Molting and Growth in Relation to Form Alternations in the Male Spiny-Cheek Crayfish *Orconectes limosus*

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Miloš Buřič, Antonín Kouba, and Pavel Kozák (2010) Molting and growth in relation to form alternations in the male spiny-cheek crayfish *Orconectes limosus*. *Zoological Studies* **49**(1): 28-38. Morphological changes associated with form alternations in the male spiny-cheek crayfish *Orconectes limosus* are described with new findings and explanations of existing equivocalness. Males were observed for 10 mo (from Feb. to Nov. 2008). Most males (84.4%) underwent 2 molts, with a 1 mo interval between molts. A small proportion (8.9%) (with a higher initial size than those that molted twice) molted once, without a form alternation. A smaller proportion (6.7%) (with a higher initial size than those that molted either once or twice) did not molt at all. Molting began after water temperatures were at least 16°C for 2 mo. Form I gonopods were longer, wider, and more robust than form II gonopods, which were similar to those of juveniles, in addition to possessing wider protopodites and shorter endopodites. Chelae of form I males were longer and wider than those in form II males. Juveniles had shorter and narrower chelae. The chelae and abdomen of form I males were more robust than those of form II males and juveniles, which did not differ. A change of body ratios did not occur in crayfish which molted once without a form alternation. Negative allometric growth of chela length (ChL) and width (ChW), and abdominal width (AbW) was observed after the molt in form II. The body size increased more at the 1st molt (to form II), than at the 2nd molt back to form I. ChL, ChW, and weight (W) increments were 3 (ChL) and 4 times (ChW and W) that of body-size increments and were dramatically higher than at the previous molt. Form alternation seemed to have a function of allowing the effective utilization of resources through which males could increase in size and be attractive to females. http://zoolstud.sinica.edu.tw/Journals/49.1/28.pdf

Key words: Invasive species, Crayfish, Intra-sex dimorphism, Growth.

*O*rconectes 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 differs from that of crayfish of the European native family, the 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 reproductive cycle in crustaceans is regulated by the endocrine system (Vogt 2002, Liu et al. 2008). Changes in characteristics associated with form alternations are the form of the hooks on the ischia of the 3rd pereiopods, body proportions, and the conformation of the gonopods (Scudamore 1948, Hobbs 2001).

A 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

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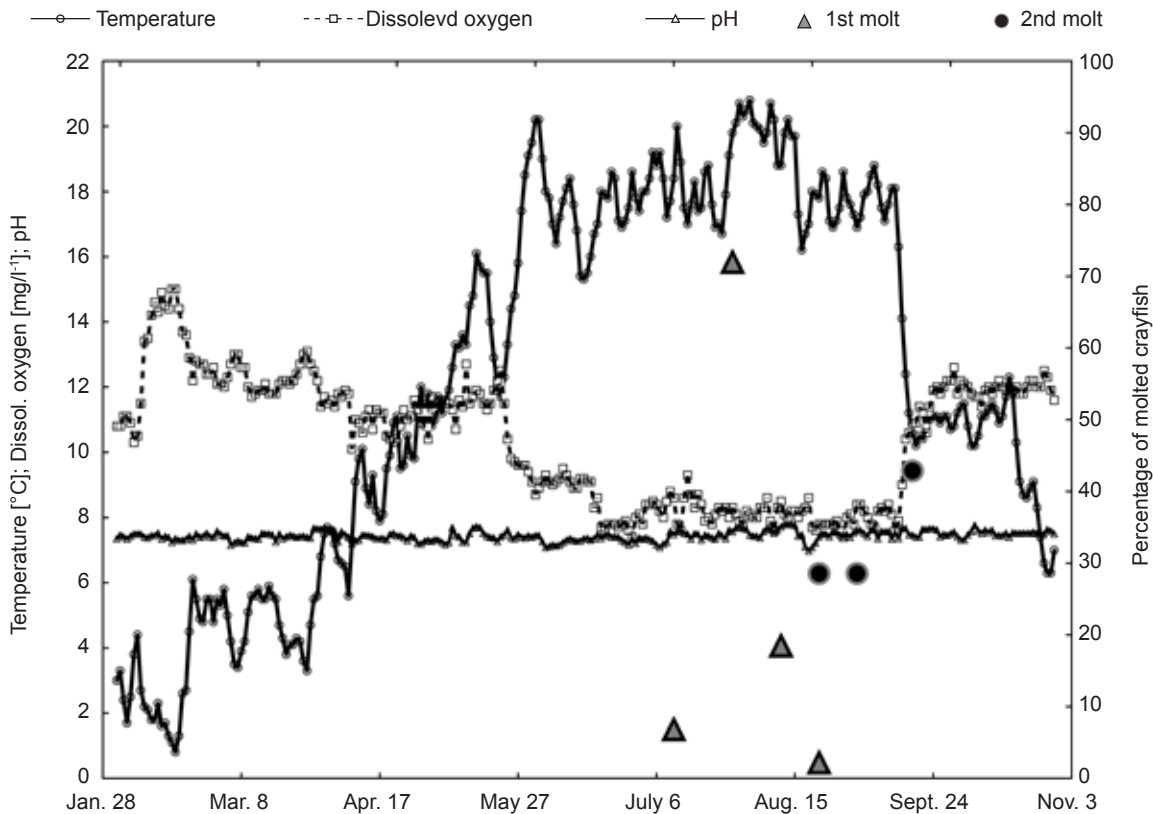E-mail:buric@vurh.jcu.cz

