Research Article

ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., Suppl. 8, 2017: 99-106

Morphometric Variability, Allometric Growth and Sexual Dimorphism in Narrow-Clawed Crayfish, *Astacus leptodactylus* Eschscholtz, 1823 (Crustacea: Decapoda) during the Ontogenesis

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Abstract: **A** study on the variability of morphometric characteristics of 927 one-summer-old, one-year-old and two-summer-old narrow-clawed crayfish, cultivated at the Institute of Fisheries and Aquaculture in Plovdiv was carried out. The weight-length relationships of the two sexes at different age groups were determined using graphic equations. Allometric growth was observed in all individuals. Significant sexual dimorphism of 12 exterior characters was recorded. Higher values of the body weight, carapace length, carapace width, chela length, chela width, chela corpulence, dactyl length, palm width and chela length (from the carpal joint to the tip) were measured in males. Concerning females, the abdomen length, abdomen width and telson width were measured.

Key words: *Astacus leptodactylus*, morphometric variability, allometry, sexual dimorphism

Introduction

The variability is a fundamental feature of the living organisms. One of its forms is the phenotypic variability of the exterior characters due to the changes in the environmental conditions or the stage of individual development as well as due to differences determined by the sexual dimorphism.

Narrow-clawed crayfish, *Astacus leptodactylus* Eschscholtz, 1823, are characterized by clearly expressed sexual dimorphism. They show well-expressed phenotypic variability enabling them to live in various water conditions, a feature determining their wide distribution and characterizing them as a species with high ecological variability (Zaikov & Hubenova 2007).

The application of morphometric methods in examination of higher crustaceans (Malacostraca) enables to analyse the varying shapes of their body and to determine the variability of individuals representing one or both sexes during ontogenesis as well as to make intrapopulation, interpopulation or interspecies comparisons. The sexual dimorphism of the exterior is inherent to many representatives of Decapoda (Romaire *et al*. 1977, Rhodes &Holdich 1979, Lindqvist & Lahti 1983, Huner *et al*. 1995, Grandjean *et al.*1997, Duris *et al*. 2001, Szaniawska *et al.* 2005, Chybowski 2007, Vlach & Valdmanova 2015). Skvortzov (1979) carried out a morpho-ecological research on two natural populations of narrow-clawed crayfish in the Middle Ural. It contained a comparative analysis on the variability of two groups of crayfish from two water bodies. Reliable differences in several morphological features were determined, which varied independently from each other between sexes within one popu-

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lation and between individuals of the two studied populations. Total variability turned out to be the lowest in that water basin, which physico-chemical parameters were most similar to species requirements towards their habitat.

Upon studies on the relations between the morphometric parameters and the water body trophic conditions carried out on three species, i.e. *Astacus astacus* (L., 1758), *A. leptodactylus* and *Orconectes limosus* (Rafinesque, 1817), sexual differences in body weight, abdomen width, chelae length and chelae width were observed in all studied populations (Burba 1996, Burba *et al.* 1999).

Morphometric studies on *A. astacus* (see Agerberg 1988, Alechnovich *et al*. 1999, Kulesh *et al*. 1999, Maguire *et al.* 2006), *A. leptodactylus* (see Agerberg, 1988), *Austropotamobius torrentium* (Schrank, 1803) and *Austropotamobius pallipes* (Lereboullet, 1858) (Grandjean *et al*. 1997, Grandjean & Souty-Grosset 2000, Streissl & Hödl, 2002, Sint *et al*. 2005, 2006, Maguire & Klobukar 2009, Parvulesku 2010, Zekic *et al*. 2013, Vlach & Valdmanova 2015) provided analysis on various exterior characters. They suggested the relationships between the body parameters; the dependency between the body weight and length was the most important for determining the growth rate and the type of growth (isometric or allometric). Isometric body growth was observed in juveniles, differing them from adult individuals, since their body grew more in weight than in length (i.e. allometric) (Nefedov & Mazanov 1973). Dependencies related to allometric growth of body parts were determined in some specimens of *A. pallipes* (Rhodes & Holdich 1979, Scalici *et al*. 2010), *Pacifastacus leniusculus* (Dana, 1852) (Guan & Wiles 1999), *Orconectes rusticus* (Girard, 1852) (Schroeder & Huber 2001), *Procambarus alleni* (Faxon, 1884) (Acosta & Perry 2000) and *Austropotamobium torrentium* (Vlach & Valdmanova 2015). Allometric chelae growth was determined for *A. astacus,* which was more clearly expressed in males than in females (Lindqvist & Lahti 1983). Ledesma *et al.* (2010) and Scalici *et al.* (2010) applied the principles of geometric morphometrics to analyse differences between juveniles and adults.

