha = 0.05$. Data are presented as averages ± standard deviation.

3. Results

3.1. Number of molts and time of molting

The majority of females (58.3%) molted twice (through a "form II" instar) during the year. The initial CL, POCL and weight of twice molted females (CL = 29.3 ± 2.57 mm; POCL = 21.6 ± 1.94 mm; W = 7.2 ± 1.85 g) and once molted females (CL = 32.5 ± 2.41 mm; POCL = 23.9 ± 1.84 mm; W = 9.7 ± 2.03 g) differed significantly ($t_{CL} = 7.01$, $P_{CL} < 10^{-6}$; $t_{POCL} = 6.79$, $P_{POCL} = < 10^{-6}$; $t_W = 7.23$, $P_W = < 10^{-6}$). All of the females were in form I (based on successful reproduction) at the beginning of the study. Form II was present for only a short period in summer. All females at the end of the observation time were in form I (based on glair gland development and mating with males).

Crayfish began molting 2 months after the water temperature reached 16 °C. Those that molted from form I to form II did so between July 11 and August 22. The second molt, back to form I, occurred between August 11 and September 18. The mean time from the release of independent juveniles to molting to form II was 44.3 ± 7.49 days or 800.5 ± 145.32 degree days (d°). The time from the 1st to the 2nd molt back to form I ranged from 12 to 39 days; the mean was 26.6 ± 6.51 days or 502.1 ± 116.22 d°. Females undergoing a single molt did so a significantly longer time ($Z_{days} = 7.38$, $P_{days} < 10^{-6}$; $Z_{d°} = 7.21$, $P_{d°} < 10^{-6}$) after release of their offspring (56.6 ± 3.58 days and 1042.8 ± 67.38 d°).

3.2. Changes in body proportions

The summary of body ratios in once molting females (initial and final values, form I) and twice molting females (initial values, and values after 1st molt and 2nd molt) demonstrated the presence of a form II stage in twice molted females after their 1st molt (Table 1). Form II was distinguished by shorter, narrower, and less robust chelae and narrower abdomens in relation to body size. At the 2nd molt, form II females' body proportions reverted to those before the 1st molt. The changes in once molted females did not happen to the ChL/PODH ratio, and decrease of ChW/POCL, ChW/ChL and AbW/POCL was at reduced. The final values for once molted and twice molted form I females did not differ. Glair gland development and successful mating was observed in both once and twice molted females.

3.3. Growth increments in successive molts

In twice molted females, the 1st molt (to form II) was characterized by a low increment of weight and ChL increase, and by reduction in AbW and ChW. Compared to growth increments of once molted females, after the 1st molt were reached higher CL and POCL increments, lower ChL, ChW and AbW increments and similar W increments. The 2nd molt was characterized by greater increases in ChL, ChW, AbW, and weight compared to both form II and once molted form I females. The CL and POCL growth increments after the 2nd molt were similar to those at the 1st molt and higher than those in once molted females. Data for successive molts are summarized in Table 2.

Differing growth rates of body parts were observed within the molts. All measured body parts grew similarly with the exception of lower PCMI of ChW (F = 11.30; $P < 10^{-6}$) after the single molt. Twice molted females showed different patterns at the two molts with more heterogeneous PCMIs among body parts. The PCMI of CL and POCL did not differ from each other, and were higher than ChL and W PCMIs, which did not differ. The lowest PCMIs found, AbW and ChW, did not differ (F = 70.81; $P < 10^{-6}$). Only CL and POCL PCMIs did not differ from each other after the 2nd molt (F = 134.52; $P < 10^{-6}$).

The PCYI values were also heterogeneous with the W increment being significantly higher, followed by lower values for ChL and ChW. The lowest values of PCYI were reached for AbW, CL, and POCL, which did not differ from each other (F = 69.42; $P < 10^{-6}$).

Table 1. Changes in ratios of body proportions in *Orconectes limosus* females before and after molting without form alternation, from form I to form II, and from form II back to form I. Data are presented as mean ± standard deviation. Values in the same row with different superscripts differ significantly (α = 0.05).

	Single molt (n = 54)		**Two molts (n = 74)**			**ANOVA**	
Body ratios	**F I →**	**F I**	**F I →**	**F II →**	**F I**	**F**	***P***
POCL/CL	0.74 ± 0.031^{ab}	0.75 ± 0.014^{a}	0.74 ± 0.014^{b}	0.74 ± 0.014^{ab}	0.75 ± 0.013^{a}	6.65	$< 10^{-4}$
ChL/POCL	0.80 ± 0.035^{a}	0.80 ± 0.038^{a}	0.78 ± 0.039^{a}	0.75 ± 0.032^{b}	0.79 ± 0.045^{a}	17.59	$< 10^{-6}$
ChW/POCL	0.35 ± 0.017^{a}	0.33 ± 0.019^{b}	0.33 ± 0.021^{b}	0.30 ± 0.018^{c}	0.33 ± 0.025^{b}	52.49	$< 10^{-6}$
ChW/ChL	0.44 ± 0.021^{a}	0.42 ± 0.017^{b}	0.42 ± 0.020^{b}	0.40 ± 0.020^{c}	0.42 ± 0.018^{b}	32.00	$< 10^{-6}$
AbW/POCL	0.73 ± 0.020^{a}	0.72 ± 0.020^{b}	0.74 ± 0.027^{a}	0.68 ± 0.018^{c}	0.73 ± 0.019^{a}	100.07	$< 10^{-6}$
CL (mm)	32.5±2.41	33.8±2.62	29.3±2.57	31.1±2.65	33.1±2.77	-	-
POCL (mm)	23.9±1.84	25.2±1.90	21.6±1.94	23.0±2.06	24.7±2.12	-	-
W (g)	9.7±2.03	10.2±2.23	7.2±1.85	7.5±1.84	9.1±2.26	-	-

Table 2. Percent molt increments (PCMI) in particular molts in *Orconectes limosus* females; without form alternation, from form I to form II, and from form II back to form I. The percent year increment (PCYI) is calculated for twice molted specimens. Data are presented as mean ± standard deviation. Values in the same row with different superscripts differ significantly (α = 0.05).

PCMI (%)	**Single molt**	**Two molts (n = 74)**			**ANCOVA**	
Parameter	**(n = 54)**	**1st molt**	**2nd molt**	**PCYI (%)**	**F**	***P***
CL	4.6 ± 1.19^{b}	6.0 ± 1.99^{a}	6.6 ± 1.45^{a}	13.1 ± 2.76	18.94	$< 10^{-6}$
POCL	5.6 ± 2.21^{b}	6.7 ± 2.41^{a}	7.3 ± 1.93^{a}	14.5 ± 3.21	7.92	$< 10^{-3}$
ChL	5.7 ± 3.00^{b}	2.7 ± 2.47^{c}	13.3 ± 4.46^{a}	16.6 ± 4.74	157.98	$< 10^{-6}$
ChW	1.4 ± 1.35^{b}	-2.5 ± 2.16^{c}	19.4 ± 6.79^{a}	16.8 ± 6.55	372.48	$< 10^{-6}$
AbW	4.0 ± 3.11^{b}	-2.1 ± 1.81^{c}	15.9 ± 3.47^{a}	13.8 ± 5.05	689.77	$< 10^{-6}$
W	4.9 ± 3.12^{b}	3.6 ± 3.54^{b}	22.8 ± 7.64^{a}	28.4 ± 9.67	161.70	$< 10^{-6}$

