Short Term, Low Temperature Preservation of the Pacific Whiteleg Shrimp *Penaeus vannamei* Spermatophores

YAMILIS CATAÑO[1,*], PABLO INTRIAGO[2], ANDREW P. SHINN[3], MARTHA PRIETO-GUEVARA[4], VÍCTOR ATENCIO-GARCÍA[4]

[1]Océanos S.A. División, Laboratorio Océanos S.A, Tolú, Colombia
[2]South Florida Farming Corp., 13811 Old Sheridan St., Southwest Ranches, FL 33330, United States of America
[3]INVE (Thailand), 471 Bond Street, Nonthaburi 11120, Thailand
[4]FMVZ/DCA/CINPIC, Universidad de Córdoba, Montería, Colombia

*E-mail: yamilis.catano@oceanos.com.co | Received: 11/05/2024; Accepted: 13/01/2025

E-ISSN: 2073-3720
https://doi.org/10.33997/j.afs.2025.38.1.001

Abstract

The study aimed to assess the efficacy of short-term (≤24 d), low-temperature preservation of Pacific whiteleg shrimp (*Penaeus vannamei*) spermatophores. A total of 432 spermatophores from 216 males, averaging 35.1 ± 3.1 g in weight, were preserved in mineral oil at temperatures of 10 °C and 24 °C. Sperm viability was monitored every 2 d over 24 d, evaluating fertility percentage, hatching rate, and nauplii deformity in females artificially inseminated with the preserved spermatophores compared with naturally inseminated females. In the control group, throughout the 24-d experiment, the average fertility, hatching rate, and nauplii count per gram of female were 66 ± 7 %, 94 ± 6 %, and 4,875 ± 148 nauplii g^{-1}, respectively. Females inseminated with spermatophores stored at both temperatures exhibited a gradual decline in all measured parameters. Although spermatophores stored at 24 °C showed a linear decrease in fertility over time, this preservation technique proved to be effective for short-term storage of sperm, with good average hatching for up to 18 d at 10 °C (>50 %) and 8 d at 24 °C (>40 %). Notably, this technique has been successfully employed for the past seven years to facilitate controlled crossbreeding, mitigating the risk of pathogen contamination.

Keywords: mineral oil, fertility assessment, hatching rate, short-term viability

Introduction

Since 2003, the Pacific whiteleg shrimp *Penaeus vannamei* has emerged as the dominant species in global shrimp aquaculture, surpassing *Penaeus monodon*. This shift can be attributed to several factors, including its heightened tolerance to white spot disease, adaptability to low salinity environments, and reduced protein requirements (Thitamadee et al., 2016).

The success of this industry hinges on various factors, including effective sanitation practices and a robust seed production program. Implementing sperm preservation in shrimp, however, has posed challenges. Developing such technology is crucial for maintaining control over genetic lines and facilitating genetic improvement programs. Additionally, it could serve as a preparatory step for selecting healthy animals and enhancing genetic diversity without transporting male broodstock. This aspect is particularly important for open thelycum species like *P. vannamei*, where mating occurs after ovarian maturation (Misamore and Browdy, 1996), in contrast to closed thelycum species such as *Penaeus monodon*, where mating takes place before ovarian maturation (Primavera, 1979). Mating occurs by depositing the male spermatophore onto the thelycum surface 4–6 h prior to spawning. During this interval, the sperm may undergo morphological modifications linked to a capacitation-like process, potentially preparing them for successful fertilisation (Aungsuchawan et al., 2011). Sperm quality is a key factor influencing fertilisation success in *P. vannamei*. Conventional assessments of sperm quality typically involve sperm counts and viability. However, Duangjai et al. (2023) demonstrated that the extent of sperm DNA damage correlates

strongly with fertilisation rates and can serve as a sensitive indicator for evaluating sperm quality in broodstock shrimp.

While several attempts at cryopreservation have been made, as reported by Lezcano et al. (2004), Nimrat et al. (2005, 2006) and Castelo-Branco et al. (2015), the results have generally been unsuccessful, with viable cells lacking fertilisation capacity. However, refrigerated storage of spermatophores is a feasible approach for managing and spawning whiteleg shrimp broodstock (Nimrat et al., 2006). The advantages of refrigerated storage for spermatophores include a more reliable supply of quality spermatophores, facilitating short-term spermatophore storage and artificial insemination activities, improving hatchery management efficiency, and allowing easy transport of male gametes in specialised breeding programmes (Nimrat et al., 2006). The objective of this study was to assess the preservation of *P. vannamei* sperm at two temperatures over short periods.

