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孵育温度对锯缘青蟹幼体质量的影响

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摘要: 研究了锯缘青蟹抱卵蟹培育和胚胎发育及孵化温度对刚孵化第 1 期溞状幼体(Z_1)干重(DW)和比能值(EC, J/mg)以及对幼体发育和存活的影响。研究结果表明: 胚胎发育随孵育温度的升高而加快, 但孵育温度与刚孵化幼体的干重和能量(E, J/ind)没有明确的相关性; 而抱卵蟹培育和胚胎发育期间孵育温度的日温差对刚孵化青蟹幼体的干重和能量有明显的影响, 并对幼体的存活和进一步发育产生影响。当孵育温度日温差≥2℃时, 胚胎发育不整齐, 孵化不同步, 死卵或孵出原溞状幼体的比例高; 孵化出的第 1 期溞状幼体一般都无法蜕皮进入第 2 期。孵育温度日温差与刚孵化幼体的干重或能量的相关性不确定, 但当孵育温度日温差≥2℃时, 刚孵化的溞状幼体的个体干重和能量的乘积($DW \times E$) < 0.746 的几率显著增加, 幼体可养活的几率极小, ($DW \times E$)可作为判断刚孵化的第 1 期溞状幼体能否正常生长发育的指标。

关键词: 锯缘青蟹; 孵育温度; 干重; 能量; 幼体质量

Effect of Brooding Temperature on Larval Quality of Mud Crab *Scylla serrata*

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Abstract: Mud crab *Scylla serrata* farming has been going on for at least three decades in south China, especially in recent decade, but it has not reached even its optimum potential. The major constraint for further expansion of mud crab culture is the limited supply of crab 'seed'. Mud crab culture still depends on wild-caught crablets, but its quantities are not sufficient to meet demand and even at the current size of mud crab culture farming.

There is a great need to develop a hatchery technology for the mass production of seed to meet the demands of mud crab farming. Mud crab culture research, particularly larval rearing, has been conducted at Department of Oceanography, Xiamen University over ten years. It has been obvious that production of eggs and newly hatched larvae is not an issue affecting the hatchery success of mud crab. Mature female broodstock and its cultivation is one key of successful larval rearing. Quality of newly hatched larvae or their inherent viability is regarded as a main issue influencing the success of hatchery production. The nutrition for broodstock was shown a considerable effect on gonad growth and fecundity, hatching and larval quality. But little is known of ecological factors such as temperature, light, salinity, bottom substrates, etc. that influence larval quality. If readily measured criteria could be used to predict the subsequent performance of larvae, it would improve the consistency of production and reduce the resources expended on larvae of inadequate viability.

The objective of this investigation is, firstly, to determine the effect of brooding temperature on dry weight and energy of newly-hatched zoea-1 of *Scylla serrata*, and on larval survival and development, and secondly to formulate a criteria that can be used as a judgement of newly hatched larval quality. Dry

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weight and energy content of newly hatched larvae of 17 broods which come from the different seasons and culturing at the different brooding temperature were measured. The embryonic development, larval production and larval development (under normal rearing conditions) from the different batches were observed.

Mature female were collected from coastal water or shrimp pond. The broodstock tank (6.5×4×2 m^3) is with sand-covered bottom and flow through seawater. The tank is maintained under low light conditions and temperature is 25℃ to 32℃, salinity ranges between 25×10^{-12} and 32×10^{-12}. The stocking density is 2～3 crabs/m^2. The broodstock are fed twice one day, in evening and in morning. The diet is consisted of crustaceans, molluscs and fish. Following ovulation the crabs were removed from broodstock tank and maintained individually in 1000 dm^3 tanks with fresh seawater and no diet. After hatching, larvae are collected for dry weight and energy content measurement and estimates of unhatched eggs, prezoea, dead zoea are made. The live zoea is transferred to rearing tank and the stocking densities of the larvae are about 200/dm^3. Seawater is settled, filtered, and formed green water by adding unicell algae (< 10^4 cells/cm^3). The tank is maintained under low light conditions at zoeal stage and temperature is 25℃ to 32℃. Salinity ranges between 25×10^{-12} and 32×10^{-12}. Zoea-1 to Zoea-2 is fed with rotifers (*Brachionus plicatilis*), Zoea-3 and following stage larvae is fed with *Artemia* nauplii. Nutritive value, especially for highly unsaturated fatty acid (HUFA) of rotifers and Artemia nauplii could be elevated through nutritional enrichment.