aggressive (Bovbjerg 1956, Tierney et al. 2008) and can be distinguished by the sclerotization, amber coloration, and lengthening of the terminal elements of the 1st pleopods (Andrews 1910, Hobbs 1989). In addition, the ischial hooks are more pronounced (Andrews 1910, Hobbs 2001), the 1st 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, terminal elements of the 1st pleopod are not as well differentiated (and never corneous), and the ischial hooks are 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 Berrill 1985). It is generally believed that form II males revert to

form I at the subsequent molt, as they normally undergo 2 molts per year (Capelli and Magnuson 1975, Smith 1981), but there is evidence that some form II males may go through 2 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 crayfish, most were based on samples from different sites. Few cambarid crayfish have been continuously studied for an extended period (Hobbs 2001). Data are lacking on molting, growth, and the number and significance of changes in body and gonopod proportions between forms in crayfish maintained in controlled conditions. These data are essential to better understand the complex cycle of cambarid crayfish.

The objective of this study was to elucidate patterns associated with male form alternation in the life history of *O. limosus*. 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

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

timing and synchrony/asynchrony; 2) to describe changes in body proportions; 3) to compare growth increments of successive molts; and 4) to describe the changes in copulatory stylet proportions.

MATERIALS AND METHODS

Animals

Specimens of *O. limosus* (both sexes) were captured (*n* = 1157) in Černovický Brook (South Bohemia, Czech Republic) in Aug. 2007. They were acclimated to laboratory conditions during autumn and winter and placed in experimental tanks in Feb. 2008. In total, the experimental period lasted 10 mo (Feb.-Nov.). The sex and reproductive state of each crayfish were identified by its 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™, Shaw I., Washington, USA) following Isely and Stockett (2001) and Buřič et al. (2008).

Experimental conditions

Crayfish were stocked in circular tanks (0.6 m in diameter, with a volume of 0.18 m³) and supplied with ~3 "shelter bricks" per crayfish. Each "shelter brick" consisted of 5 levels of hexagonal pipes (250 mm long) in groups of 6. Each side of a regular hexagon measured 30 mm. The photoperiod and water temperature was natural ambient, with natural daylight and a flow-through water supply (head-race of the River Blanice in Vodňany). The head-race of the River Blanice is very similar to the site at Černovický Brook (similar stream morphology, flow rate, shading by trees, and fish stocks). The water temperature was the same as in outdoor conditions. The inlet water was filtered through a drum filter. Tanks were cleaned regularly, and dissolved oxygen (DO) was measured twice daily using an oximeter (Oxi 315i, WTW, Weilheim, Germany). The water temperature was measured every 3 h using data loggers (RT-F53, Qi Analytical, Prague, Czech Republic), and the pH was measured daily (pH 315i, WTW). The water temperature, DO level, and pH are shown in figure 1. Crayfish were fed an excess of fish pellets, frozen chironomid larvae, and minced carrot 2-5 times/wk (depending on the season and amount of uneaten food).