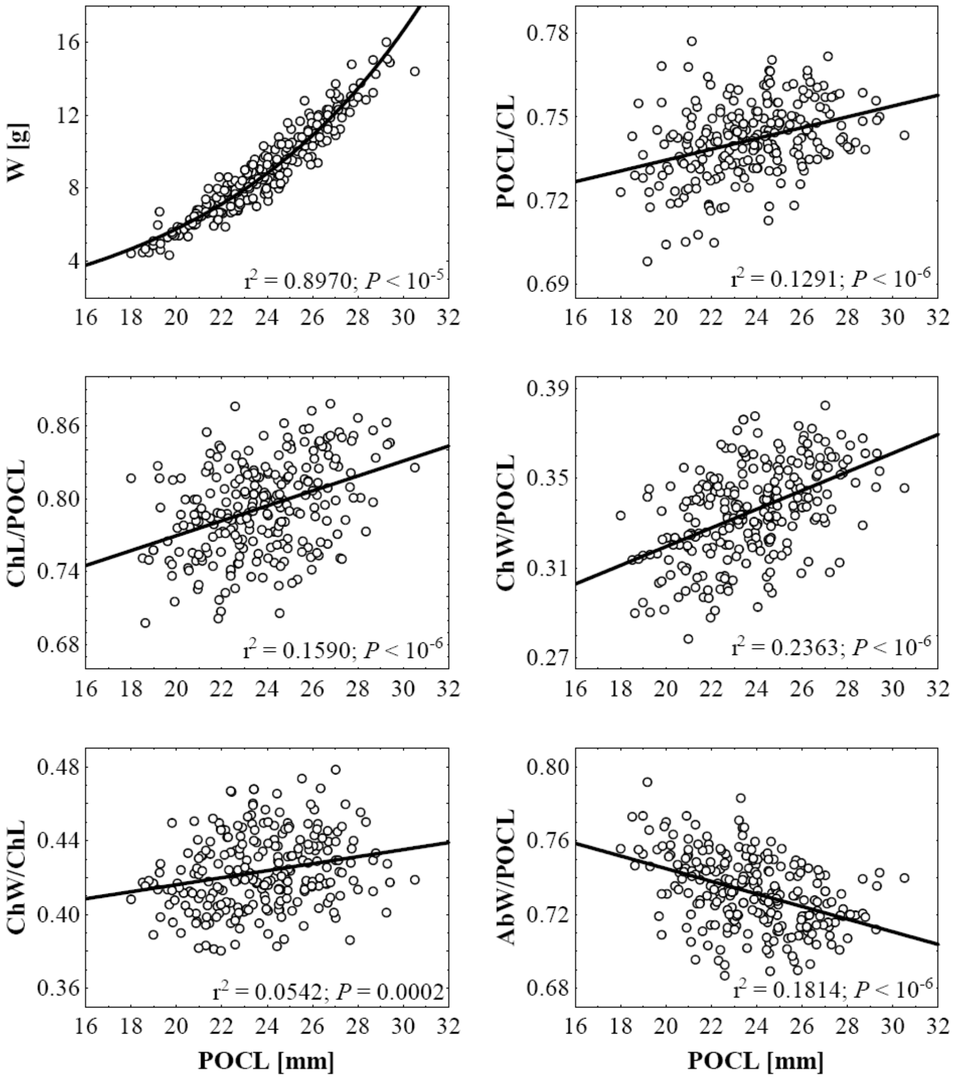


Figure 1. Relationships of crayfish body size to different body ratios and weight in form I females. Coefficient of determination and *P* - value are shown in the figures.

3.4. Body ratios vs. body size

Regression analysis of body size to body part ratios was processed for form I females (Fig. 1). Weight (F = 23.02; $P < 10^{-5}$; $y = 0.6974e^{0.1057x}$), POCL/CL (F = 35.74; $P < 10^{-6}$; $y = 0.6956 + 0.0019x$), ChL/POCL (F = 46.71; $P < 10^{-6}$; $y = 0.6469 + 0.0061x$), ChW/POCL (F = 77.97; $P < 10^{-6}$; $y = 0.2363 + 0.0042x$) and ChW/ChL (F = 14.22; $P < 10^{-3}$; $y = 0.3781 + 0.0019x$) ratios increased with increasing POCL. The AbW/POCL ratio decreased (F = 54.40; $P < 10^{-6}$; $y = 0.8133 + 0.0034x$) with increasing POCL.

4. Discussion

This study provides evidence for the suggestion that cambarid females undergo cyclic dimorphism, alternating between sexually active and sexually inactive stages, demonstrating that adult female *O. limosis* possess an ecological tool in their life-history similar to that of the conspecific males (Hobbs 1989).

In contrast with the predominant opinion that adult females of genus *Orconectes* usually undergo a single annual molt (Van Deventer 1937), we found the majority of adult females molted twice. Scudamore (1948) and Weagle and Ozburn (1972) observed a small proportion of females to molt twice per year, but they assumed that these did not reproduce in that year. Hence we used only oviposited females in the present study. The assumption that females do not alternate forms has been based on the delay in the spring molt of egg-bearing females, which was presumed to leave too short time for a second molt (Scudamore 1948). Egg-bearing females commonly molt 2–3 weeks after their young have left the pleopods, 5 or 6 weeks later than males and non-reproducing females (Hobbs 2001). We revealed a more extended time between juvenile independence and female molting. Molting occurred after about two months of warmer water temperatures, in mid-July, and was completed by mid-September. To avoid the ambiguity associated with described molting periods, we calculated the time between juvenile independence and molting and time between molts, not only in days, but also in degree days.

The delay in molting due to the presence of eggs and juveniles did not inhibit a second molt, since females were able to molt twice in a short time, much shorter than reported 8 weeks for twice molting adult males (Hobbs 2001). However, Pieplow (1938) reported three molts per year in both sexes of *O. limosus*, based on captures and observations of gastrolith occurrence. Hence, Pieplow (1938) probably observed two molts of form alternating females as 1st and 3rd molt, and the females which molted only once as 2nd molt in the growing season because once molted ones molt later than occurred molt to form II and earlier than molt back to form I.

All the females at the beginning and end of the present study were sexually active form I (based on the presence of attached eggs and dependent offspring, glair gland development, and mating behavior), which demonstrated that form I females undergoing a single molt and not altering form were reproductively active. This contradicts Wetzel (2002), who concluded that once molted females did not complete a cycle and were not able to reproduce.

The reasons for a single molt might be low pH conditions preventing normal mineralization during molting (Greenaway 1985), water temperature (Mundahl and Benton 1990), or the influence of food quality and availability (Olsson et al. 2008). These do not apply to the present study, where crayfish were held under identical conditions of optimal pH, natural water temperature, high levels of dissolved oxygen, an adequate number of shelters, and sufficient food. The single molt in this case may be explained by higher initial body size of single molted females which size was close to the maximum size in the source population. Such large females have reached sufficient capacity for egg production and are similar in size to large males in the population. This suggests decreasing growth with increasing size and age via lower molting frequency and lower molt increment. This strategy can positively influence the survival of large fecund females, since frequent-molting crayfish are more vulnerable to cannibalism and predation (Nyström 2002).

Two molts alone do not confirm form alternation in females, and requires evidence of significant morphological changes. The occurrence of a difference in abdomen width, as reported by Wetzel (2002), is insufficient evidence for form alternation. We presumed form alternation in females, if it exists, to exhibit patterns similar to those of males. Therefore, form I females should have, in addition to the wider abdomen, larger, wider, and more robust chelae (Hobbs 1981; Hamr and Berrill 1985). Males have proportionally larger chelae than females (Holdich 2002). We therefore expected smaller differences between female forms.

Reversible changes in four body ratios were associated with form alternation. Form II females exhibited shorter, narrower, and less robust chelae in relation to body size, in contrast with the findings of Stein (1976), who claimed that chelae of females do not undergo seasonal changes. The narrower abdomen was confirmed as a further accompanying characteristic of form II females (Wetzel 2002). These differences reverted to ratios characteristic of form I during the ensuing molt. Once molted crayfish did not exhibit differences between initial and final values of chela length, but the chela was only slightly narrower, and less robust, and the abdomen was narrower in relation to body size. The slightly narrower abdomen may have prompted Wetzel's (2002) statement that once molted females are in sexually inactive form II. However, the final values for once molted and twice molted form I females did not differ. Glair gland development and successful mating was observed in both once and twice molted females.

The mechanisms for alteration in body ratios are differing growth increments in successive molts. The molt to form II provides only slight increments in weight and chelae length, and a reduction of abdomen and chela width. Minimal (but not negative) growth increments of male chelae were reported by Pieplow (1938) and Stein (1976). Conversely, body size growth increments were high, suggesting that the 1st molt increased body size while conserving energy for greater growth of all body parts during the 2nd molt. The high body size increase and several times higher increase of chela length and width, abdomen width, and weight entails regaining and further increasing size of important body structures, along with body growth. Body size increments were higher than those in once molted females. The growth of body parts in once molted females is more or less uniform, indicating maintenance of body dimensions. In general, we recorded isometric growth - except in ChW (single molt), as well as negative (1st molt) and positive (2nd molt) allometric growth of measured body parts in adult females. Those findings refute the hypothesis of isometric growth of female chelae during their life (Rhodes and Holdich 1979; Hobbs 2001).

A broad abdomen is an effective shield enabling protection of attached eggs (Holdich 2002). During the non-reproductive period a wider abdomen offers no benefit, but body size increase has a positive influence on fecundity of crayfish females (Smith 1981). In general, less energy is invested in abdomen growth when it has no practical function, resources are utilized for increasing fitness and body size.

Similarly, dimorphism of chelae may be connected to their most important use. This includes prey capture and manipulation, burrowing, defense against predators, inter- and intra-specific interactions, and reproduction (Holdich 2002). Large chelae are advantageous in competition for limited resources and in aggressive behavior (Nakata and Goshima 2003); males with large chelae for example are more successful in copulating with larger females (Stein 1976).

The form II with small chelae occurs during summer, when water temperatures are high and food availability should be maximal (Nyström 2002). Large chelae do not appear to be necessary for prey capture and manipulation and may even be disadvantageous for this purpose (Stein 1976). Form II females face minimal predatory risk, are sexually inactive, and are less often exposed to aggressive encounters, so large chelae may not be beneficial.

On the other hand, females use visual stimuli during mate selection (Aquiloni et al. 2009), and males choose larger females (Aquiloni and Gherardi 2008). Larger chelae may also be a mark of "female quality." Female chelae may play an important role in reproductive selection, since a non-preferred mate, such as a small male, or a male with smaller chelae (Aquiloni and Gherardi 2008), could not overpower a female with larger chelae (Andrews 1910; Mason 1970). Higher exposure of mating pairs to potential predation (Berrill and Arsenault 1984) may also increase the importance of large chelae in form I females, since, in interaction with predator fish, crayfish with larger chelae have an advantage (Stein 1976). The minimal chela growth during molting to form II means less energy is expended in growing, as well as carrying, large chelae having no practical function.

Chelae show positive allometric, not isometric (Rhodes and Holdich 1979), growth. Abdomen width is probably not such important as body size (with associated increasing fecundity), because it grow slowly than body size. Rhodes and Holdich (1979) reported positive allometric growth of the female abdomen. However, females display positive allometry prior to maturation and a pronounced increase in relative abdomen size at the final juvenile molt (Rhodes and Holdich 1979). Subsequently, abdominal width can decrease.

