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# An investigation on the sperm number and reproductive parameters of males in wild caught freshwater crayfish (*Astacus leptodactylus*, Eschscoltz)

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**Abstract**

In the present study, the sperm number of wild caught *Astacus leptodactylus* was counted in the beginning of reproduction season. In addition, the reproductive system, testes, and vasa deferentia were weighed, and gonado-somatic index and testicular index were calculated. Twenty-five adult males of *A. leptodactylus* were used for the study. Significant linear regressions were found between body length (and weight) and sperm count, reproductive system weight, testes weight and vasa deferentia weight ($P < 0.05$). The mean sperm number of vas deferens for the size range of 41-56 mm carapace length crayfish was $5.72 \pm 4.54 \times 10^9$ sperm/distal vas deferens (DVD) section. Sperm number ranged from $4 \times 10^8$ to $8.5 \times 10^9$ sperm/DVD section. The results revealed that an increase in body weight causes an increase in sperm number. For example, mean sperm number was $3.87 \pm 2.87 \times 10^9$ for the weight range of 15-24 g crayfish, $5.01 \pm 2.62 \times 10^9$ for the weight range of 25-35 g crayfish and $7.37 \pm 2.87 \times 10^9$ for the weight range of 36-42 g crayfish. In conclusion, this study provides some data on the male reproduction characters and needs to be improved with details about the reproductive performance of males (i.e., sperm number and quality) in order to reach a maximum reproductive efficiency in captivity.

**Keywords**

*A. leptodactylus*; crayfish; sperm number; sperm production

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## Introduction

*Astacus leptodactylus* is one of the most important crayfish species in Europe due to its aquaculture potential, economic value and wide consumer demand (Harlıoğlu and Holdich, 2001; Wickins and Lee, 2002; Harlıoğlu, 2011). It originates from western Asia and eastern Europe, e.g., Turkey (Koksal, 1988). It was also widely introduced into many countries, e.g., Poland, Italy, Germany, England, Spain and France (Skurdal and Taugbol, 2001; Holdich et al., 2009; Harlıoğlu and Güner, 2007). In recent years, *A. leptodactylus* has been stocked in many freshwater resources throughout Turkey because of its economic importance and to restore crayfish stocks previously devastated by crayfish plague (Harlıoğlu, 2008; Bök et al., 2010).

Turkey was the largest supplier of *A. leptodactylus* in Europe until 1986. In 1984 nearly 8000 tons were harvested, the majority has been exported (Köksal, 1988; Holdich, 1993). After 1985, the harvest of *A. leptodactylus* was dramatically diminished in most populations in Turkey as a result of crayfish plague, which was caused by the fungus-like organism, *Aphanomyces astaci* (Köksal, 1988; Rahe and Soylu, 1989). In 1991, the production was less than 350 tons. Although there has been a steady increase in the crayfish production of Turkey in recent years, the harvest of *A. leptodactylus* is still under pressure of the plague (Harlıoğlu, 2008). It was 750 tons in 2007, 783 tons in 2008 and 734 tons in 2009 (Anonymous, 2007, 2008, 2009).

It is therefore clear that crayfish production increased in Turkey, and that there is a need to improve reproduction of *A. leptodactylus* under controlled conditions. However, *A. leptodactylus* is not reproduced in Turkey at present. All production is obtained from wild harvest.

Under natural conditions, the majority of *A. leptodactylus* reaches first sexual maturity at the age of three years (Harlıoğlu, 1996). In the west of Turkey, mating occurs during October and November when the water temperature is 7-12°C (Köksal, 1988). However, in the east of Turkey, because of climatic conditions (such as temperature), mating and spawning of crayfish may occur after December (Harlıoğlu, 1999). Via the vas deferens the sperm masses, which are packaged into spermatophores, transferred from male to female during mating. The male's sperm enter the female at the bottom of her legs, where the eggs are fertilized and then released (Holdich, 2002). Research on the anatomy and physiology of the male reproductive system is important in order to better understand the biology of crayfish. Sperm number is also a very important factor for natural or artificial spawning (Krol et al., 2006).

