Nauplius
THE JOURNAL OF THE BRAZILIAN CRUSTACEAN SOCIETY

e-ISSN 2358-2936
www.scielo.br/nau
www.crustacea.org.br

ORIGINAL ARTICLE

# Sperm viability in wild-caught males of *Macrobrachium tenellum* (Smith, 1871) (Decapoda: Caridea: Palaemonidae) fed with different diets

Omar Alejandro Peña-Almaraz[1,2] orcid.org/0000-0003-3223-6598
Edilmar Cortés-Jacinto[3] orcid.org/0000-0002-6098-0640
Fernando Vega-Villasante[1] orcid.org/0000-0003-4208-265X
G. Fabiola Arcos-Ortega[3] orcid.org/0000-0002-6098-3569
Daniel Badillo-Zapata [1,4] orcid.org/0000-0003-0522-1163
Manuel Alejandro Vargas-Ceballos[1,3] orcid.org/0000-0002-3999-3965

1 Laboratorio de Calidad de Agua y Acuicultura Experimental, Universidad de Guadalajara. Av. Universidad 203, Delegación Ixtapa, 48280 Puerto Vallarta, Jalisco 48280, Mexico.
OAPA E-mail: mcomr.tc@gmail.com
FVV E-mail: fernandovega.villasante@gmail.com
DBZ E-mail: danielbad00@hotmail.com
MAVC E-mail: m.alejandrovargas.ceballos@gmail.com

2 Universidad de Guadalajara, Centro Universitario de la Costa, Programa de Doctorado BEMARENA. Puerto Vallarta, Jalisco, México.

3 Programa de Acuicultura, Centro de Investigaciones Biológicas del Noroeste, S.C. A. Instituto Politécnico Nacional, 195, La Paz, Baja California Sur 23096, Mexico.
ECJ E-mail: ecortes04@cibnor.mx
GFAO E-mail: farcos04@cibnor.mx

4 Cátedras CONACyT, Consejo Nacional de Ciencia y Tecnología. Ciudad de México, México.

ZOOBANK: http://zoobank.org/urn:lsid:zoobank.org:pub:A6F241B6-735B-484F-AB9C-476A8177CAD3

## ABSTRACT

The relationship of body weight and length with sperm load and spermatophore weight was evaluated in wild males of *Macrobrachium tenellum* (Smith, 1871). In addition, the influence of a commercial and natural feed on the load and viability of sperm was determined in wild males. In 28 wild males spermatophore extraction was performed by electrostimulation 24 h after capture to analyze the relationship between body length/weight and sperm load and viability, respectively. Spermatophore weight (SW), total number (TS), dead (DS) and abnormal sperm cells (AS) were used as indicators of sperm viability. The results obtained show that there is a positive correlation between TS per spermatophore compared to body length and weight of the male. To study the effect of different diets on sperm load, three diets were

Corresponding Author
Manuel Alejandro Vargas-Ceballos
m.alejandrovargas.ceballos@gmail.com

SUBMITTED 17 November 2020
ACCEPTED 28 September 2021
PUBLISHED 11 April 2022

DOI 10.1590/2358-2936e2022007

employed: Diet A, commercial shrimp feed; Diet B, natural feed (mixture of squid, sardine and clam); and Diet C, combination of natural and commercial diet. To assess the initial reproductive status of the males at the beginning of the experiment, sperm viability was determined in a subsample of specimens after capture. Broodstock were fed *ad libitum* once a day during 70 days. TS, DS, AS and SW were evaluated. The results of the bioassay did not show differences between treatments in the parameters analyzed, except DS, which was higher in the initial group. In conclusion, the present work suggests that it is feasible to substitute fresh feed for an artificial diet for the maintenance in captivity of male *M. tenellum* broodstock shrimp.

## Keywords

Broodstock, nutrition, reproductive biology, shrimp, spermatophore

## Introduction

*Macrobrachium tenellum* (Smith, 1871) is considered a species with production potential in aquaculture, due to its high population densities in natural conditions and its broad capacity to adapt and tolerate diverse environments; even compared to larger species of the genus, such as *Macrobrachium americanum* (Spence Bate, 1868) and *Macrobrachium carcinus* (Linnaeus, 1758) (García-Guerrero *et al.*, 2013). *Macrobrachium tenellum* is exploited by artisanal fishers for self-consumption and/or for sale in local markets (García-Guerrero *et al.*, 2013). Several studies have focused on its biology, ecology (*e.g.*, Román-Contreras, 1979; 1991; Guzmán-Arroyo *et al.*, 1981; López-Uriarte *et al.*, 2020) and experimental culture (Vega-Villasante *et al.*, 2011; López-Uriostegui *et al.*, 2014; Peña-Herrejón *et al.*, 2019). It has omnivorous and detritivorous habits with a wide trophic spectrum; consumption of insects, larvae, small crustaceans and mollusks, annelids, organic detritus, remains of organisms and seeds (Espinosa-Chaurand *et al.*, 2011). However, despite its economic importance the main limitation to its production in commercial aquaculture is the lack of information on the reproductive cycle of this species under controlled conditions, due to the paucity of studies related to broodstock nutrition, which influences gonadic maturation, sperm viability, and larval quality (Pérez-Velázquez *et al.*, 2002; Coman *et al.*, 2007; Sui *et al.*, 2011).

Nutrition is one of the most important factors in crustacean growth (D'Abramo *et al.*, 1997; Mendez-Martinez *et al.*, 2017). In the case of decapods, most research analyzing the effect of different feeds and nutrients on sperm quality and viability has been conducted on penaeid shrimp species, due to their commercial importance (Coman *et al.*, 2007; Braga *et al.*, 2010; Leelatanawit *et al.*, 2014). For example, Leelatanawit *et al.* (2014) reported that sand polychaetes improved sperm weight and sperm count in *Penaeus monodon* (Fabricius, 1798), whereas percentage of abnormal sperm cells and percentage of acrosome reaction of the polychaete-fed group were significantly lower and higher than those fed only with pellets, respectively. On the other hand, in the freshwater crayfish *Astacus leptodactylus* (Eschscholtz, 1823) different rates of long chain n-3 polyunsaturated fatty acid significantly increase the sperm number (Harlıoğlu *et al.*, 2013). However, usually penaeid broodstock are fed fresh feed (mollusks, fishes, crustaceans, marine polychaetes) with or without the addition of a commercial diet to promote maturation and reproduction (Harrison, 1997; Browdy, 1998; Rothlisberg, 1998; Peixoto *et al.*, 2005; Braga *et al.*, 2010; Leelatanawit *et al.*, 2014).

There is a limited number of studies on the reproductive performance of species of *Macrobrachium* (Spence Bate, 1868), and the vast majority of these studies are focused on females, limiting the role of the male only to egg fertilization (Cavalli *et al.*, 1999; Cavalli *et al.*, 2001; Asusena *et al.*, 2012; Olele *et al.*, 2012; Rashmi and Pandey, 2014). *Macrobrachium rosenbergii* (De Man, 1879) is the most studied species in aspects of culture and diet in broodstock of both sexes (Cavalli *et al.*, 1999; Marques *et al.*, 2000; Sagi and Aflalo, 2005; Nhan *et al.*, 2009). On the other

hand, the work of Pérez-Rodríguez *et al.* (2019) is the most important precedent for the present study, since they studied sperm viability and regeneration of the *M. americanum* spermatophore, a species native to basins of the Pacific region, with a similar distribution to *M. tenellum* (from Baja California, Mexico to northern Peru) (Holthuis, 1952). These authors did not find significant differences in the mean biochemical composition, weight and sperm count of the spermatophores of *M. americanum* among diets supplied (*i.e.*, fresh feed, commercial feed and a mixture of both diets).

Methods for evaluating sperm quality consist of determining the morphology, counting the number of sperm cells with the aid of optical microscopy, and the weight and regeneration of spermatophores (Coman *et al.*, 2007; Leelatanawit *et al.*, 2014; Harlıoğlu and Farhadi, 2017). The most widespread technique for spermatophore extraction in decapods is electrostimulation, since it is fast and can provide complete expulsions (Goldberg and Oshiro, 2000; Viana da Costa *et al.*, 2016). This technique was first used in *M. rosenbergii* (see Harris and Sandifer, 1986), however, the use of electrostimulation may be a limiting factor for subsequent sperm extractions (Baskar *et al.*, 2004), since there is evidence that it causes melanization of reproductive tissue (Harris and Sandifer, 1986). Melanization of reproductive tissue can cause a decrease in the number of sperm cells in new spermatophores (Rosas *et al.*, 1993; Nakayama *et al.*, 2008).