The unique cycle form alternation in females involves potential benefits (increased potential for growth and regeneration of lost appendages, effective use of resources to increase fitness and appearance), and costs (increased frequency of vulnerability during molting). In general, form alternation seems to enable the female to utilize resources effectively to increase size as well as provide an appearance attractive to males.

Acknowledgements

This investigation was financially supported by the Czech Ministry of Education (project USB RIFCH No. MSM6007665809), and the Czech Science Foundation (projects GAAV IAA601870701). We also deeply appreciate the assistance of the Lucidus Consultancy for correcting this manuscript.

References

Andrews E.A., 1910. Conjugation in the crayfish *Cambarus affinis*. J. Exp. Zool. 9: 235-364.

Aquiloni L., Gherardi F., 2008. Mutual mate choice in crayfish: large body size is selected by both sexes, virginity by males only. J. Zool. 274: 171-179.

Aquiloni L., Massolo A., Gherardi F., 2009. Sex identification in female crayfish is bimodal. Naturwissenschaften 96: 103-110.

Berrill M., Arsenault M., 1984. The breeding behaviour of a northern temperate orconectid crayfish, *Orconectes rusticus*. Anim. Behav. 32: 333-339.

Brewis J.M., Bowler K., 1982. Growth of the freshwater crayfish *Austropotamobius pallipes* in Northumbria. Freshw. Biol. 12: 187-200.

Buřič M., Kozák P., Vích P., 2008. Evaluation of different marking methods for spiny-cheek crayfish (*Orconectes limosus*). Knowl. Manage. Aquat. Ecosyst. 389: 1-8.

Greenaway P., 1985. Calcium balance and molting in the crustacean. Biol. Rev. Camb. Philos. Soc. 60: 425-454.

Guiasu R.C., 2002. *Cambarus*. In: Holdich D.M. (ed.): Biology of freshwater crayfish, Blackwell Science Ltd., Oxford, UK, pp. 609-634.

Hamr P., 2002. *Orconectes*. In: Holdich D.M. (ed.): Biology of freshwater crayfish, Blackwell Science Ltd., Oxford, UK, pp. 585-608.

Hamr P., Berrill M., 1985. The life histories of north-temperate populations of the crayfish *Cambarus robustus* and *Cambarus bartoni*. Can. J. Zool. 63: 2313-2322.

Hobbs H.H. Jr., 1974. Synopsis of the families and genera of crayfishes (Crustacea: Decapoda). Smithson. Contrib. Zool. 164: 32 pp.

Hobbs H.H. Jr., 1981. The crayfishes of Georgia. Smithson. Contrib. Zool. 318: 549 pp.

Hobbs H.H. Jr., 1989. An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae). Smithson. Contrib. Zool. 480: 236 pp.

Hobbs H.H. III, 2001. Decapoda. In: Thorp J.H., Covich A.P. (eds): Ecology and Classification of North American Freshwater Invertebrates, Academic press, London, pp. 955-1001.

Holdich D.M., 2002. Background and Functional Morphology. In: Holdich D.M. (ed.): Biology of freshwater crayfish, Blackwell Science, Oxford, pp. 3-29.

Holdich D.M., Haffner P., Noël P., Carral J., Füderer L., Gherardi F., Machino Y., Madec J., Pöckl M., Śmietana P., Taugbol T., Vigneux E., 2006. Species files. In: Souty-Grosset

C., Holdich D.M., Noël P., Reynolds J.D., Haffner P. (eds): Atlas of Crayfish in Europe, Publications Scientifiques du MNHN, Paris, pp. 49-130.

Mason J.C., 1970. Copulatory behavior of the crayfish, *Pacifastacus trowbridgii* (Stimpson). Can. J. Zool. 48: 969-976.

Mundahl N.D., Benton M.J., 1990. Aspects of the thermal ecology of the rusty crayfish *Orconectes rusticus* (Girard). Oecologia 82: 210-216.

Nakata K., Goshima S., 2003. Competition for shelter of preferred sizes between the native crayfish species *Cambaroides japonicus* and the alien crayfish species *Pacifastacus leniusculus* in Japan in relation to prior residence, sex difference, and body size. J. Crust. Biol. 23: 897-907.

Nyström P., 2002. Ecology. In: Holdich D.M. (ed.): Biology of freshwater crayfish. Blackwell Science, Oxford, UK, pp. 192-235.

Olsson K., Nyström P., Stenroth P., Nilsson E., Svensson M., Granéli W., 2008. The influence of food quality and availability on trophic position, carbon signature, and growth rate of an omnivorous crayfish. Can. J. Fish. Aquat. Sci. 65: 2293-2304.

Pieplow U., 1938. Fischereiwissenschaftliche Monografie von *Cambarus affinis* Say. Zeitschrift für Ficherei und deren Hilfswissenschaften 36: 349-440.

Reynolds J.D., 2002. Growth and reproduction. In: Holdich D.M. (ed.): Biology of freshwater crayfish, Blackwell Science, Oxford, UK, pp. 152-191.

Rhodes C.P., Holdich D.M., 1979. On size and sexual dimorphism in *Austropotamobius pallipes* (Lereboullet), a step in assessing the commercial exploitation potential of the native British freshwater crayfish. Aquaculture 17: 345-358.

Scudamore H.H., 1948. Factors influencing molting and sexual cycles in the crayfish. The Biol. Bull. 95: 229-237.

Smith D.G., 1981, Life history parameters of the crayfish *Orconectes limosus* (Raf.) in southern New England. Ohio J. Sci. 81: 169-172.

Stein R.A., 1976. Sexual dimorphism in crayfish chelae: functional significance linked to reproductive activities. Can. J. Zool. 54: 220-227.

Suko T., 1960. Studies on the development of the crayfish. VI. The reproductive cycle. Sci. Rep. Saitama Univ. 3B: 79-91.

Tierney A.J., Gunarante C., Jennison K., Monroy V., Donnelly L., 2008. Behavioral correlates and alternate male forms (form I and form II) in the crayfish *Orconectes rusticus*. J. Crust. Biol. 28: 596-600.

Van Deventer W.C., 1937. Studies on the biology of the crayfish, *Cambarus propinquus* Girard. Ill. Biol. Monograph 15: 7-57.

Weagle K.V., Ozburn G.W., 1972. Observation on aspects of the life history of the crayfish, *Orconectes virilis* (Hagen), in Northwestern Ontario. Can. J. Zool. 50: 366-370.

Wetzel J.E., 2002. Form Alteration of Adult Female Crayfishes of the genus *Orconectes* (Decapoda: Cambaridae). Am. Midl. Nat. 147: 326-337.

Chapter 11

General Discussion
English Summary
Czech Summary
Acknowledgements
List of Publications
Training and Supervision Plan during Study
Curriculum Vitae

General Discussion

Despite the fact that *Orconectes limosus* life history was studied in area of its original (Smith 1981) as well as secondary area of distribution (Van Den Brink 1988; Chybowski 2007), a lot of obscurities and unrecognized parts in its biology still remain for this invasive crayfish. It is widely known that *O. limosus* acts as a vector of crayfish plague (Holdich et al. 1999; Söderhäll and Cerenius 1999; Oidtmann et al. 2002), but we can only imagine the factors supporting fast continuous spread of *O. limosus* when the population is not infected by crayfish plague pathogen. Considerable goal of present dissertation was therefore to elucidate these shadowy nooks in *O. limosus* biology and to study factors influencing its invasive spread as reproduction success, growth patterns, form alternation, migration ability, behavior, habitat preferences, or effect of water temperature.

Invasive species are often very successful in reproduction (Linqvist and Huner 1999) and *O. limosus* can be, following our results, also marked as very successful. *Orconectes limosus* was found as a highly fecund species with rising fecundity affected by increasing body size confirming results of e.g. Pieplow (1938) or Stypińska (1973). *Orconectes limosus* has a higher average fecundity than native European crayfish (Stucki 2002; Saéz-Royuela et al. 2006), and comparable to non-native American species present in Europe (Guiasu 2002; Lewis 2002). The observed spring spawning and short egg incubation (about 46 days) suggested the great advantage compared to native crayfish, spawning in autumn and incubating more than 8 months (Skurdal and Taugbøl 2002). The early sexual maturation and also high number of juveniles at the 3rd stage per female gives this species a good predisposition for quick establishment in new localities. The small *O. limosus* females are able to produce a large number of offspring than two years older native species (Skurdal and Taugbøl 2002).

We hypothesized that the *O. limosus* high juvenile production can affect strong competition between juveniles if another crayfish species occur. The study was conducted to estimate the intra- and inter-specific interactions of *O. limosus* and *Astacus astacus*, the most common native crayfish in Europe (Holdich et al. 2006). We found that hatchlings, as well as first independent instars, of *O. limosus* are much smaller than those in native *A. astacus*. This factor influenced very low survival rate of *O. limosus* in the communal stock with *A. astacus*, due to direct predation. That was an expected result of the first phase of rearing, found e.g. for *Procambarus acutus acutus* and *Procambarus clarkii* communal stock (Mazlum and Eversole 2005), resulting from body size as a major determinant of dominance in crayfish (Butler and Stein 1985). But the high intra-specific cannibalism makes the survival of *O. limosus* juveniles low anyway, also in separated stock. *Astacus astacus* specimens showed much lower level of cannibalism than *O. limosus*, and even lower than is reported by Gydemo and Westin (1993). However, the growth of *O. limosus* juveniles was much faster than that of *A. astacus*, and the size and weight of both species, were equivalent already in 11 weeks after hatching. That leads to the decrease of *A. astacus* survival similar to *O. limosus* in communal stock as a result of inter-specific competition between similar sized crayfish. In addition, at the end of the growing season, 19-week-old *O. limosus* attained a much higher mean size and weight than *A. astacus*. Gydemo and Westin (1989) found a tendency in males to attain larger sizes after 6 and 9 months of rearing. This is in contrast to *O. limosus* in our study where we found the biggest individuals in each aquarium to be females. Those specimens were even mature at the end of their 1st growing season. It suggests the high importance of growth in females, which can reproduce at the end of their fist year. In general *O. limosus* exhibit rapid growth rate, which could be an advantage in competition with other species. The only disadvantage was lower overall survival of *O. limosus* communal stock, which could be balanced by higher fecundity and early maturation of this species.