In Bulgaria, the studies on the exterior characters of narrow-clawed crayfish of various age are limited. Several authors specified the weight reached by juveniles of *A. leptodactylus* upon different experiments: various diets (Zaikov *et al.* 2000), under various stocking density conditions and upon cultivation of one-summer-old crayfish in a polyculture with carps and bighead carp (Zaikov *et al.* 2001). In addition, an analysis of the length-weight relationship for one-summer-old narrow-clawed crayfish was carried out (Hubenova *et al.* 2001). Sexual dimorphism of narrow-clawed crayfish from the natural population of the Kardzhali artificial lake was determined in a previous study (Vasileva *et al.* 2005).

The purpose of the present article is to study morphometric variability, type of body growth and sexual dimorphism of narrow-clawed crayfish of different age, cultivated at the Institute of Fisheries and Aquaculture, during the ontogenesis.

Material and Methods

Material, experimental animals and growing conditions

The work was carried out in experimental facilities, aquarium and laboratories of the Institute of Fisheries and Aquaculture, Plovdiv.

Exterior characteristics were studied on specimens of different age, representing a pond population. The following age groups and number of specimens were analysed: the first age group consisting of 149 female and 175 male one-summer-old specimens, the second age group consisting of 145 female and 163 male one-year-old specimens and the third age group consisting of 154 female and 141 male two-summer-old specimens. Sixteen body parameters were measured and analysed according to the scheme applied by Rhodes & Holdich (1979) and Skvortsov (1979). We also added the measurement of the length of palm (Fig. 1).

The one-summer-old, the one-year-old and the two-summer-old crayfish were grown in earthen carp ponds, up to 1 meter deep, overgrown with aquatic vegetation, providing them with shelter and food. During their vegetation season, crayfish were additionally fed once a week with grind sunflower and wheat seeds.

Morphometric methods

The morphometric studies on the exterior of different age groups were conducted (Fig. 1). Measurements were taken by means of mechanical callipers to the accuracy of 0.1 cm. The body weight was measured on an analytical scale with an accuracy of 0.01 g.

Statistical methods

The relative values of the exterior features were calculated on the basis of their absolute values versus body length (total length). Data from all studies were statistically processed by means of software for Microsoft Office. The average arithmetic value – (X) of the parameters with its error – (Sx),

