

学校编码: 10384  
学号: 22420111151354

密级\_\_\_\_\_

廈門大學

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*

董燕妮

指导教师姓名: 林琮武 教授  
专业名称: 海洋生物学  
论文提交日期: 2014 年 5 月  
论文答辩时间: 2014 年 5 月

2014 年 5 月

## 厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下，独立完成的研究成果。本人在论文写作中参考其他个人或集体已经发表的研究成果，均在文中以适当方式明确标明，并符合法律规范和《厦门大学研究生学术活动规范（试行）》。

另外，该学位论文为（ 浮游组 ）课题（组）的研究成果，获得（ 浮游组 ）课题（组）经费或实验室的资助，在（ 周隆泉楼B2-205 ）实验室完成。（请在以上括号内填写课题或课题组负责人或实验室名称，未有此项声明内容的，可以不作特别声明。）

声明人（签名）：

2014年05月29日

## 厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文，并向主管部门或其指定机构送交学位论文（包括纸质版和电子版），允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索，将学位论文的标题和摘要汇编出版，采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于：

1.经厦门大学保密委员会审查核定的保密学位论文，  
于 年 月 日解密，解密后适用上述授权。

2.不保密，适用上述授权。

（请在以上相应括号内打“√”或填上相应内容。保密学位论文应是已经厦门大学保密委员会审定过的学位论文，未经厦门大学保密委员会审定的学位论文均为公开学位论文。此声明栏不填写的，默认为公开学位论文，均适用上述授权。）

声明人（签名）：

2014年05月

摘要.....	IX
Abstract.....	XI
<b>第一章 绪论</b> .....	<b>1</b>
1.1 日本囊对虾生殖生物学简介.....	1
1.1.1 分类与分布.....	1
1.1.2 养殖发展历程.....	1
1.1.3 生殖习性.....	2
1.1.3.1 雌性生殖系统.....	2
1.1.3.2 交配.....	3
1.1.3.3 产卵.....	4
1.2 国内外相关的研究概况.....	5
1.2.1 切眼柄在虾蟹促熟中的应用.....	5
1.2.2 切除眼柄在虾蟹摄食、生长、存活和能量转化等方面的研究概况.....	6
1.2.3 单侧和双侧切除眼柄对对虾促熟的影响.....	7
1.2.4 虾蟹生殖内分泌调节的相关研究.....	8
1.2.5 5-HT 在虾蟹体内的生理作用.....	10
1.2.6 对虾生殖生物学及其繁殖技术.....	11
1.2.7 虾蟹卵母细胞的发育形态.....	12
1.3 问题的提出、研究的内容及其研究思路.....	14
1.4 本研究的目的是和意义.....	16
<b>第二章 材料与方法</b> .....	<b>17</b>
2.1 实验材料.....	17
2.1.1 亲虾驯养实验的时间与地点.....	17
2.1.2 亲虾和沙蚕的来源.....	17
2.1.3 亲虾驯养的容器及其配套设施.....	17
2.1.4 实验主要使用的仪器和试剂.....	17
2.2 实验方法.....	18

2.2.1 亲虾驯养与促熟.....	18
2.2.2 5-HT 溶液的配制及其进入雌虾体内方式的说明.....	19
2.2.3 实验设置.....	20
2.2.3.1 亲虾切眼柄促熟的实验.....	20
2.2.3.2 亲虾 5-HT 促熟实验.....	20
2.2.3.3 5-HT 和切眼柄协同促熟实验.....	24
2.2.3.4 卵母细胞、脑、卵巢、视神经节、胸神经节的离体培养实验.....	25
2.2.4 石蜡切片的制备与染色.....	26
2.2.5 相关参数的观测方法与说明.....	26
2.2.6 影响促熟的因子组合.....	27
2.2.7 实验数据的处理.....	28
<b>第三章 结果与分析.....</b>	<b>29</b>
3.1 5-HT 对日本囊对虾促熟的影响.....	29
3.1.1 注射 5-HT 对亲虾促熟的影响.....	29
3.1.1.1 注射两种浓度的 5-HT 对亲虾促熟的比较.....	29
3.1.1.2 5-HT 注射的次数对亲虾促熟的影响.....	30
3.1.2 5-HT 通过药饵途径对亲虾促熟效果的影响.....	32
3.1.2.1 喂以两种剂量 5-HT 对亲虾促熟效果的影响.....	32
3.1.2.2 5-HT 喂食的次数对亲虾促熟的影响.....	33
3.1.3 药浴 5-HT 对亲虾促熟的影响.....	35
3.1.3.1 药浴 5-HT 两种浓度对亲虾促熟的影响.....	35
3.1.3.2 5-HT 药浴的次数对亲虾促熟的影响.....	36
3.1.4 在相同条件下, 5-HT 的三种处理对亲虾促熟的结果.....	38
3.2 切眼柄对亲虾促熟的影响.....	39
3.3 5-HT 和切眼柄对亲虾协同促熟效应的评析.....	40
3.3.1 5-HT 和切眼柄与亲虾的产卵量和重复(多次)产卵次数的关系.....	40
3.3.2 5-HT 和切眼柄与受精率和孵化率的关系.....	41
3.3.3 5-HT 在卵母细胞离体培养中的诱导作用.....	42
3.3.4 5-HT 和切眼柄对亲虾协同促熟的影响.....	44