Most studies on the nutritional requirements for optimal sexual maturation in marine and freshwater shrimp are focused on females (Samuel *et al.*, 1999; Braga *et al.*, 2010; Harlıoğlu *et al.*, 2018; Viana da Costa *et al.*, 2021). However, research is needed to analyze the effect of diet on sperm quality and viability in males. There is evidence that nutritional deficiencies may be one of the reasons for reproductive problems in males (Samuel *et al.*, 1999) that could compromise larval production (Braga *et al.*, 2010). In the present work, the influence of feed (*i.e.*, commercial marine shrimp and natural fresh) on the reproductive performance of *M. tenellum* was analyzed, as currently there are no studies existing that address this issue in wild males.

## Material and Methods

### *Capture and acclimatization of broodstock*

A total of 150 adult male *M. tenellum* were captured in the Ameca River, Jalisco, Nayarit (20°41'10.2"N 105°16'11.5"W) and in the El Ermitaño estuary located in the town of La Cruz de Loreto, Tomatlán, Jalisco (19°58'37.4"N 105°28'25.1"W), Mexico, between May and August 2019. Capture was performed with the help of cast nets, spoon nets and electro-fishing (SAMUS 725MP, Special Electronic, Bialystok, Poland).

The specimens were transported in 80 L plastic containers or a 1000 L capacity tank and provided with constant aeration during their transfer to the Water Quality and Experimental Aquaculture Laboratory (LACUIC – University of Guadalajara, Puerto Vallarta, Jalisco, Mexico). In the laboratory, they were kept in 1000 L plastic containers connected to a biofilter and exposed to a 12 h light/12 h dark photoperiod until the beginning of the experiments. A prophylactic dose of Terramycin (oxytetracycline 2 ppm for 24 h) dissolved in the water was applied to prevent bacterial infections (Ma *et al.*, 2020). During this 30-day acclimation period residues were removed with a siphon and 30 % of the water was replaced every third day and the specimens were fed with Azteca® brand shrimp feed (30 % crude protein, 5 % lipids, 12 % ash, 12 % water content). Only males used for the experiment which studied the effect of different diets on sperm viability were acclimatized. Previous to each experiment, the wet weight of each male was recorded with a digital scale (Ohaus®, model NV622, Parsippany, NJ, USA; 0.01 g accuracy) and the total body length was measured from the posterior margin of the ocular orbit to the tip of the telson using a Vernier Calliper (Gearwrench®, model 3759D, Lancaster, Pennsylvania, USA; 0.01 mm accuracy).

### *Voltage evaluation for electrostimulation*

Four treatments were evaluated with different voltages (1.5, 3.0, 6.0 and 9.0 V direct current; 10 specimens per treatment), to determine the voltage that would present the best results for spermatophore expulsion. Specimens were placed on an illuminated table with the ventral region exposed. The electrodes were placed in contact with both gonopores of the male

(at the base of the fifth pair of pereiopods, shocks of 1-s duration), until the extraction of the spermatophore was achieved (Harris and Sandifer, 1986). 6.0 and 9.0 V were the only ones that achieved ejaculation of the spermatic sac, with 3 and 9 males expelling at least one spermatophore, respectively. Therefore, it was established that the most effective voltage was 9.0 for further use in the experiment.

*Evaluation of sperm viability*

In order to evaluate sperm viability, the spermatophore was extracted by electrostimulation. The weight of each spermatophore (SW) was recorded with an analytical balance (Adam®, model NBL 214i, Kingston, Milton Keynes, U.K.; 0.0001 g accuracy) and stored in 2 mL plastic microtubes containing 0.5 mL of distilled water. These were carefully macerated using a glass tissue grinder to obtain complete release and homogenization of the sperm cells. Later, spermatic counts were performed using a Neubauer chamber (AmScope, model B020c, Irvine, CA, USA) (Viana da Costa *et al.*, 2016). The percentage of dead sperm cells was determined through staining cells with Trypan blue (solution 0.1 %) (Ceballos-Vázquez *et al.*, 2003). After 10 min, blue cells (dead sperm cells) were counted under light microscopy. Normal sperm cells appeared like an inverted-umbrella with a long spike projecting from its convex surface as was previously observed for *M. rosenbergii* (see Poljaroen *et al.*, 2010) and *M. americanum* (see Pérez-Rodríguez *et al.*, 2019). Abnormal sperm cells were distinguished from normal ones by malformed bodies or by a bent, short or missing spike (Leung-Trujillo and Lawrence, 1987). The number of sperm cells contained in each observed spermatophore (TS), as well as the percentage of abnormal sperm cells (AS) and percentage of dead sperm cells (DS) were estimated using the following formulas:

$$DS = \left(\frac{Ds}{TS}\right) \times 100$$

Where: Ds is the number of dead sperm cells counted and TS is the number of total sperm cells counted.

$$AS = \left(\frac{As}{TS}\right) \times 100$$

Where: As is the number of abnormal sperm cells counted and TS the number of total sperm cells counted (Pérez-Rodríguez *et al.*, 2019).

*Sperm viability in wild males*

Twenty-eight wild males (3.6–21.2 g body weight and 54.0–97.7 mm body length) were used for a comparative experiment between body weight, body length and sperm viability. Spermatophore extraction was carried out 24 h after capture and TS, DS and AS were estimated. Subsequently, males were divided according to their body length and body weight into small males (n = 9; 3.6–7.2 g, 54.0–67.5 mm) and large males (n = 19; 10.0–21.2 g, 60.7–97.7 mm). The large males on which the first spermatophore extraction was performed were kept in four 600 L tanks (60 days), with constant aeration, daily siphoning, replenishment of 90 % of water every 4 days and daily feeding (Azteca®) at 5 % of the biomass, in order to compare the effect of electrostimulation on a second spermatophore extraction.

*Effect of diet on sperm viability*

The Experimental Units (EUs) consisted of 48 plastic tanks each with an 80 L capacity distributed in three treatments (16 EUs per treatment). One male was confined per EU and a group of males were kept in the same conditions to replace possible deaths during the experiment. Water was maintained with constant aeration and daily 5 % water replacements after residue removal (feces and unconsumed feed) from the bottom and replacement of 100 % of water was performed every third day. During the bioassay the experimental units were exposed to a 12 h light/12 h dark photoperiod. Daily pH (Ohaus, model ST20), temperature and dissolved oxygen (YSI, model 550A, Yellow Springs Instruments, Yellow Springs, Ohio, USA) were recorded.

The following diets were evaluated: Diet A, Azteca® commercial shrimp feed; Diet B, natural feed (squid, sardine and clam at a 1:1:1 ratio) (Tab. 1); and Diet C, combination of natural and commercial diet at a 1:1 ratio. The components of the diets were quantified according to standard procedures of the Association of Official Analytical Chemists (AOAC, 1995): humidity by means of the difference of wet weight

and dry weight; ashes by means of sample calcination; proteins by the Kjeldahl method; and determination of total fats by Soxhlet extraction. The bioassay was performed on a 70-day period. To assess the initial reproductive status of the males at the beginning of the experiment, sperm viability was determined for a subsample of specimens after capture (24 h) (initial group). These data were used to determine the initial condition of the broodstock males, and are presented for comparison only. Broodstock was fed *ad libitum* (approximately 5 % of total weight) once a day at noon and additionally molts and mortality were recorded.

**Table 1.** Composition of fresh feed (squid, sardine and clam in a ratio of 1: 1: 1) and commercial diet (Azteca®) supplied to the *Macrobrachium tenellum* shrimps during the bioassay period.

| Composition | Fresh feed | Commercial feed |
|---|---|---|
| Moisture (%) | 23.84 ± 0.61 | 5.84 ± 0.15 |
| Protein total (%) | 51.67 ± 0.50 | 29.59 ± 1.29 |
| Lipids total (%) | 8.27 ± 0.20 | 7.03 ± 0.29 |
| Ashes (%) | 1.98 ± 0.30 | 10.80 ± 0.83 |
| NFE* | 38.08 | 53.30 |

* Nitrogen Free Extract = 100 – (Σ Protein total + Lipids total + Ashes).

The 48 males of *M. tenellum* used in the experiment had an initial body weight range of 6.8–14.6 g (average: 9.19 ± 1.83 g). Groups of similar weights and sizes were assigned to each treatment: diet A, diet B and diet C.