Materials and Methods

Ethical approval

The practices align with ethical principles regarding the treatment of animals in commercial shrimp production and scientific research and adhere to the animal welfare regulations in Ecuador and wider. All procedures regarding the collection of spermatophores were conducted by experienced staff and additionally overseen by a veterinarian. In all cases, the handling and sacrifice of the animals was carried out following the Rules of Conduct for the Use of Animals in Teaching and Research of the Faculty of Veterinary Medicine and Animal Husbandry of the University of Córdoba, Colombia.

Selection and maintenance of broodstocks, collection of spermatophore and artificial insemination

All specimens used in this study were sourced from commercial ponds at Oceanos S.A farm in Cartagena, Colombia, with an average weight of 35 ± 3.1 g. Subsequently, the animals were transported via truck to the hatchery in Tolú, Sucre, Colombia. Upon arrival, the shrimp were acclimated in 10 m^3 tanks within a maturation unit, with natural photoperiod and recirculation, maintaining a salinity of 30 ± 1.0 ppt, temperature of 28 ± 0.5 °C, dissolved oxygen levels of 6.2 ± 0.1 mg L^{-1}, pH of 7.9 ± 0.1 adhering to procedures outlined by Intriago et al. (2012). The stocking density was maintained at 12 animals m^{-3}, with a female-to-male ratio of 1:1. Broodstocks were fed at a rate of 24 % of the shrimp biomass, divided into six equal rations d^{-1} using fresh frozen food. The fresh diet was composed of 43 % squid (*Doryteuthis opalescens*), 22 % adult *Artemia* sp., 9 % polychaetes (*Alitta virens*) and 26 % mussel (*Mytilus chilensis*). The system's daily maintenance included siphoning out feed residue once a day and conducting a water exchange equivalent to 15 % of the total volume.

Tanks were monitored daily for moulting and, females with ovaries at stage IV, characterised by green olive or dark orange ripened ovaries, were selected for artificial insemination. Spermatophores were selected based on their external characteristics, such as colour, absence of melanisation, and thickness. A total of 432 spermatophores were used from 216 *P. vannamei* males.

Spermatophores were manually ejaculated and withdrawn using forceps. For artificial insemination, females were gently restrained ventral side up, and tissue paper was delicately inserted into each side of the thelycum to absorb any fluid. A single spermatophore (one per female) was then gently squeezed and inserted into the thelycum; the spermatophore was fixed with a clamp to the thelycum. Following insemination, females were individually transferred to a 300 L spawning tank.

Experimental protocol for fertilisation and embryonic development

Ideally, embryos would have been sampled within 1 h of spawning (i.e., soon after first cleavage) to determine the relative male parental contribution as soon as possible after fertilisation. However, as the accuracy of genotyping embryos is only reliable 7 h after spawning (Rao et al., 2010), this 7 h time point was the earliest embryonic age possible post-fertilisation. Consequently, the primary assumption of the paper is that 'consistent proportions of 7-h-old embryos and 1-h-old embryos' indicate that male influence is negligible. It is noteworthy that, hereafter, embryo development rate refers to the percentage of fertilised embryos at 7-h post-spawning that hatched into nauplii (and not as typically measured from the initial first cleavage). A sample of 500 embryos collected 7-h post-spawning was used to evaluate embryo viability and estimate the fertilisation rate (Table 2). In most spawnings from wild broodstock, between 30 % and 80 % of eggs are fertilised, resulting in a standard error of the estimated proportion of approximately 2 % (binomial distribution).

Assessment of temperature regime for spermatophore preservation

Two temperatures (10 and 24 °C) were evaluated to investigate the effect of preserving spermatophores on reproductive success and assessed every 2 d until d 24. The spermatophores (2 per vial) were packed in 1.5 mL Eppendorf tubes using mineral oil as an extender (Sigma-Aldrich, USA). Spermatophores were acclimated before artificial insemination (AI) by immersing the Eppendorf tubes in a serological bath (WNB 7, Memmert, Germany) at 30 °C for one min. Before artificial insemination, spermatophores were washed with sterile seawater as a prophylactic measure.