第四章 讨论.....	47
4.1 虾蟹亲体切除眼柄的促熟效应.....	47
4.2 5-HT 在虾蟹促熟中的应用.....	48
4.2.1 虾蟹亲体促熟中 5-HT 注射法和药浴法的促熟效果.....	48
4.2.2 摄食 5-HT 对亲虾促熟的影响.....	50
4.2.3 5-HT 进入虾蟹体内三种用药方式的促熟效果比较.....	51
4.3 5-HT 和切眼柄在虾蟹促熟过程中的协同作用.....	54
4.4 5-HT 在虾蟹促熟过程中的生殖内分泌调节作用.....	55
第五章 论文结语.....	59
5.1 主要成果.....	59
5.2 本研究的创新点与特色.....	60
5.3 不足之处.....	60
5.4 展望.....	61
参考文献.....	63
在学期间参加的科研活动和发表的文章.....	79
致谢.....	80



## Contents

Abstract(in Chinese).....	IX
Abstract(in English).....	XI
Chapter 1. Introduction.....	XIV
1.1 Brief introduction to the reproductive biology of <i>Marsupenaeus japonicus</i> ....	1
1.1.1 Taxonomic position and distribution.....	1
1.1.2 Aquaculture and development course.....	1
1.1.3 Reproductive habits.....	2
1.1.3.1 Female genital system.....	2
1.1.3.2 Mating.....	3
1.1.3.3 Spawning.....	4
1.2 Related research of domestic and foreign.....	5
1.2.1 Applications of eyestalk ablation technology to promoting ovarian maturation of shrimps and crabs.....	5
1.2.2 Research situation on applications of eyestalk ablation to feeding, growth, survival, and energy conversion etc of shrimps and crabs.....	6
1.2.3 The comparison between effects of unilateral and bilateral eyestalk ablation on promoting ovarian maturation of shrimps.....	7
1.2.4 Research about reproductive endocrine regulation of shrimps and crabs.....	8
1.2.5 Research about the physiological role of 5-HT in shrimps and crabs.....	10
1.2.6 Reproductive Biology and breeding technology of shrimps.....	11
1.2.7 Morphological Observation on oocyte development of shrimps and crabs.	12
1.3 Problems to be put forward, research content and research idea.....	14
1.4 Purpose and significance of the research.....	16
Chapter 2. Materials and Methods.....	16
2.1 Materials.....	17
2.1.1 Time and location of the female broodstock domestication experiment.....	17
2.1.2 Source of the female broodstock and clam worm.....	17