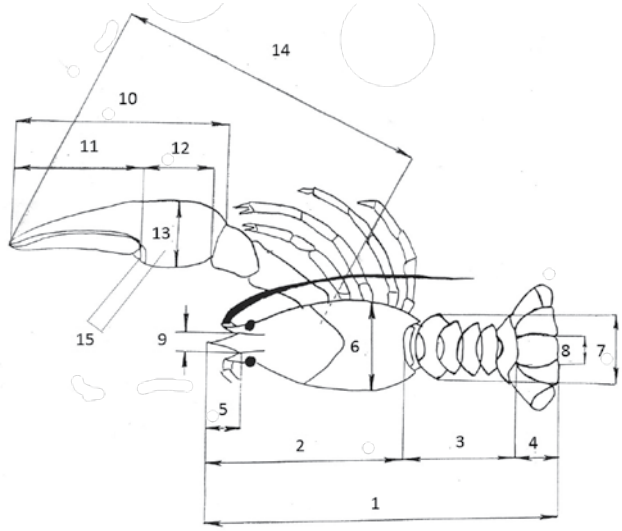

Fig. 1. Exterior parameters of narrow-clawed crayfish *Astacus leptodactylus* Esch. Total body length (1), Carapace length (2), Abdomen length (3), Telson length (4), Rostrum length (5), Carapace width (6), Abdomen width (7), Telson width (8), Rostrum width (9), Chela length (10), Dactyl length (11), Palm length (12), Chela width (13), Chelae length from its carpal join to its tip (14), Chela corpulence (15)

the standard deviation – (SD) and the coefficient of variation (Cv, %), as a major index, characterizing biological variability, were calculated. Some dependencies were determined, and the correlation coefficients between the exterior features body weight and zoological body length were specified. The reliable differences upon the comparison of two samples were analysed by means of a Student's t-test where the degree of probability was $p<0.05$*, $p<0.01$**, $p<0.001$***.

Results and Discussion

Variability of body weight and zoological length absolute values

The linear and weight body growth was observed in dynamics, with the three age groups analysed separately (Table 1).

Body weight

The absolute value of the body weight of one-summer-old male crayfish varied from 1.52 to 24.06 g (10.24±4.99 g), and that of the female crayfish from 0.69 to 19.55 g (7.79±3.95 g) (Table 1). The coefficient of variation of this parameter had very high values (48.7% and 50.65%) (Table 1). The absolute value of the body weight of the one-year-old male crayfish varied from 1.8-33.95 g (11.13±6.45 g), and that of female from 1.69 to 19.51 g (8.35±4.04 g) (Table 1). The absolute value of the body weight of the two-summer-old male crayfish varied from 14.59 to 46.56 g (28.89±6.6 g), and that of female crayfish from 14.74 to 35.1 g (25.08±4.81g) (Table 1). Significant differences in this parameter were determined between individuals of both sexes for the whole analyzed samples of different age-groups ($p<0.001$***); the values for the male specimen were higher.

Zoological body length (Total body length)

The absolute value of the total body length for the one-summer-old male crayfish varied from 4.0 to 9.3 cm (6.86 cm), and for the female crayfish from 3.1 to 9.0 cm (6.39 cm) (Table 1). The absolute value of the total body length for the one-year-old male crayfish varied from 4.2 to 10.2 cm, and those for the female crayfish from 4.0 to 8.8 cm (Table 1). The values of this parameter for the two-summer-old male crayfish varied from 8.0 to 11.1 cm, and those for the females from 8 to 11 cm. The variation of this parameter ranged from 6.61 % to 20.2 %. The higher values were observed for first age group, and this parameter become lower with increasing the age of individuals.The feature length of the body demonstrated a lower variation's degree in comparison to the value of the parameter weight of the body.

Allometric growth

The relationship of the weight and length of the body for the three studied age groups, expressed by means of power equations for both sexes, is presented in Fig. 2. The exponent b had values ranging from 2.85 to 3.34, which showed that the growth of the crayfish individuals of all age-groups was regulated by Huxley's principle of allometric growth (Uzunov & Kovachev 2002). According to this equation, the growth was considered isometric where the coefficient b=3. In cases where b≠3, the growth was considered allometric, which corresponded to data established by other authors (Acosta & Perry, 2000, Vlach & Valdmanova 2015). In our study, we found negative allometry in two-summer-old females (b=2.85). This means that the individuals grew more on length than on weight. In all other age groups and in two-summer-old males the allometry was positive.

Relative values. Sexual dimorphism

In addition to the specified differences, based on to the body weight and body length found of male and female one-summer-old crayfish (first age group), sexual dimorphism was determined based on 11 additional exterior features (Table 2).

Table 1. Absolute values of the exterior parameters Body weight and Total body length of narrow-clawed crayfish *Astacus leptodactylus* Esch. from different age groups.

