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硕 士 学 位 论 文

日本囊对虾(*Marsupenaeus japonicus*)精荚人工移植及其繁殖性能的研究

Studies on Artificial Spermatophore Transplantation and Reproductive Performance of *Marsupenaeus japonicus*

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目 录

Contents

摘 要

日本囊对虾（*Marsupenaeus japonicus*）是我国海水对虾养殖的重要经济品种之一。本研究采用实验生态学、形态学、海水养殖技术、外科手术以及个体标记等方法，初步筛选出精荚获取方法的最佳组合技术；查明了影响精荚移植的因素；攻克了精荚人工移植的技术难关，已实现经产蜕壳雌亲虾精荚再植成功率达 95%以上；在此基础上，跟踪观测了精荚人工移植雌虾的产卵潜伏期、产卵时间间隔、产卵能力和繁殖周期等生殖性能，并对其受精率、孵化率、卵径和无节幼体体长等生殖质量参数进行了测定与分析。以期为海水对虾养殖业的可持续、健康发展提供可靠的理论依据，并为对虾生殖生态学和遗传育种学的研究积累基础资料。主要结果和结论如下：

1. 精荚获取技术组合的优化

日本囊对虾精荚获取的最佳方法为 7.5-10.5 V 电刺激法，精荚排出率为 55-90%，精荚破损率<11.11%，随着电压的上升，精荚破损率和亲虾死亡率明显增加，12V 时亲虾出现死亡，30 V 时亲虾全部死亡，精荚全部破裂。亲虾左右两侧精荚的排出率没有差异(P>0.05)。

以精荚排出率和重量为精荚成熟时间的评判依据，5 d 精荚基本成熟，9 d 达最大值（排出率为 100%，重量 0.336±0.041 g），精荚再成熟时间平均为 5.67 d。

两种精荚获取方法（电刺激法和夹取法）相比，在所获取精荚的质量（重量、精子总数、活精子数、精子畸形率和精荚黑化率）和对亲虾身体伤害（亲虾死亡率和精荚再成熟率）方面都有显著差异（P<0.01），前者优于后者。电刺激法和夹取法所取精荚植入雌虾，其繁殖的卵子在受精率和孵化率方面，前者影响不大，在不同批次之间波动也不明显（P>0.05）；而后者影响较大，尤以连续多次进行精荚取样，降幅极为明显（P<0.01）。

2. 精荚人工移植

日本囊对虾精荚人工移植首获成功！精荚植入雌虾蜕壳的最佳时间为雌虾蜕壳后 12-48 h，成功率为 100%，亲虾为零死亡率；<12 h，成功率为 0，亲虾死亡率上升至 40%；>48 h，成功率仅为 20%。

精荚双侧或单侧都能成功植入，然而单侧植入者虽产卵，但受精率较低；“废

旧”精荚也能成功植入蜕壳雌虾纳精囊，但不能受精，原因待查；

水温、盐度、光照强度等环境因素对精荚移植的影响不明显。

3. 经产蜕壳雌亲虾再植精荚的繁殖性能

经产蜕壳雌亲虾和海捕雌亲虾的 Fulton 状态指数分别为 1.262 和 1.228，两者虽均>1，但是前者略高于后者。

精荚再植雌亲虾的产卵潜伏期为 18-54 h，平均为 32.4 h；产卵量为 95.14-230.37 万粒/每尾，平均为 112.99 万粒/每尾；每尾亲虾每次产卵数量为（36.33±4.13）-（57.37±3.77）万粒，平均为（46.76±4.11）万；多次产卵次数为 2-5 次，平均 3.4 次，两次产卵的时间间隔为 48-96 h，平均为 65 h；繁殖周期为 8-15 d。

精荚再植雌亲虾繁殖的受精率和孵化率，卵径和无节幼体体长虽呈波动变化，但个体之间和产卵批次之间无显著差异。

精荚成熟度（精荚获取的时间间隔）会影响再植雌亲虾繁殖的受精率，间隔 3 d，受精率为 33.24%，间隔 5-11 d,受精率为 74.33-76.24%，但不影响其孵化率，始终保持在 86.50-91.73%之间。

