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厦门大学

硕 士 学 位 论 文

5-羟色胺和切眼柄对日本囊对虾卵巢协同促熟效应的研究

Study on Collaborative Maturation-Promoting Effect of 5-Hydroxytryptamine and Eyestalk Ablation on the Ovary of Female *Marsupenaeus japonicus*

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摘要

本研究从生殖生态学角度，结合生产实践，采用实验生态学、形态学、组织学、海水养殖学和组织离体培养等多学科的方法和技术，以亲虾 GSI，卵巢成熟率，手术效应期，产卵次数，产卵时间间隔，产卵量，受精率，孵化率，无节幼体数，卵径和无节幼体体长等 11 项生殖性能参数评价促熟的效果，研究了 5-羟色胺（5-HT）和切眼柄对日本囊对虾亲虾分别的促熟效果和它们之间的协同促熟效应，以及 5-HT 促熟的生殖内分泌调控机制，以期丰富海产虾蟹生殖生态学基础资料，为日本囊对虾无节幼体的繁育生产提供更安全、可靠和高效的促熟新技术。主要结果如下：

1、（1）亲虾注射两种剂量 15ug/g 和 50ug/g 5-HT 均显促熟增效（P<0.05），其中主要参数产卵量分别增加 57.26%和 85.22%，无节幼体产量分别增加 64.02%和 102.53%，相比之下，50ug/g 5-HT 的促熟效果明显优于（P<0.05）15ug/g 5-HT。同时以 50ug/g 5-HT 剂量为基础，注射次数分 1 次、2 次和 3 次，研究结果显示随着注射次数的增加产卵量、无节幼体产量和受精率均呈正相关关系（P<0.05），注射 3 次效果最佳，重复（多次）产卵次数达 5 次，产卵量和无节幼体产量提高 104%和 103%，而孵化率略呈负相关关系。

（2）通过药饵途径两种含量 15ug/g 和 50ug/g 5-HT 对亲虾促熟具有增效效应，尤以 50ug/g 5-HT 药饵的促熟效果更佳（P<0.05），产卵量提高 101.97%，无节幼体产量提高 188.19%。在不同用药次数的情况下，产卵量、受精率、孵化率和无节幼体产量均随着摄食药饵次数的增加而增加，呈正相关关系（P<0.05），峰值均出现在 3 次用药组。

（3）5-HT 通过药浴途径对亲虾促熟也具有增效作用，剂量 15ug/g 5-HT 仅略显增效，剂量 50ug/g 5-HT 的促熟效果显著（P<0.05）：后者的产卵量提高 107.91%，无节幼体产量提高 103.86%。在不同用药次数的情况下，产卵量、受精率、和无节幼体产量均随着药浴次数的增加而增加，呈正相关关系（P<0.05），而孵化率略呈负相关关系，即略下降态势，峰值均出现在 3 次用药组。

（4）注射法、药饵法、药浴法三种处理方式在相同条件下的促熟效果比较，剂量为 50ug/g 5-HT 药饵法 3 次用药的促熟效果最佳，产卵量提高 217.45%，达

（69.49±4.12）万粒，无节幼体产量提高了 268.31％，达（38.96±1.16）万尾；5-HT 对亲虾促熟的最佳处理模式是：药饵法、剂量为 50ug/g、用药 3 次。

2、经切眼柄处理的雌亲虾，产卵时间提前 106.00h，产卵的时间间隔缩短 128.93h，产卵量增加 159.78%，无节幼体产量增加 192.72％，可见，切眼柄的促熟效果明显（P<0.05）。

3、在相同实验条件下，与未切眼柄组相比，亲虾的产卵量和无节幼体产量，5-HT 单因素组分别提高了 202.06%和 306.15%，切眼柄单因素组提高了 168.50%和 208.83%，切眼柄+5-HT 双因素组分别提高了 259.45%和 368.25%，可见都具有显著的促熟作用（P<0.05）。同时，两单因素组之间的整体促熟效果也有一定的差异，5-HT 组优于切眼柄组，但不显著（P>0.05），而双因素组的促熟效果比两单因素组的高值还要高，表明这两个单因素组合在一起时发生交互作用，产生协同促熟效应。

4、经组织离体培养，卵巢组织单独没促熟效应；卵巢组织单加视神经节，或脑，或胸神经节，促熟效应不明显（P>0.05），卵巢组织+脑+视神经节，或卵巢组织+胸神经节+视神经节联合培养时有促进卵巢发育的作用，但效果不显著（P> 0.05），只有卵巢组织+脑+胸神经节联合培养时，卵母细胞直径达到最大值（0.2665±0.047）mm，表明 5-HT 不是直接作用于卵巢组织，而是通过脑和胸神经节的神经内分泌活动，进而促进卵巢的发育。

关键词：日本囊对虾；卵巢；5-羟色胺；切眼柄；促熟；生殖性能；产卵量；无节幼体产量

ABSTRACT

Via experimental ecology, morphology, microanatomy, marine aquaculture, *in vivo* and *in vitro* techniques approaches, and using ovarian index, proportion of spawning females, time to ovarian maturation, times of spawning frenquency, spawning time interval as main indicators of quality of spawns, this study aimed to explore the effects of exogenous 5-HT and eyestalk ablation on the reproductive performance of the *Marsupenaeus japonicus*. The findings of this study would provide theoretical guidance to healthy, safe and sustainable development for aquaculture industry, and would contribute basic data to the study of shrimp reproductive ecology and shrimp genetic and breeding.