### *Statistical analysis*

A Shapiro-Wilk normality ($p < 0.05$) and Levene's test of homoscedasticity ($p < 0.05$) analysis were performed on the data obtained from all measured variables. A linear regression model was used to represent the correlation of the body weight and body length of the wild males with the SW and the TS, as well as between the SW and the TS. The comparison between the body size/weight and the sperm cell load of wild specimens of *M. tenellum* was made and analyzed 24 h after its collection. A one-way ANOVA test ($p < 0.05$) was performed to determine differences between treatments. In the case of differences, a Tukey test ($p < 0.05$) was applied to determine where the differences are. Data that did not meet the assumptions of normality and homoscedasticity were analyzed using the non-parametric Kruskal-Wallis one-way test ($p < 0.05$). Since there was a significant correlation between body size and the TS, an ANCOVA ($p < 0.05$) with body weight as a covariable was also applied to compare between treatments (adjusted means were computed and compared). Data on abnormal and dead sperm cells percentages were transformed using the arcsine function prior to the above tests (Zar, 1996). All statistical analyses were performed with IBM® SPSS® Statistics software v.24.0.

## Results

### *Sperm viability in wild males*

No significant correlation was observed between body weight and SW ($R^2 = 0.0781$; Fig. 1A). Neither a significant correlation was detected between TS and SW ($R^2 = 0.094$; Fig. 1B) nor between SW and body length ($R^2 = 0.1021$; Fig. 1C). In contrast, a significant positive correlation was observed between TS and body weight ($R^2 = 0.523$; Fig. 1D). A similar positive correlation occurred between TS and body length ($R^2 = 0.5041$; Fig. 1E).

Analyzed wild shrimps were classified in three categories according to their body size and body weight as follows: small males (n = 9): 3.6–7.2 g and 54.0–67.5 mm; large males (n = 19): 10.0–21.2 g and 60.7–97.7 mm, and some second spermatophore extraction large males surviving the first extraction after 60 days (n = 12), with ranges of 12.8–24.4 g, 76.6–101.6 mm (Tab. 2).

There were no significant differences in SW (ANOVA, $F = 0.59$, df = 1, $p > 0.05$) and AS (ANOVA, $F = 0.31$, df = 1, $p > 0.05$) between the small and large male groups. However, TS (ANOVA, $F = 24.00$, df = 1, $p < 0.05$) and DS (ANOVA, $F = 15.40$, df = 1, $p < 0.05$) were different, with a higher amount in the large male group, for both parameters. However, ANCOVA with body weight as the covariable did not detect differences in TS (ANCOVA, $F = 1.06$, df = 1, $p > 0.05$) (Tab. 2).

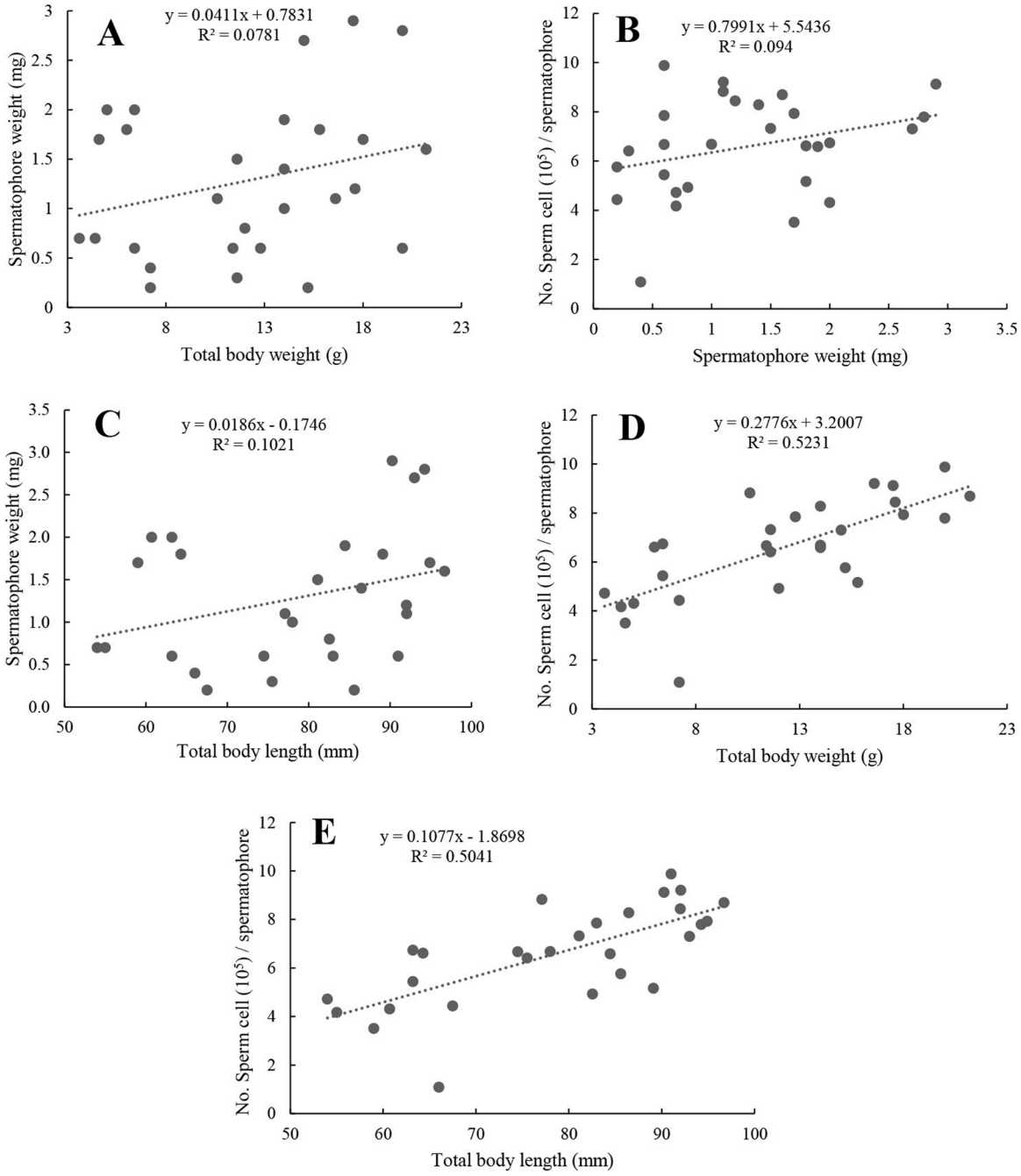


**Figure 1.** Relationship between (**A**) body weight and spermatophore weight, (**B**) sperm cell number and spermatophore weight, (**C**) spermatophore weight and body length, (**D**) sperm cell number and body weight, and (**E**) sperm cell number and body length of wild *Macrobrachium tenellum*, analyzed after collection.

Between the large males and second extraction large males groups, there was no significant difference in SW (ANOVA, $F = 1.99$, df = 1, $p > 0.05$), however, TS was different (ANOVA, $F = 40.61$, df = 1, $p < 0.05$), with a higher amount reported in the large males and a lower amount in the large males group to which a second spermatophore extraction was performed (Tukey, $q = 3.05$, $p < 0.05$). With respect to DS and AS, the treatment with the significantly highest count was the second spermatophore extraction of large males, with 15.30 % (ANOVA, $F = 11.61$, df = 1, $p < 0.05$) and 8.30 % (ANOVA, $F = 16.43$, df = 1, $p > 0.05$), respectively. The same result was obtained when an ANCOVA was done (Tab. 2).

**Table 2.** Comparison of sperm viability indicators in wild males of *Macrobrachium tenellum*: small males vs large males vs large males second spermatophore extraction after 60 days (mean ± standard deviation).

| Parameters | Small males | Large males | Large males, second spermatophore extraction |
|---|---|---|---|
| Body weight (g) | 5.64 ± 1.29$^{a}$ | 15.21 ± 3.20$^{bA}$ | 16.77 ± 3.80$^{A}$ |
| Body length (mm) | 61.43 ± 4.68$^{a}$ | 86.40 ± 6.93$^{bA}$ | 88.45 ± 8.21$^{A}$ |
| Spermatophore weight (mg) | 1.12 ± 0.74$^{a}$ | 1.35 ± 0.81$^{aA}$ | 2.26 ± 2.61$^{A}$ |
| | (1.69 ± 0.43)$^{a}$ | (1.53 ± 0.33)$^{aA}$ | (1.97 ± 0.47)$^{A}$ |
| Sperm cells / spermatophore ($10^{5}$) | 4.56 ± 1.70$^{a}$ | 7.52 ± 1.39$^{bA}$ | 4.12 ± 1.54$^{B}$ |
| | (5.77 ± 0.81)$^{a}$ | (6.95 ± 0.45)$^{aA}$ | (4.02 ± 0.41)$^{B}$ |
| Dead sperm cells (%) | 4.00 ± 2.45 | 10.40 ± 4.53 | 15.30 ± 2.66 |
| Arcsine transformed | 4.01 ± 2.00$^{a}$ | 10.37 ± 4.40$^{bA}$ | 15.33 ± 2.57$^{B}$ |
| | (3.50 ± 2.30)$^{a}$ | (10.70 ± 1.30)$^{bA}$ | (15.40 ± 1.10)$^{B}$ |
| Abnormal sperm cells (%) | 4.30 ± 2.35 | 4.80 ± 1.72 | 8.30 ± 3.11 |
| Arcsine transformed | 4.33 ± 2.33$^{a}$ | 4.74 ± 1.69$^{aA}$ | 8.33 ± 3.08$^{B}$ |
| | (4.10 ± 1.10)$^{a}$ | (4.88 ± 0.69)$^{aA}$ | (8.40 ± 0.59)$^{B}$ |

