



不同育性三倍体长牡蛎性腺发育过程中的营养成分比较

王朔¹, 薛茗元¹, 杨琼¹, 于红^{1,2*}, 李琪^{1,2}

(1. 中国海洋大学, 海水养殖教育部重点实验室, 山东 青岛 266003;

2. 青岛海洋科学与技术国家实验室, 海洋渔业科学与食物产出过程功能实验室, 山东 青岛 266237)

摘要: 为阐明不同育性三倍体长牡蛎性腺发育与营养成分变化的关系, 实验对不育型和可育型三倍体长牡蛎性腺发育过程中的主要营养成分(糖原、总蛋白质和总脂肪含量)进行分析, 并与二倍体长牡蛎进行比较。结果显示, 三倍体长牡蛎性腺—内脏团、闭壳肌和外套膜3种组织中的糖原含量均显著高于同时期的二倍体长牡蛎, 性腺—内脏团和闭壳肌中的总蛋白质含量则显著低于同时期的二倍体。随着性腺发育, 可育型三倍体长牡蛎性腺—内脏团的糖原含量下降了31.88%, 二倍体长牡蛎下降82.41%, 而不可育型三倍体长牡蛎糖原含量下降了0.55%, 这与糖原为配子发生供能密切相关。此外, 不育型三倍体长牡蛎性腺—内脏团的糖原、总蛋白质和总脂肪含量在繁殖季节中均没有发现明显的波动, 而可育型三倍体长牡蛎由于性腺一定程度的发育, 其营养成分含量的变化趋势与二倍体类似。研究表明不育型和可育型三倍体长牡蛎在繁殖季节营养成分存在明显的差异, 不育型三倍体的糖原品质性状优于可育型, 这为长牡蛎育性控制育种提供了重要参考依据。

关键词: 长牡蛎; 三倍体; 不同育性; 营养成分

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牡蛎是世界上养殖范围最广、产量最高的贝类, 其中长牡蛎(*Crassostrea gigas*)又被称为太平洋牡蛎, 具有环境适应力强、生长速率快等优点, 是我国海水贝类养殖的主导品种^[1]。长牡蛎的繁殖期一般在春夏季节, 随着性腺发育和精卵的排放, 长牡蛎软体部由于糖原含量的急剧下降而导致品质下降, 同时还会伴随抗逆性下降、生长速率减慢等现象, 是牡蛎养殖业面临的一个棘手问题, 因此育性控制育种一直是牡蛎遗传育种的重要研究方向。目前, 人工诱导三倍体牡蛎是长牡蛎育性控制的主要技术手段。研究者通过化学或物理以及杂交等方法,

诱导获得了具有生长快速、肉质鲜美、成活率高、育性差等优点的三倍体长牡蛎^[2-4], 一定程度上缓解了二倍体牡蛎繁殖季节过后不能上市的问题, 填补了夏季牡蛎的市场空白。因此自三倍体牡蛎诱导成功后就受到了世界各国研究人员的关注, 现已在中国、韩国及欧美等国家实现了规模化养殖。

在自然界中, 三倍体水产动物通常是不育的, 例如三倍体合浦珠母贝(*Pinctada martensii*)、海湾扇贝(*Argopecten irradians*)等^[5-6], 部分三倍体鱼类虽然可以发育形成正常精巢, 却不能形成可育配子产生后代, 如三倍体大菱鲆(*Scoph-*

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第一作者: 王朔(照片), 从事贝类遗传育种研究, E-mail: 1165018093@qq.com

通信作者: 于红, E-mail: hongyu@ouc.edu.cn



thalmus Maximus)、斑点叉尾鲷(*Ictalurus punctatus*)、大西洋鲑(*Salmo salar*)等^[7-9]。然而有研究报道三倍体长牡蛎性腺发育存在可育型和不可育型两种情况。大部分三倍体长牡蛎个体不能产生或者产生极少量的配子,称为 β 型三倍体长牡蛎($3n\beta$);约25%的三倍体长牡蛎个体可以产生大量成熟配子,称为 α 型三倍体长牡蛎($3n\alpha$)^[10]。笔者在长牡蛎三倍体培育过程中,也发现了部分三倍体长牡蛎在繁殖季节能够产生大量的精卵。不同育性三倍体长牡蛎的存在,引起了研究者的关注^[11-12],也为研究者开展贝类多倍体性腺发育的研究提供了重要材料。

贝类的性腺发育与软体部的生化成分变化密切相关,在长牡蛎配子发生过程中,软体部蛋白质和脂肪含量略有上升,而糖原含量显著下降,长牡蛎配子发生需要储存的糖原提供能量^[13]。Allen等^[14]对长牡蛎软体部糖原含量的研究发现,在繁殖季节,二倍体牡蛎的糖原含量大幅度下降,而三倍体糖原含量下降幅度不大。曾志南等^[15]和孔令锋等^[16]的研究中也发现了类似结果。然而这些研究中,都没有具体区分可育型和不可育型三倍体长牡蛎,有关不同育性的三倍体长牡蛎性腺发育过程中生化成分的含量变化是否存在差异还未见报道。此外,糖原含量作为三倍体牡蛎优良品质的重要指标,其是否随育性而发生变化也是研究者关注的一个重要问题。