The main results were as follows:

1、(1) As an experimental treatment, 5-HT solution was injected into domesticated *M. japonicus* at 50 ug/g body weight and 15 ug/g body weight. All the 5-HT injection females reached the spawning condition（P<0.05）, the injection of 15 ug/g and 50 ug/g of 5-HT demonstrated that the amount of eggs increased by 57.26％ and 85.22％, respectively. The increases of nauplii amount were 64.02％ and 102.53％, respectively. The results suggests that injection of 5-HT at 50ug/g BW is more effective in inducing ovarian maturation and spawning in female *M. japonicus* broodstock, compared to the injection of 5-HT-injected at 15ug/g BW（P<0.05）.For 5-HT injection at 50ug/g, with 4-day intervals (day 1, 5 and 9), the neurotransmitter was applied for once, twice, third, respectively. The highest amount of eggs per spawn, fertilization rate and number of nauplii per spawn showed that 3 times 5-HT injection was more effective than any other groups（P<0.05）. Spawning occurred 5 times within 12 days in 3 times 5-HT injection treatment. Additionally, the amount of eggs and the amount of nauplii produced per spawner also increased by 104％ and 103％ in 3 times 5-HT injection treatment, respectively. The hatching rate of the 3 times 5-HT injection treatment, however, declined slightly.

(2) 5-HT solution was ingested by domesticated *M. japonicus* at 50 ug/g body

weight and 15 ug/g body weight.5-HT induced ovarian maturation and spawning at both dosis tested, generating more spawnings at 50 ug/g B W compared to that at 15ug/g BW（P<0.05）. The amount of eggs and the amount of nauplii produced per spawner were also increased by 101.97％ and 188.19％ in the ingestion of 50 ug/g of 5-HT, respectively. For 5-HT ingestion treatment at 50ug/g, with 4-day intervals (day 1, 5 and 9), the neurotransmitter was applied once, twice and 3 times, respectively. The result showed that the amount of eggs per spawn, hatching rate, fertilization rate and number of nauplii per spawn of the 3 times 5-HT-ingestion treatment were higher than other treatments（P<0.05）.

(3) 5-HT solution was permeated into domesticated *M. japonicus* at 50 ug/g body weight and 15 ug/g body weight and ovarian maturation and spawning were recorded. All of the 5-HT permeating treatment females reached the spawning condition （P<0.05）. The ovarian maturation and spawning of the 5-HT permeating treatment at 15ug/g BW increased insignificantly, while those of the treatment at 50ug/g BW increased significantly. The amount of eggs and the amount of nauplii produced per spawner increased by 107.91％ and 103.86％ in the 5-HT permeating treatment at 50ug/g BW, respectively. In the case of 5-HT using frequency, the amount of eggs per spawn, fertilization rate and number of nauplii per spawn of 3 times 5-HT permeating treatment were higher than other groups（P<0.05）. The hatching rate of the 3 times 5-HT permeating treatment, however, declined insignificantly.

(4) Under the same conditions, the 5-HT injection, 5-HT ingestion and 5-HT permeating treatment groups had a comparable performance in their effects on development of ovarian maturation. 5-HT induced ovarian maturation and spawning at three treatments, generating most spawnings at 3 times 5-HT ingestion at 50ug/g animals. The amount of eggs produced in each spawn was（69.49±4.12）$\times 10^4$, and the amount of nauplii produced was（38.96±1.16）$\times 10^4$, increasing by 217.45% and 268.31%, respectively. The finding that 3 times 5-HT-ingestion at 50ug/g treatment produced highest amount of eggs than others also suggests that the former could maintain its reproductive capability to a longer period.

2、Compared with the control group, the eyestalk ablation treatment induced a sooner and a higher rate of maturation and spawning. The number of hours to reach ovarian maturation is calculated from the beginning of treatment (eyestalk ablation) to the first spawing event. It was ahead of 106 hours by eyestalk ablation treatment *M. japonicus*. 128.93 hours of the spawning interval was cut down. The amount of eggs produced by eyestalk ablation treatment group increased by 159.78%, the amount of nauplii increased by 192.72％.

3、The effects of combined 5-HT ingestion and eyestalk ablation on *M. japonicus* maturation and spawning quality, compared to single eyestalk ablation or single 5-HT-ingested（$P<0.05$）were evident. The amount of eggs and the amount of nauplii of 5-HT ingestion treatment alone increased by 202.06% and 306.15％. The amount of eggs and the amount of nauplii of eyestalk ablation treatment alone increased by 208.83％.

On the other hand, the amount of eggs and the amount of nauplii of 5-HT ingestion and eyestalk ablation combined treatment increased by 259.45% and 368.25%, respectively. The effects of the eyestalk ablation and the 5-HT ingestion treatments differed insignificantly ($P> 0.05$), although the effects of the latter group was higher. The amounts of eggs and the amounts of nauplii of 5-HT injection and eyestalk ablation combined treatment increased by 57.89% and 62.10%, respectively. These results demonstrated that 5-HT injection and eyestalk ablation combined treatment, in *M. japonicus*, stimulated ovarian maturation, spawning and the release of maturation promoting pheromones, induced a sooner and a higher rate of maturation and spawning.

4、*In vitro* study showed that:With regard to treatment A consisted of ovary with two dosis of 5-HT, the result showed that the 5-HT could increased M. japonicus oocyte diameter insignificantly. Treatment B made up of ovary with single optical or brain or thoracic ganglion, no statistical differences were found. Treatment C consisting of ovary and optical with brain or thoracic ganglion also had no statistical differences, but it induced the increasing of oocyte diameter. While in treatment D, which consisted of ovary, brain and thoracic ganglion, the diameter of oocyte was the