Since body weight was used as a covariable, the adjusted means are shown in parenthesis. Superscripts with different letters show significant differences ($p < 0.05$) between small males and large males (lowercase letters) and between large males and second extraction large males (uppercase letters). For percentage values, the first row corresponds to untransformed values, and the second and third rows correspond to arcsine transformed values analyzed by ANOVA or ANCOVA, respectively.

*Effect of diet on sperm viability*

Spermatophore extraction was possible in 8 shrimps fed with diet A, 13 males of diet B, 9 of diet C and in 16 recently collected wild shrimps (initial group). There were significant differences in the DS (ANCOVA, $F = 5.43$, df = 3, $p < 0.05$), which was different in the initial group with 8.32 % compared to diet A with 3.00 %, but there were no differences between the three experimental diets tested. No differences were found in TS (ANCOVA, $F = 0.92$, df = 3, $p > 0.05$), AS (ANCOVA, $F = 0.49$, df = 3, $p > 0.05$), SW (ANCOVA, $F = 1.27$, df = 3, $p > 0.05$) between the three experimental treatments and the initial group (Tab. 3). At the end of the bioassay there were no significant differences in body weight and size gain of shrimps from the different diets used (Kruskal-Wallis, $H = 2.84$; $p > 0.05$) (Tab. 3). Water quality parameters remained relatively constant during the experimental period of 70 days in all treatments. Temperature was between 27.0–30.0 °C, pH between 7.3–8.3 and dissolved oxygen ranged from 4.9–6.0 mg/L.

*Effects of electrostimulation*

Although effective, 9.0 V electrostimulation is counterproductive in the long term for the health of the male shrimp. Nineteen wild males of 10.0–21.2 g and 60.7–97.7 mm were electrostimulated. Sixty-three percent of survival (only 12 survivors) occurred after 60 days of captivity, after which a second spermatophore extraction was made. Melanization was present in one of the spermatophores of a stimulated male (Fig. 2). This spermatophore presented a 98 % mortality of the contained sperm cells.

## Discussion

*Sperm viability in wild males*

In general, the lowest values of sperm viability (less TS, higher DS and AS) were found in the group of wild specimens that were electrostimulated for the second time compared to the first electrostimulation. This suggests that repeated electrostimulation in *M. tenellum* is counterproductive in the long term, as has been reported in other species of the genus: *M. americanum* (see Pérez-Rodríguez *et al.*, 2019), *Macrobrachium malcolmsonii* (Milne-Edwards, 1844) (see Samuel *et al.*, 1999), *M. rosenbergii* (see Harris and Sandifer, 1986), as well as in marine shrimp (Rosas *et al.*, 1993). In all cases a decrease in sperm viability is mentioned when comparing the first extraction with subsequent ejaculations. In the case of *M. malcolmsonii*, a significant decrease in sperm cells count is reported after the third week of electrostimulation, reaching zero at week seven and eight (Samuel *et al.*, 1999).

The AS only differed in males in which a second spermatophore extraction was performed, showing 8 % of sperm cells with abnormal morphology, compared to the two remaining groups. In these latter groups, the percentage did not vary from an average of 4 %. This differs from what was reported in the

**Table 3.** Initial and final values of body weight, body length and sperm viability values in *Macrobrachium tenellum* fed with three different diets over a period of 70 days and an initial group of newly collected males (mean ± standard deviation).

| Parameters | Diet A | Diet B | Diet C | Initial group |
|---|---|---|---|---|
| Initial body weight (g) | 9.29 ± 1.91 | 9.16 ± 1.99 | 9.12 ± 1.76 | 10.00 ± 2.46 |
| Final body weight (g) | 10.23 ± 2.03 | 10.12 ± 2.50 | 9.91 ± 2.03 | N/A |
| Weight gain (g) | 0.80 ± 0.50 | 0.85 ± 0.50 | 0.80 ± 0.48 | N/A |
| Initial body length (mm) | 71.10 ± 4.49 | 71.15 ± 4.44 | 71.35 ± 4.50 | 72.46 ± 7.68 |
| Final body length (mm) | 73.93 ± 4.51 | 73.76 ± 5.39 | 72.03 ± 4.07 | N/A |
| Length gain (mm) | 2.83 ± 1.91 | 2.60 ± 1.87 | 1.32 ± 0.90 | N/A |
| Spermatophore weight (mg) | 1.73 ± 1.73<br>(1.74 ± 0.36) | 0.89 ± 0.74<br>(0.90 ± 0.28) | 1.07 ± 0.94<br>(1.05 ± 0.34) | 1.01 ± 0.67<br>(1.00 ± 0.25) |
| Sperm cells ($10^5$) / spermatophore | 7.31 ± 4.13<br>(7.21 ± 1.08) | 6.51 ± 2.87<br>(6.40 ± 0.85) | 5.01 ± 3.41<br>(5.15 ± 1.02) | 5.29 ± 2.26<br>(5.35 ± 0.76) |
| Dead sperm cells (%)<br>Arcsine transformed | 3.00 ± 1.19<br>3.01 ± 1.19[a]<br>(3.20 ± 1.20[a]) | 4.53 ± 1.71<br>4.50 ± 1.72[ab]<br>(4.75 ± 0.94)[ab] | 4.30 ± 1.57<br>4.31 ± 1.57[ab]<br>(4.03 ± 1.14)[ab] | 8.32 ± 5.39<br>8.35 ± 5.42[b]<br>(8.23 ± 0.85)[b] |
| Abnormal sperm cells (%)<br>Arcsine transformed | 5.00 ± 1.06<br>5.10 ± 1.07<br>(4.95 ± 0.70) | 5.80 ± 1.99<br>5.76 ± 2.00<br>(5.71 ± 0.55) | 6.00 ± 2.08<br>5.97 ± 2.09<br>(6.03 ± 0.66) | 4.58 ± 2.17<br>4.56 ± 2.19<br>(4.61 ± 0.50) |
| Electrostimulated males | 8 | 13 | 9 | 16 |

A= pellet; B= fresh feed; C= mix pellet and fresh feed; Initial group= newly collected and analyzed males. Since body weight was used as a covariable, the adjusted means are shown in parenthesis. Superscripts with different letters show significant differences ($p < 0.05$). For percentage values, the first row corresponds to untransformed values, and the second and third rows correspond to arcsine transformed values analyzed by ANOVA or ANCOVA, respectively.

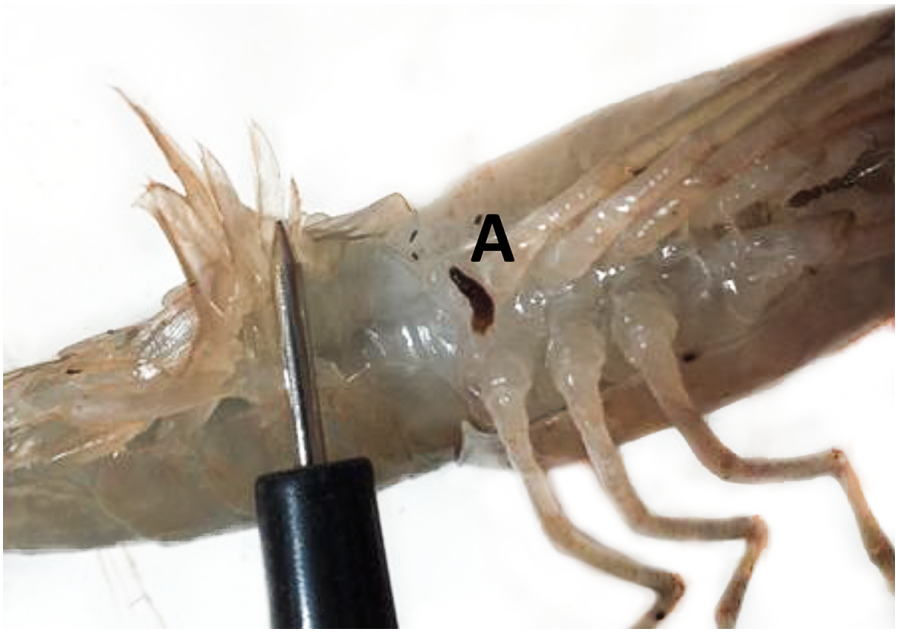


**Figure 2.** *Macrobrachium tenellum* adult male who underwent a second spermatophore extraction using the electrostimulation technique. A= The dark brown, melanized spermatophore is different from that observed in healthy males.

