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Evaluation of reproductive parameters of males in wild freshwater crayfish (*Astacus leptodactylus*)

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Abstract
The mature crayfish (*A. leptodactylus*) were caughted with pinter from Keban Dam Lake Koçkale hunting area at the breeding season. The crayfish were stored for 20 minutes at 20°C and anesthetized. Carapace lengths were measured by caliper and their weights were weighed. Then the crayfish were dissected and the carapace removed. Macroscopic observations were realized in the reproductive system. All reproductive system, testes and the vasa deferans were weighed. The testicular index (TI) and gonado-somatic index (GSI) were calculated. The means of TI and GSI was 0.27±0.17 and 1.66±0.99, respectively. Consequently, the present study would be useful for reproductive management and efficiency.

Keywords: *A. leptodactylus,* crayfish, reproductive parameters.

INTRODUCTION

The crustaceans (Crustacea) sub-branch of the animal kingdom belong to the Astacidae family and naturally spread in the inland waters of the northern hemisphere [1, 2]. Astacus leptodactylus Eschscholtz, which is called "narrow claw crayfish" Turkish crayfish in inland waters of our country, is distributed as a single species in 1823. Crayfish are benthic organisms that have an important function in inland water ecosystems due to their accelerating effects on organic matter conversion. Crayfish is a shellfish species produced by hunting, which is not cultivated in our country. *A. leptodactylus* is the most preferred crayfish species in Europe in terms of taste and is one of our important inland products, which have been hunted and exported for 35-40 years [3].

To date, numereous studies have been conducted about distribution of crayfish populations and taxonomic determination [4, 5], body parts [6], artificial production [7], embryonic development periods [8], hunting, productivity [9, 10], disease states and crayfish plague [11, 12, 13, 14], meat yield according to height and weight, abdomen and kelipet meat rates [15, 16, 17], bioecological and morphometric properties [16, 18], production, aquaculture and marketing status [19], life of populations cycle [20], the relationship between female individuals and egg lengths [21], egg incubation period, survival rate growth and meat-nutrient content the effects of temperature, eyelid cutting and stocking density [22], potential egg productivity [23].

Researchs about biology of crayfish anatomy and physiology are important to better understand and production. Sperm count is a very important factor for natural or artificial insemination [24].The main purpose in aquaculture is to achieve maximum yield and develop special techniques for reproduction and growth under controlled conditions. Therefore, a few studies have been conducted on the performance the breeding of crayfish [25]. For these reasons, culture studies are needed better understanding of male reproductive development associated with sperm quality. There are numerous research about reproductive performance of female for *A. leptodactylus* species [8, 21, 23, 26, 27, 28]. In addition, studies have been realized in crayfish about the effects of different substances added to food and toxic substances studies on antioxidant status in tissues and ovaries [29, 30]. However, information about the reproductive biology of *A. leptodactylus* was about anatomy system and, formation and transfer of spermatophore [3, 31]. In this context, in this study, we aimed to determine gonad weight, vasa deferens weight, gonado-somatic index and testicular index, reproductive system weight of freshwater crayfish *A. leptodactylus*,

MATERIAL AND METHOD

The study was performed with crayfish (n=30) obtained from Keban Dam Lake Koçkale hunting area (Figure 1). Crayfish were caught in start of reproduction period, mid-reproduction period and at the end of the breeding period.

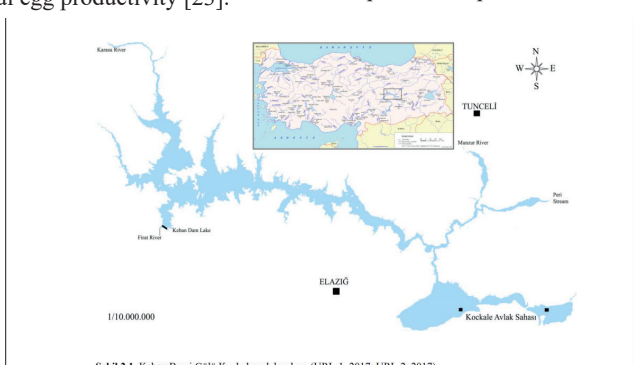

Figure 1. Keban Dam Lake Koçkale hunting areas.