Data collection

Crayfish were measured before and after molting (when the exoskeleton was fully hardened). Carapace length (CL, from the tip of the rostrum to the posterior edge of the 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 the carpal joint), chela width (ChW, at the widest part), and abdominal 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) (Fig. 2A). 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 and Sohn, Balingen, Germany). Molting and form alternations were recorded. The growth increment was calculated for each molted crayfish, with the equation (Brewis and Bowler 1982):

$$L_i = (L_a - L_b) \times \frac{100}{L_b} \ [\%];$$

where L_i is the length increment, L_a is the length after molting, and L_b is the length before molting.

There was a need to determine the body dimensions to calculate the ratios of body proportions. The 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 the carapace length is more reliable, since it is a rigid immovable structure, but rostrum length may vary among individuals, and the rostrum can also 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 to measure the copulatory stylet. Form I and juveniles (~1 yr old) were taken at the beginning of the experiment, and form II

crayfish were taken on 22 July. The stylets were dissected, fixed in 96% ethanol, labeled, and stored for later processing. The mesial and lateral surfaces of fixed stylets were photographed under a stereomicroscope (Olympus, Lens, France) coupled to an adapter with a digital camera (Olympus). Measurements of the stylet were made to the nearest 0.001 mm with the QuickPHOTO CAMERA 2.2 program (Olympus, Hamburg, Germany). These comprised the total length of the gonopodium (GL, from the base to the tip of the copulatory stylet), base width (BW, the width of the gonopod base), maximum width of the gonopodium (GW), endopodite length (EL) and width (EW), and protopodite width (PW) (Fig. 2B). The ratios, GL/POCL, GW/POCL, BW/GL, GW/GL, EL/GL, and PW/GL were also calculated.
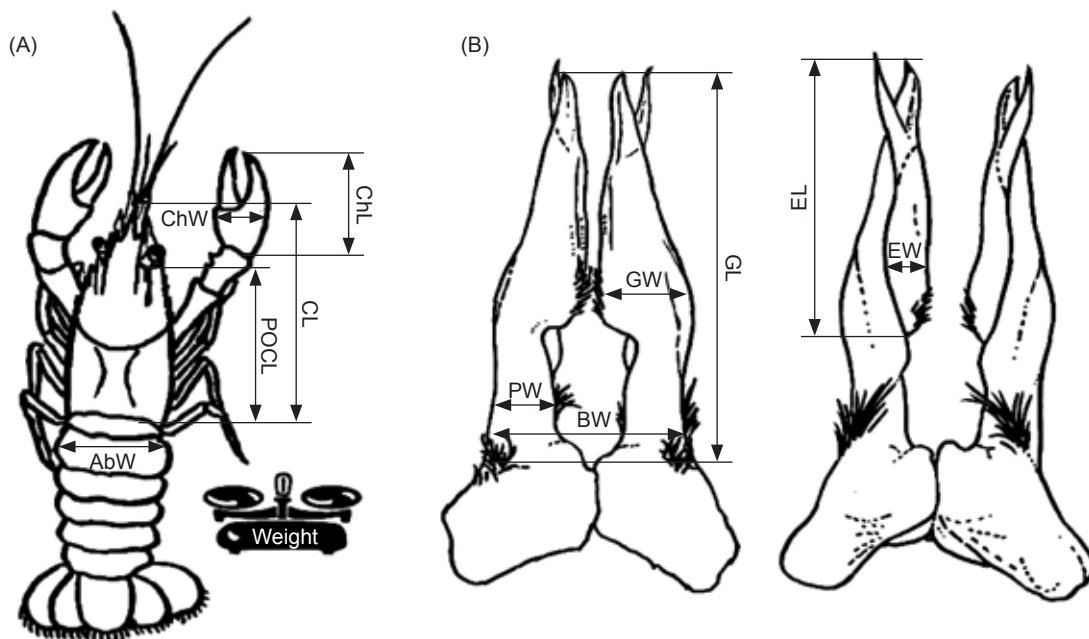
Data analysis