Followed by a need to find an applicable method of marking, for further research of *O. limosus* life cycle as well as for field mark-recapture observations, 4 different marking methods were evaluated. These methods were chosen with regards to their suitability for crayfish. Some widely used methods could be though unfitting, especially for small sized *O. limosus*. We evaluated marking methods using passive integrated transponders (following e.g. Bubb et al. 2002), visible implant elastomers (following e.g. Pilollo et al. 2008), visible implant alphanumeric tags (following e.g. Isely and Stockett 2001), and cauterization (following e.g. Abrahamsson 1973). High survival was obtained with all methods tested. The use of visible implant alphanumeric tags seems to be the most applicable method for laboratory as well as field studies, thanks to easy manipulation, high tag retention and very good readability. In addition, other methods have some disadvantages. Concretely: cauterization negatively affects growth and the readability is time-limited; application of visible implant elastomers is more laborious and the readability can be influenced by tags moving or smudging; the use of passive integrated transponders is limited by required big size of specimens, as reported also by Bubb et al. (2002), which eliminates its usability for animals with short life span as *O. limosus*. The most suitable method was therefore applied in following studies of form alternation in *O. limosus* and in monitoring crayfish populations.

The high fecundity and rapid growth are not all factors supporting the successful establishment of *O. limosus* in many freshwater waterbodies. This species also disposes of the cycle which economically exploits resources in a different way depending on the season. Orconectid males are thus dimorphic, alternating between a reproductively active state (form I), and a reproductively inactive state (form II) (Scudamore 1948; Hobbs 1989). These forms occur in all known *Orconectes* species (Hobbs 1974). Form alternation occurs during semi-yearly molts (Hobbs 2001; Guiasu 2002), hence we had to follow individuals throughout the year to observe morphological changes associated with form alternation. The cycle with new findings and explanations of existing equivocalness were carefully described. Most males underwent 2 molts with form alternation, which is usual (Boyd and Page 1978; Hamr and Berrill 1985), but with a half time interval between molts than reported (Stein 1976). A small proportion (higher initial size than twice molting) molted once, without form alternation. A smaller proportion (higher initial size than twice and once molted) did not molt at all. Chelae of form I males were longer and wider than in form II. Juveniles had even shorter and narrower chelae. These finding are in accordance with previous studies (Andrews 1910; Pieplow 1938). The chelae and abdomen of form I were more robust than those of form II and juveniles, which did not differ. The key to the morphological changes is allometric growth. Concretely, negative allometric growth of chela length and width and abdomen width after the molt to form II and positive allometric growth of the same body parts after successive molt back to form I.

To conclude, body size increments are higher after molt to form II and chelae and abdomen growth is minimal. That suggests that the priority of the 1st molt is to increase body size while conserving energy. The minimal chelae growth during molting to form II therefore makes ecological sense. Less energy is expended in growing, as well as carrying, large chelae which would have no practical function. If they are not necessary for prey capture and manipulation or for predator defense (Stein 1976), there is no reason to grow massive chelae or a wider abdomen. But, there is a need to increase body size before the 2nd molt, since body size is the most important attribute for success in aggressive interactions, mating success, including more frequent copulation and interruption of copulating pairs (Berrill and Arsenault 1984; Aquiloni and Gherardi 2008b). Cambarid crayfish have to increase their size through only two molts annually (Stein 1976; Reynolds 2002), and must use resources effectively.

Hence they invest more in growth at the molt to form II. Based on data obtained, form alternation seems to have a function in utilizing resources effectively to increase size as well as attractive appearance for females because females prefer large males with large chelae (Aquiloni and Gherardi 2008a).

There was a possibility that the similar system exists also in *O. limosus* females. By contrast to predominant opinion that females molt only once a year (Van Deventer 1937; Hobbs 2001) some authors observed females molted twice (Scudamore 1948; Weagle and Ozburn 1972). Unfortunately, they assumed that the females did not reproduce in that year. Only Wetzel (2002) tried to settle this issue for some species of genus *Orconectes* and found female able to undergo two molts each year with "form alternation". But Wetzel's (2002) opinion was promoted only by wider abdomens of form I females. The significant cyclic changes observed in chelae length, width and robustness, as well as in abdomen width in present study clearly demonstrate the form alternation similar to that in conspecific males. Females were able to alter between sexually active and sexually inactive form by way of short time span between successive molts as well as different growth patterns of various parts of body. Some females that molted only once without distinctive morphological changes were also seen. All the females (once and twice molted) were determined as sexually active form I (by glair gland development and mating with males), which demonstrates that once molted form I females did not need to alter the form and also can participate in reproduction, which corrects the Wetzel's (2002) opposite opinion.

But, why do females need to alter between two distinctive forms? Form alternation is probably an ecological tool simply changing different body parts, the use of which is influenced by reproductive season, chelae and abdomen. Broad abdomen is an effective shield enabling protection of attached eggs (Holdich 2002; Reynolds 2002). During non-reproductive period it is not necessary to build wider abdomen, but body size increase is required as the positive factor influencing fecundity of crayfish females (Reynolds 2002; Larsson and Magoulick 2008). Chelae also have important functions, e.g. prey capture, defense against predators, and reproduction (Stein 1976; Holdich 2002). Similarly as females, males choose attractive females i.e. larger ones (Aquiloni and Gherardi 2008a), as a need to mate with more fecund females. Larger chelae could also be the mark of "female quality". Also, females chelae probably play an important role in reproductive selection because small male, or male with smaller chelae, i.e. non-preferred mate (Aquiloni and Gherardi 2008a), cannot overpower female with larger chelae (Andrews 1910; Mason 1970). In addition, the minimal chelae growth, which would have no practical function, during molting to form II conserving resources for next molt. In general, less energy is expended in chelae and abdomen growth when it has no practical function. Form alternation by females involves potential benefits (as increased potential for growth and regeneration of lost appendages, effective use of resources increasing fitness and appearance), and costs (as increased frequency of extreme vulnerability during molting). In general, form alternation seems to have an ecological function in utilizing resources effectively to increase size as well as attractive appearance for suitable males.

In connection, there is a part of *O. limosus* spatial and temporal patterns description. In the logical way, it was better to start with laboratory study before we moved research in the field conditions. The laboratory study was conducted as a comparison of day and night activity estimation in three experimental groups consisting of *A. astacus* only, *O. limosus* crayfish only, and a mixture of both species. The results show noble crayfish in separated stock as typical nocturnal creature as known also by Lozan (2000) or Skurdal and Taugbøl (2002). Higher day and night activity was found for *O. limosus* than for noble crayfish, with more active *O. limosus* females than males. A high activity of *O. limosus* was described also by Lozan (2000), Stucki (2002) and Pieplow (1938). In accordance to results obtained in natural conditions with noble crayfish (Cukerzis 1988; Hamrin 1987), activity peaked for both species in separated stocks soon after the start of night time hours. The activity of both species was modified when both species were kept together, with an increased day activity found in noble crayfish, and night activity similar between species. Bubb et al. (2006) also referred this pattern as a possible mechanism of displacement of native crayfish species by invading crayfish i.e. caused just by diurnal displacement of the native species from refuge (Bubb et al. 2006), which could lead to higher vulnerability to predation (Lodge and Hill 1994). Our results suggest that there are inter-specific interactions between studied species, which could contribute to noble crayfish displacement. However, there is probably a complexity of mechanisms that influenced the chances of IC.

Radio-telemetry observation was evaluated as the most descriptive method for study of wide range of patterns in crayfish movements, behavior and ecology (e.g. Bubb et al. 2004; Gherardi et al. 2002). We carried out three radio-telemetry studies in different season (spring, growing season, autumn) supported by data from mark-recaptures. As expected, radio-telemetry provided a broad range of results, which are important to understanding of *O. limosus* successful spreading.