Assessment of fecundity rate

After spawning, females were returned to the maturation tank, and eggs were collected via siphoning, washed with 100 ppm formaldehyde (37 %), and transferred to the spawning tank. Once a spawning was detected and completed, the females were immediately removed from the tank and returned to the maturation tank.

Three samples of 1 mL each were randomly taken from the pool of collected eggs (n = 100) using a graduated pipette. The number of eggs was estimated by volumetrics, with three repetitions performed per experimental unit.

Fecundity (eggs per female) was estimated by multiplying the average number of oocytes per sample by the total volume of the container. Subsequently, relative fecundity (*Fr*) was calculated based on the number of eggs laid per unit weight of the female (eggs g^{-1}).

Three samples of eggs from each individual spawn were evaluated. Egg samples (n = 100 eggs) were placed in a Petri dish and examined under a stereo microscope (Wild MZ8, Leica, Germany) 8-h after spawning, with the presence of gastrulation used to distinguish fertilised from unfertilised eggs. The proportion of successfully fertilised eggs (fertility, *F*) was calculated as follows:

$$F = Number\ of\ fertilised\ eggs - Total\ number\ of\ eggs$$

The number of eggs per spawning was estimated based on the samples count and the tank volume. The eggs were then left in the tanks to hatch. Approximately 1–2 h after the first observation of nauplii, the tanks were thoroughly mixed to ensure homogeneity of the eggs and nauplii within the tank. Subsequently, 3 × 250 mL samples were taken (Table 1). The number of nauplii per spawning was estimated from the samples count and the tank volume. The percentage of eggs that hatched was calculated by dividing the number of nauplii by the number of eggs. Deformities in nauplii were determined by examining the number of deformed appendages. The parameters (relative fecundity, fertility rate, hatching rate, hatched nauplii, deformity nauplii) evaluated in inseminations with preserved spermatophores were compared every 2 d with parameters obtained with naturally copulated females from the same breeding batches.

Statistical analyses

A completely randomised design was used. All parameters were submitted to normality (Shapiro-Wilk test) and homoscedasticity (Levene test) tests and then analysed using ANOVA, followed by Tukey's multiple range test. When the assumptions of normality and homogeneity of variance were not met, the non-parametric Kruskal-Wallis and Dunn tests were performed. In all cases, $P < 0.05$ was considered statistically significant. All results were expressed as mean ± standard deviation. All statistics tests were analysed using SAS version 9.1 for Windows (2004, SAS Institute Inc., USA).

Results

Fecundity rate vs insemination

The average fecundity rate (*Fr*) of the females ranged from 7366 ± 29.5 (10 °C, d 2) to 7435 ± 24.4 eggs g^{-1} (control, d 12) (Table 1). It was observed that on d 2 of the experiment, artificial insemination (IA) females with spermatophores at 24 °C had significantly higher mean *Fr* (7377 ± 27.1 eggs g^{-1}) than IA females with spermatophores at 10 °C (7366 ± 29.5 eggs g^{-1}) ($P < 0.05$), but not significantly different to the *Fr* of the control females (7370 ± 30.4 eggs g^{-1}). However, the *Fr* of the females used between the fourth and tenth days did not show significant differences, nor in the last four days of the experiment ($P > 0.05$).

Spermatophore quality and fertilisation potential

During the first 4 d of the experiment, fertility did not exhibit statistical differences between treatments using preserved spermatophores (10 °C and 24 °C) and natural copulation ($P > 0.05$). However, from the sixth day onwards, fertilities achieved through natural copulation exceeded those obtained with spermatophores preserved at 10 °C ($P < 0.05$), and the latter, in turn, exceeded those obtained with spermatophores preserved at 24 °C ($P < 0.05$).