Carapace length, length and width of cheliped, length and width of abdomen of the crayfish were measured with a caliper (±0.01 g). Sex discrimination was made in individuals. For determination of length-weight relationship of crayfish with regression equations and growth constants, exponential linear relationship model $W = aL^b$ [32, 33]. LogW = Log a + bLog L a full logarithmic relationship model was used [1]. The length-weight relationship was examined in terms of the relationship between the total length-live weight (TLW) and in captured individuals, the length and sex and regression equations, curves and correlation coefficients were assessed. The crayfish were anesthetized by standing at -20°C for 20 minutes. Then, crayfish were dissected and carapaces removed.

Macroscopic observations were made in the reproductive system. Reproduction system and testes were weighed. Vasa deferens (VDS) parts in male subjects were separated and weighed. Vasa deferens (VDS) weight = reproductive system weight - testicular weight. Gonado-somatic index (GSI) was assessed as reproductive system weight/body weight × 100. Sperm count (Spermatozoa / VDV) was determined using Eosin dye with Thoma slide. EXCEL and SPSS 24.0 program was used in the evaluation of the data.

RESULTS

The values of the male body parts are presented in Table 1.

Table 1. Some morphological features *of A. leptodactylus* captured from Koçkale hunting area

	N	Min.	Max.	Mean	SD
Carapace length	30	55	74	63,75	6,12
Carapace width	30	28	38	33,49	3,31
Abdomen length	30	55	73	64,71	5,35
Abdomen width	30	24	38	28,35	3,50
Chela length	30	48	82	63,36	10,62
Chela width	30	17	29	21,87	3,68
Weight	30	35,50	104,54	64,76	19,66

A linear relationship was found between height and weight of male individuals ($r^2 = 0.726$) (Figure 2).

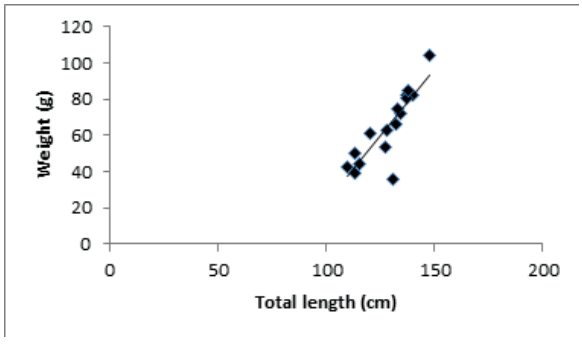

Figure 2. Relationship length-weight of *A. leptodactylus.*

Mean values and distributions of Vas deferens weight, testicular weight, reproductive system weight and testicular index (TI) of male subjects are given in Table 2. The testicular index of male subjects ranged from 0.02 to 0.67, with a mean of 0.27 ± 0.17.

Table 2. Mean values of Vas deferens weight, testicular weight, reproductive system weight and testicular index (TI) of *A. leptodactylus.*

Parameters	N	Min.	Max.	Mean	SD
Vas deferens weight (g)	30	0,52	2,70	1,35	0,63
Testis weight (g)	30	0,02	0,55	0,19	0,16
Reproductive system weight (g)	30	0,58	3,20	1,55	0,71
Testicular index (TI)	30	0,02	0,67	0,27	0,17
Sperm count (x10⁹)	30	2,91	9,25	4,28	1,97

The linear regression between the weight of crayfish and testis weight is given in Figure 3. There was a weak relationship between weight and Vas deferens weight ($r^2 = 0.198$).

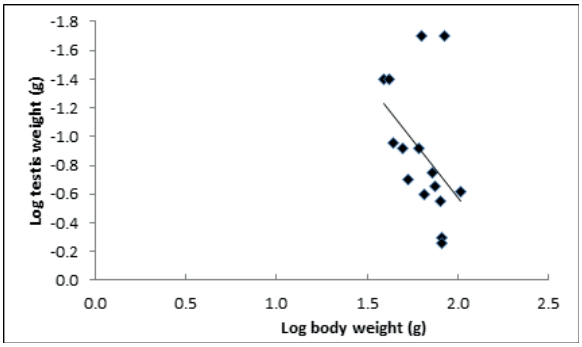

Figure 3. Relationship between weight and testis weight *Astacus leptodactylus*.

The linear regression between the weights of crayfish and Vas deferens weight is presented in Figure 4. There was a weak relationship between weight and Vas deferens weight ($r^2 = 0,183$). The linear regression between the weight and the reproductive system weight is given in Figure 4. There was a weak relationship between weight and reproductive system weight ($r^2 = 0.271$). The linear regression between the total length and the sperm count is given in Figure 5. There was a weak relationship between total length and sperm count ($r^2 = 0.079$). The linear regression between the weights and sperm count is shown in Figure 6. There was a weak relationship between weight and sperm count ($r^2 = 0.025$). The mean GSI values were 2.34 ± 0.91.