Data were analyzed with Statistica 8.0 (StatSoft, Tulsa, Oklahoma, USA). All values were examined for a normal distribution (by the Kolmogorov-Smirnov test) and homoscedacity (by the Levene test). A *t*-test was used to compare molting times and molt increments; a paired *t*-test was used to compare body proportions in individual crayfish before and after molting; 1-way analysis of variance (ANOVA) with Tukey's post-hoc test was used to compare body proportions and copulatory stylet proportions of form I, form II, and juveniles; and Spearman's rank correlation was used to test for a correlation of AbW/POCL with body size. The null hypothesis was rejected at α = 0.05. Data are presented as the average ± standard deviation (SD).

RESULTS

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 form I. A small proportion of crayfish (8.9%) molted just once during the year but remained in the reproductively active form I. A smaller proportion (6.7%) did not molt and remained in form I. The initial CL and POCL values 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) significantly differed (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. All form I males (including twice-

Fig. 2. Designation of measurements of *Orconectes limosus* (A) body dimensions and (B) copulatory stylet dimensions (lateral and mesial views). CL, carapace length; POCL, postorbital carapace length; ChL, chela length; ChW, chela width; AbW, abdominal width; GL, total length of gonopodium; BW, base width; GW, maximal width of gonopodium; EL, endopodite length; EW, endopodite width; PW, protopodite width.

molted, once-molted and even non-molted crayfish) were observed to successfully mate. Males began molting 2 mo after the water temperature had increased to at least 16°C. Crayfish that molted from form I to form II did so between 11 July and 22 Aug., and the highest proportions of newly molted crayfish were seen on 28 July (72.1%) and 11 Aug. (18.6%). The 2nd molt, back to form I, occurred between 22 Aug. and 18 Sept. All of the males that molted only once did so between 11 and 22 Aug. The time between the end of mating (when females oviposited) and molting to form II was 65.8 ± 7.10 d 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 d or 590.2 ± 99.82 d°. Males that underwent 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 d and 1499.8 ± 94.42 d°).

Changes in body proportions

A summary of ratios of body proportions in form I, form II, and juveniles is given in table 1. It is evident that form I males had longer and wider chelae than form II males and juveniles, and form II males had longer and wider chelae than juveniles. In addition, form I males had broader abdomens and a higher ChW/ChL ratio than form II males 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 a form alternation (form I → form I), with the exception of the AbW/POCL ratio, which decreased.

Growth increments in successive molts

The 1st molt (to form II) can be characterized by a low increment of weight increase, and in AbW and ChL, and a 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 in the 2nd molt was greater than that in the 1st molt and was similar to increments in CL and POCL in the 2nd molt. Increments in ChL, ChW, and weight were significantly higher than those observed in the 1st molt and higher than growth increments in CL, POCL, and AbW in the 2nd molt. The AbW/POCL ratio decreased with an increasing body size (Spearman rank correlation, r_S = -0.5873, $p < 0.05$). Data are summarized in table 3.

Changes in morphology of copulatory stylets

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

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

DISCUSSION

The life history of *O. limosus* was 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 observations of individual crayfish) describing molting and growth patterns associated with form alternation are still lacking. Most existing data were gathered from samples rather than continuous observations.