The embryonic development of mud crab is expedited with brooding temperature rising, embryonic developmental time is short under high brooding temperature. And there are no specific relations between dry weight (*DW*), or energy content (*EC*, J/mg) of newly-hatched zoea-1 and brooding temperature. But effects of the daily difference in temperature during embryonic development on dry weight, energy (*E*, J/ind) of newly-hatched zoea-1 are obviously, and larval survival and development are affected subsequently. When the daily difference in brooding temperature ≥2℃, three phenomenon will detected. First, mud crab embryonic development or hatching is irregular, the proportion of prozoea and died egg production is higher. second, the newly hatched zoea-1 could not exuviate to zoea-2. Third, the energy content of newly hatched zoea-1 is lower than 0.10 J/ind. The newly hatched zoea-1 which dry weight is lower (< 6.0 μg/ind) and energy content is high (> 25 J/mg) of mud crab could not exuviate to zoea-2 when brooding temperature (> 30℃) and the daily difference in brooding temperature (≥2℃) is high during some day of brooding time. There some exceptions that the newly hatched zoea-1 which dry weight and energy content is lower could not exuviate to zoea-2 when brooding temperature is normal, this may be related with quality of berried female mud crab.

There are three characters of abnormal or poor quality newly hatched zoea-1 of mud crab: (1) Dry weight is low and energy content is high. (2) Energy and energy content is also low. (3) Dry weight and energy is also low. There are no certain relations between dry weight or energy of newly-hatched zoea-1 and difference in brooding temperature. But the probability of ($DW\times E$) < 0.75 of newly-hatched zoea-1 increases significantly when the daily difference in brooding temperature ≥2℃, and viability of the larvae is worse. ($DW\times E$) of the newly hatched larvae might be an indicator of larval quality or viability.

Key words: *Scylla serrata*; brooding temperature; dry weight; energy; larval quality

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锯缘青蟹(*Scylla serrata*)是我国南方地区主要的经济蟹类之一, 名优养殖品种。随着青蟹养殖技术的改进和养殖规模的扩大, 特别是虾蟹混养模式迅速的推广, 捕捞自然蟹苗已远不能满足生产的需求, 青蟹

苗种已成为养殖青蟹、甚至南方部分地区虾池养殖的主要限制因子, 对大规模的苗种培育技术要求越来越迫切。亲蟹的驯养、产卵及孵化是青蟹人工育苗的第一步, 林琼武等实验研究了锯缘青蟹亲蟹的驯养[1]; 周友富等报道了全人工培育锯缘青蟹抱卵技术[2]。锯缘青蟹人工育苗中, 最困难的不是如何获得足够数量孵化的幼体, 而是如何获得质量好的或可养活性(viability)高的刚孵化幼体。在青蟹人工育苗生产实践中, 经常遇到第一期溞状幼体(Z_1)无法蜕皮为 Z_2 而倒池, 造成人工、饵料和场地的浪费, 提高了育苗成本。刚孵化青蟹幼体的质量将直接影响幼体的生长发育和存活率, 从而影响人工育苗的效果。Mann 等研究了来自不同季节的锯缘青蟹亲蟹所产卵和幼体的差异、切除眼柄对卵和幼体的影响、可预示幼体可养活性的一些特征[3]。Millamena 等报道了饲喂不同饵料对锯缘青蟹亲蟹繁殖效果的影响, 发现多种饵料混合或交替投喂的亲蟹繁殖效果好[4]。

本文实验测定了来自不同季节的抱卵蟹, 在不同的抱卵蟹培育、胚胎发育温度下 17 尾锯缘青蟹亲蟹刚孵化幼体的干重(DW)和比能值(EC, J/mg), 观察记录了胚胎发育和孵化的情况及各批幼体的生长发育(提供正常的温、盐、饵料等青蟹幼体培育条件)结果; 初步研究了青蟹刚孵化幼体的干重和能值与抱卵蟹培育、胚胎发育温度的关系, 及与幼体生长发育存活的关系; 探讨刚孵化青蟹幼体的干重和能量的乘积($DW \times E$)可否作为衡量幼体质量好坏的指标。