The main objective in aquaculture is to achieve maximum reproduction readiness year round, and to develop specific techniques for reproduction and growth in captivity. Hence, a few studies concerning reproduction performance of crayfish have been conducted (Bugnot et al., 2009a). Therefore, a better understanding of male reproductive development and associated sperm quality is necessary for culture studies. For *A. leptodactylus*, the female reproductive performance has been

intensively studied (Köksal, 1988; Harlıoğlu and Türkgülü, 2000; Harlıoğlu et al., 2002, 2004; Harlıoğlu and Barım, 2004; Harlıoğlu and Duran, 2010). However, as concerns male *A. leptodactylus*, the knowledge about the reproductive biology of males is limited to the anatomy of the reproductive system and formation and transfer of the spermatophore (Erkan et al., 2009). Within this framework, the objective of the present study is to determine the sperm number of wild caught freshwater crayfish *A. leptodactylus* in the beginning of the reproductive season. In addition, the present study was also conducted to investigate the reproductive system weight, testes weight, vasa deferentia weight, gonado-somatic index and testicular index of this species.

## Material and methods

Twenty-five adult males of *A. leptodactylus* (mean weight: 28.26 ± 7.25 g, weight range: 15-42 g, mean carapace length: 48.52 ± 0.41 mm, carapace size range: 41-56 mm) were obtained in the beginning of reproduction season from Keban Dam Lake, Turkey between January 3 and January 20, 2011.

In the laboratory, the carapace length of crayfish was measured using a digital caliper. They were weighed and cold anaesthetized at −20°C during 20 minutes. Then, the crayfish samples were dissected, removing the carapace and macroscopic observations of the reproductive system were made, determining relative size, colour and consistency of the sperm.

The whole reproductive system and testes were weighed. Vasa deferentia (VD) weight was estimated as the difference between reproductive system and testes weight; gonado-somatic index (GI) was calculated as: (reproductive system weight/body weight × 100) and testicular index (TI) was calculated as: (testes weight/body weight × 100). A modified protocol developed by Leung-Trujillo and Lawrence (1987) was employed. A 1-cm section of the distal vas deferens (DVD) was disaggregated in 1 ml of physiological solution for crustaceans (Van Harreveld, 1936). This section corresponds to the approximated size of the spermatophore transferred to the female during mating (López Greco et al., 2007; López Greco and Lo Nostro, 2008). Then, spermatozoa were counted by using a Neubauer camera. Sperm count was expressed as spermatozoa/DVD section (Bugnot and López Greco, 2009a).

## Results

Crayfish samples were sacrificed in the beginning of the reproductive season. They had white distal vas deferens. This color was found in different intensities in VD. These VD were also harder in consistency than normal ones; they had the elasticity of the muscular sheath and broke easily under manipulation. In addition, under light microscopic analysis, the sperm of crayfish samples did not show any morphological abnormalities.

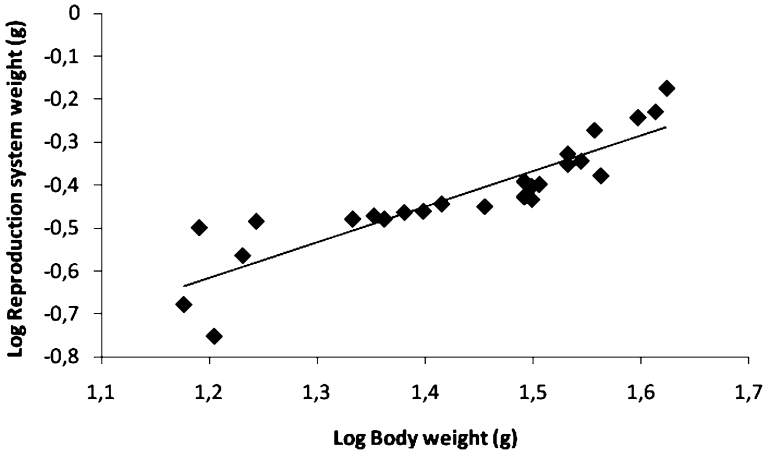


**Figure 1.** Linear regression between body weight and reproductive system weight of *A. leptodactylus*.

In the present study, significant ($P < 0.05$) linear regressions were found between body weight and reproductive system weight (fig. 1), testes weight (fig. 2) vasa deferentia weight (fig. 3).

For the size range of 41-56 mm carapace length crayfish, mean reproduction system weight was 392.5 ± 11.26 mg, mean testes weight was 167.4 ± 4.05 mg and mean vas deferentia weight was 225.1 ± 7.25 mg. The statistical parameters of the linear regression analysis are detailed in table 1 for the effect of length and table 2 for the effect of weight on the reproductive performance (sperm count, reproductive system weight, testes weight, vasa deferentia weight, gonado-somatic index and testicular index) of *A. leptodactylus*.