The typical life cycle of males of *Orconectes*, reported by Hobbs (2001) in North America, is a

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

A 1st molt	Form I (before molting)	Form II (after molting)	Residual	t	p
POCL/CL	0.73 ± 0.013[b]	0.74 ± 0.01.48[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]	-0.30 ± 0.011	14.69	< 10^{-6}

B 2nd molt	Form II (before molting)	Form I (after molting)	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]	0.090 ± 0.011	-20.49	< 10^{-5}
ChW/POCL	0.31 ± 0.034[b]	0.36 ± 0.031[a]	0.040 ± 0.010	-11.35	< 10^{-4}
ChW/ChL	0.33 ± 0.017[a]	0.34 ± 0.013[a]	0.010 ± 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 molting)	Form I (after molting)	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]	-0.010 ± 0.018	1.08	0.3581
ChW/POCL	0.39 ± 0.015[a]	0.37 ± 0.024[a]	-0.020 ± 0.019	2.40	0.0959
ChW/ChL	0.35 ± 0.024[a]	0.34 ± 0.026[a]	-0.010 ± 0.026	0.59	0.5945
AbW/POCL	0.63 ± 0.012[a]	0.60 ± 0.012[b]	-0.030 ± 0.007	8.70	0.0032

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

Increment (%)	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}

periodic form alternation throughout life, associated with 2 molts/yr, in spring (May/June) and summer (Aug.). 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-Aug., later than was previously reported (Van Den Brink et al. 1988, Larson and Magoulick 2008), despite the breeding season having occurred at the usually observed time (Smith 1981, Kozák et al. 2006). The 2nd molt took place between mid-Aug. and mid-Sept. The time between successive molts was therefore shorter than the 8 wk reported by Stein (1976) and Hobbs (2001). Muck et al. (2002) reported the 1st molting in water temperatures up to 9°C (Apr./May) and the 2nd molting at temperatures to 8°C (Aug./Sept.).

In the present study, crayfish first molted following 2 mo of water temperatures at up to 16°C. The last crayfish underwent the 2nd molt in temperatures exceeding 10°C. It can be assumed that *O. limosus* has higher temperature requirements, which is documented in degree-days for the first time in the present study. This surprising finding raises questions about differences between North American and European *O. limosus* habitats, which could alter its requirements. However, 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 in the subsequent molt (Stein et al. 1977, Smith 1981) was confirmed. However, there were 2 exceptions to this common
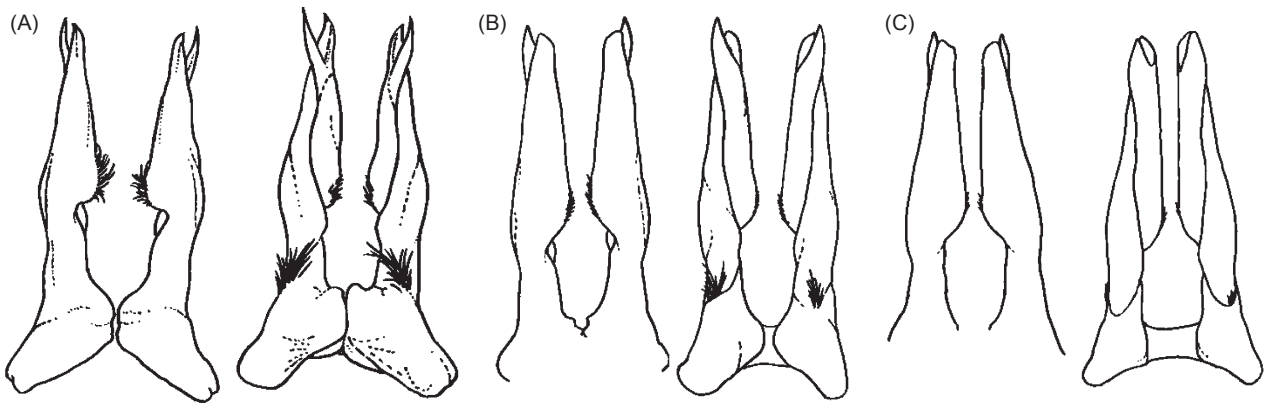

Fig. 3. Lateral and mesial views of the 1st pleopod of male *Orconectes limosus* (A) form I, (B) form II, and (C) juvenile.