Age groups	Parameters	Body weight, g		Total body length, cm	
		Male	Female	Male	Female
One-summer-old crayfish	x±SD	10.24±4.99	7.79±3.95	6.86±1.14	6.39±1.29
	Cv, %	48.7	50.65	16.68	20.2
	lim	1.52÷24.06	0.69÷19.55	4.0÷9.3	3.1÷9.0
One-year-old crayfish	x±SD	11.13±6.45	8.35±4.04	6.99±1.29	6.59±1.07
	Cv, %	57.97	48.39	18.51	16.27
	lim	1.8÷33.95	1.6÷19.51	4.2÷10.2	4.0÷8.8
Two-summer-old crayfish	x±SD	28.89±6.6	25.08±4.81	9.71±0.69	9.78±0.65
	Cv, %	22.83	19.17	6.89	6.61
	lim	14.59÷46.56	14.74÷35.10	8.0÷11.1	8.0÷11.0

Table 2. Absolute and relative values of exterior parameters in one-summer-old crayfish

Features	Absolute values ± SD		Relative values ± SD	
	Males	Females	Males	Females
Carapace length	3.53 ± 0.62	3.20 ± 0.64	51.4 ± 1.24***	50.27 ± 2.06
Abdomen length	2.39 ± 0.4	2.30 ± 0.49	34.81 ± 2.1	35.88 ± 1.31***
Telson length	0.95 ± 0.16	0.89 ± 0.18	13.9 ± 0.88	13.93 ± 0.2
Rostrum length	0.52 ± 0.09	0.50 ± 0.1	7.64 ± 0.83	7.86 ± 0.83*
Carapace width	1.74 ± 0.33	1.59 ± 0.37	25.29 ± 1.23***	24.67 ± 1.28
Abdomen width	1.51 ± 0.28	1.50 ± 0.34	22.03 ± 0.97	23.39 ± 1.1***
Telson width	0.85 ± 0.14	0.81 ± 0.17	12.34 ± 0.61	12.59 ± 0.76**
Rostrum width	0.38 ± 0.07	0.36 ± 0.08	5.59 ± 0.55	5.65 ± 0.55
Chela length	2.38 ± 0.62	1.82 ± 0.45	34.22 ± 3.95***	28.24 ± 2.11
Dactyl length	1.32 ± 0.36	1.01 ± 0.26	18.89 ± 2.45***	15.64 ± 1.35
Palm length	0.78 ± 0.2	0.63 ± 0.15	11.2 ± 1.42***	9.86 ± 1.0
Chela width	0.89 ± 0.21	0.76 ± 0.2	12.79 ± 1.4***	11.79± 1.3
Chela total length	4.73 ± 1.09	3.83 ± 0.86	68.17 ± 5.66***	59.60 ± 3.54
Chela corpulence	0.49 ± 0.12	0.4 ± 0.1	7.02 ± 0.94***	6.21 ± 0.66

The features, which characterize the chelas of the crayfish – length, width, corpulence, length of the dactyl**,** length of the palm and length of the chelae from its carpal join to its tip, and carapace length and width have reliably higher values for males compared to females. The values of the features length and width of the abdomen and width of the telson are significantly higher for females.

By studying the relative values of exterior parameters of one-year-old crayfish (Second age group)and two-summer-old crayfish, sexual dimorphism was identified based on the same exterior features (Tables 3 and 4).

The values of the indices, characterizing the crayfish chelas – length, width, corpulence, length of the dactyl**,** length of the chelae from its carpal join to its tip and palm length**,** and carapace length and width were significantly higher for male one-year-old and two-summer-old crayfish compared to those obtained from females of the same age. The values obtained for the length and width of the abdomen and the width of the telson were also significantly higher of the one-year-old and two-summer-old females compared to males of the same age-groups.