The mean sperm number in the vas deferens for the size range of 41-56 mm carapace length crayfish was 5.72 ± 4.54 × $10^9$ sperm/DVD section. The lowest

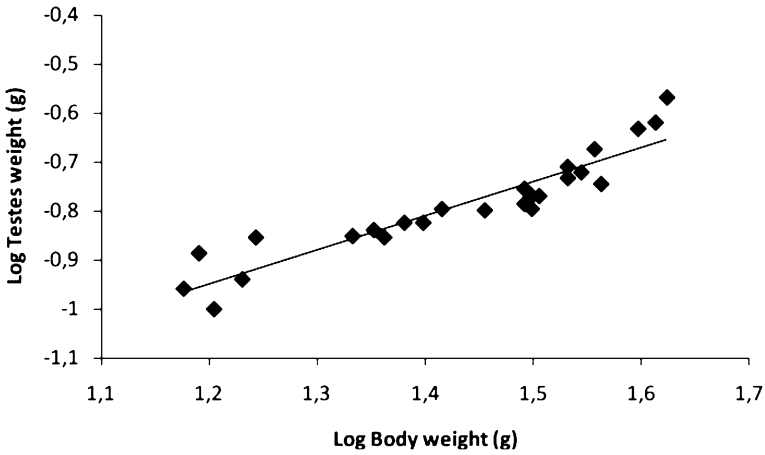


**Figure 2.** Linear regression between body weight and testes weight of *A. leptodactylus.*

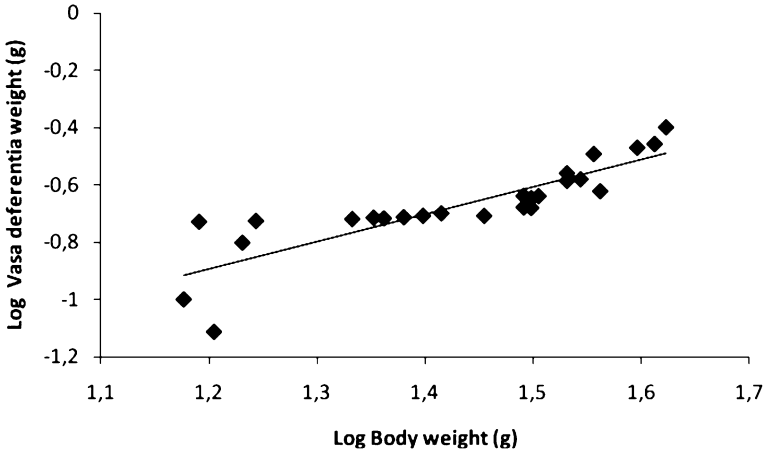


**Figure 3.** Relationship between body weight and vasa deferentia weight of *A. leptodactylus*.

sperm number ($4 \times 10^8$) was obtained from 42 mm carapace crayfish. The highest sperm number ($8.5 \times 10^9$) was obtained from 50 mm carapace crayfish. The relationship between carapace length and sperm number of crayfish is presented in fig. 4. Sperm count was weakly correlated to carapace length ($P > 0.05$). The relationship between body weight and sperm number of crayfish is shown in fig. 5.

The results also showed that in general an increase in body weight causes an increase in sperm number. For example, mean sperm number was $3.87 \pm 2.87 \times 10^9$ for the weight range of 15-24 g crayfish, $5.01 \pm 2.62 \times 10^9$ for the weight range of 25-35 g crayfish and $7.37 \pm 2.87 \times 10^9$ for the weight range of 36-42 g crayfish.

In the present study, it was determined that GI varied from 1.10 to 2.04 with a mean index of $1.41 \pm 0.21$. In addition, TI varied from 0.49 to 0.83 with a mean index of $0.60 \pm 0.08$.