Table 4. Ratios of copulatory stylet proportions in form I, form II, and juvenile male *Orconectes limosus*. Data are presented as the 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	Juvenile 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)	31.50 ± 3.36[a]	32.90 ± 2.63[a]	19.00 ± 2.04[b]	252.84	< 10^{-6}
POCL (mm)	22.30 ± 2.60[a]	24.30 ± 2.05[a]	13.60 ± 1.49[b]	253.75	< 10^{-6}
Weight (g)	9.10 ± 3.36[a]	9.10 ± 2.18[a]	1.70 ± 0.59[b]	250.02	< 10^{-6}

life-history model. A small proportion of males molted only once and did not change form. Suko (1960) also reported that a small number of *Procambarus clarkii* (Girard 1852) males molted only once. Suggested reasons were low pH conditions that may have prevented normal mineralization during molting (Hobbs 2001), water temperature (Mundahl and Benton 1990), and the influence of food quality and availability (Reynolds 2002, Olsson et al. 2008). These do not appear to apply to the present study, where crayfish were held under identical conditions, consisting of an optimal pH (Malley 1980, Nyström 2002), natural water temperature (without high divergences from seasonal normal values), high levels of DO, an adequate number of shelters, and sufficient food. Those crayfish not changing form underwent the molt later and showed higher initial CL and POCL values than did males that molted twice. Belated molting of once-molted males could explain the assumption of Pieplow (1938), based on the presence of the gastrolith, that *O. limosus* molts 3 times/yr.

The 2nd exception is that a small proportion of males did not molt throughout the year. The initial CL and POCL values of the non-molting males were higher than those in twice- and once-molted males. These 2 exceptions could point to an explanation for the 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 values of non-molted males were close to maximum values in the source population. This suggests decreasing growth, as a function of the number of molts, with increasing size and age. This strategy may 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, as even non-molted crayfish successfully mated.

The twice-molted 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 once-molting crayfish). Molting in synchrony and form alternation were 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 males 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). These patterns were 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 discrepancies 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 the water temperature, DO 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 the morphology of the copulatory stylets, was the change in chela dimensions relative to the body size. Form I males had longer and wider chelae than did form II ones. Juveniles had shorter and narrower chelae than both form I and form II males. The ChW/ChL and AbW/POCL ratios in form I males were also higher (i.e., chelae and abdomen were more robust) than those of form II males and juveniles, which did not differ from each other. Chelae have important functions, including prey capture and manipulation, defense against predators, inter- and intraspecific interactions, and reproduction (Stein 1976, Holdich 2002). Chela dimorphism may be connected to their use. Form I males are characterized by higher inter-male aggression (Bovbjerg 1956, Tierney et al. 2008), greater competition for females (Hobbs 2001), and higher exposure to potential predation (Berrill and Arsenault 1984). Large chelae are advantageous in competition for limited resources and 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 successfully copulate 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 males.

Following the breeding season, form I males molt to form II and exhibit 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 males from encounters with aggressive form I ones. 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 these purposes (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 is 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 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.

There is a need to increase the body size before the 2nd molt. First, 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 other 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 2 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 3 (ChL) and 4 times (ChW) higher than 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, and the annual increment was therefore lower than the POCL increment. This resulted in a slow decrease in the AbW/POCL ratio with increasing body size, possibly due to the lower importance of the 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 was reported in other species (Boyd and Page 1978, Acosta and Perry 2000). Changes in body proportions that occurred in form alternation seem to have a function of utilizing resources effectively to increase in size as well as to appear attractive to 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 3 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 males due to lengthening of the terminal elements. One of these terminal elements is soft and flexible, whereas the 2nd is rigid and ends in a deep, 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 showed a different structure, with both terminal elements being shorter, sclerotized, calcified, and rounded. In general, form II gonopods were similar to juvenile gonopods, but had 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 and complementary data about form alternation of cambarid crayfishes, which can contribute to a better understanding of the life history of widely distributed crayfish in North American and European inland waters, and probably also other species in the genus *Orconectes*.

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