Skvortsov (1979) specified significant differences in *A. leptodactylus* exterior between specimens of both sexes in the carapace length, telson length, chelae length, chelae corpulence and dactyl length, abdomen width, carapace width and rostrum width. The male stone crayfish *A. torrentium* also reached higher weight and had longer carapace and bigger chelas than females (Streissl & Hödl 2002, Zaikov *et al*. 2011). Chybowski (2007) and Szaniawska *et al*. (2005) specified higher values for the carapace length, the claw length and width for the male individuals as well as higher values for the abdomen length and width for females of *O. limosus*. In their study, Grandjean *et al.* (1997) determined that mature males of *A. pallipes* were characterized by bigger chelas, and females had wider abdomen.

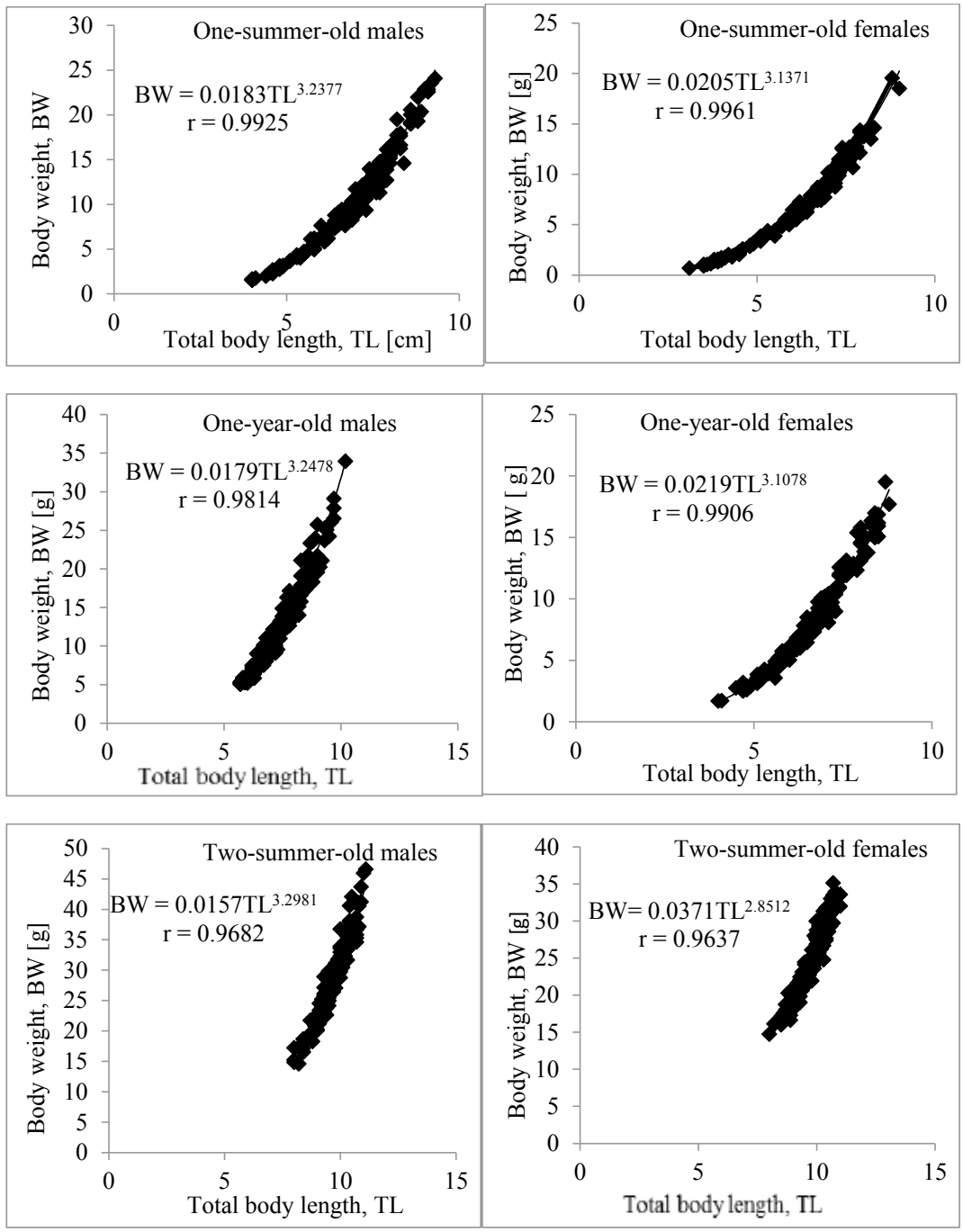

Fig. 2. The relationship of the weight and length of the body for the three studied age groups in *Astacus leptodactylus* Ecsh