The mean fertility rate (*FR*) obtained through natural copulation ranged between 50.8 ± 3.7 % (d 2) and 74.5 ± 7.0 % (d 12), with statistically significant differences between these values. Fertility obtained with spermatophores preserved at 10 °C progressively declined from d 2 (65.5 ± 7.0 %) to d 22 (4.8 ± 6.0 %), ultimately losing its fertilising capacity by d 24. Spermatophores preserved at 24 °C maintained fertility above 40 % during the first four days. However, they experienced a drastic decrease between the sixth and eighth days, reaching 15 %, and ultimately lost their fertilising capacity by d 14 (Table 2).

Hatching of post spawn embryos

On the second day of the experiment, hatching percentages ranged from 97.3 ± 5.0 % (control) to 74.3 ± 9.0 % (24 °C), with no statistically significant difference observed between these values ($P > 0.05$). Between the fourth and sixth day, hatching rates obtained with spermatophores preserved at 10 °C did not differ statistically from those of the control or those maintained at 24 °C. From days 12 to 18, no statistical difference was observed in hatching percentages between the control treatment and 10 °C ($P < 0.05$). However, from day 20 onwards, a statistical difference was observed between the hatching rates

Table 1. Mean relative fecundity (eggs g^{-1} ± SD) of Pacific whiteleg shrimp, *Penaeus vannamei*, from females inseminated with preserved spermatophores at two different temperatures and through natural copulation.

Storage days	Treatment		
	Natural mating*	10 °C	24 °C
2	7370 ± 30.4^{ab}	7366 ± 29.5^{b}	7377 ± 27.1^{a}
4	7370 ± 26.9^{a}	7369 ± 29.3^{a}	7370 ± 30.0^{a}
6	7400 ± 22.1^{a}	7399 ± 28.4^{a}	7401 ± 25.4^{a}
8	7402 ± 25.0^{a}	7400 ± 29.9^{a}	7402 ± 26.3^{a}
10	7412 ± 30.6^{a}	7411 ± 29.8^{a}	7413 ± 31.1^{a}
12	7435 ± 24.4^{a}	7431 ± 22.9^{b}	7430 ± 26.3^{b}
14	7411 ± 25.9^{a}	7396 ± 27.5^{b}	7398 ± 28.5^{b}
16	7423 ± 27.6^{a}	7416 ± 26.1^{b}	7414 ± 28.2^{b}
18	7401 ± 24.2^{a}	7401 ± 26.2^{a}	7403 ± 29.3^{a}
20	7410 ± 32.4^{b}	7418 ± 30.0^{a}	7417 ± 29.8^{a}
22	7409 ± 34.0^{a}	7408 ± 29.9^{a}	7406 ± 30.2^{a}
24	7420 ± 33.1^{a}	7423 ± 29.3^{a}	7422 ± 26.7^{a}

*is natural mating on the days that artificial inseminations were performed with preserved spermatophores.
Values with different letters in the same row indicate a statistically significant difference between treatments ($P < 0.05$).

Table 2. Mean fertility rate (% ± SD) of Pacific whiteleg shrimp, *Penaeus vannamei*, obtained through natural copulation and artificial insemination with spermatophores preserved at 10 °C and 24 °C.

Storage days	Treatment		
	Natural mating*	10 °C	24 °C
2	50.8 ± 3.7^{a,5}	65.5 ± 7.0^{a,1}	46.2 ± 5.1^{a,1}
4	65.3 ± 7.7^{a,5}	59.8 ± 9.0^{a,2}	41.7 ± 4.1^{a,1}
6	64.3 ± 7.6^{a,3}	40.5 ± 7.0^{b,3}	15.7 ± 6.6^{c,2}
8	67.2 ± 3.8^{a,3}	34.8± 10.5^{b,3}	15.5 ± 5.4^{b,2}
10	62.7 ± 5.0^{a,3}	28.8 ± 6.7^{b,4}	1.7 ± 4.1^{c,2}
12	74.5 ± 7.0^{a,1}	35.5 ± 7.0^{b,2}	2.7 ± 2.9^{c,2}
14	70.5 ± 3.9^{a,2}	31.2 ± 9.0^{b,4}	
16	58.5 ± 3.7^{a,4}	27.5 ± 9.5^{b,4}	
18	71.3 ± 7.7^{a,4}	21.2 ± 8.8^{b,4}	
20	71.1 ± 7.1^{a,2}	18.0 ± 8.0^{b,4}	
22	62.7 ± 8.0^{a,3}	4.8 ± 6.0^{b,5}	
24	70.5 ± 4.0^{a,3}		

*corresponds to natural mating on the days that artificial inseminations were performed with preserved spermatophores.
Different letters indicate statistically significant differences between columns ($P < 0.05$), and different numbers indicate a statistically significant difference between rows ($P < 0.05$).

of the control treatment (98.0 ± 8.1 %) and those obtained with spermatophores preserved at 10 °C (40.0 ± 14.0 %) ($P < 0.05$).