本研究以可育型和不可育型三倍体长牡蛎为材料,分析其性腺—内脏团、闭壳肌和外套膜组织的3个主要营养成分(糖原、总脂肪和总蛋白质)在繁殖季节中的含量变化,并与二倍体长牡蛎进行比较,旨在查清不同育性三倍体长牡蛎营养成分随性腺发育的变化特性,评估不同育性三倍体长牡蛎的营养价值。研究结果将为长牡蛎育性控制育种提供重要信息。

1 材料与方法

1.1 实验材料

实验所用二倍体和三倍体长牡蛎均取自山东即墨田横岛养殖海区,为人工养殖的2龄长牡蛎。其中三倍体长牡蛎是由四倍体与二倍体长牡蛎杂交获得,二倍体是同期同海区养殖的商业群体。

1.2 实验方法

样品处理 从4—7月,每月采集二倍体长牡蛎(壳高为77.91~81.38 cm)和三倍体长牡蛎(壳高为85.13~107.31 cm)各30只。样品活体解剖,先取小部分鳃丝和性腺用于倍性检测和组织切片分析,然后将外套膜、闭壳肌和剩余的性腺—内脏团放入液氮中速冻,再移至 -80°C 超低温冰箱保存。

倍性检测 取1~2根鳃丝于PBS缓冲液中,手术剪剪碎,用1 mL注射器吹打数十次,通过300目筛绢过滤至提前预冷的无水乙醇中, 4°C 固定4 h以上,取1 mL沉淀离心后弃上清液,用1 mL PBS缓冲液重悬细胞,用荧光染料碘化丙啶(propidium iodide, PI)对细胞DNA染色30 min,采用流式细胞仪(Beckman CytoFLEX)进行倍性检测,激发光波长为488 nm,荧光通道选用585/42 BP,测定DNA分子荧光强度,以二倍体长牡蛎为对照,待测峰值为二倍体的1.5倍时视为三倍体。

组织学分析 取约 5 mm^3 大小的性腺组织置于波恩氏液中固定24 h,75%酒精置换出波恩氏液,组织经75%、80%、85%、90%、95%、100%酒精梯度脱水,二甲苯透明,石蜡包埋后进行切片,切片厚度为 $5\text{ }\mu\text{m}$ 。用苏木精—曙红(H.E)染色法染色,封片晾干后用光学显微镜(Olympus BX51)观察并拍照。

营养成分检测 将鲜重约5 g的外套膜、性腺—内脏团和闭壳肌转移到塑料自封袋中,冷冻干燥48 h后,将样品研磨成粉末,并用80目(孔径为 $177\text{ }\mu\text{m}$)筛网过滤,放在干燥器中待用。每月每种类型的牡蛎取3只作为一组,设置3个生物学重复,根据Wang等^[17]构建的近红外反射光谱(NIR)分析模型,使用傅里叶变换NIR光谱仪(Thermo Fisher, Antaris MX)分析样品透射或反射光线的光密度,根据不同物质的含量与近红外区内多个不同的波长点吸收峰呈线性关系的理论进行各样品的糖原含量、总蛋白质含量和总脂肪含量的检测,利用TQ Analyst (Version 9.1.17, USA)软件处理采集的光谱数据。