**Table 1.**
Statistical parameters of the regression analysis for the effect of length in the reproductive performance of *Astacus leptodactylus* (sperm count (number: $\times 10^9$ $mL^{-1}$), reproductive system weight, testes weight and vas deferens weight, gonado-somatic index and testicular index).

| Variable | $F$ | $P$ | $r^2$ |
|---|---|---|---|
| Reproductive system weight | 256.855 | 0.00 | 0.918 |
| Testes weight | 355.544 | 0.007 | 0.939 |
| Vas deferens weight | 194.493 | 0.000 | 0.849 |
| GI | 1.042 | 0.422 | 0.043 |
| TI | 1.393 | 0.523 | 0.319 |

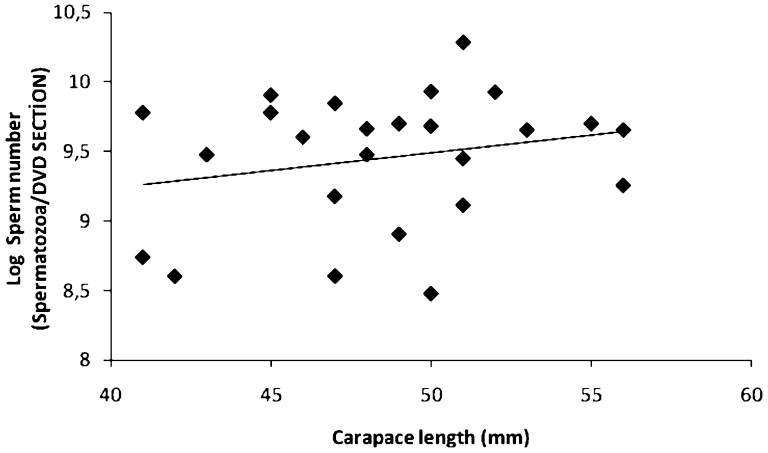


**Figure 4.** Relationship between carapace length and sperm number of *A. leptodactylus.*

## Discussion

Although sperm number is one of the most important sperm quality parameters, there is a lack of information in the literature on the sperm number of crayfish species. To the best of our knowledge, except Bugnot and López Greco's studies (2009a, 2009b) on *Cherax quadricarinatus*, no other research has investigated the relationship of sperm number with body size of crayfish. Bugnot and López Greco (2009a, 2009b) observed $10 \times 10^6$-$1 \times 10^9$ sperm/DVD section in *C. quadricarinatus* which is similar to the present study.

In comparison with crayfish species, relatively more studies have been conducted on the sperm number in shrimp. The comparison of mean sperm number and body weight between crayfish and shrimp species is given in table 3.

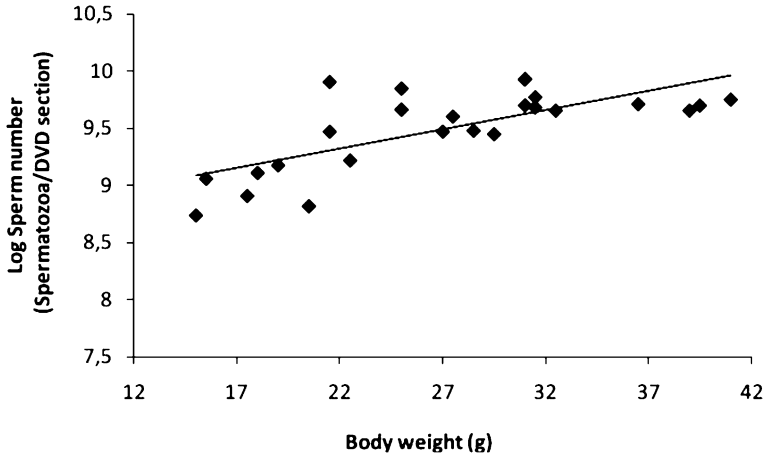


**Figure 5.** Relationship between body weight and sperm number of *A. leptodactylus.*

**Table 2.**
Statistical parameters of the regression analysis for the effect of weight in the reproductive performance of *Astacus leptodactylus* (sperm count (number: $\times 10^9$ $mL^{-1}$), reproductive system weight, testes weight and vas deferens weight, gonado-somatic index and testicular index).

| Variable | $F$ | $P$ | $r^2$ |
|---|---|---|---|
| Reproductive system weight | 110.286 | 0.035 | 0.800 |
| Testes weight | 151.941 | 0.025 | 0.866 |
| Vas deferens weight | 88.891 | 0.025 | 0.735 |
| GI | 3.536 | 0.289 | 0.133 |
| TI | 2.082 | 0.534 | 0.475 |

Nevertheless, only a few studies have examined the male reproductive system and sperm production in crayfish (López Greco et al., 2007; López Greco and Lo Nostro, 2008; Bugnot and López Greco, 2009b; Erkan et al., 2009). For example, in a study on the sperm production in the red claw crayfish (*Cherax quadricarinatus*), Bugnot and López Greco (2009a) stated that male size has important effects on sperm production and the structure of the reproductive system. They also stated that sperm number in *C. quadricarinatus* showed a significant regression with body length. In contrast with this result, we found that sperm numbers are weakly related to male length. However, in the present study, sperm number in *A. leptodactylus* presented a significant regression to body weight.

