The effect of water temperature on the number of moults and growth of juvenile signal crayfish *Pacifastacus leniusculus* Dana

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ABSTRACT: The growth rate, frequency of moulting, and intermoult intervals of juvenile *Pacifastacus leniusculus* were studied under experimental conditions over a period of 3 months. Juveniles were reared individually in small boxes at two different temperatures: 14.31 ± 0.64°C (cold water) and 20.54 ± 0.69°C (warm water). Although the average weight and length of consecutive stages were similar at both temperatures, juveniles in warm water attained more moults. Within the age group juveniles achieved a higher length and weight in warm water than in cold water. Three month-old juveniles reached 147 mg and 18.5 mm in cold water at the 6th stage of development, and 259 mg and 22.2 mm in warm water at the 8th stage. Specific growth rate (SGR) decreased (5.6 and 10.4 for cold and warm water in the first stage, respectively) and reached final values of 1.4 and 1.5 after 3 months of growth. SGR was significantly higher in warm water and showed negative correlations with the number of days after hatching and number of moults. Duration of intermoult periods was significantly influenced by water temperature, with five moults attained in cold water compared to seven in warm water. All intermoult periods were significantly longer in cold water than in warm water. The average percent weight and length increments decreased with increasing number of moults, length, weight and number of days after hatching. In warm water increments were higher (89.8% and 21.8% for weight and length, respectively) than in cold water (68.5% and 20.3% for weight and length, respectively), and the decrease was faster. However, the final values were similar at both temperatures (about 36% and 11% for weight and length, respectively). The mean absolute weight and length moult increments were not significantly influenced by water temperature. Although the temperature influenced growth due to the number of moults, the duration of individual intermoult periods did not affect the weight and length of juveniles in particular stages.

Keywords: *Pacifastacus leniusculus*; moulting; developmental stage; growth; temperature

Signal crayfish *Pacifastacus leniusculus* has a typical life cycle of a member of the crayfish family Astacidae. Its life cycle is also typical of a cool temperature zone species, although it grows faster and reaches a greater final size than comparative species (Lewis, 2002; Holdich et al., 2006). In laboratory experiments, signal crayfish was found to have a greater tolerance to temperature compared to *Austropotamobius pallipes* and *Astacus leptodactylus*, and a higher growth rate than those two

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species at different tested temperatures (Holdich et al., 2006).

The regulation of seasonal growth by water temperature is common for temperate species, including signal crayfish. Some seasonal growers are cool temperature species, growing best at < 22°C, whereas others are adapted to warmer waters, which is generally associated with restricted levels of dissolved oxygen (Reynolds, 2002). Signal crayfish grows best at temperatures between 20 and 25°C (Firkins and Holdich, 1993).

The growth pattern in crustaceans is a discontinuous process with successive moults (Lowery, 1988; Ackefors et al., 1995). While the internal physiological growth is continuous, a rapid increase in length and weight occurs only at the moult. Since growth is accomplished through the process of moulting, the growth rate is a product of the moult increment and frequency (Reynolds, 2002). The moult frequency appears to vary according to temperature, food availability (Price and Payne, 1984; Lowery and Holdich, 1988), individual growth rate (Price and Payne, 1984) and age (Lewis, 2002). Moults are numerous in the first months of juvenile life and decrease as the crayfish approaches the sexual maturity (Reynolds, 2002). Juveniles of signal crayfish could undergo as many as 11 moults during their first year (Holdich et al., 2006). According to Cukerzis (1979) signal crayfish moults eight or nine times in their first year. Moulting of juveniles usually occurs during the summer (Stucki, 2002).

The present study aimed at the evaluation of the time span between moults during the first three months after hatching. The main objectives were to assess the impact of two different temperatures on growth, moulting frequency, intermoult period, and length and weight increments per moult.