1　材料和方法

1.1　亲蟹和抱卵蟹的培育

从产地或市场选购性腺较饱满的雌蟹经消毒处理后放养于铺垫 10cm 泥沙、沙和不铺垫的水泥池($6.5 \times 4 \times 2m^3$)内, 放养密度< 3 只/m^2, 池水深度不小于 1m, 海水盐度> 20, 并逐步升高, 培育温度< 32℃, 日温差不大于 1.5℃; 每天于傍晚和凌晨投喂鲜活鱼、贝, 投喂量不大于 5% 亲蟹重; 每隔几天清池检查, 取出抱卵蟹, 清理残饵和尸体。抱卵蟹单独培养于孵化池内, 盐度> 25, 日温差为 1.0、1.5、2.0 或 2.5℃, 不予饵料, 检查胚胎发育情况, 在估计孵化前 2d 换上消毒海水; 记录孵化情况。

1.2　青蟹幼体培育

从青蟹孵化池收集活力好的幼体用消毒海水清洗后投放育苗池, 第 1 期溞状幼体(Z_1)密度> 50 ind/dm^3; 育苗用海水是沙滤消毒海水、经 5μm 孔径的滤水袋过滤入池, 盐度范围是 25～30; 幼体培育温度范围是 25～31℃, 温度日变幅不大于 1.5℃; Z_1、Z_2 投喂轮虫(*Brachionus plicatilis*), 轮虫密度 40～60 ind/cm^3, 此期间适当投放单细胞藻类(单细胞藻类密度< 10^4/cm^3)形成微绿水。$Z_2 \rightarrow Z_3$ 或 Z_3 开始时投喂卤虫(*Artemia* sp.)无节幼虫, 密度 3～10ind/cm^3, 并视幼体摄食情况增减; 大眼幼体(M)的培育温度可> 30℃, M 后期盐度也可逐渐适当降低, 除投喂卤虫无节幼虫外, 并可辅以鱼糜和贝肉羹。记录不同孵育温度下孵化幼体的发育情况。

1.3　幼体干重和比能值测定

取刚孵化的锯缘青蟹幼体, 用砂滤再经网滤的海水洗涤 2 次, 用滴管挑数 4000 尾幼体于 200 目预先称重的尼龙筛网上, 用去离子水洗涤 2 次, 在 60℃下烘干 24h 至恒重后, 取出存放于干燥器或- 20℃下的冰箱内待分析。幼体的干重(DW, μg/ind)用 Sartorius BP211D 型电子天平称量(0.01mg), 比能值(EC, J/mg)用美国 *PARR* 1266-EF 半微量氧弹式量热仪的微型氧弹 1107 型氧弹测定(标准差 3%)。

2　结果

2.1　孵育温度与胚胎发育速度的关系

锯缘青蟹抱卵蟹的培育及胚胎发育期间温度高, 抱卵至孵化的时间短, 温度低则抱卵至孵化的时间长; 在实验温度范围内, 青蟹的胚胎发育时间与孵育温度的关系基本符合关系式: $D = 522(T - 117)^{-1.38}$ [5] (表 1)。

2.2　孵育温度与刚孵化幼体干重和比能值或能量的关系

从表 1 看, 孵育温度与刚孵化幼体的干重和比能值或能量没有明确的相关性。表 1 中温度的正负范围并不是青蟹抱卵蟹培育和胚胎发育期间平均温度的标准差, 而是表示青蟹抱卵蟹培育和胚胎发育期间某些天的日温差。实验发现, 当抱卵蟹培育和胚胎发育期间某些天的日温差≥2℃, 一方面胚胎发育不整齐,

孵化不同步,死卵和孵出原溞状幼体的比例高;另一方面,孵化的第 1 期溞状幼体一般都无法蜕皮转变进入第 2 期;第三,刚孵化的溞状幼体的能量低,一般都< 0.10J/ind。青蟹抱卵蟹培育和胚胎发育的温度高,并且期间某些天的日温差大,刚孵化的溞状幼体的干重低(< 6.0 μg/ind),幼体的比能值高(> 25 J/mg),这样的青蟹第 1 期溞状幼体更无法蜕皮转变进入第 2 期。此外,有些批次的青蟹抱卵蟹,其胚胎发育期间的培育温度正常,但刚孵化的溞状幼体的干重和比能值都偏低,这样的幼体也很难正常生长发育。

表 1 不同批次锯缘青蟹孵化幼体的干重(DW, μg/ind)和能量(E, J/ind; EC, J/mg)

Table 1 The dry weight (DW, μg/ind) and energy (E, J/ind) or energy content (EC, J/mg DW) of *Scylla serrata* newly hatched larvae from different batch