marine shrimp *Litopenaeus vannamei* (Boone, 1931), in which the proportion of sperm cells with abnormal morphology is less as the weight of the broodstock increases (Ceballos-Vazquez *et al.*, 2003). In large males (10.0–21.2 g) a higher DS and a higher TS were observed compared to small males (3.6–7.2 g). This indicates that, although there is a higher number of dead sperm cells in larger shrimp, this is compensated for by the higher TS. A higher DS in large males in *M. tenellum* could signify a process of reproductive senecence, as reported for *Austropotamobius italicus* (Lereboullet, 1858) (see Rubolini *et al.*, 2006; 2007).

For *M. rosenbergii*, Daniel *et al.* (2010) mentioned that older males might provide less viable sperm for mating and that the reproductive capacity decreased over time. There is evidence that sperm quality and production decrease with age in various taxa (Wolf *et al.*, 2000; Kidd *et al.*, 2001; Schäfer and Uhl, 2002). However, more studies are needed to corroborate this hypothesis. It has been observed in other decapods that females tend to select males of equal or greater size than themselves for mating (Robertson and Butler, 2013). However, when large males are not available, females mate with smaller males, which may result in mating difficulty, increased mating time, and reduced fertility rates (Daniels *et al.*, 2010; Robertson and Butler, 2013). In this study, the minimum size of sexual maturity for males was not assessed; however, the smallest male that had its spermatophore successfully retrieved presented a body weight of 3.6 g and a body length of 54.0 mm. The reduced AS present in small males of *M. tenellum*, could indicate the possibility of successful oocyte fertilization without any problems. On the other hand, Sato *et al.* (2008) mentioned that the presence of spermatophores in the *vas deferens* is an indicator of sexual maturity in decapods, which infers a physiological maturity, however, it does not always imply that such a male is sexually active. Therefore, it is important to evaluate in future research work the effectiveness of reproduction in small *M. tenellum* males.

The low TS found in *M. tenellum* (compared to other species of the same genus), and the low percentage of dead and abnormal cells, suggests that the viability of the sperm cells, and not the quantity, exerts a greater influence on the reproductive success of the species. The maximum number of eggs reported for females of this species is 10,384 (Vargas-Ceballos *et al.*, 2018). Therefore, the sperm cells values found in this study exceed, at least 40 times, the number of eggs, without taking into account that the counts were made with only one spermatophore, so the total number of sperm cells that could be delivered in copulation could be double.

As for the relationship between the body length and weight of wild broodstock males and sperm cells viability, it was determined that there is no trend in the indicators of spermatophore weight and body weight, nor TS and SW against body length. Similarly, for example in *Macrobrachium acanthurus* (Wiegmann, 1836) the relationship between SW and body weight is low ($p > 0.05$) (Viana da Costa *et al.*, 2016), verifying that large shrimp do not necessarily produce heavier spermatophores. Likewise, no differences in SW and AS in relation to the body weight of *M. americanum* were found (Pérez-Rodríguez *et al.*, 2019). On the other hand, in the present work, the TS compared to the length and weight of the male were the indicators that presented a positive correlation, equal to what was mentioned for *M. americanum* (see Pérez-Rodríguez *et al.*, 2019). Different studies with other decapods reported that in general, larger males have a higher reproductive performance (SW, TS, AS and live sperm percentage), compared to smaller males in marine shrimp (Alfaro, 1993; Ceballos-Vazquez *et al.*, 2003), crayfish (Bugnot and López-Greco, 2009; Harlıoğlu *et al.*, 2012; Farhadi *et al.*, 2019) and crabs (Sato *et al.*, 2008; Sato *et al.*, 2010). However, Paschoal and Zara (2018) stated that sperm cell production was not correlated with somatic growth in *Macrobrachium amazonicum* (Heller, 1862), occurring independently of any body size increase. This is not the case for *M. tenellum*, at least in sperm cells per spermatophore, showing more sperm cells in specimens with greater body weight and length.

*Effect of diet on sperm viability*

One of the two possible spermatophores was extracted in 75 % of the cases from any one male. Only in two cases simultaneous expulsion of both spermatophores occurred, and it was not possible to extract spermatophores from all the specimens that were electrostimulated. The reason why the electrostimulation was not successful was only evident in two cases, one in which the shrimp presented a premolt state and another in which the shrimp had just concluded ecdysis, with an exoskeleton in the process of calcification. The life of crustaceans is based around the molt cycle (Vega-Villasante *et al.*, 2007) but molting is not a physiologically benign process and so it deeply affects the life of decapods (Yamasaki-Granados *et al.*, 2012). Pre and post-molting specimens are therefore in a metabolic state that limits their normal

processes and gives energetic priority to the molting development or the recovery of the exoskeleton, not to sperm cells production. In *L. vannamei*, Parnes *et al.* (2006) mentioned that during pre-molting the spermatophore is degraded by alteration of the extracellular matrix and phagocytosis of sperm cells so that during molting the spermatophore is not present.

The average SW (1.17 ± 0.38 mg) did not vary with the treatments of the experimental specimens nor in the initial group specimens. The proportion of SW of the total male body weight was 0.01 % (males average body weight: 9.94 ± 0.14 g). This falls within the range reported by Pérez-Rodríguez *et al.* (2019) in *M. americanum* males, with a proportion of SW with respect to body weight of 0.0005 to 0.02 % (body weight range: 31.0–314.0 g). The body weight/spermatophore weight proportion in males of *M. rosenbergii* is 0.016 % (average body weight: 23.7 ± 9.8 g; Harris and Sandifer, 1986). Similarly, the SW ratio value in *M. acanthurus* is 0.007% (average body weight: 10.63 ± 4.70 g; Viana da Costa *et al.*, 2016), which is slightly lower than in *M. tenellum*. These small differences may be due to the type of single spermatophore present in males of the genus *Macrobrachium*, which varies considerably in size and shape with each ejaculate and regeneration (Paschoal and Zara, 2018). It may also be due to the process of electrostimulation, which can cause a large percentage of incomplete or partial expulsions, generating a possible sampling error (Sandifer and Lynn, 1980; Sandifer *et al.*, 1984).

The average TS calculated in this study for the experimental diets was $6.02x10^5 \pm 7.9x10^4$, a low value compared to *M. americanum*, a species with which *M. tenellum* shares a distribution. In this former species an average of 12.1 million sperm cells per spermatophore is reported (body weight range: 31–314 g; Pérez-Rodríguez *et al.*, 2019). Other species of the genus also present higher values than those reported in *M. tenellum*. For example, $2.7x10^8$ sperm cells in males of *M. malcolmsonii* (average body weight: 33.21 ± 1.3 g; Samuel *et al.*, 1999), $6.0x10^6$ sperm cells (body weight: 16.0 g; Revathi *et al.*, 2012) and $6.0 \pm 4.0x10^6$ sperm cells (average body weight: 45.0 ± 4.0 g; Claudet *et al.*, 2016); both in males of *M. rosenbergii*.

The DS was considerably low, although it varied between the initial group (wild shrimp) and in the experimental diets, obtaining a higher DS in the initial group (8.3 ± 5.3 %) and a lower percentage (3.0 ± 1.1 %) in the experimental males fed the Azteca® commercial diet. The above suggests that some environmental variable at the collection site may be acting on sperm viability, since environmental stress produces a direct effect on the metabolism, which affects the flow of energy (Davis and McEntire, 2009). By providing feed to a shrimp under controlled conditions, this stress is probably reduced or lifted. On the other hand, Meunpol *et al.* (2005) described high degeneration of wild male *P. monodon* sperm cells possibly due to changes in the environment resulting from water quality at the collection site, among other things. In consecutive spermatophore extractions from *M. malcolmsonii* males (33.21 ± 1.3 g), the percentage of live sperm cells per spermatophore decreased significantly (Samuel *et al.*, 1999). Their results showed 97.5 % of live sperm cells in the first spermatophores extracted, but only 49.5 % by week four of the experiment, followed by a total absence of viable sperm during the remaining study period. Their results at first extraction are similar to those found in the present study (97 ± 1.19 %), but the DS observed by Pérez-Rodríguez *et al.* (2019) in *M. americanum* was generally higher (35 % ± 1.75 %), indicating that one third of sperm cells per spermatophore are not viable. This high proportion could be linked to the larger total number of sperm cells in this species which are up to 20 times higher in total sperm cells counts, compared to *M. tenellum*.