MATERIAL AND METHODS

Rearing conditions

Juveniles of *P. leniusculus* in the 1st developmental stage (hatchlings) were separated from females and placed individually in small boxes made from clear plastic. Each box, 40 mm in height, was divided into18 separated chambers, with the bottom area of each individual chamber 45 × 30 mm. Juveniles were reared at two different temperatures: 14.31 ± 0.64°C (cold water) and 20.54 ± 0.69°C (warm water). The temperature was continuously monitored with temperature sensors. Altogether 36 juveniles (2 × 18) were stocked at each temperature.

Juveniles were fed daily in excess with diced frozen chironomid larvae and algae *Cladophora sp.* Uneaten food from the previous day was removed. Throughout the experiment the photoperiod was maintained to 12 hours dark and 12 hours light. Dissolved oxygen (> 7.5 mg/l) and pH (7.1–7.6) were measured on a daily basis.

The experiment was conducted over a 3-month period (from the end of May to the beginning of September).

Measurements and data analysis

Juveniles were daily evaluated. In order to allow the crust to harden and to get an exact value of the new length and weight, body wet weight and length were measured two days after each moult. Each crayfish was dried on filter paper to remove excess water trapped between the branchiostegites and appendages before weighing with an electronic balance (Kern and Sohn GmbH, Balingen, Germany) to the nearest 0.0001 g. Total length was measured from the tip of the rostrum to the end of the telson to the nearest 0.5 mm. The first length measurements were done at the 2nd developmental stage.

Measurements included:

Specific growth rate (*SGR*)

$$SGR = (\ln(W_t) - \ln(W_i)) \times 100/T \text{ (\% per day)}$$

where:
W_t = weight at time t (g)
W_i = initial weight (g)
T = time (days)

Intermoult period (T_{im})

$$T_{im} = T_{n+1} - T_n \text{ (days)}$$

where:
T_n = time of *n* moult
T_{n+1} = time of *n*+1 moult (Jussila and Evans, 1996)

Weight increment at moult (W_m)

$$W_m = (W_a - W_b) \times 100/W_b \text{ (\%)}$$

where:
W_a = weight after moult
W_b = weight before moult (Brewis and Bowler, 1982)

Length increment at moult (L_m)

$$L_m = (L_a - L_b) \times 100/L_b \text{ (\%)}$$

Table 1. Age (days and day degrees (d°)), weight and length at different developmental stages in juvenile *Pacifastacus leniusculus* (mean ± standard deviation), means in the same column with different superscripts are significantly different ($P < 0.05$)

		Developmental stage							
		1	2	3	4	5	6	7	8
Age (days)	cold	–	9.6 ± 1.9^a	29.8 ± 3.5^a	48.8 ± 4.1^a	70.7 ± 5.3^a	94.4 ± 7.8^a	–	–
	warm	–	6.0 ± 0.0^b	16.6 ± 1.2^b	28.1 ± 1.6^b	41.0 ± 2.0^b	57.3 ± 3.0^b	73.9 ± 4.2	93.7 ± 4.9
Age (d°)	cold	–	139.7 ± 27.8^a	435.1 ± 47.3^a	710.4 ± 59.1^a	$1\,042.4 \pm 72.0^a$	$1\,419.9 \pm 88.7^a$	–	–
	warm	–	125.7 ± 0.0^b	338.3 ± 24.6^b	578.0 ± 33.4^b	849.2 ± 43.8^b	$1\,198.4 \pm 75.4^b$	1 539.9 ± 81.9	1 947.8 ± 102.7
Weight (mg)	cold	15.8 ± 1.5^a	26.5 ± 3.1^a	42.8 ± 6.6^a	68.7 ± 12.9^a	110.3 ± 22.6^a	147.0 ± 27.4^a	–	–
	warm	15.7 ± 1.5^a	29.9 ± 4.5^a	48.9 ± 7.3^a	65.4 ± 10.0^a	99.5 ± 17.9^a	144.7 ± 30.5^a	193.3 ± 45.6	258.6 ± 49.9
Length (mm)	cold	–	9.8 ± 0.6^b	11.8 ± 0.6^b	14.2 ± 0.8^a	16.6 ± 1.0^a	18.5 ± 0.6^a	–	–
	warm	–	10.3 ± 0.6^a	12.4 ± 0.5^a	14.3 ± 0.8^a	15.9 ± 0.9^a	17.6 ± 1.1^a	20.0 ± 1.6	22.2 ± 1.2