Cyclic seasonal migrations of *O. limosus* between brook and reservoir were observed (by sixteen captures and two radio-telemetry studies) in the small tributary of a large reservoir. The hypothesis is supported by a significant trend of downstream migration to the reservoir found in autumn and gradual abundance increasing in spring months. A similar cyclic migration has never been observed in crayfish. Crayfish inhabit the brook only during the growing season, and over-winter in the reservoir. The water temperature is commonly known as a factor influencing crayfish life cycle, movement and behavior (Mundahl and Benton 1990; Gherardi 2002). Lower water temperature in the brook throughout the year can be therefore, with higher fish predation pressure in reservoir, a possible factor explaining this pattern. Mark-recaptures data simply correlate with the influence of water temperature. *Orconectes limosus* is able to move rapidly downstream and even upstream (> 100 m.day-1) through small stream. The motional activity was observed even higher during spring mating period, since the hectic searching of suitable mate was found as a stronger factor than an influence of water temperature. When water temperature increased up to 10 °C, crayfish spring migration suddenly collapsed because of the end of mating and the start of spawning. In addition, increased diurnal activity was confirmed, especially in mating season, compared to native crayfish (Lozan 2000). The night activity was found increasing in cloudy weather i.e. there is strong moonlight avoidance. The possible explanation of this pattern is that moonlight provides better conditions for night predation, and cloudy weather can be a factor that minimizes the risk of being preyed on. The high ability to move rapidly in small watercourses can signify a threat of penetration of native crayfish habitats and enable the transmission of crayfish plaque. The threat is very high especially in mating season, due to increased motional activity. The spatial and temporal behavior of invasive species illustrates their danger.

Orconectes limosus was observed displaying homing behavior, and occupy a simple system of shelters. The most exploited shelter was often centrally located as a hub for movements within the home area. This finding differs from results of Robinson et al. (2000), who did not observe a return of displaced animals to home sites. We can conclude that *O. limosus* is capable of homing like some other decapods (Vannini and Cannicci 1995). *Orconectes limosus* also showed a significant preference of sandy/silt substrata, as also described by Hamr (2002) or Holdich et al. (2006), and deeper parts of the brook, but always in the presence of a sufficient number of shelters such as big stones, rocks or driftwood. The preference for home areas with an abundance of organic matter suggested that the crayfish choose places with high food availability. Organic matter is, in itself, an adequate food source for crayfish, and also provides a habitat for smaller invertebrates, also the prey of crayfish (Guan and Wiles 1998; Nyström 2002).

At the end, it can be concluded, that *O. limosus* was found as highly active, very movable, fast growing, highly fecund species, able to utilize resources effectively, and able to influence native crayfish. We can suggest only one limiting factor which can limit spreading of *O. limosus*, low water temperature. *Orconectes limosus* has surely a considerable impact on other freshwater organisms, especially on members of indigenous crayfish, but the populations inhabiting upper parts of cooler brooks have a real chance to live undisturbed. On condition that human activities will not be a supporting factor in invaders spread and habitat degradation.

Major conclusions

- *Orconectes limosus* is a highly fecund species advantaged by short egg incubation. The small *O. limosus* females are able to produce a higher egg counts than two years older native species, thanks to early maturation.
- *Orconectes limosus* juveniles grow much faster than those of *A. astacus*, and can influence survival of *A. astacus* juveniles. High fecundity is reduced by high mortality and cannibalism in *O. limosus* as usual for *r*-selected species.
- The marking by visible implant alphanumeric tags was found as most suitable for laboratory as well as field studies of small sized *O. limosus*.
- *Orconectes limosus* population was observed migrating seasonally between reservoir and its small tributary. It inhabits the tributary during growing season and over-winter in reservoir.
- *Orconectes limosus* is active also in day hours and it can modify activity patterns of native *A. astacus* (day time activity increase) when both species occurred.
- *Orconectes limosus* is able to move very fast in upstream and downstream direction, which enables it to fast penetration of new localities.
- The night activity of *O. limosus* is positively affected by cloudy weather.
- *Orconectes limosus* is capable to homing, using a simple system of shelters in home area.
- When *O. limosus* can choose, it prefers sandy/silt bottomed, deeper parts with a presence of organic matter.
- Spring mating period in *O. limosus* increases its mobility, with 10 times higher distance moved during mating period than after that.
- The effect of water temperature on *O. limosus* movements and also on inhabiting studied locality was observed. The search of suitable mate was found as a stronger factor than water temperature during mating season.
- Morphological changes associated with form alternation in male *O. limosus* were described with new findings and explanations of existing equivocalness.
- The unique cycle of *O. limosus* females form alternation was first described. The significant cyclic changes, observed in chelae length, width and robustness, as well as in abdomen width, clearly demonstrate the form alternation similar to that in conspecific males.

Future perspectives

- The importance of autumn or spring mating period in connection with reproduction success. Participation of individual males in offspring.

- To find the way of fertilization process in *O. limosus* with respect to long term storage of spermatophores in females *annulus ventralis*.
- Behavioral responses of *O. limosus* to conspecific males and females, to members of another crayfish species, and to fish predators.
- Genetic structure of *O. limosus* populations.

References

Abrahamsson S.A.A., 1973. The crayfish *Astacus astacus* in Sweden and the introduction of the American crayfish *Pacifastacus leniusculus*. Freshwater Crayfish 1: 27-39.

Andrews E.A., 1910. Conjugation in the crayfish *Cambarus affinis*. J. Exp. Zool. 9: 235-364.

Aquiloni L., Gherardi F., 2008a. Mutual mate choice in crayfish: large body size is selected by both sexes, virginity by males only. J. Zool. 274: 171-179.

Aquiloni L., Gherardi F., 2008b. Evidence of female cryptic choice in crayfish. Biol. Lett. 4: 163-165.

Berrill M., Arsenault M., 1984. The breeding behaviour of a northern temperate orconectid crayfish, *Orconectes rusticus*. Anim. Behav. 32: 333-339.

Boyd J.A., Page L.M., 1978. The life history of the crayfish *Orconectes kentuckiensis* in Big Creek, Illinois. Am. Midl. Nat. 99: 398-414.

Bubb D.H., Lucas M.C., Thom T.J., Rycroft P., 2002. The potential use of PIT telemetry for identifying and tracking crayfish in their natural environment. Hydrobiologia 483: 225-230.

Bubb D.H., Lucas M.C., Thom T.J., 2004. Movement and dispersal of the invasive signal crayfish *Pacifastacus leniusculus* in upland rivers. Freshwat. Biol. 49: 357-368.

Bubb D.H., Thom T.J., Lucas M.C., 2006. Movement, dispersal and refuge use of co-occuring introduced and native crayfish. Freshwat. Biol. 51: 1359-1368.

Butler M.J., Stein R.A., 1985. An analysis of mechanism governing species replacement in crayfish. Oceologia 66: 168-177.

Chybowski L., 2007. Morphometrics, fecundity, density, and feeding intensity of the spiny-cheek crayfish, *Orconectes limosus* (Raf.) in natural conditions. Arch. Pol. Fish. 15: 175-241.

Cukerzis J.M., 1988. *Astacus astacus* in Europe. In: Holdich D.M., Lowery R.S. (eds.): Freshwater Crayfish: Biology, Management and Exploitation, Croom Helm, London, pp. 309-340.

Gherardi F., 2002. Behaviour. In: Holdich, D.M. (ed.): Biology of Freshwater Crayfish. Blackwell Science Ltd., London, pp. 258-290.

Gherardi F., Tricarico E., Ilhéu M., 2002. Movement patterns of an invasive crayfish, *Procambarus clarkii*, in temporary stream of southern Portugal. Ethol. Ecol. Evol. 14: 183-197.

Guan R., Wiles R.P., 1998. Feeding ecology of the signal crayfish *Pacifastacus leniusculus* in the British lowland river. Aquaculture 169: 177-193.

Guiasu R.C., 2002. *Cambarus*. In: Holdich D.M. (ed.): Biology of freshwater crayfish, Blackwell Science Ltd., Oxford, UK, pp. 609-634.

Gydemo R., Westin L., 1989. Growth and survival of juvenile Astacus astacus L. in optimized water temperature. In: De Pauw N., Jaspers E., Ackefors H., Wilkins N. (eds.): Aquaculture – a biotechnology in progress. European Aquaculture Society, Bredene, Belgium, pp. 383-391.

Gydemo R., Westin L., 1993. Effects of starvation, constant light and partial dactylotomy on survival of noble crayfish, *Astacus astacus* (L.), under high density laboratory conditions. Freshwater Crayfish 9: 79-83.

Hamr P., 2002. *Orconectes*. In: Holdich D.M. (ed.): Biology of freshwater crayfish, Blackwell Science Ltd., Oxford, UK, pp. 585-608.

Hamr P., Berrill M., 1985. The life histories of north-temperate populations of the crayfish *Cambarus robustus* and *Cambarus bartoni*. Can. J. Zool. 63: 2313-2322.

Hamrin S., 1987. Seasonal crayfish activity as influenced by fluctuating water levels and presence of fish predators. Holarctic Ecol. 10: 45-51.

Hobbs H.H. Jr., 1974. Synopsis of the families and genera of crayfishes (Crustacea: Decapoda). Smithson. Contrib. Zool. 164: 32 pp.

Hobbs H.H. Jr., 1989. An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae). Smithson. Contrib. Zool. 480: 236 pp.

Hobbs H.H. III, 2001. Decapoda. In: Thorp J.H., Covich A.P. (eds): Ecology and Classification of North American Freshwater Invertebrates, Academic press, London, pp. 955-1001.

Holdich D.M., 2002. Background and Functional Morphology. In: Holdich D.M. (ed.): Biology of freshwater crayfish, Blackwell Science, Oxford, pp. 3-29.

Holdich D.M., Ackefors H., Gherardi F., 1999. Native and alien crayfish in Europe: Some conclusions. In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 281-292.

Holdich D.M., Haffner P., Noël P., Carral J., Füderer L., Gherardi F., Machino Y., Madec J., Pöckl M., Śmietana P., Taugbol T., Vigneux E., 2006. Species files. In: Souty-Grosset C., Holdich D.M., Noël P., Reynolds J.D., Haffner P. (eds): Atlas of Crayfish in Europe, Publications Scientifiques du MNHN, Paris, pp. 49-130.