The studied groups of one-summer-old and one-year-old narrow-clawed crayfish demonstrated higher values of the rostrum length index of females. The analysis does not prove sexual dimorphism in the next age group. Females of two-summer-old crayfish had longer length of the telson than that in males. The younger crayfish do not have differences in this parameter. These results define the exterior changes during ontogeny. The length of the carapace, length of the abdomen, length of the telson and length of the rostrum are defining factors for the formation of the body length. Analyzing the percentage differences in the increase of these body parts in the two sexes, it is determined that for the separate weight groups the length of the carapace increases more for males than for females. For the female individuals, the length of the abdomen and the length of the telson increase more compared to those of males.

Males of the first and the second age-groups have significantly longer body due to their longer carapace. There were no differences between both

Table 3. Absolute and relative values of exterior parameters in one-year-old crayfish

Features	Absolute values ± SD		Relative values ± SD	
	Males	Females	Males	Females
Carapace length	3.8 ± 0.55	3.28 ± 0.57	51.03 ± 1.35***	49.79 ± 1.39
Abdomen length	2.61 ± 0.35	2.38 ± 0.38	35.07 ± 1.5	36.15 ± 1.9***
Telson length	1.03 ± 0.15	0.93 ± 0.17	13.88 ± 0.82	14.09 ± 1.06
Rostrum length	0.56 ± 0.08	0.52 ± 0.1	7.59 ± 0.8	7.89 ± 0.76***
Carapace width	1.89 ± 0.3	1.63 ± 0.3	25.29 ± 1.0***	24.67 ± 1.0
Abdomen width	1.64 ± 0.25	1.56 ± 0.2	22.04 ± 0.85	23.63 ± 1.27***
Telson width	0.92 ± 0.13	0.84 ± 0.14	12.37 ± 0.66	12.81 ± 0.68***
Rostrum width	0.42 ± 0.07	0.38 ± 0.07	5.61 ± 0.57	5.80 ± 0.62**
Chela length	2.63 ± 0.63	1.88 ± 0.40	34.95 ± 4.36***	28.34 ± 2.11
Dactyl length	1.48 ± 0.36	1.05 ± 0.22	19.71 ± 2.41***	15.78 ± 1.23
Palm length	0.89 ± 0.19	0.68 ± 0.13	11.91 ± 1.37***	10.27 ± 0.87
Chela width	0.97 ± 0.2	0.78 ± 0.17	12.93 ± 1.38***	11.69 ± 1.14
Chela total length	5.1 ± 1.04	3.94 ± 0.75	69.27 ± 5.6***	59.51 ± 2.93
Chela corpulence	0.53 ± 0.11	0.41 ± 0.09	7.1 ± 0.8***	6.21 ± 0.67

Table 4. Absolute and relative values of exterior parameters in two-summer-old crayfish