Hatching percentages with spermatophores preserved at 10 °C remained above 70 % until the eighth day, above 50 % until day 18, and no hatching was observed on day 24. With spermatophores preserved at 24 °C, hatching rates remained above 40 % until the eighth day, sharply declining from day 10, and no hatching was achieved from day 14 onwards. The hatching percentages of the control treatment did not exhibit statistical differences throughout the trial ($P > 0.05$), ranging between 98.0 ± 8.1 % (day 20) and 82.0 ± 4.0 % (day 24) (Table 3).

Hatched nauplii

Throughout the 24 days of the experiment, natural copulation consistently yielded higher mean numbers of nauplii per gram of female compared to those obtained with preserved spermatophores ($P < 0.05$). Nauplii per gram obtained through natural copulation ranged between 4463 ± 1040 nauplii g^{-1} (day 18) and 4998 ± 800 nauplii g^{-1} (day 12). When using spermatophores preserved at 10 °C, higher mean numbers of nauplii per gram were observed (470–4,779 nauplii g^{-1}) compared to those maintained at 24 °C (0–3,733 nauplii g^{-1}, $P < 0.05$) (Table 4). At 24 °C, no nauplii hatched after day 12 and at 10 °C none hatched after day 22 (Table 4).

Table 3. The hatching rate (% ± SD) of Pacific whiteleg shrimp, *Penaeus vannamei*, obtained through natural copulation and insemination with spermatophores preserved at 10 °C and 24 °C.

Storage days	Treatment		
	Natural mating*	10 °C	24 °C
2	97.3 ± 5.0^{a,1}	85.0 ± 9.0^{a,1}	74.3 ± 9.0^{a,1}
4	96.2 ± 6.0^{a,1}	86.5 ± 12.0^{ab,1}	41.7 ± 12.9^{b,12}
6	94.5 ± 5.0^{a,1}	82.8 ± 13.7^{ab,1}	44.5 ± 13.6^{b,123}
8	96.3 ± 5.0^{a,1}	71.0 ± 11.2^{a,12}	44.7 ± 15.4^{ab,123}
10	95.2 ± 6.0^{a,1}	53.2 ± 13.5^{ab,123}	14.2 ± 8.9^{b,23}
12	97.7 ± 7.0^{a,1}	65.5 ± 15.0^{a,12}	15.0 ± 12.9^{b,23}
14	82.0 ± 7.0^{a,1}	53.2 ± 14.0^{a,12}	
16	97.1 ± 8.0^{a,1}	54.5 ± 14.1^{a,12}	
18	97.8 ± 8.0^{a,1}	50.4 ± 14.1^{a,123}	
20	98.0 ± 8.1^{a,1}	40.0 ± 14.0^{b,123}	
22	95.2 ± 9.0^{a,1}	13.8 ± 12.0^{b,23}	
24	82.0 ± 4.0^{a,1}		

*corresponds to natural mating on the days that artificial inseminations were performed with preserved spermatophores.
Different letters indicate statistically significant differences between columns ($P < 0.05$), and different numbers indicate a statistically significant difference between rows ($P < 0.05$).

Table 4. Hatched nauplii (nauplii g^{-1} female ± SD) of Pacific whiteleg shrimp, *Penaeus vannamei*, obtained through natural copulation and insemination with spermatophores preserved at 10 °C and 24 °C.