Isely J.J., Stockett P.E., 2001. Tag retention, growth, and survival of red swamp crayfish marked with a visible implant tag. N. Am. J. Fish. Manage. 21: 422-424.

Larson E.R., Magoulick D.D., 2008. Comparative life history of native (*Orconectes eupunctus*) and introduced (*Orconectes neglectus*) crayfishes in the Spring River drainage of Arkansas and Missouri. Am. Midl. Nat. 160: 323-341.

Lewis S.D., 2002. *Pacifastacus*. In: Holdich D.M. (ed.): Biology of freshwater crayfish, Blackwell Science, Oxford, pp. 511-540.

Lindquist O., Huner J.V., 1999. Life history characteristcs of crayfish: What makes some of them good colonizers? In: Gherardi F., Holdich D.M. (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield, pp. 23-28.

Lodge D.M., Hill A.M., 1994. Factors governing species composition, population size and productivity of cool-water crayfishes. Nord. J. Freshwat. Res. 69: 111-136.

Lozan J.L., 2000. On the threat to the European crayfish: A contribution with the study of the activity behaviour of four crayfish species (Decapoda: Astacidae). Limnologica 30 (2): 156-166.

Mason J.C., 1970. Copulatory behavior of the crayfish, *Pacifastacus trowbridgii* (Stimpson). Can. J. Zool. 48: 969-976.

Mazlum Y., Eversole A.G., 2005. Growth and survival of *Procambarus acutus acutus* (Girard, 1852) and *P. clarkii* (Girard, 1852) in competitive settings. Aquacult. Res. 36: 537-545.

Mundahl N.D., Benton M.J., 1990. Aspects of the thermal ecology of the rusty crayfish *Orconectes rusticus* (Girard). Oecologia 82: 210-216.

Nyström P., 2002. Ecology. In: Holdich D.M. (ed.): Biology of freshwater crayfish. Blackwell Science, Oxford, UK, pp. 192-235.

Oidtmann B., Heitz E., Rogers D., Hoffmann R.W., 2002. Transmission of crayfish plague. Dis. Aquat. Org. 52: 159-167.

Pieplow U., 1938. Fischereiwissenschaftliche Monografie von *Cambarus affinis* Say. Zeitschrift für Ficherei und deren Hilfswissenschaften 36: 349-440.

Pilollo F., Free G., Crosa G., Sena F., Ghiani M., Cardoso A.C., 2008. The invasive crayfish *Orconectes limosus* in Lake Varese: Estimating abundance and population size structure in the context of habitat and methodological constrains. J. Crust. Biol. 28: 633-640.

Reynolds J.D., 2002. Growth and reproduction. In: Holdich D.M. (ed.): Biology of freshwater crayfish, Blackwell Science, Oxford, UK, pp. 152-191.

Robinson C.A., Thom T.J., Lucas M.C., 2000: Ranging behaviour of a large freshwater invertebrate, the white-clawed crayfish *Austropotamobius pallipes*. Freshwat. Biol. 44: 509-521.

Saéz-Royuela M., Carral J.M., Celada J., Perez J.R., Gonzales A., 2006. Pleopodal egg production of the white-clawed crayfish *Austropotamobius pallipes* Lereboullet under laboratory conditions: Relationship between egg number, egg diameter and females size. Bull. Fr. Peche Piscic. 380-381: 1207-1213.

Scudamore H.H., 1948. Factors influencing molting and sexual cycles in the crayfish. The Biol. Bull. 95: 229-237.

Skurdal J., Taugbøl T., 2002. *Astacus*. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish. Blackwell Science Ltd., London, pp. 467-510.

Smith D.G., 1981, Life history parameters of the crayfish *Orconectes limosus* (Raf.) in southern New England. Ohio J. Sci. 81: 169-172.

Söderhäll K., Cerenius L., 1999. The crayfish plague fungus: History and recent advances. Freshwater Crayfish 12: 11-35.

Stein R.A., 1976. Sexual dimorphism in crayfish chelae: functional significance linked to reproductive activities. Can. J. Zool. 54: 220-227.

Stucki T.P., 2002. Differences in live history of native and introduced crayfish species in Switzerland. Freshwater crayfish 13: 463-476.

Stypińska M., 1973. The fecundity of three types of crayfish inhabiting Polish waters. Rocz. Nauk. Rol. 95 (1): 147-156.

Van Den Brink F.W.B., Velde G., Geelen J.F.M., 1988. Life history parameters and temperature-related activity of an American crayfish, *Orconectes limosus* (Rafineque, 1817) (Crustacea, Decapoda), in the area of the major rivers in The Netherlands. Arch. Hydrobiol. 114: 275-289.

Van Deventer W.C., 1937. Studies on the biology of the crayfish, *Cambarus propinquus* Girard. Ill. Biol. Monograph 15: 7-57.

Vannini M., Cannicci S., 1995. Homing behaviour and possible cognitive maps in crustacean decapods. J. Exp. Mar. Biol. Ecol. 193: 67-91.

Weagle K.V., Ozburn G.W., 1972. Observation on aspects of the life history of the crayfish, *Orconectes virilis* (Hagen), in Northwestern Ontario. Can. J. Zool. 50: 366-370.

Wetzel J.E., 2002. Form Alteration of Adult Female Crayfishes of the genus *Orconectes* (Decapoda: Cambaridae). Am. Midl. Nat. 147: 326-337.

English summary

Biology of spiny-cheek crayfish (*Orconectes limosus*, Rafinesque, 1817) under conditions of the Czech Republic and the study of factors influencing its invasive spreading

Miloš Buřič

The fecundity and time of embryonic development were described in *O. limosus*. Linear relationships between female size and ovarian fecundity, pleopodal fecundity, and production of juveniles were confirmed. The number of eggs ranged widely, between 46 and 505. The value of the female gonadosomatic index before spawning was 4.2±1.8%. The mean egg diameter was 1.8±0.2 mm. Mean incubation time was 46±3.8 days, i.e. 647±39.9 degree days. Mean production of juveniles at the 3rd stage was found to be 135.7±67.24 juveniles. The early sexual maturation and also high number of juveniles at the 3rd stage per female gives this species a good predisposition for quick establishment in new localities.

Astacus astacus and *O. limosus* juveniles were reared from the stage of independence until the end of the growing season. The first period revealed very low survival *O. limosus* rate in the communal stock caused by predation of *A. astacus*. Separately reared *O. limosus* proved higher survival rate (~50%). *Astacus astacus* showed the highest survival rate (~90%). The significant effect of bigger size of *A. astacus* than that of *O. limosus* and higher intra-specific cannibalism of *O. limosus* were reflected during the first phase of rearing. Size and weight of both species were equivalent 11 weeks after hatching. The higher cumulative survival rate was reached in the intra-specific stock of *A. astacus* (90%) while low cumulative survival of both species was found in communal stock (<50%) in the second phase of rearing. At the end of the growing season, *O. limosus* attained a higher mean size and weight. Growth of *O. limosus* was much faster than that of *A. astacus*.

Four methods of marking were evaluated for *O. limosus* under laboratory conditions: passive integrated transponders, visible implant elastomers, visible implant alphanumeric tags and cauterization. High survival (>90%) was obtained with all methods tested. Tag retention and readability were evaluated, and molt increments and number of molts were recorded. Cauterization negatively affects the percent molt increment of crayfish. The use of visible implant alphanumeric tags seems to be the most applicable method for laboratory as well as field studies (easy manipulation, high tag retention and very good readability).

Seasonal migrations of *O. limosus* between brook and reservoir were observed. Sixteen captures (between April 2007 and October 2008) and one radio-telemetry study (from October to November) were conducted. A significant trend of downstream migration to the reservoir was found in autumn. *Orconectes limosus* showed the ability for rapid downstream movements (>100 m per day). Seasonal fluctuations in crayfish density were observed and suggested that the occurrence of crayfish in the brook goes through periodic temporal changes. A lower water temperature in the brook throughout the year, and possible fish predation pressure in reservoir, could contribute to this phenomenon.

We compared day and night activity of *A. astacus* and *O. limosus* under laboratory conditions. Three groups were included: *A. astacus* only, *O. limosus* only, and a mixture of both species. Higher day and night activity was found for *O. limosus* than *A. astacus*, with more active *O. limosus* females, and higher night activity in *A. astacus*, when kept as individual species. These patterns were modified in a mixed stock, with an increased day activity found in *A. astacus*, and night activity similar between species. A higher activity during the day might influence the vulnerability of *A. astacus* to day time predators.

Orconectes limosus adults were radio-tracked in two seasons, early summer and late autumn. High nocturnal activity (33.3%) and relatively high diurnal activity (18.2%) were observed, with a positive effect of cloudy weather on nocturnal movements. Crayfish showed the ability for rapid upstream and downstream movements during short time periods. The maximum daily movement was 139 m. The average movement distances per day were 7.2 and 6.7 m in spring and autumn, respectively. A significant trend of downstream migration was observed in autumn. Crayfish displayed homing behavior, with the use of a system of shelters. A preference for soft bottomed, deeper areas of the brook like pools, with the presence of organic matter was observed. Obtained data suggest that crayfish inhabit the brook during the growing season and over-winter in the reservoir. This study provides information on the spatial and temporal behavior of invasive species in suitable habitat for native crayfish.