Table 2. Percent and absolute increments (days and day-degrees), and specific growth rate (*SGR*) at different developmental stages in juvenile *Pacifastacus leniusculus* (mean ± standard deviation)

		Moult						
		1	2	3	4	5	6	7
Percent weight increment (%/moult)	cold	68.5 ± 19.5^b	62.1 ± 25.4^a	60.8 ± 27.1^a	52.3 ± 29.4^a	35.6 ± 18.2^b	–	–
	warm	89.8 ± 28.5^a	66.0 ± 28.9^a	36.0 ± 18.6^b	40.1 ± 17.0^a	58.8 ± 19.6^a	31.4 ± 18.5	37.9 ± 23.1
Absolute weight increment (mg/moult)	cold	11.6 ± 2.9^b	16.2 ± 6.0^a	26.3 ± 11.0^a	39.7 ± 17.1^a	41.3 ± 16.2^a		
	warm	15.0 ± 4.4^a	19.0 ± 7.2^a	17.5 ± 8.2^b	26.1 ± 11.9^b	53.2 ± 19.4^a	45.4 ± 27.2	70.0 ± 37.8
Percent length increment (%/moult)	cold	–	20.3 ± 6.8^a	19.9 ± 7.8^a	14.3 ± 5.9^a	11.6 ± 5.3^a	–	–
	warm	–	21.8 ± 8.1^a	14.9 ± 5.9^b	11.8 ± 2.9^a	11.2 ± 5.4^a	13.1 ± 8.3	11.1 ± 5.2
Absolute length increment (mm/moult)	cold	–	2.0 ± 0.6^a	2.3 ± 0.9^a	2.2 ± 1.1^a	1.9 ± 0.8^a	–	–
	warm	–	2.2 ± 0.7^a	1.8 ± 0.7^b	1.6 ± 0.6^b	1.7 ± 0.9^a	2.3 ± 1.4	2.2 ± 1.0
SGR	cold	5.6 ± 2.1^b	2.4 ± 1.0^b	2.4 ± 0.9^a	2.1 ± 1.2^a	1.4 ± 0.8^b	–	–
	warm	10.4 ± 3.7^a	4.7 ± 1.6^a	2.6 ± 1.1^a	2.7 ± 1.1^a	2.9 ± 1.1^a	1.6 ± 0.7	1.5 ± 0.6

means in the same column with different superscripts differ ($P < 0.05$)

where:
L_a = length after moult
L_b = length before moult

Statistical analysis

Differences in weight, length, moult increments, *SGR* and duration of intermoult periods as influenced by different temperatures were tested by nonparametric Mann and Whitney tests. Spearman rank correlations were calculated between variables. All statistical analyses were conducted using Statistica 7.0 (StatSoft, Inc.). The null hypothesis was rejected at a significance level of α = 0.05, and data are presented as mean ± standard deviation.

RESULTS

Growth

The average weight and length were similar ($P > 0.05$) within the different development stages (Table 1). However, juveniles in warm water grew faster and showed more moults during the same