Storage days	Treatment		
	Natural mating*	10 °C	24 °C
2	4792 ± 850^{a,8}	4779 ± 1020^{b,1}	3733 ± 1100^{c,1}
4	4799 ± 700^{a,7}	4505 ± 1100^{b,2}	2917 ± 1060^{c,2}
6	4862 ± 970^{a,6}	4128 ± 1050^{b,3}	1439 ± 1080^{c,3}
8	4951 ± 960^{a,2}	3713 ± 900^{b,4}	1089 ± 1070^{c,4}
10	4997 ± 1040^{a,1}	3272 ± 1045^{b,5}	138 ± 1100^{c,5}
12	4998 ± 800^{a,1}	2916 ± 1030^{b,7}	126 ± 150^{c,6}
14	4926 ± 1200^{a,3}	2864 ± 1050^{b,7}	
16	4887 ± 900^{a,5}	2281 ± 800^{b,8}	
18	4463 ± 1040^{a,9}	2173 ± 1300^{b,9}	
20	4907 ± 1021^{a,4}	1750 ± 1200^{b,10}	
22	4997 ± 900^{a,1}	470 ± 100^{b,11}	
24	4926 ± 1004^{a,3}		

*corresponds to natural mating on the days that artificial inseminations were performed with preserved spermatophores.
Different letters indicate statistically significant differences between columns ($P < 0.05$), and different numbers indicate a statistically significant difference between rows ($P < 0.05$).

Naupliar deformities

The deformity values did not differ significantly between the treatments nor among the evaluation days of the study ($P > 0.05$). The estimated deformity ranged from 1.8 ± 0.1 % (natural copulation) to 5.0 ± 1.0 % (preserved at 10 °C) (Table 5).

Discussion

The relative fecundity rate (*Fr*) obtained in this study (ranging from 7,366 to 7,435 eggs g^{-1}) between the female's natural mating and the females inseminated to low-temperature preservation spermatophores showed consistency and low variability, attributable to the use of females from the same batch with similar reproductive characteristics. Comparable *Fr* values were reported by Intriago et al. (2012) (6,127 eggs g^{-1}), while Pérez (2005) reported slightly lower *Fr* for *Litopenaeus schmitti*. These findings suggest that the females in the experiment exhibited good reproductive performance in terms of *Fr*.

During the initial four days of the experiment, fertility rates (ranging from 59.8 % to 41.7 %) using spermatophores preserved in mineral oil at 10 °C or 24 °C did not differ significantly compared to fertility rates obtained through natural copulation (65.3 %) ($P < 0.05$). Similarly, the hatching percentages did not significantly differ during the first two days (74.3 % - 97.3 %). Subsequently, the fertility of preserved spermatophores declined, with the fertilising capacity

Table 5. Mean percentage of deformity (% ± SD) of Pacific whiteleg shrimp, *Penaeus vannamei*, nauplii obtained through natural copulation and insemination with spermatophores preserved at 10 °C and 24 °C.

Storage days	Treatment		
	Natural mating*	10 °C	24 °C
2	1.8 ± 0.1^{a,1}	2.8 ± 0.5^{a,1}	2.1 ± 0.6^{a,1}
4	2.0 ± 0.3^{a,1}	3.2 ± 0.7^{a,1}	2.3 ± 0.8^{a,1}
6	1.8 ± 0.5^{a,1}	2.6 ± 0.8^{a,1}	2.2 ± 0.9^{a,1}
8	2.2 ± 0.4^{a,1}	2.9 ± 0.7^{a,1}	2.2 ± 0.9^{a,1}
10	2.0 ± 0.4^{a,1}	3.5 ± 0.5^{a,1}	3.0 ± 0.3^{a,1}
12	2.2 ± 0.3^{a,1}	3.0 ± 0.9^{a,1}	3.1 ± 1.1^{a,1}
14	3.1 ± 0.6^{a,1}	3.2 ± 0.8^{a,1}	
16	2.0 ± 0.7^{a,1}	3.0 ± 0.9^{a,1}	
18	2.6 ± 0.5^{a,1}	3.0 ± 0.7^{a,1}	
20	2.6 ± 0.6^{a,1}	4.6 ± 0.8^{b,1}	
22	2.0 ± 0.4^{b,1}	5.0 ± 1.0^{a,1}	
24	2.0 ± 0.2^{a,1}		

*corresponds to natural mating on the days that artificial inseminations were performed with preserved spermatophores. Different letters indicate statistically significant differences between columns ($P < 0.05$), and different numbers indicate a statistically significant difference between rows ($P < 0.05$).

lasting until day 22 at 10 °C and approximately half that duration at 24 °C. For commercial purposes, the preservation of spermatophores at 10 °C can be recommended until day 16 (27.5 % fertility, with hatching of 54.4 %), whereas preservation at 24 °C is advisable only until day 4 (41.7 % fertility, with hatching rate of 41.7 %).