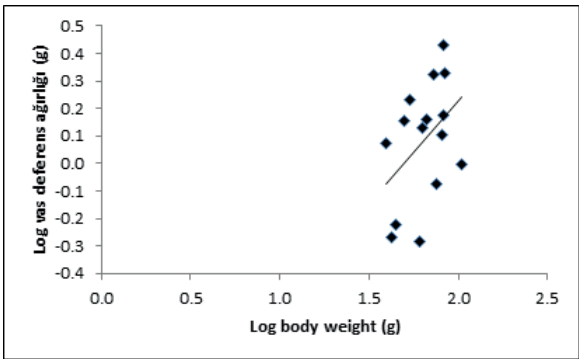

Figure 3. Relationship between weight and Vas deferens weight *Astacus leptodactylus*.

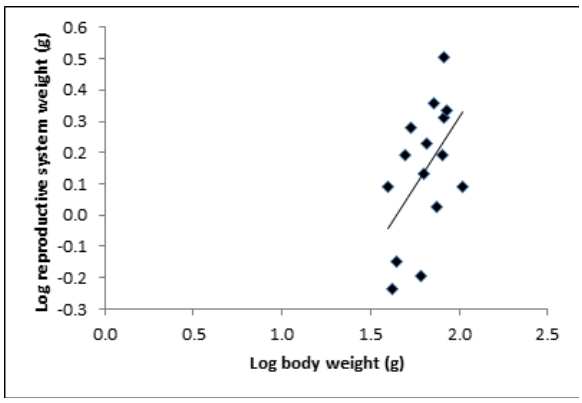

Figure 4. Relationship between weight and the reproductive system weight *Astacus leptodactylus*.

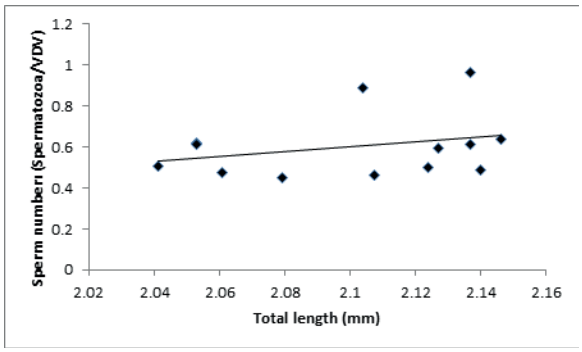

Figure 5. Relationship between total length and sperm count of *Astacus leptodactylus.*

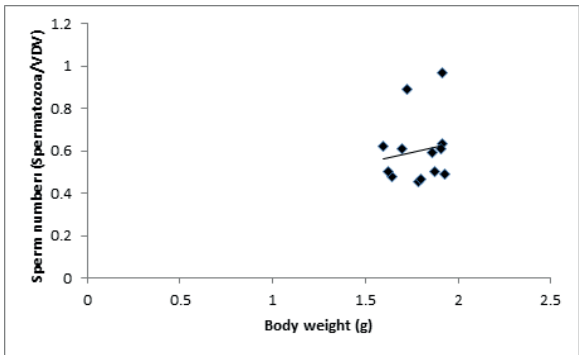

Figure 6. Relationship between weight and sperm count of *Astacus leptodactylus.*

DISCUSSION

To date, many studies have been conducted on the female reproductive performance of *A. leptodactylus* species [8, 21, 23, 26, 27, 28], studies on reproductive characteristics of male individuals are limited [3, 31, 34, 35, 36, 37].