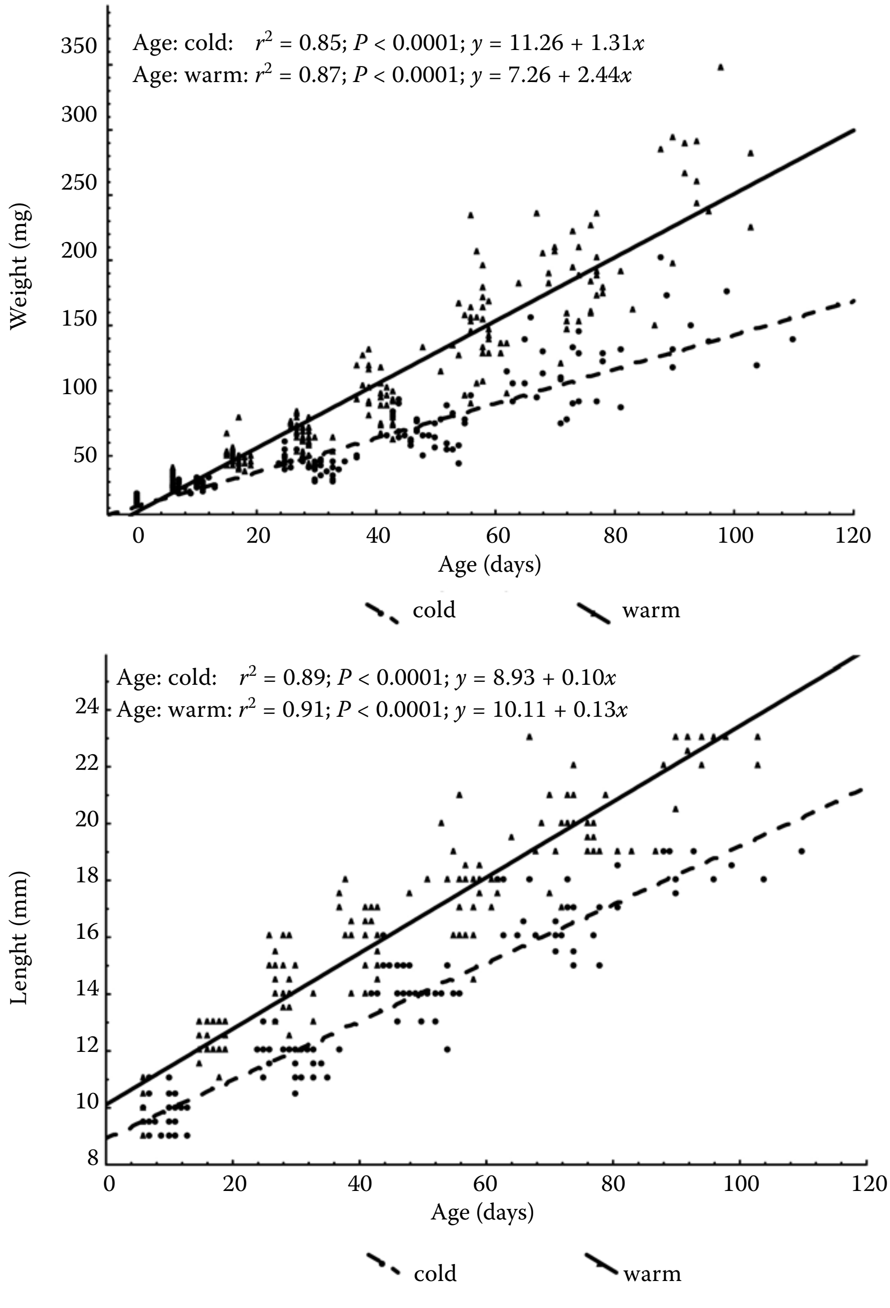

Figure 1. Linear relationships between body weight and age (A), and total length and age (B), of juvenile *Pacifastacus leniusculus* at different water temperatures

Table 3. Spearman rank correlation coefficients ($P < 0.05$) between increments and number of moults, age in days, length and weight in juvenile *P. leniusculus*

	Percent weight increment		Percent length increment		Absolute weight increment	
	warm	cold	warm	cold	warm	cold
Number of moults	–0.467	–0.324	–0.429	–0.383	0.711	0.766
Age (days)	–0.468	–0.321	–0.429	–0.400	0.694	0.715

period. Weight and length at both temperatures showed a linear relationship with the number of days after hatching (Figure 1).