There were no differences in the AS of *M. tenellum* between the different experimental diets and the initial group, obtaining an average of 5.3 ± 0.7 %. The most observed abnormal sperm cell morphology was an unusual base shape, only one sperm cell with a bent spike was observed and none with an absent base or spike or with a short spike. Samuel *et al.* (1999) reported a significant increase in the AS after the third week of continuous electrostimulation, indicating an increase in abnormal cells related to the use of electrostimulation and the reproductive exhaustion it causes (Perez-Rodriguez *et al.*, 2019). The type and quality of feed are characteristics that favor sexual

maturation and sperm cells viability in decapods (Perez-Velazquez *et al.*, 2002; Meunpol *et al.*, 2005; Harlioğlu *et al.*, 2013). Commonly, a mixture of fresh feeds is used as a diet for maturation in reproductive males and to favor increased sperm cells viability (Meunpol *et al.*, 2005; Harlioğlu *et al.*, 2018). Good sperm load and viability have also been reported in the penaeid shrimp when fed with a mixture of fresh and granulated feed (Alfaro and Lozano, 1993). However, this did not occur in *M. tenellum*. Similar results to those obtained in the present investigation are mentioned by Perez-Rodriguez *et al.* (2019) for *M. americanum*. Males devote only a small portion of energy to the production of gametes and sperm load, since most of the energy is used to increase body size and develop, or maintain, large chelipeds for fighting and mate guarding (Correa and Thiel, 2003; Paschoal and Zara, 2018; Pérez-Rodríguez *et al.*, 2019). Conversely, the sperm cells viability of males of *M. malcolmsonii* can be improved by supplementing the granulated diet with natural feed (Samuel *et al.*, 1999) and these results indicate that the relationship between diet and sperm cells viability is complicated and needs further analysis.

*Effects of electrostimulation*

Electrostimulation with 9 V turned out to be the most effective way to expel male spermatophores in *M. tenellum*, although it was evident that the decrease in the sperm cells count caused after such stimulation, resulted in a 40 % sperm cells mortality and to one case of melanization in a spermatophore. Viana da Costa *et al.* (2016) reported sperm cell percentage mortality at 71.4 % in *M. acanthurus* stimulated with 4.5 V, and 42.9 % in males electrostimulated with 6.0 V after 60 days, coinciding with the mortality reported in the present study with 9.0 V. There is also evidence of melanization of reproductive tissue caused by electroejaculation in *M. rosenbergii* (Harris and Sandifer, 1986). Moreover, continuous electrostimulations caused a decrease in spermatophore size and temporary sterility in *M. carcinus* (Moreno *et al.*, 2000). Likewise, electrostimulation of males of *Litopenaeus setiferus* (Linnaeus, 1767) caused male reproductive system melanization (MRSM) and the male reproductive tract degenerative syndrome (MRTDS) (Rosas *et al.*, 1993). These conditions are both characterized by a gradual decrease in sperm count and an increase in the percentage of dead and abnormal cells (Harlioğlu *et al.*, 2018).

## Conclusion

There were no differences between body weight and size increase at the end of the experimental diets period. No significant differences were detected in sperm viability indicators using a combined diet of fresh feed mixed with commercial feed in a 1:1 ratio, compared to diets that included only commercial or fresh feed.

According to the information obtained from the electrostimulation tests, it can be concluded that more precise analyses are required to determine the optimal voltage for spermatophore extraction in *M. tenellum* males, without it considerably affecting the health of the males.

A positive correlation between the TS and the body length and body weight of wild *M. tenellum* males was detected. Larger males also contained a higher DS, and this phenomenon could be related to natural reproductive senescence of the specimens.

The present study showed that this native species, presents good sperm viability, even without a specific diet. However, we recommend that wild males of *M. tenellum* broodstock should be fed pelleted feed because it is cheaper and easier to supply than fresh feed. We expect that the information generated will help to better understand the male reproductive biology of this species and provide tools to achieve a maximum reproductive capacity in captivity.

## Acknowledgments

This research was financed by Project FC2016/2930 – Consejo Nacional de Ciencia y Tecnología (CONACyT) of ECJ. MAVC is a CONACyT postdoctoral fellow (2020-000022-01NACV-00177). OAPA thanks CONACyT for the scholarship for doctoral research.

## References


Alfaro, J. 1993. Reproductive Quality Evaluation of Male *Penaeus stylirostris* from a Grow-Out Pond. *Journal of the World Aquaculture Society*, 24: 6–11.

Alfaro, J. and Lozano, X. 1993. Development and deterioration of spermatophores in pond-reared *Penaeus vannamei*. *Journal of the World Aquaculture Society*, 24: 522–529.

AOAC. 1995. Official Methods of Analysis 16th ed. Association of Official Analytical Chemists. Arlington, VA, USA, 684p.

Asusena, A.C.J.; Carlos, S.H.J.; Arturo, F.C.J. and Genaro, D. P. 2012. The effects of eyestalk ablation on the reproductive and immune function of female *Macrobrachium americanum*. *Journal of Aquaculture Research and Development*, 3: 156.

Baskar, N.V.; Mariappan, S.; Surendraraj A. and Venkataramani, V.K. 2004. Broodstock development in freshwater prawn *Macrobrachium rosenbergii* by artificial insemination. *Indian Journal of Fisheries*, 51: 517–520.

Braga, A.L.; Nakayama, C.L.; Martins, J.G.; Colares, E.P. and Wasielesky, W. 2010. Spermatophore quality of the pink shrimp *Farfantepenaeus paulensis* (Decapoda, Dendrobranchiata) broodstock fed with different maturation diets. *Aquaculture*, 307: 44–48.

Browdy, C.L. 1998. Recent developments in penaeid broodstock and seed production technologies: improving the outlook for superior captive stocks. *Aquaculture*, 164: 3–21.

Bugnot, A.B. and López-Greco, L.S. 2009. Sperm production in the red claw crayfish *Cherax quadricarinatus* (Decapoda, Parastacidae). *Aquaculture*, 295: 292–299.

Cavalli, R.O.; Lavens, P. and Sorgeloos, P. 1999. Performance of *Macrobrachium rosenbergii* broodstock fed diets with different fatty acid composition. *Aquaculture*, 179: 387–402.

Cavalli, R.O.; Lavens, P. and Sorgeloos, P. 2001. Reproductive performance of *Macrobrachiurn rosenbergii* females in captivity. *Journal of the World Aquaculture Society*, 32: 60–67.

Ceballos-Vazquez, B.P.; Rosas, C. and Racotta, I.S. 2003. Sperm quality in relation to age and weight of white shrimp *Litopenaeus vannamei*. *Aquaculture*, 228: 141–151.

Claudet, V.P.; Narasimman, S. and Natesan, M. 2016. Effect of cryoprotectants and cooling rates on fertility potential of sperm in the giant freshwater prawn, *Macrobrachium rosenbergii* (De Man). *Animal Reproduction Science*, 171: 49–57.

Coman, G.J.; Arnold, S.J.; Callaghan, T.R. and Preston, N.P. 2007. Effect of two maturation diet combinations on reproductive performance of domesticated *Penaeus monodon*. *Aquaculture*, 263: 75–83.

Correa, C. and Thiel, M. 2003. Mating systems in caridean shrimp (Decapoda: Caridea) and their evolutionary consequences for sexual dimorphism and reproductive biology. *Revista Chilena de Historia Natural*, 76: 187–203.

D'Abramo, L.; Conklin, D. and Akiyama, D.L. 1997. Crustacean nutrition. p. 71–84. In: L.R. D'Abramo, D.E. Conklin and D.M. Akiyama (eds), Advances in World Aquaculture. Baton Rouge, Louisiana, USA, World Aquaculture Society.

Daniels, W.H.; Cavalli, R.O.; Smullen, R.P. 2010. Broodstock management. p. 40–54. In: M.B. New, W.C. Valenti, J.H. Tidwell, L.R. D'abramo, and M.N. Kutty (eds), Freshwater Prawns: Biology and Farming. Wiley-Blackwell, Oxford, England.