抱卵时间 Date	孵育温度 T (℃)	发育时间 (d)	干重 DW (μg/ind)	能量 E (J/ind)	比能值 EC (J/mg)	$DW \times E$	幼体发育存活情况
1999-04-19	24.5±1	12	6.32	0.118	18.68	0.746	至 Z_{4-3} 停电死亡
1999-09-03	29±0.5	9	6.20	0.159	25.69	0.986	至M 和部分仔蟹 C, 1%
1999-09-21	30±1	8	4.07	0.121	29.62	0.492	Z_1 死亡
1999-09-28	30±2	7	3.57	0.119	33.22	0.425	Z_1 死亡
1999-10-20	26±1	12	10.03	0.175	17.48	1.755	至 Z_3 无控温放弃
2000-05-16	26±0.5	11	11.3	0.132	11.69	1.492	至 C 存活率 25%
2000-05-17	28±0.5	9	8.40	0.113	13.48	0.949	至 C 存活率 30%
2000-05-29	27±2.5	9	5.60	0.073	12.98	0.409	Z_1 死亡
2000-05-29	27±2.5	10	7.00	0.090	12.88	0.630	Z_1 死亡
2000-05-30	27±0.5	10	9.80	0.125	12.78	1.225	* * 至 C 存活率 3%
2000-06-02	27.5±2	10	8.60	0.097	11.29	0.834	存活率极低至 Z_2 放弃
2000-06-04	28.5±2	9	8.20	0.095	11.58	0.779	存活率极低至 Z_2 放弃
2000-06-07	27±2	10	8.80	0.099	11.31	0.871	存活率极低至 Z_3 放弃
2000-06-10	29±1	8	9.33	0.175	18.74	1.633	* 至 C 存活率 5%
2000-06-11	29±2	10	4.67	0.136	29.21	0.635	Z_1 死亡
2000-06-12	29±1	8	12.20	0.222	18.18	2.708	* 至 C 存活率 1%
2000-06-25	26±1	12	9.25	0.153	16.57	1.420	* 至 C 存活率 5%

* 培育青蟹幼体的条件发生变化,达不到技术要求的条件; * * $Z_2 \rightarrow Z_3$ 时大量死亡;表中±℃表示日温差;C 表示青蟹仔蟹

2.3 孵育温度日温差与幼体质量的关系

不能正常发育的锯缘青蟹刚孵化的第 1 期溞状幼体的干重和能量或比能值有 3 个特征,一是干重低,而幼体比能值高;二是幼体能量和比能值都偏低;三是幼体干重和能量都偏低;(表 1)。但这过高和过低的"度"很难确定,因为样品数有限。通过分析发现,刚孵化的青蟹第 1 期溞状幼体的个体干重和能量的乘积可作为判断刚孵化的第 1 期溞状幼体能否正常生长发育的可能的指标,而这一指标结合了幼体干重和能量两个参数;当日温差≥2℃时,($DW \times E$)< 0.746 的几率显著增加(图 1),幼体可养活的几率极小,因此抱卵蟹培育和胚胎发育期间应注意温度的变化,尽可能把日温差控制在 2℃以内。从表 1 中 $DW \times E$ 的数值看,刚孵化的第 1 期溞状幼体能够正常生长发育的($DW \times E$)低限是 0.746,如果($DW \times E$)< 0.746,同时幼体比能值偏高或偏低,那么,这种刚孵化的第 1 期溞状幼体不能正常生长发育,必须放弃。

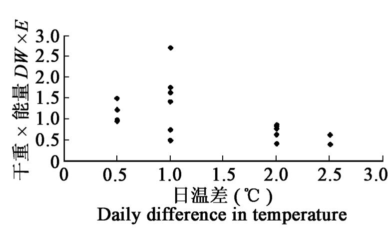

图 1 孵育温度日温差与锯缘青蟹刚孵化幼体干重和能量的关系

Fig. 1 The relation between dry weight, energy of newly hatched larvae and daily difference in brooding temperature during embryonic development of *S. serrata*