Specific growth rate (*SGR*)

Values for *SGR* at the different developmental stages are presented in Table 2. *SGR* was found to be significantly higher in warm water compared to cold water at the 1st, 2nd and 5th stages. *SGR* decreased with the developmental stage (Spearman rank correlation; warm: $r_s = –0.696$; cold: $r_s = –0.625$; $P < 0.05$), age (Spearman rank correlation; warm: $r_s = –0.709$; cold: $r_s = –0.645$; $P < 0.05$) and weight (Spearman rank correlation; warm: $r_s = –0.636$; cold: $r_s = –0.470$; $P < 0.05$).

Intermoult periods

Moulting occurred at a higher rate in warm water than in cold water. Whilst all the juveniles attained the 8th developmental stage in warm water, some juveniles did not reach the 6th stage in cold water (Table 2). The temperature has a significant influence on the moult frequency, being higher in warm water compared to cold water.

All intermoult periods were longer ($P < 0.05$) in cold water compared to warm water, while the 6th developmental stage was reached in around 57 days (1 195 day degrees) and 94 days (1 353 day degrees) in warm and cold water, respectively. The temperature significantly influenced the duration of intermoult periods, as presented by the number of days to attain specific developmental stages (Table 1).

Weight and length increments at moult

In both warm and cold water the average percent weight and length increments decreased with increasing number of moults, number of days after hatching (Table 3). As a consequence of the faster decrease in warm water, final values were similar for both temperatures (Table 2). Absolute weight increments showed positive linear correlations with increasing number of moults, number of days after hatching at both temperatures (Table 3).

DISCUSSION

The findings of the present study indicate that the processes of moulting, moult frequency and growth are increased by warm water compared to cold water, which is in agreement with previous studies on noble crayfish *Astacus astacus* (Ackefors et al., 1995) and other crayfish species (Price and Payne, 1984; Lowery, 1988; Lowery and Holdich, 1988; Tamkeviciene, 1988; Verhoef and Austin, 1999; Reynolds, 2002). According to Ackefors et al. (1995) the average number of days between moults increased in noble crayfish from about 11 at stage 3 to 21 at stage 7. In the present study, the intermoult periods for signal crayfish juveniles increased in a similar pattern to the above between stages 3 and 7 in warm water, but were much longer in cold water.

Various researchers have presented different values for the weight and length of juvenile signal crayfish. Savolainen et al. (2004) found the weight of ca. 100 days old signal crayfish juveniles (reared at a temperature of 16.4–19.4°C) higher (from 0.294 to 0.690 g) than in the current study. A higher mean size and weight were reported for summerlings in wild or pond culture by Tulonen et al. (1995) (17.9 mm carapace length; 1.45 g), Westman et al. (1993) (around 30 mm total length), and Tamkeviciene (1988) (around 26 mm and 0.4 g). However, the number of moults attained during the first summer in the above studies was in agreement with the present results. According to Tamkeviciene (1988) 4 month-old signal crayfish juveniles underwent 8 to 9 moults, whereas Lewis (2002) found 11 moults in the 1st year of the life cycle. The small bottom area for each holding cell could contribute to a slower growth of juveniles in the present study, as reported by Ackefors (1995) and Goyert and Avault (1979). However, this did not have an influence on the aims of the study.

Ackefors et al. (1995) recorded a comparable average increase (16–19% from 2nd to 3rd stage and 10–14% from stage 3 to stage 7) in carapace length for noble crayfish. However, we observed a higher percent in weight increment than around 40% reported by Ackefors et al. (1995). This difference could be attributed to differences in morphological parameters between species.

Specific growth rate (*SGR*) decreased considering the sequential stages. The influence of water temperature on *SGR* shortened intermoult periods, with similar increments per moult attained in a shorter time period increasing *SGR* in warm water.

The present study showed that the temperature influenced growth increasing the number of moults and duration of individual intermoults periods, but did not affect the weight and length of juveniles at particular stages.

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