Features	Absolute values ± SD		Relative values ± SD	
	Males	Females	Males	Females
Carapace length	4.99 ± 0.38	4.78 ± 0.33	51.31 ± 1.06***	48.8 ± 0.82
Abdomen length	3.43 ± 0.26	3.65 ± 0.28	35.33 ± 1.37	37.29 ± 1.26***
Telson length	1.3 ± 0.11	1.36 ± 0.11	13.42 ± 0.69	13.85 ± 0.65***
Rostrum length	0.67 ± 0.08	0.67 ± 0.08	6.92 ± 0.68	6.83 ± 0.6
Carapace width	2.53 ± 0.21	2.43 ± 0.18	26.01 ± 0.93***	24.85 ± 0.95
Abdomen width	2.15 ± 0.18	2.67 ± 0.24	22.08 ± 0.82	27.25 ± 1.4***
Telson width	1.19 ± 0.09	1.24 ± 0.09	12.22 ± 0.46	12.65 ± 0.44***
Rostrum width	0.51 ± 0.05	0.52 ± 0.05	5.26 ± 0.45	5.27 ± 0.44
Chela length	3.92 ± 0.54	2.94 ± 0.32	40.2 ± 3.61***	30.01 ± 2.08
Dactyl length	2.27 ± 0.34	1.66 ± 0.2	23.28 ± 2.36***	16.92 ± 1.48
Palm length	1.25 ± 0.17	1.0 ± 0.11	12.79 ± 1.65***	10.17 ± 0.86
Chela width	1.32 ± 0.21	1.19 ± 0.14	13.58 ± 1.67***	12.11 ± 0.98
Chela total length	7.43 ± 0.84	5.97 ± 0.52	75.86 ± 7.94***	60.96 ± 2.58
Chela corpulence	0.74 ± 0.11	0.63 ± 0.07	7.63 ± 0.84***	6.44 ± 0.55

sexes in the total body length in the two-summer-old crayfish. The abdomen, rostrum and telson were longer in females of two-summer-old crayfish and compensate the longer carapace in males of the same age group. This corresponds with the negative allometric growth of the two-summer-old females (Fig. 2).

Several studies on *A. torrentium* (Streiss & Hödl 2002) and *A. astacus* (Cucerzis, 1979) confirmed the rule that female crayfish reach greater length than males at the same age.

The sexual dimorphism as characterised on the external morphological features is related mainly to peculiarities of the connection with the specific functions in both sexes during the reproduction. Dissections of two-summer-old crayfish in the course of the present study revealed that the ovocytes have started to accumulate yolk and the sperm ducts of males contained spermatic fluid, which during the copulation would be deposited as a spermatophore on ventral surface of females (on the coxae of the third periopods). This was demonstrated in the two-summer-old crayfish living in the conditions of South Bulgaria: they matured at the two-summer-old age and can reproduce in the second year of their life. This fact was evidenced by the greater values of the length and width of the abdomen in females, which are related to presence and protection of eggs. The values of the characters associated with the chelae size, which had an

important role during the copulation, were higher in males. Male crayfish develop longer and wider carapace, probably due to the bigger chelas connected with it. These data correspond to the results of Woodlock & Reynolds (1988) on *A. pallipes,* in which bigger males copulated more often than smaller individuals. Our results also correspond to the data of Sint *et al.* (2005)and to our previous study (Vasileva *et al.* 2005) demonstrating that the sexual dimorphism in the exterior was related to the process of copulation.

The sexual dimorphism in the two-summer-old crayfish was confirmed on the basis of the same twelve parameters recorded in a natural population of the Kardzhali artificial lake (Vasileva *et al*. 2005). In view that all analysed two-summer-old crayfish of the cultivated population as well as those from the Kardzhali artificial lake (Vasileva *et al*. 2005) were mature, we may conclude that this rule is valid for mature individuals of various populations.

Conclusions

In all age-groups, male narrow-clawed crayfish reach significantly higher average body weight compared to females.

In the equations expressing the relationship between the weight and the length of the body, the exponent b had values ranging from 2.85 to 3.34, which demonstrate that the crayfish of all age groups have allometric growth.

Eleven morphometric characters demonstrate the sexual dimorphism of the one-summer-old, one-year-old and two-summer-old narrow-clawed crayfish. The characters with higher values in males are the length and the width of the carapace; the length, the width and the corpulence of the chelae; the length of the dactyl; the length of the chelae from its carpal join to its tip and the length of the palm. The characters with higher values in females are the length and the width of the abdomen and the length and the width of the telson.

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