Although sperm count is one of the important sperm quality parameters, there are few studies. Bugnot and López Greco's [25, 37] examined the relationship between *Cherax quadricarinatus* body size and sperm count. In their study, they determined the number of sperm to be 10×10^6-1×10^9 sperm / DVD. Harlıoğlu et al. [3], the sperm count of freshwater crayfish (*A. leptodactylus*) caught from nature at the beginning of the breeding season was determined in their study. 25 mature male freshwater crayfish (*A. leptodactylus*) were used in the study. The average sperm count in vas deferens was determined as $5.72 \pm 4.54 \times 10^9$ sperm / distal vas deferens (DVD) for crayfish with 41-56 mm carapace length. The sperm count ranged from 4×10^8 to 8.5×10^9 sperm / DVD. The results revealed that the number of sperm increased as the body weight increased. For example, the average sperm count in individuals with a weight range of 15-24 g was $3.87 \pm 2.87 \times 10^9$, while the average sperm count in individuals with a weight range of 36-42 g was $7.37 \pm 2.87 \times 10^9$. In this study, the mean sperm count in vas deferens was determined as $4.28 \pm 1{,}97 \times 10^9$ sperm / distal vas deferens (DVD) in individuals with a size of 55-74 mm carapace and weight range of 35-104 g. It was between 25 x 10^9 sperm / DVD.

Harlıoğlu et al. [3] reported that vas deferens, testis and reproductive system weights of crayfish with a size of 41-56 mm carapace were 0.225 ± 7.25, 0.167 ± 4.05 and 0.392 ± 11.26, respectively. When compared with the previous study, it was found that the average weight of the vas deferens, testis and reproductive system of crayfish with a length of 55-74 mm carapax was 1.35 ± 0.63, 0.19 ± 0.16 and 1.55 ± 0.71, respectively. This difference may be related to the size of crayfish.

Mirheydari et al. [38] examined the annual reproductive cycle of male individuals of *A. leptodactylus* in Aras Dam Lake, external appearance of testicular and vas deferentia, and vasa deferentia was divided into three structural parts: proximal vas deferens (PVD), middle vas deferens (MVD) and distal vas. deferens (DVD) and 139,64, 140,07, 100,29, 118,04 mm and 88,28, 99,58, 58,72, 64,58 mg, respectively. Harlıoğlu et al. [3] determined the sperm count of freshwater crayfish (*A. leptodactylus*) caught from nature at the beginning of the breeding season. In addition, weights of reproductive system, testis and vas deferens were determined and gonado-somatic index and testicular index were calculated. 25 mature male freshwater crayfish (*A. leptodactylus*) were used in the study. Significant linear regression was between sperm number, length and reproductive system weight, testicular weight and vas deferens weight ($p < 0.05$). In this study, it was determined that there was a weak relationship between weight and reproductive system weight, testis weight and vas deferens weight.

Harlıoğlu et al. [36] stated that there was a significant difference between the average sperm count of crayfish fed with LC n-3 PUFA supplemented diets [D1 (1%), D2 (2%), D3 (3%)] and crayfish in the control group (P). <0.05). The highest sperm count was in the group fed with D3 ration ($6.27 \pm 0.14 \times 10^8$). The results also showed that the weight of the reproductive system of crayfish fed D3 and D2 was significantly higher than the control and D1 diet groups ($P < 0.05$). On the other hand, they determined no significant difference in testicular weight and vas deferens weight between control and D1-fed crayfish and D2 and D3-fed crayfish. They found that gonadosomatic index of crayfish fed D3 and D2 were significantly higher than control and D1 ($P < 0.05$), but they did not find a significant difference in the testicular index of control D1, D2 and D3 ($P > 0.05$). Harlıoğlu et al. [3] reported in their study that the testicular index of freshwater crayfish (*A. leptodactylus*) caught from nature at the beginning of the breeding season ranged from 0.49 to 0.83 on average, which was 0.60 ± 0.08. In the present study, the testicular index ranged from 0.02 to 0.67 and the mean was 0.27 ± 0.17.

Mirheydari et al. [38] examined the annual reproductive cycle of male individuals of *A. leptodactylus* in Aras Dam Lake and seasonal variation of fluctuations in gonado-somatic index (GSI), indicating that GSI changes in a seasonal distribution range (0,50, 0,52, 1,21-1,12). Harlıoğlu et al. [3] reported that the average GSI of male crayfish with a size of 41-56 mm carapace was 0.225 ± 7.25. In this study, the average GSI value of male crayfish with a length of 55-74 mm carapace was 2.34 ± 0.91. These differences may be caused by differences in the size and habitat of the crayfish.

In conclusion, to date, in different water resources in our

country and around the world, a numerous studies have been conducted on growth, morphological characteristics of *A. leptodactylus*, but studies on reproductive characteristics are limited. This study will contribute to the literature. Determination of growth and reproductive characteristics is important for sustainable hunting and protection of populations. The data obtained in this study especially will be useful in the planning of hunting strategies.

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