Adult *O. limosus* males were radio-tracked during and after spring mating period, from April to June. Higher distances moved per day were observed, when mating occurred (14.9±21.4 m). Maximum distances moved upstream and downstream were 118 m and 95 m per day, respectively. Crayfish were observed active even during day hours. When water temperature increased up to 10°C, crayfish spring migration collapsed, due to the end of mating and the start of spawning.

Morphological changes associated with form alternation in male *O. limosus* were described with new findings and explanations. Most males (84.4%) underwent 2 molts, with a one month interval between molts. Bigger males molted once without form alternation (8.9%) or did not molt at all (6.7%). Chelae of form I males were longer and wider than in form II. Juveniles had shorter and narrower chelae. The chelae and abdomen of form I were more robust than those of form II and juveniles. Negative allometric growth of chela length (ChL) and width (ChW) and abdomen width (AbW) occurred at the molt to form II. Body size increased more at the molt to form II. The ChL, ChW and weight (W) increments were 3 (ChL) and 4 (ChW, W) times that of body size increments at the molt back to form I. The unique cycle of form alternation was also first described in *O. limosus* females. The significant cyclic changes, observed in ChL, ChW and robustness, as well as in AbW, clearly demonstrate the form alternation similar to that in conspecific males. Females are able to alter between forms by way of short time span between successive molts as well as different growth patterns of various parts of body. Larger females, once molting, do not have to alter between forms and remain sexually active. They only grow slowly and lose chelae robustness. Form alternation seems to have an ecological function in utilizing resources effectively to increase size of important body parts and also attractive appearance for males.

Present work elucidates the unrecognized parts of *O. limosus* biology and discusses factors influencing its invasive spread as reproduction success, growth patterns, form alternation, migration ability, behavior, habitat preferences, or effect of water temperature. Several presented successful experiments show a wide range of interesting results which open the new areas in crayfish research.

Czech Summary
Souhrn

Biologie raka pruhovaného (*Orconectes limosus*, Rafinesque, 1817) v podmínkách ČR a studium faktorů ovlivňujících jeho invazní šíření

Miloš Buřič

Plodnost a embryonální vývoj raka pruhovaného byly popsány se zjištěnou lineární závislostí ovariální, pleopodální a pracovní plodnosti na velikosti samic. Plodnost byla velmi variabilní a pohybovala se v rozmezí 46–505 vajíček o průměrné velikosti 1.8±0.2 mm. Gonadosomatický index samic před kladením dosahoval 4.2±1.8%. Inkubační doba dosahovala 46±3.8 dní, resp. 647±39,9d°. Průměrná pracovní plodnost dosahovala průměrně 135.7±67.2 ráčat. Časná pohlavní dospělost a vysoká pracovní plodnost samic poskytují raku pruhovanému dobré predispozice pro rychlé osídlení nových lokalit.

Ráčata raka říčního a raka pruhovaného byla odchovávána od osamostatnění od samic do konce vegetační sezóny. V první fázi odchovu bylo patrné velmi nízké přežití raka pruhovaného způsobené predací většího raka říčního. Oddělená obsádka raka pruhovaného měla vyšší přežití (~50%). Rak říční měl v obou obsádkách nejlepší přežití (~90%). V první fázi odchovu se tak projevila vyšší počáteční velikost raka říčního a vysoký stupeň kanibalismu u raka pruhovaného. Velikost a hmotnost obou druhů se ale vyrovnala již 11 týdnů po líhnutí. Ve druhé fázi odchovu dosahovala vysokého přežití oddělená obsádka raka říčního (90%), zatímco nízkého přežití dosahovaly oba druhy ve společné obsádce (<50%). Na konci vegetační sezóny dosahoval rak pruhovaný větší velikosti i hmotnosti. Rak pruhovaný roste v první vegetační sezóně mnohem rychleji než rak říční.

V laboratorních podmínkách proběhlo testování použitelnosti čtyř metod značení pro raka pruhovaného: čipováním, implantovanými alfanumerickými štítky, elastomery a pájkou. U všech metod značení bylo dosaženo vysokého přežití (>90%). Hodnocena byla ztrátovost a čitelnost značek, počet svlékání a přírůstek za svlékání. Značení pájkou negativně ovlivňovalo růst raků. Nejvhodnější metodou pro laboratorní i terénní studie se zdá být použití alfanumerických štítků.

U raka pruhovaného byla pozorována sezónní migrace mezi vodní nádrží a jejím přítokem. Celkem proběhlo 16 odlovů (od dubna 2007 do října 2008) a podzimní radio-telemetrická studie. Na podzim byl pozorován jasný trend poproudové migrace do nádrže. Rak pruhovaný byl schopný rychlé poproudové migrace (>100 m za den). Sezónní fluktuace v abundanci raků naznačují, že populace raků v toku prochází periodickými změnami. Nižší teplota vody v potoce během roku a vyšší predační tlak v nádrži mohou být faktory zodpovědnými za tyto změny.

V laboratorních podmínkách bylo provedeno porovnání denní a noční aktivity raka říčního a pruhovaného ve třech typech obsádek: rak říční samostatně, rak pruhovaný samostatně a společná obsádka obou druhů. V samostatných obsádkách rak pruhovaný vykazoval vyšší denní i noční aktivitu než rak říční, samice raka pruhovaného byly aktivnější než samci a rak říční byl aktivní téměř výhradně v noci. Ve společné obsádce byla situace jiná. Noční aktivita byla podobná pro oba druhy, ale rak říční vykazoval zvýšenou denní aktivitu v porovnání se samostatnou obsádkou. Zvýšená denní aktivita raka říčního může vést ke zvýšení predace na tomto druhu.

Na konci jara a v pozdním podzimu byly provedeny radio-telemetrické studie migrace raka pruhovaného. Sledována byla vysoká noční (33.3%) i denní aktivita (18.2%),

s pozitivním efektem oblačného počasí na noční aktivitu raků. Byla pozorována schopnost rychlé poproudové i protiproudové migrace (max. 139 m za den). Průměrná uražená vzdálenost za den byla na jaře a na podzim 7.2, resp. 6.7 m. Na podzim byl pozorován trend poproudové migrace do nádrže. Raci byli schopní opakovaně používat systém úkrytů ve svém domovské okrsku. U sledovaných raků byla patrná preference písčito-hlinitého dna a hlubších partií toku (tůní) s přítomností organického materiálu. Z dat je možné usuzovat, že rak pruhovaný využívá potok pouze během vegetační sezóny a přezimuje v nádrži. Studie přináší informace o chování raka pruhovaného v habitatu vhodném pro původní druhy raků.

Další radio-telemetrická studie probíhala během a po skončení období páření raka pruhovaného. Vyšší migrační aktivita byla sledována během období páření (14.9±21.4 m). Max. vzdálenost uražená po proudu a proti proudu byla zjištěna 95, resp. 115 m za den. Raci byli aktivní i přes den. Po zvýšení teploty vody nad 10°C migrační aktivita prudce klesla, protože samice již začaly klást vajíčka a období páření skončilo.

Byla nalezena nová vysvětlení a zjištění k morfologickým změnám během cyklické změny reprodukční formy samců raka pruhovaného. Většina samců (84.4%) prodělala 2 svlékání s přibližně měsíčním odstupem. Větší samci se svlékali pouze jednou bez změny formy (8.9%) nebo se nesvlékali vůbec (6.7%). Samci ve formě I mají delší a širší klepeta než ve formě II. Juvenilové mají ještě kratší a užší klepeta. Klepeta a abdomena FI samců jsou robustnější než u FII samců a juvenilů. Negativní allometrický růst délky a šířky klepet a šíře abdomenu je typický pro svlékání do formy II, kdy je ale možné pozorovat vyšší přírůstek velikosti těla. Při svlékání zpět do formy I dosahují přírůstky délky a šířky klepet a hmotnosti 3–4násobku přírůstku velikosti těla. Unikátní cyklus změny formy byl rovněž poprvé popsán u samic raka pruhovaného. Významné cyklické změny pozorované u délky, šířky a robustnosti klepet, stejně jako u šíře abdomenu, jasně demonstrovaly změnu formy podobnou jako u samců. Samice jsou schopny měnit formu díky krátkému intervalu mezi svlékáními a různému růstu různých částí těla. Větší samice, které se svlékají jen jednou, formu nemění a zůstávají sexuálně aktivní. Růst těchto samic je pouze pomalejší a jejich klepeta nepatrně ztrácí na robustnosti. Změna formy má viditelně ekologickou funkci v efektivním využití zdrojů pro růst těla a jeho důležitých částí.

Tato disertační práce osvětluje nepoznaná nebo sporná místa v biologii raka pruhovaného a diskutuje faktory jež ovlivňují jeho invazivní šíření jako reprodukční úspěšnost, růst, změna reprodukční formy, migrační schopnosti, chování, preference habitatu nebo vliv teploty vody. Několik prezentovaných úspěšných experimentů předkládá širokou škálu zajímavých výsledků, které otevírají potenciální nové oblasti ve výzkumu raků.

Acknowledgements

I would like to thank my supervisor **Assoc. Prof. Dipl.-Ing. Pavel Kozák, Ph.D.**, who initiated me into crayfish research, and supported me positively and humanly during my experimental work. I am glad that I could work with him and get important professional experience as well as friendly advice.

I also have to thank all my colleagues from former **Department of Aquaculture and Hydrobiology** as well as the staff of the whole **Research Institute of Fish Culture and Hydrobiology** in Vodňany.