Davis, K. and McEntire, M. 2009. Comparison of the cortisol and glucose stress response to acute confinement among white bass, *Morone chrysops*, striped bass, *Morone saxatilis* and sunshine bass, *Morone chrysops* x *Morone saxatilis*. *Journal of the World Aquaculture Society*, 40: 567–572.

Espinosa-Chaurand, L.; Vargas-Ceballos, M.; Guzmán-Arroyo, M.; Nolasco-Soria, H.; Carrillo-Farnés, O.; Chong-Carrillo, O. and Vega-Villasante, F. 2011. Biología y cultivo de *Macrobrachium tenellum*: estado del arte. *Hidrobiológica*, 21: 99–117.

Farhadi, A.; Harlıoğlu, M.M. and Harlıoğlu, A.G. 2019. The relationship between weight and length of male broodstock with gamete production in the narrow-clawed crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823). *Aquaculture Research*, 50: 1380–1383.

García-Guerrero, M.U.; Becerril-Morales, F.; Vega-Villasante, F. and Espinosa-Chaurand, L.D. 2013. Los langostinos del género *Macrobrachium* con importancia económica y pesquera en América Latina: conocimiento actual, rol ecológico y conservación. *Latin American Journal of Aquatic Research*, 4: 651–675.

Guzmán-Arroyo, M.; Rojas, G.J.L. and González, G.L.D. 1981. Ciclo anual de maduración y reproducción del "Chacal" *Macrobrachium tenellum* y su relación con factores ambientales en las lagunas costeras de Mitla y Tres Palos, Guerrero, México (Decapoda: Palaemonidae). *Anales del Instituto de Ciencias del Mar y Limnología*, 9: 67–80.

Goldberg, R.S. and Oshiro, L.M.Y. 2000. Eficiência da eletroejaculação de morfotipos machos do camarão-de-água-doce *Macrobrachium rosenbergii*. *Revista Brasileira de Zootecnia*, 29: 1–5.

Harlıoğlu, M.M. and Farhadi, A. 2017. Factors affecting the reproductive efficiency in crayfish: implications for aquaculture. *Aquaculture Research*, 48: 1983–1997.

Harlıoğlu, M.M.; Farhadi, A. and Gür, S. 2018. Determination of sperm quality in decapod crustaceans. *Aquaculture*, 490: 185–193.

Harlioğlu, M.M.; Köprücü, K.; Harlioğlu, A.G.; Yonar, S.M.; Duran, T.Ç.; Çakmak, M.N.; Aksu, Ö.; Özcan, S.; Kutluyer, F. and Gündoğdu, H. 2013. Effect of dietary n-3 series fatty acids on sperm production in the freshwater crayfish, *Astacus leptodactylus* (Eschscholtz) (Astacidae). *Aquaculture International*, 21: 273–282.

Harlıoğlu, M.M.; Kutluyer, F. and Gür, S. 2012. An investigation on the sperm number and reproductive parameters of males in wild caught freshwater crayfish (*Astacus leptodactylus*, Eschscholtz). *Animal Biology*, 62: 409–418.

Harris, S.E.G. and Sandifer, P.A. 1986. Sperm production and the effects of electrically induced spermatophore expulsion in the prawn *Macrobrachium rosenbergii* (de Man). *Journal of Crustacean Biology*, 6: 633–647.

Harrison, K. 1997. Broodstock nutrition and maturation diets. p. 390–408. In: L.R. D'Abramo, D.E. Conklin, D.M. Akiyama (eds), Crustacean Nutrition. Advances in World Aquaculture. Baton Rouge, USA, The World Aquaculture Society.

Holthuis, L.B. 1952. A general revision of the Palaemonidae (Crustacea: Decapoda: Natatia) of the Americas. II. The subfamuly Palaemonidae. *Allan Hancock Foundation Occasional Papers*, 12: 11–132.

Kidd, S.A.; Eskenazi, B. and Wyrobek, A.J. 2001. Effects of male age on semen quality and fertility: a review of the literature. *Fertility and Sterility*, 75: 237–248.

Leelatanawit, R.; Uawisetwathana, U.; Khudet, J.; Klanchui, A.; Phomklad, S.; Wongtripop, S.; Angthoung, P.; Jiravanichpaisal, P. and Karoonuthaisiri, N. 2014. Effects of polychaetes (*Perinereis nuntia*) on sperm performance of the domesticated black tiger shrimp (*Penaeus monodon*). *Aquaculture*, 433: 266–275.

Leung-Trujillo, J.R. and Lawrence, A.L. 1987. Observations on the decline in sperm quality of *Penaeus setiferus* under laboratory conditions. *Aquaculture*, 65: 363–370.

López-Uriarte, E.; Vega-Villasante, F.; Wehrtmann, I.S.; Cortés-Jacinto, E.; García-Guerrero, M.U.; Badillo- Zapata, D.; Chong-Carrillo, O. and Vargas-Ceballos, M.A. 2020. Reproductive biology of the freshwater shrimp *Macrobrachium tenellum* (Smith, 1871) (Decapoda: Caridea: Palaemonidae) in Mexico. *Journal of Crustacean Biology*, 40: 746–755.

López-Uriostegui, F.; Ponce-Palafox, J.T.; Arredondo-Figueroa, J.L.; Benítez-Mandujano, M.A.; Gómez, M.G.U.; Vargasmachuca, S.C. and Esparza-Leal, H.M. 2014. Effect of stocking density on growth and survival of the prawn *Macrobrachium tenellum* cultured in a cage-pond system. *North American Journal of Aquaculture*, 76: 164–169.

Ma, R.; Wang, Y.; Zhao, L.; Zhou, J.; Zhao, S.; Li, X.; Dai, X. and Fang, W. 2020. Exploration of the death causes in zoea stages of *Macrobrachium rosenbergii* based on microbial diversity analysis. *Aquaculture*, 519: 734710

Marques, H.D.A.; Lombardi, J.V. and Boock, M.V. 2000. Stocking densities for nursery phase culture of the freshwater prawn *Macrobrachium rosenbergii* in cages. *Aquaculture*, 187: 127–132.

Méndez-Martínez, Y.; Yamasaki-Granados, S.; García-Guerrero, M.U.; Martínez-Córdova, L.R.; Rivas-Vega, M.E.; Arcos-Ortega, F.G. and Cortés-Jacinto, E. 2017. Effect of dietary protein content on growth rate, survival and body composition of juvenile cauque river prawn, *Macrobrachium americanum* (Bate 1868). *Aquaculture Research*. 48: 741–751.

Meunpol, O.; Meejing, P. and Piyatiratitivorakul, S. 2005. Maturation diet based on fatty acid content for male *Penaeus monodon* (Fabricius) broodstock. *Aquaculture Research*, 36: 1216–1225.

Moreno, C.A.; Graziani, C.A. and Orta, T.J. 2000. Reproducción natural y artificial del camarón de río *Macrobrachium carcinus* (L.). *Interciencia*, 25: 249–253.

Nakayama, C.L.; Peixoto, S.; Lopes, D.; Vita, G.; Krummenauer, D.; Foes, G.; Cavalli, R.O. and Wasielesky, W. 2008. Métodos de extrusão manual e elétrica dos espermatóforos de reprodutores selvagens do camarão-rosa *Farfantepenaeus paulensis* (Decapoda: Penaeidae). *Ciência Rural*, 38: 2018–2022.

Nhan, D.T.; Wille, M.; Hung, L.T. and Sorgeloos, P. 2009. Comparison of reproductive performance and offspring quality of giant freshwater prawn (*Macrobrachium rosenbergii*) broodstock from different regions. *Aquaculture*, 298: 36–42.

Olele, N.F.; Tawari-Fufeyin, P. and Okonkwo, J.C. 2012. Reproductive biology of freshwater prawn *Macrobrachium vollenhovenii* (Herklots, 1857) caught in Warri river. *Banats Journal of Biotechnology*, 3(6).

Parnes, S.; Raviv, S.; Shechter, A. and Sagi, A. 2006. Males also have their time of the month! Cyclic disposal of old spermatophores, timed by the molt cycle, in a marine shrimp. *Journal of Experimental Biology*, 209: 4974–4983.

Paschoal, L.R.P. and Zara, F.J. 2018. Sperm count of *Macrobrachium amazonicum* (Heller, 1862) populations with distinct life histories, with introduction of a simple counting method. *Aquaculture*, 491: 368–374.

Peixoto, S.; Cavalli, R.O. and Wasielesky, W. 2005. Recent developments on broodstock maturation and reproduction of *Farfantepenaeus paulensis*. *Brazilian Archives of Biology and Technology*, 48: 997–1006.