It has been found that within a species, sperm numbers were high in the whole range of evaluated size compared to the female's fecundity (Jones, 1997; Bugnot

**Table 3.**
Comparison of mean sperm number and body weight between crayfish and shrimp species.

| Species | Sperm number (sperm/DVD section) | Body weight (g) | Researcher |
|---|---|---|---|
| *Cherax quadricarinatus* | 10 000 000-1 000 000 000 | 8.55-270.64 | Bugnot and López Greco (2009a) |
| *Cherax quadricarinatus* | $1.77 \pm 0.4 \times 10^8$ | – | Bugnot and López Greco (2009a) |
| *A. leptodactylus* | $3.87 \pm 2.87 \times 10^9$ | 15-24 | This study |
| | $5.72 \pm 4.54 \times 10^9$ | 24-35 | |
| | $7.37 \pm 2.87 \times 10^9$ | 36-42 | |
| *Litopenaeus vannamei* | $1.04\text{-}4.57 \times 10^6$ | 24.7-38.0 | Ceballos Vázquez et al. (2003) |
| *Litopenaeus vannamei* | $18.6 \times 10^6$ | – | Perez Velazquez et al. (2001) |
| *Penaeus muelleri* | $2.22\text{-}5.62 \times 10^6$ | 5-20 | Díaz et al. (2001) |
| *Farfantepenaeus paulensis* | $1.23\text{-}2.27 \times 10^6$ | – | Peixoto et al. (2004) |
| *Litopenaeus vannamei* | $1.5\text{-}1.9 \times 10^6$ | – | Alfaro and Lozano (1993) |
| *Penaeus monodon* | $2.48 \times 10^6$ | 61-90 | Pratoomchat (1993) |

**Table 4.**
Comparison of sperm number, reproduction system weight, testes weight and vas deferens weight between *C. quadricarinatus* and *A. leptodactylus*.

| | Sperm count (sperm/DVD section) | Reproduction system weight (mg) | Testes weight (mg) | Vas deferens weight (mg) |
|---|---|---|---|---|
| *C. quadricarinatus* | | | | |
| Summer | $4.05 \pm 3 \times 10^8$ | $83.6 \pm 35.9$ | $7.2 \pm 2.1$ | $76.3 \pm 34.3$ |
| Autumn | $1.88 \pm 0.3 \times 10^8$ | $42.1 \pm 19.4$ | $9.4 \pm 3.4$ | $32.7 \pm 18.3$ |
| Winter | $1.77 \pm 0.4 \times 10^8$ | $58.2 \pm 12.8$ | $13.7 \pm 3.6$ | $44.5 \pm 10$ |
| Spring | $1.63 \pm 0.4 \times 10^8$ | $47.5 \pm 10.6$ | $9.3 \pm 2.8$ | $38.2 \pm 9$ |
| *A. leptodactylus* | | | | |
| Winter | $5.72 \pm 4.54 \times 10^9$ | $392.5 \pm 11.26$ | $167.4 \pm 4.05$ | $225.1 \pm 7.25$ |

et al., 2009a). Similarly, our results also indicated that sperm numbers in *A. leptodactylus* were high (up to $8.5 \times 10^9$ sperm/DVD section) compared to the female's fecundity of 200 to 400 eggs per spawning (Köksal, 1988; Harlıoğlu and Türkgülü, 2000).

Dissimilar reproduction system weight, testes weight and vasa deferentia weight were found for *C. quadricarinatus* in different seasons by Bugnot and López Greco (2009a). Those of *A. leptodactylus* observed in the present study are relatively higher (table 4) than the findings of Bugnot and López Greco (2009a). This difference may be due to the sample size and weight of the crayfish species.

In conclusion, this study represents data on the male reproduction characters (sperm number, reproductive system weight, testes weight, vasa deferentia weight, gonado-somatic index and testicular index) of wild caught *A. leptodactylus*. We expect that this information will help to understand and improve reproductive performance of males (i.e., sperm number and quality) in order to reach a maximum reproductive capacity in captivity. Therefore, it will now be interesting to investigate whether the sperm number of this species can be increased by feeding under controlled conditions.

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