Early findings on the conservation of spermatophores for short periods were reported by Chow (1982), who demonstrated the fertilising capacity of *Macrobrachium rosenbergii* spermatophores preserved for nine days in Ringer's solution at 2 °C with medium renewal every two days. Ishida et al. (1986) found that *Homarus americanus* females inseminated with spermatophores stored in paraffin oil at 4 and 7 °C for 108 days showed some fertilisation capacity. Bray and Lawrence (1998) observed fertilisation capacity in *P. vannamei* spermatophores after 36 h of preservation in seawater and calcium-free saline solution at 15 °C, suggesting storage temperatures between 5 and 12 °C. Nimrat et al. (2005) preserved *P. monodon* spermatophores in mineral oil at 2 and 4 °C, achieving high fertility (88.3 %) and hatching (87.6 %) after seven days. Nimrat et al. (2006) reported greater viability of *P. vannamei* spermatophores stored in mineral oil at 2 and 4 °C with antibiotic supplementation after 35 d, suggesting mineral oil as the most suitable medium for refrigerated spermatophore preservation without adverse effects on sperm viability. Richardson et al. (2002) suggested that mineral oil functions as a moisturising agent, preventing cell dehydration, while Nimrat et al. (2006) proposed that mineral oil may protect the integrity of spermatophores and sperm membranes in *P. vannamei*. The present study indicates the effective conservation of whiteleg shrimp spermatophores in mineral oil at 10 °C for commercial purposes up to d 16, yielding 2,281 nauplii g^{-1} female. In contrast, preservation at 24 °C is recommended until d 4, producing 2,917 nauplii g^{-1}. Notably, viable nauplii were obtained until d 22 at 10 °C and until d 12 at 24 °C.

Mineral oil has been previously utilised for spermatophore conservation in tiger shrimp *Penaeus monodon* at 2 and 4 °C, maintaining adequate viability, particularly when supplemented with antibiotics. For instance, Nimrat et al. (2005) achieved a hatching rate of 87.6 % until days 7-8 of conservation with 0.1 % or 3 % antibiotic supplementation. This study observed similar results with hatching percentages of spermatophores preserved at 10 °C (71.0 ± 11.2 % on d 8) and 24 °C (above 40 % until the eighth day).

The deformity values of nauplii were not statistically different ($P > 0.05$) between those obtained with preserved spermatophores (at 10 and 24 °C) and natural copulation, remaining below 5 % in all cases. This result suggests that the conservation method employed in this study did not influence nauplii deformity, which primarily manifested as broken antennae and furcal spines.

Conclusion

The findings suggest that preserving whiteleg shrimp spermatophores in mineral oil at 10 °C is suitable for commercial purposes up to d 16, yielding 2,281 nauplii g^{-1} female. In contrast, preservation at 24 °C is feasible only until d 4, producing 2,917 nauplii g^{-1} of female.

Acknowledgements

The authors acknowledge Oceanos S.A. and South Florida Farming Corp for funding this study.

Conflict of interest: The authors declare that they have no conflict of interest.

Author contributions: Yamilis Cataño: Conceived the study, directed the research writing, editing and interpretation of the results. Pablo Intriago: Conceived the study, directed the research writing, editing and interpretation of the results. Andrew P. Shinn: Writing and editing. Martha Prieto-Guevara: Editing and directed the research. Víctor Atencio-García: Editing and directed the research.