2.1.3 The container and its supporting facility of the female broodstock domestication.....	17
2.1.4 Relative equipments and regents of this syudy.....	17
2.2 Methods.....	18
2.2.1 Domestication and promoting maturation of the broodstock.....	18
2.2.2 The configuration of 5-HT solution and handing instructions to the way of 5-HT into the female shrimp body .....	19
2.2.3 Experiment design.....	20
2.2.3.1 The maturation-promoting experimental design of the femaels by ablating eyestalk.....	20
2.2.3.2 The maturation-promoting experimental design of the femaels by means of 5-HT.....	20
2.2.3.3 Maturation-promoting experimental design of the females by ablating eyestalk and with feeding 5-HT.....	24
2.2.3.4 <i>In vitro</i> culture experiment of oocytes, brain, ovary, optic nerve and the thoracic ganglia.....	25
2.2.4 The preparation and dyeing of paraffin section.....	26
2.2.5 Measuring method and calculation of experimental parameters.....	26
2.2.6 The combination of factors affecting promoting maturation.....	27
2.2.7 Statistical analysis of experimental data.....	28
Chapter 3. Results.....	29
3.1 The influence of 5-HT on the ovarian maturation of <i>M japonicus</i> .....	29
3.1.1 The influence of 5-HT by injecting on the ovarian maturation of <i>M.japonicus</i> .....	29
3.1.1.1 The influence of two dosis of 5-HT by injecting on the ovarian maturation of <i>M japonicus</i> .....	29
3.1.1.2 The influence of 5-HT by injecting with different times on the ovarian maturation of <i>M. japonicus</i> .....	30
3.1.2 The influence of 5-HT by feeding on <i>M japonicus</i> ovarian maturation .....	32
3.1.2.1 The influence of 5-HT by feeding at two dosis on the ovarian	

maturation of <i>M. japonicus</i> .....	32
3.1.2.2 The influence of 5-HT by feeding with different times on the ovarian maturation of <i>M. japonicus</i> .....	33
3.1.3 The influence of 5-HT by bathing on the ovarian maturation of <i>M.japonicus</i> .....	35
3.1.3.1 The influence of 5-HT by bathing at two dosis on the ovarian maturation of <i>M. japonicus</i> .....	35
3.1.3.2 Difference times of 5-HT by bathing on the ovarian maturation in the <i>M.japonicus</i> .....	36
3.1.4 The results on the promoting ovarian maturation of <i>M.japonicus</i> with three treatment ways of 5-HT under the same conditions.....	38
3.2 The influence of eyestalk ablation on the ovarian maturation of <i>M. japonicus</i> ..	39
3.3 Evaluation on the collaborative maturation- promoting effects of 5-HT and eyestalk ablation on female <i>M. japonicus</i> .....	40
3.3.1 Spawning quantity and spawning frequency of female <i>M. japonicus</i> under the condition of different maturation-promoting ways.....	40
3.3.2 Fertility rate and hatching rate of eggs spawned by female <i>M. japonicus</i> under the condition of different maturation-promoting ways.....	41
3.3.3 Effect of 5-HT on oocyte maturation, <i>in vitro</i> culture.....	42
3.3.4 The collaborative maturation-promoting effects of 5-HT and eyestalk ablation on female <i>M. japonicus</i> .....	44
Chapter 4. Discussion.....	47
4.1 Maturation-promoting effect of eyestalk ablation on the females of shrimps and crabs .....	47
4.2 Applications of 5-HT to promoting ovarian maturation of shrimps and crabs .....	48
4.2.1 Maturation-promoting effects of 5-HT injections and water bath on the females of shrimps and crabs.....	48
4.2.2 Maturation-promoting effect of 5-HT by feeding on the females of shrimps	

and crabs .....	50
4.2.3 Comparison among maturation-promoting effects of 5-HT used by three kinds of ways on the females of shrimps and crabs.....	51
4.3 Synergy effect of 5-HT and eyestalk ablation on the promoting maturation of the female shrimps and crabs.....	54
Chapter 5. Epilogue.....	59
5.1 The main results of the present research.....	59
5.2 Innovation and characteristic of the present research.....	60
5.3 Shortage of the present research.....	60
5.4 Prespects of the present research.....	61
References.....	63
Research activities and paper published.....	79
Acknowledgement.....	80

## 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

( $69.49 \pm 4.12$ ) 万粒, 无节幼体产量提高了 268.31%, 达 ( $38.96 \pm 1.16$ ) 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 ( $0.2665 \pm 0.047$ ) 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 frequency, 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 doses 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  $(69.49 \pm 4.12) \times 10^4$ , and the amount of nauplii produced was  $(38.96 \pm 1.16) \times 10^4$ , 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 spawning 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



Degree papers are in the "[Xiamen University Electronic Theses and Dissertations Database](#)". Full texts are available in the following ways:

1. If your library is a CALIS member libraries, please log on <http://etd.calis.edu.cn/> and submit requests online, or consult the interlibrary loan department in your library.
2. For users of non-CALIS member libraries, please mail to [etd@xmu.edu.cn](mailto:etd@xmu.edu.cn) for delivery details.

廈門大學博碩士論文摘要庫