关键词：日本囊对虾；亲虾；获取精荚的方法；精荚移植；繁殖性能

Abstract

Marsupenaeus japonicus is an important economic species in China shrimp farming. This research, by the use of the methods of experimental ecology, morphology, marine aquaculture and individual markings, preliminarily screened out the optimal combination technology of obtaining spermatophore methods, and ascertained factors that influence the spermatophore transplantation. A technical difficulty of artificial spermatophore transplantation was overcome, and the success rate of transplantation spermatophores to multiparous molting female broodstock reached over 95%. On the basis, spawning latency, spawning time interval, fecundity and reproductive cycle of female broodstock transplanted spermatophores were observed. Parameters of the reproductive quality, such as fertilization rate, hatching rate, egg diameter and nauplius length of these female broodstock, were determined and analysed. These findings will provide theoretical guidance to healthy, safe and sustainable development for aquaculture industry, and will contribute basic data to the study of shrimp reproductive ecology and shrimp genetic and breeding. The main results and conclusions were as follows:

1. Optimized technical combination of obtaining spermatophores

The most suitable method to obtain *Marsupenaeus japonicus* spermatophores was electrical stimulation with voltages of 7.5-10.5 V. In these voltages, excretion rates of spermatophores were 55-90%, and damage rate was less than 11.11%. The excretion rate and the mortality rate increased rapidly with the voltage rising. However male broodstock began to die when the voltage rose to 12 V. There were no left of male broodstock and undamaged spermatophores when the voltage was 30 V. And there was no significant difference on spermatophore excretion rates between left and right.

The time interval of regeneration of many spermatophores was 5 d, and the peak period was 9 d with the excretion rate of 100% and the average weight of (0.336±0.041) g. The average spermatophore regeneration time was 5.67 d.

There was significant difference in quality of spermatophore obtained by electrical stimulation and clip, such as weight, total sperm count, living sperm count and sperm deformity rate. And there was significant difference in the injury degree of male broodstock, such as the survival rate and the maturation rate, after they were obtained spermatophores by

electrical stimulation and clip. Therefore the electrical stimulation to obtain spermatophores was better than clip.

Fertilization rates and hatching rates of female broodstock transplanted spermatophores were little influenced by the obtaining method of electrical stimulation. And there was no significant difference among different obtaining times. But the influenced by the obtaining method of the clip was more and more obvious with obtaining times increasing, and decreasing ranges of the fertilization rate and the hatching rates were significant difference.

2. Artificial spermatophore transplantation of *Marsupenaeus japonicus*

The paper first reported the artificial spermatophore transplantation of *Marsupenaeus japonicus*! The best time to insert spermatophores was 12-48 h after female broodstock molting. The success rate and the mortality rate was respectively 100% and zero. The success rate and the mortality rate were respectively 20% and 40%, when spermatophores were inserted in less than 12 h. And the success rate was 20% when spermatophores were inserted after more than 48 h.

Although one or two spermatophores could be successfully inserted in the seminal receptacle of female broodstock and these female could spawn , these female broodstock with only one inserted spermatophore had low fertilization rates. Wasted spermatophores could be also successfully inserted in the seminal receptacle of female broodstock, but eggs spawned by these shrimps were not fertilized, the reason being unclear.

The success rate of spermatophore transplantation had little relation to temperature, salinity and light.

The best way to insert spermatophores was female broodstock, that were domesticated under low light (500 Lux), sea water of 22℃, salinities more than 30, were inserted one spermatophore after 12-48 h of female broodstock molting. It was beneficial to daily management and performed operations to female broodstock. It also increased the success rate of spermatophore transplantation.

3. Reproductive performance of multiparous molting female broodstock transplanted spermatophores

Fulton condition factors of female broodstock from wild and multiparous molting shrimp were 1.262 and 1.228, respectively. So the former was a little better that the latter.

Spawning latencies of female broodstock transplanted spermatophores were 18-54h, and the average was 32.4 h. The quantity of eggs was 954.1-2303.7 thousand grains per female

shrimp, and the average quantity were 1129.9 thousand grains per female shrimp. The quantity of eggs were 363.3-573.7 thousand grains per female each time. Spawning times of these prawns were 2-5, and the average was 3.2 times. Spawning time intervals of these prawns were 48-96 h, and the average was 65 h. Reproductive cycles of these prawns were 8-15 d.

Fertilization rates, hatching rates, egg diameters and nauplius length of female broodstock transplanted spermatophores had fluctuation changes, but there was no significant difference in different individuals and different spawning numbers.

The fertilization rate was influenced by the spermatophore maturity (the time interval of obtaining spermatophores). The fertilization rate was 33.24% when time intervals were 3d, and fertilization rates were 74.33-76.27% when time intervals were 5-11 d. Thereupon the spermatophore regeneration could be regarded as more than 5 d. The hatching rate was not influenced by the time interval of obtaining spermatophores, and hatching rates ranged from 86.50% to 91.73%.

Keywords: *Marsupenaeus japonicas*; Broodstock; Spermatophore obtaining method; Spermatophore transplantation; Reproductive performance