3 讨论

3.1 影响青蟹幼体质量的因素

刚孵化的青蟹溞状幼体质量或幼体内在的可养活性是青蟹幼体培育成败的关键, 人们对影响青蟹幼体质量的因素知之甚少, 减少刚孵化幼体的不稳定性而获得最好质量的刚孵化幼体的尝试大都尚未获得成功, 如果能制定一种指标来预示刚孵化幼体的可养活性, 无疑将提高青蟹育苗的稳定性并可减少用于不可养活幼体的开支。Mann 等[3]分析了①产卵时间与孵化率、②卵径与原溞状幼体的比例、不可养活幼体的比例、③孵化率与卵数、溞状幼体数、原溞状幼体数、不可养活幼体数、④卵数与原溞状幼体数、不可养活幼体数等 4 组特征与溞状幼体大小的相关性, 发现以上各组特征与特征间相关性显著, 大都达到极显著水平, 因此难以确定作为预示幼体可养活性指标特征; 但他们认为季节(温度)和切除眼柄将影响幼体质量[3,4]。深海红蟹(*Chaceon quinquedens*)[6]卵的脂肪和蛋白质比(L/P)与亲蟹中肠腺和卵巢中的L/P有关, 而其胚胎发育中卵黄消耗的类型与卵黄的L/P有关, 因此胚胎发育期间蛋白质的代谢与卵黄沉积类型有关, 因而幼体质量受到繁殖亲蟹营养状况的影响[4]; 有些批次的青蟹抱卵蟹, 其胚胎发育期间的培育温度正常, 但刚孵化的溞状幼体的干重和比能值都偏低, 这可能与亲蟹的营养状况有关。亲体孵育幼体的质量除与卵的内在本质有关外, 孵育温度是影响幼体质量的最重要因子[7,8]。不同季节、不同锯缘青蟹亲蟹所产受精卵的质量和幼体的质量都存在差别, 所以不同批次幼体在相同的培育条件下的培育效果是不一样的[7,9]。

3.2　孵育温度影响幼体质量的原因

一般认为, 水生甲壳动物由于没有排氮的障碍, 胚胎倾向于利用蛋白质为主要的能源物质, 锯缘青蟹[10,11]胚胎蛋白质含量高、脂肪低, 在胚胎发育过程中蛋白质降低的百分数最大, 青蟹胚胎发育主要以蛋白质为能源; 双齿扇蟹(*Xantho bidentatus*)胚胎发育期间干重和有机物质下降, 水分和灰分增加, 脂肪在胚胎发育后期才减少, 可溶性的蛋白质的增加是转变为结构蛋白质, 非可溶性的蛋白质下降或是作为能源物质或是转变为可溶性的蛋白质, 胚胎发育期间以蛋白质为主要的能源物质[12]; 鼹蟹(*Emerita holthuisi*)[13]和大鲎(*Tachypleus gigas*)[14]胚胎发育期主要的能量来源也是蛋白质; 同时蛋白质也是组织器官形成的基石。大西洋鳕鱼(*Gadus morhua*)在胚胎发育的自然温度(0～6.1℃)范围内, 温度升高 6℃, 胚胎蛋白质生长速度提高 1.65 倍, 而胚胎发育时间缩短了 2.7 倍, 孵化出膜时总的蛋白质含量降低了 1.65 倍, 孵出幼体蛋白质含量随温度的升高而下降[8]。因此抱卵蟹培育和胚胎发育的温度将直接影响幼体的质量, 从而影响幼体的进一步生长发育和存活率。中华绒螯蟹(*Eriocheir sinensis*)和斑节对虾(*Penaeus monodon*)胚胎及幼体的生长发育随温度升高而加快, 但幼体的大小和蜕皮存活率随温度的升高而降低, 孵育温度对幼体大小和蜕皮存活率的影响, 首先决定于升温方式, 然后才是温度高低[7]。

3.3　预示青蟹幼体质量的可能指标

不能正常发育的锯缘青蟹刚孵化的第 1 期溞状幼体的干重和能量和比能值有 3 个特征, 一是干重低, 而幼体比能值高; 二是幼体能量和比能值都偏低; 三是幼体干重和能量都偏低。第 1 种情况一般是胚胎发育温度高, 幼体不能正常发育, 可能是由于幼体的细胞分化和组织发生不完善; 第 2 种情况可能与胚胎发育期间日温差大、能量消耗过大、使幼体存储能量不足有关, 也可能与抱卵蟹或卵的质量有关; 第 3 种情况可能主要是由于胚胎发育期间日温差过大造成能量消耗大和幼体组织结构发育不完善。通过分析发现, 刚孵化的青蟹第 1 期溞状幼体干重和能量的乘积($DW \times E$)可作为判断刚孵化的第 1 期溞状幼体能否正常生长发育的指标。当孵育温度日温差≥2℃, 刚孵化青蟹幼体$DW \times E < 0.746$出现的几率明显增大, 幼体可养活性或幼体质量差。因此, 为获得质量稳定可养活的幼体, 锯缘青蟹抱卵蟹培育和胚胎发育期间日温差控制在 2℃以内是必要的, 降温和升温可以采取螺旋式方法。由于样品数少, 因此本文提出的预示青蟹幼体质量的指标有待进一步实验验证。

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