References

Aungsuchawan, S., Browdy, C.L., Withyachumnarnkul, B. 2011. Sperm capacitation of the shrimp *Litopenaeus vannamei*. Aquaculture Research 42:188–195. https://doi.org/10.1111/j.1365-2109.2010.02579.x

Bray, W.A., Lawrence, A.L. 1998. Male viability determinations in *Penaeus vannamei*: Evaluation of short-term storage of spermatophores up to 36 h and comparison of Ca-free saline and seawater as sperm homogenate media. Aquaculture 160:63–67. https://doi.org/10.1016/S0044-8486(97)00225-1

Castelo-Branco, T., Batista, A.M., Pessoa Guerra, M.M., Soares R, Peixoto, S. 2015. Sperm vitrification in the white shrimp *Litopenaeus vannamei*. Aquaculture 436:110–113. https://doi.org/10.1016/j.aquaculture.2014.11.005

Chow, S. 1982. Artificial insemination using preserved spermatophores in the palaemonid shrimp *Macrobrachium rosenbergii*. Bulletin of the Japanese Society of Scientific Fisheries 48:1693–1695. https://doi.org/10.2331/suisan.48.1693

Duangjai, E., Umyoo, P., Meunpol, O., Puanglarp, N. 2023. Sperm quality assessment of white shrimp (*Litopenaeus vannamei*) broodstock using comet assay. Trends in Sciences 20:4768. https://doi.org/10.48048/tis.2023.4768

Intriago, P., Espinoza, J., Cabrera, J., Sanchez, A., Navarrete, A. 2012. Nitrogen flow in a recirculating operation of *Litopenaeus vannamei* maturation in Ecuador. Journal of Aquaculture Research & Development 3:153. https://doi.org/10.4172/2155-9546.1000153

Ishida, T., Talbot, P., Kooda-Cisco, M. 1986. Technique for the long-term storage of lobster (*Homarus*) spermatophores. Gamete Research 14:183–195. https://doi.org/10.1002/mrd.1120140302

Lezcano, M., Granja, C., Salazar, M. 2004. The use of flow cytometry in the evaluation of cell viability of cryopreserved sperm of the marine shrimp (*Litopenaeus vannamei*). Cryobiology 48:349–356. https://doi.org/10.1016/j.cryobiol.2004.03.003

Misamore, M.J., Browdy, C.L. 1996. Mating behavior in the white shrimps *Penaeus setiferus* and *P. vannamei*: a generalized model for mating in *Penaeus*. Journal of Crustacean Biology 16:61–70. https://doi.org/10.1163/193724096X00289

Nimrat, S., Sangnawakij, T., Vuthiphandchai, V. 2005. Preservation of black tiger shrimp *Penaeus monodon* spermatophores by chilled storage. Journal of the World Aquaculture Society 36:76–86. https://doi.org/10.1111/j.1749-7345.2005.tb00133.x

Nimrat, S., Siriboonlamom, S., Zhang, S., Xu, Y., Vuthiphandchai, V. 2006. Chilled storage of white shrimp (*Litopenaeus vannamei*) spermatophores. Aquaculture 261:944-951. https://doi.org/10.1016/j.aquaculture.2006.08.018

Pérez Jar, L. 2005. Fisiología y calidad reproductiva de machos de camarón blanco *Litopenaeus schmitti* en condiciones de cautiverio. PhD thesis. Centro de Investigaciones Biológicas del Noroeste S.C., La Paz, Mexico. 150 pp. https://aquadocs.org/handle/1834/1544 (in Spanish).

Primavera, J.H. 1979. Notes on the courtship and mating behavior in *Penaeus monodon* Fabricius (Decapoda, Natantia). Crustaceana 37:287–292. https://doi.org/10.1163/156854079X00609

Rao, M., Arnold, S.J., Cowley, J.A. 2010. High-throughput DNA extraction for PCR-based genotyping of single *Penaeus monodon* embryos and nauplii. Aquaculture 310:61–65. https://doi.org/10.1016/j.aquaculture.2010.10.001

Richardson, G.F., Gardiner, Y.T., McNiven, M.A. 2002. Preservation of rainbow trout (*Oreochromis mykiss*) eyed eggs using a perfluoro chemical as an oxygen carrier. Theriogenology 58:1283–1290. https://doi.org/10.1016/S0093-691X(02)00955-X

Thitamadee, S., Prachumwat, A., Srisala, J., Jaroenlak, P., Vinu Salachan, P., Sritunyalucksana, K., Flegel, T.W., Itsathitphaisarn, O. 2016 Review of current disease threats for cultivated penaeid shrimp in Asia. Aquaculture 452:69–87. https://doi.org/10.1016/j.aquaculture.2015.10.028