Peña-Herrejón, G.A.; Sánchez-Velázquez, J.; García-Trejo, J.F.; Soto-Zarazúa, G.M. and Rico-García, E. 2019. Effect of stocking density on growth and survival of the prawn *Macrobrachium tenellum*, cultured in a recirculating aquaculture system. *Latin American Journal of Aquatic Research*, 47: 342–348.

Pérez-Rodríguez, J.C.; Gómez-Gutiérrez, J.; López-Greco, L.S. and Cortés-Jacinto, E. 2019. Spermatophore production and sperm quality of the river prawn *Macrobrachium americanum* (Spence Bate, 1868) fed with different diets. *Aquaculture Research*, 50: 1–13.

Perez-Velazquez, M.; Lawrence, A.L.; Gatlin, D.M.; González-Félix, M.L. and Bray, W.A. 2002. Replacement of fresh dietary components by a dry feed for successful maturation of male *Litopenaeus vannamei* (Boone) broodstock. *Aquaculture Research*, 33: 1091–1095.

Poljaroen, J.; Vanichviriyakit, R.; Tinikul, Y.; Phoungpetchara, I.; Linthong, V.; Weerachatyanukul, W. and Sobhon, P. 2010. Spermatogenesis and distinctive mature sperm in the giant freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879). *Zoologischer Anzeiger*, 249: 81–94.

Rashmi, T. and Pandey, A.K. 2014. Anatomical, histological and ultrastructural studies on reproductive system of freshwater prawn, *Macrobrachium dayanum* (Crustacea: Decapoda). *Journal of Experimental Zoology, India*, 17: 631–647.

Revathi, P.; Iyapparaj, P.; Munuswamy, N. and Krishnan, M. 2012. Vitellogenesis during the ovarian development in freshwater female prawn *Macrobrachium rosenbergii* (De Man). *International Journal of Aquatic Science*, 3: 13–27.

Robertson, D. N. and Butler IV, M. J. 2013. Mate choice and sperm limitation in the spotted spiny lobster, *Panulirus guttatus*. *Marine Biology Research*, 9: 69–76.

Román-Contreras, R. 1979. Contribución al conocimiento de la biología y ecología de *Macrobrachium tenellum* (Smith) (Crustacea, Decapoda, Palaemonidae). *Anales del Instituto de Ciencias del Mar y Limnología*, 6: 137–160.

Román-Contreras, R. 1991. Ecología de *Macrobrachium tenellum* (Decapoda: Palaemonidae) en la laguna Coyuca, Guerrero, Pacífico de México. *Anales del Instituto de Ciencias del Mar y Limnología*, 18: 87–96.

Rosas, C.; Sanchez, A.; Eugenia-Chimal, M.; Saldaña, G.; Ramos, L. and Soto, L. 1993. The effect of electrical stimulation on spermatophore regeneration in white shrimp *Penaeus setiferus*. *Aquatic Living Resources*, 6: 139–144.

Rothlisberg, P.C. 1998. Aspects of penaeid biology and ecology of relevance to aquaculture: a review. *Aquaculture*, 164: 49–65.

Rubolini, D.; Galeotti, P.; Ferrari, G.; Spairani, M.; Bernini, F. and Fasola, M. 2006. Sperm allocation in relation to male traits, female size, and copulation behaviour in a freshwater crayfish species. *Behavioral Ecology and Sociobiology*, 60: 212–219.

Rubolini, D.; Galeotti, P.; Pupin, F.; Sacchi, R.; Nardi, P.A. and Fasola, M. 2007. Repeated matings and sperm depletion in the freshwater crayfish *Austropotamobius italicus*. *Freshwater Biology*, 52: 1898–1906.

Sagi, A. and Aflalo, E.D. 2005. The androgenic gland and monosex culture of freshwater prawn *Macrobrachium rosenbergii* (De Man): a biotechnological perspective. *Aquaculture Research*, 36: 231–237.

Samuel, M.J.; Kannupandi, T. and Soundarapandian, P. 1999. Nutrional effects on male reproductive performance in the freshwater prawn *Macrobrachium malcolmsonii* (H. Milne Edwards). *Aquaculture*, 172: 327–333.

Sandifer, P.A. and Lynn, J.W. 1980. Artificial insemination of caridean shrimp. p. 271-288. In: W.H Clark Jr, and H.S. Adams (eds), Recent Advances in Invertebrate Reproduction. Amsterdam, Elsevier.

Sandifer, P.A.; Lawrence, A.L.; Harris, S.A.; Chamberlain, G.W.; Stokes, D.A. and Bray, W.A. 1984. Electrical stimulation of spermatophore expulsion in marine shrimp, *Penaeus* spp. *Aquaculture,* 41: 181–187.

Sato, T.; Yoseda, K.; Abe, O. and Shibuno, T. 2008. Male maturity, number of sperm, and spermatophore size relationships in the coconut crab *Birgus latro* on Hatoma Island, southern Japan. *Journal of Crustacean Biology*, 28: 663–668.

Sato, T.; Yoseda, K.; Okuzawa, K. and Suzuki, N. 2010. Sperm limitation: possible impacts of large male-selective harvesting on reproduction of the coconut crab *Birgus latro. Aquatic Biology*, 10: 23–32.

Schäfer, M.A. and Uhl, G. 2002. Determinants of paternity success in the spider *Pholcus phalangioides* (Pholcidae, Araneae): the role of male and female mating behaviour. *Behavioral Ecology and Sociobiology*, 51: 368–377.

Sui, L.Y.; Sun, H.X.; Wu, X.G.; Wille, M.; Cheng, Y.X. and Sorgeloos, P. 2011. Effect of dietary HUFA on tissue fatty acid composition and reproductive performance of Chinese mitten crab *Eriocheir sinensis* broodstock. *Aquaculture*, 19: 269–282.

Vargas-Ceballos, M.A.; López-Uriarte, E.; García-Guerrero, M.U.; Wehrtmann, I.S.; Ríos-Jara, E. and Vega-Villasante, F. 2018. Fecundity, egg volume and reproductive output of *Macrobrachium tenellum* (Crustacea: Palaemonidae) from the northern coast of Jalisco, Mexico. *Latin American Journal of Aquatic Research*, 6: 502–511.

Vega-Villasante, F.; Cortés-Jacinto, E. and García-Guerrero, M. 2007. Contribution to the knowledge of moulting and growth of *Callinectes arcuatus* (Brachyura; Portunidae) Ordway (1863), in Baja California Sur, México. *Crustaceana*, 80: 769–778.

Vega-Villasante, F.; Martínez-López, E.A.; Espinosa-Chaurand, L.D.; Cortés-Lara, M.C. and Nolasco-Soria, H. 2011. Crecimiento y supervivencia del langostino (*Macrobrachium tenellum*) en cultivos experimentales de verano y otoño en la costa tropical del Pacífico mexicano. *Tropical and Subtropical Agroecosystems*, 14: 581–588.

Viana da Costa, T.; López-Greco, L.S.; Oshiro, L.M.Y.; Melo, E.P.; Reis, H.F. 2021. Diets for *Macrobrachium acanthurus* breeders: Sperm evaluation. *Ciência Animal Brasileira*, 21: e-60022

Viana da Costa, T.; Oshiro, L.M.Y.; López-Greco, L.; Melo, P.; Antunes de Mattos, L. and Bambozzi-Fernandes, A. 2016. Determinación del voltaje y el tamaño del animal óptimos para la extracción de espermatóforos en el camarón de agua dulce *Macrobrachium acanthurus*. *Latin American Journal of Aquatic Research*, 44: 422–428.

Wolf, K.N.; Wildt, D.E.; Vargas, A.; Marinari, P.E.; Kreeger, J.S.; Ottinger, M.A. and Howard, J.G. 2000. Age-dependent changes in sperm production, semen quality and testicular volume in the blackfooted ferret (*Mustela nigripes*). *Biology of Reproduction*, 63: 179–187.

Yamasaki-Granados, S.; Ruíz-Fregozo, M.; Vega-Villasante, F.; Espinosa-Chaurand, L.D.; Cortés-Jacinto, E. and García-Guerrero, M. 2012. Contributions to the biology of molting and growth of the longarm river prawn *Macrobrachium tenellum* (Decapoda: Palemonidae) in Mexico. *Archives of Biological Sciencies, Belgrade*, 64: 651–658.

Zar, J.H. 1996. Biostatistical Analysis, 3rd ed. Upper Saddle River, NJ, Prentice-Hall, 662p.