Special thanks belong to **Dipl.-Ing. Antonín Kouba** for his help in my experiments, mental support, and unceasing and often deadpan humor.

Finally, I would like to thank heartily to my family, which sometimes had to endure my physical as well as psychical absence at home, and which gave me love and life in delight.

The Ph.D. work was supported by:

1) Research plans of USB RIFH No. MSM6007665809 financed by Ministry of Education, Youth and Sport of the Czech Republic (responsible leader: Prof. Dipl.-Ing. Otomar Linhart, DSc.)

2) Czech Science Foundation project No. GAAV IAA601870701 (responsible leader for USB RIFCH: Assoc. Prof. Dipl.-Ing. Pavel Kozák, Ph.D.).

3) Internal Grant Agency of the University of South Bohemia – Grant No. 88/2006/P-VÚRH (responsible leader: Dipl.-Ing. Miloš Buřič).

List of Publications

Peer-reviewed papers

Buřič, M., Kouba, A., Kozák, P., 2009. Intra-sex dimorphism in female *Orconectes limosus*. (submitted Journal of Limnology). (IF2007=2.375)

Buřič, M., Kouba, A., Kozák, P., 2009. Spring mating period in *Orconectes limosus*: The reason for movement! (under review Aquatic Sciences). (IF2007=1.646)

Buřič, M., Kouba, A., Kozák, P., 2009. Moulting and growth in relation to form alternation of male spiny-cheek crayfish, *Orconectes limosus*. (submitted Zoological Studies). (IF2007=0.731)

Buřič, M., Kozák, P., Kouba, A., 2009. Movement patterns and ranging behaviour of the invasive spiny-cheek crayfish in a small reservoir tributary in the Czech Republic. (in press Fundamental and Applied Limnology/Archiv für Hydrobiologie). (IF2007=1.409)

Buřič, M., Kouba, A., Kozák, P., 2009. Seasonal migration of *Orconectes limosus* (Rafinesque, 1817) to a small tributary of large river. (under review Freshwater Crayfish 17).

Kouba, A., **Buřič, M.**, Kozák, P., 2009. Bioaccumulation and effects of heavy metals in crayfish: a review. (submitted Ecotoxicology). (IF2007=2.405)

Kozák P., **Buřič M.**, Kanta J., Kouba A., Hamr P., Policar T., 2009. The effect of water temperature on number of moults and growth of juvenile signal crayfish *Pacifastacus leniusculus* Dana. (in press Czech Journal of Animal Science). (IF2007=0.633)

Musil, M., **Buřič, M.**, Policar, T., Kouba, A., Kozák, P., 2009. Comparison of day and night activity between noble (*Astacus astacus*) and spiny-cheek crayfish (*Orconectes limosus*). (under review Freshwater Crayfish 17).

Hulák, M., Kašpar, V., Kozák, P., **Buřič, M.**, Filipová, L., Petrusek, A., 2009. Cross-species amplification of microsatellite markers in the invasive spiny-cheek crayfish (*Orconectes limosus*): assessment and application. (submitted Journal of Applied Genetics). (IF2007=0.967)

Kouba, A., Carral, J.M., **Buřič, M.**, Mráz, J., Policar, T., Kozák, P., 2009. Artificial incubation of noble crayfish (*Astacus astacus*) eggs in a partial recirculating system using formaldehyde as antifungal treatment (submitted Aquaculture). (IF2007=1.735)

Kouba, A., Kozák, P., **Buřič, M.**, Kanta, J., 2009. The effect of water temperature on number of moults and growth of juvenile noble crayfish (*Astacus astacus*). (under review Freshwater Crayfish 17).

Buřič, M., Kozák, P., Vích, P., 2008. Evaluation of different marking methods for spiny-cheek crayfish *(Orconectes limosus)*. Knowledge and Management of Aquatic Ecosystems/Bulletin Français de la Pêche et de la Pisciculture 389 (02), 1 – 8. (IF2007=0.571)

Aquiloni, L., **Buřič, M.**, Gherardi, F., 2008. Crayfish females eavesdrop on fighting males before choosing the dominant. Current Biology 18 (11), 462 – 463. (IF2007=10.539)

Kozák P., **Buřič M.**, Policar T., Hamáčková J., Lepičová A., 2007. The effect of inter- and intra-specific competition on survival and growth rate of native juvenile noble crayfish *Astacus astacus* and alien spiny-cheek crayfish *Orconectes limosus*. Hydrobiologia 590, 85 – 94. (IF2007=1.201)

Kozák, P., **Buřič, M.**, Policar T., 2006. The fecundity, time of egg development and juveniles production in spiny-cheek crayfish (*Orconectes limosus*) under controlled conditions. Bulletin Français de la Pêche et de la Pisciculture 380 – 381*,* 1171 – 1182. (IF2006=0.719)

Proceedings and abstracts

Buřič, M., Kouba, A., Kozák, P., 2008. Seasonal migration of *Orconectes limosus* (Rafinesque, 1817) to a small tributary of large river. In: 17th Symposium of International Association of Astacology (IAA), Kuopio, Finland, August 4 – 8, 2008. s.70.

Kouba, A., Kozák, P., **Buřič, M.**, Kanta, J., 2008. The effect of water temperature on number of moults and growth of juvenile noble crayfish (*Astacus astacus*). In: 17th Symposium of International Association of Astacology (IAA), Kuopio, Finland, August 4 – 8, 2008. s.74.

Kozák, P., Musil, M., **Buřič, M.**, Policar, T., Kouba, A., 2008. Comparison of day and night aktivity between noble (*Astacus astacus*) and spiny-cheek crayfish (*Orconectes limosus*). In: 17th Symposium of International Association of Astacology (IAA), Kuopio, Finland, August 4 – 8, 2008. s.40.

Kozák, P., **Buřič, M.**, Kanta, J., Kouba, A., Policar, T., Hamr, P., 2007. The Effect of Water Temperature on Number of Moults and Growth for Invasive Crayfish *Pacifastacus leniusculus* D. during the First Three Months of their Life. In: 15th International Conference on Aquatic Invasive Species, Nijmegen, Nederland, September 23 – 27, 2007. s. 97.

Kozák, P., **Buřič, M.**, Policar T., 2005. The fecundity and juveniles production in spiny-cheek crayfish (*Orconectes limosus*). In: „European crayfish as heritage species – liking research and management strategies to conservation and socio-ekonomics.“ Craynet-Final Conference, Firenze, Italy, May 2-5, 2005, 27 – 28.

Training and Supervision Plan during Study

Name	Miloš Buřič
Research Department	Laboratory of Ethology and Nutrition of Fish and Crayfish
Daily supervisor	
Supervisor	Assoc. Prof. Dipl.-Ing. Pavel Kozák, Ph.D.
Period	1st October 2006 until 30th September 2009

PhD courses	**year**
Biostatistics	2007
Crayfish Culture	2007
Applied Hydrobiology	2008
Fish Ecology	2008
English language	2009

Scientific seminars	**year**
Scientific seminar at USB RIFCH 3/2007	2007
Ecology of spiny cheek crayfish at UNIFI, Florence, Italy	2007
Scientific seminar at USB RIFCH 11/2008	2008
Scientific seminar at USB RIFCH 2/2009	2009

International Conferences	**year**
15th International Conference on Aquatic Invasive Species, September 23–27, 2007, Nijmegen, Nederland. (*Poster presentation*)	2007
17th Symposium of International Association of Astacology (IAA), August 4–8, 2008, Kuopio, Finland. (*Poster presentation*)	2008

Foreign stays during PhD study at RIFCH	**year**
University of Florence, Department of Animal Biology and Genetics, Dr. Francesca Gherardi, Florence, Italy.	2007

Curriculum Vitae

Surname: Buřič

First name: Miloš

Title: Dipl.-Ing.

Born: 17th March, 1982, Prague

Nationality: Czech

Present position: Ph.D. student, USB RIFCH, Laboratory of Ethology and Nutrition of Fish and Crayfish, Vodňany, Czech Republic.

Education:

1997–2001 High School of Fishery in Vodňany.

2001–2006 Dipl.-Ing (M.Sc.), University of South Bohemia in České Budějovice, Faculty of Agriculture, specialization: Fisheries.

Since 2006 Ph.D. (fishery) - USB RIFCH, Laboratory of Ethology and Nutrition of Fish and Crayfish, Vodňany, Czech Republic.

Professional experience:

2004–present – Ph.D. student USB RIFCH, Laboratory of Ethology and Nutrition of Fish and Crayfish

2007 – Short term attachment on the Department of Animal Biology and Genetics at the University of Florence (Italy)

2007 – Responsible leader in the project of the Grant Agency of USB no. 88/2006/P-VÚRH (*The analysis of migration ability in alien spiny-cheek crayfish Orconectes limosus*).

Ph.D. courses: Biostatistics, Crayfish Culture, Applied Hydrobiology, Fish Ecology, English language.

Current Research Program: Crayfish biology with special interest on alien crayfish species.

Languages: English – in word and writing.

Pedagogical experiences: Seminars for Ms.C. students of Mendel University of Agriculture and Forestry in Brno (2008, 2009); Seminar days at USB RIFCH (2007, 2008, 2009); Seminar for members of Czech Fishing Union; Seminar: Ecology of spiny cheek crayfish at University of Florence, Italy; Consultant of one bachelor thesis, and four diploma thesis.