1.3 数据分析

数据统计分析采用SPSS 21.0软件处理,用单因素方差分析(One-Way ANOVA)对营养成分的组间差异进行显著性检验,多重比较采用

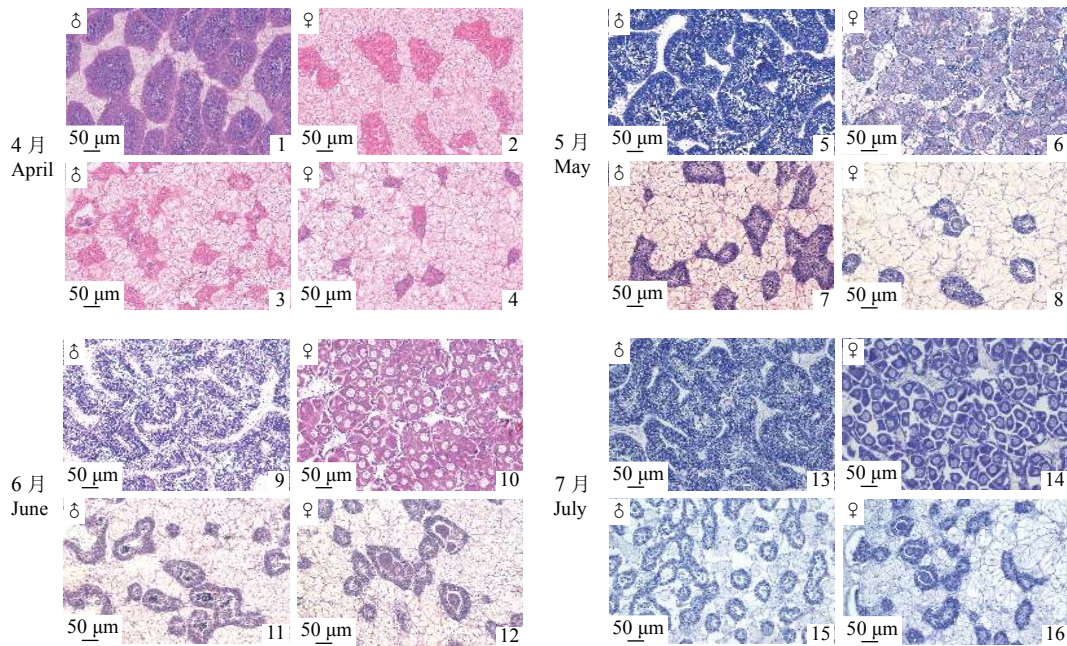
Dunnnett 氏 T_3 检验, 显著性水平为 $P < 0.05$ 。

2 结果

2.1 性腺组织石蜡切片形态观察

通过石蜡切片结果可以看出, 4月, 三倍体可育型 ($3n\alpha$) 长牡蛎的性腺中结缔组织较少, 卵巢滤泡腔中出现较多卵母细胞, 精巢滤泡腔中

出现从精原细胞到精子各个发育阶段的细胞, 主要为精母细胞 (图版)。三倍体不育型 ($3n\beta$) 长牡蛎的性腺中, 滤泡腔大小明显小于 $3n\alpha$ 型的滤泡腔, 且卵巢滤泡腔中主要是卵原细胞及极少量的卵母细胞, 精巢滤泡腔中也主要为精原细胞和极少量的精母细胞。5—7月, $3n\alpha$ 型性腺持续发育, 出现成熟的精子和卵子; 而 $3n\beta$ 型性腺几乎没有出现明显的变化, 一直保持滞育的状态。



图版 不同育性三倍体长牡蛎的性腺发育情况

1、2、5、6、9、10、13、14. 三倍体可育型长牡蛎 ($3n\alpha$); 3、4、7、8、11、12、15、16. 三倍体不可育型长牡蛎 ($3n\beta$)

Plate Gonadal development of different fertility of triploid *C. gigas*

1, 2, 5, 6, 9, 10, 13, 14. fertile triploid *C. gigas* ($3n\alpha$); 3, 4, 7, 8, 11, 12, 15, 16. sterile triploid *C. gigas* ($3n\beta$)

2.2 糖原含量变化

二倍体长牡蛎 3 种组织中糖原含量在 4—7 月呈显著下降趋势, 性腺—内脏团、闭壳肌和外套膜糖原含量 7 月比 4 月分别下降了 82.41%、60.45% 和 85.21%; $3n\alpha$ 型长牡蛎 3 种组织中糖原含量在 4—7 月也出现了下降趋势, 性腺—内脏团、闭壳肌和外套膜糖原含量 7 月比 4 月分别下降了 28.39%、52.67% 和 47.99%, 下降幅度均低于二倍体; 5—7 月, 同月份 3 种组织中的糖原含量均显著高于二倍体。 $3n\beta$ 型长牡蛎, 性腺—内脏团、闭壳肌和外套膜糖原含量 7 月比 4 月分别下降了 0.55%、40.29% 和 52.68% (图 1)。

2.3 总脂肪含量变化

3 种类型长牡蛎性腺—内脏团的总脂肪含量

均高于闭壳肌和外套膜的总脂肪含量。二倍体长牡蛎性腺—内脏团的总脂肪含量在 5 月上升之后开始显著下降 ($P < 0.05$), 7 月下降至 5 月的 76.46%; $3n\alpha$ 型长牡蛎性腺—内脏团的总脂肪含量在 6 月达到最高值后开始显著下降 ($P < 0.05$), 下降幅度为 22.74%; $3n\beta$ 型长牡蛎性腺—内脏团的总脂肪含量 5—6 月显著升高, 4 月和 7 月没有显著差异 (图 2)。二倍体长牡蛎闭壳肌的总脂肪含量在 4—6 月均显著 ($P < 0.05$) 高于三倍体, 7 月含量相差不大。3 种类型长牡蛎闭壳肌的总脂肪含量均呈下降趋势, 二倍体牡蛎下降最多, 下降了 39.34%; $3n\alpha$ 和 $3n\beta$ 型牡蛎分别下降了 14.83% 和 20.71%。二倍体牡蛎外套膜的脂肪含量一直呈下降状态, $3n\alpha$ 和 $3n\beta$ 型牡蛎外套膜脂肪变化趋势相反。在性腺发育期间, $3n\beta$ 型牡蛎

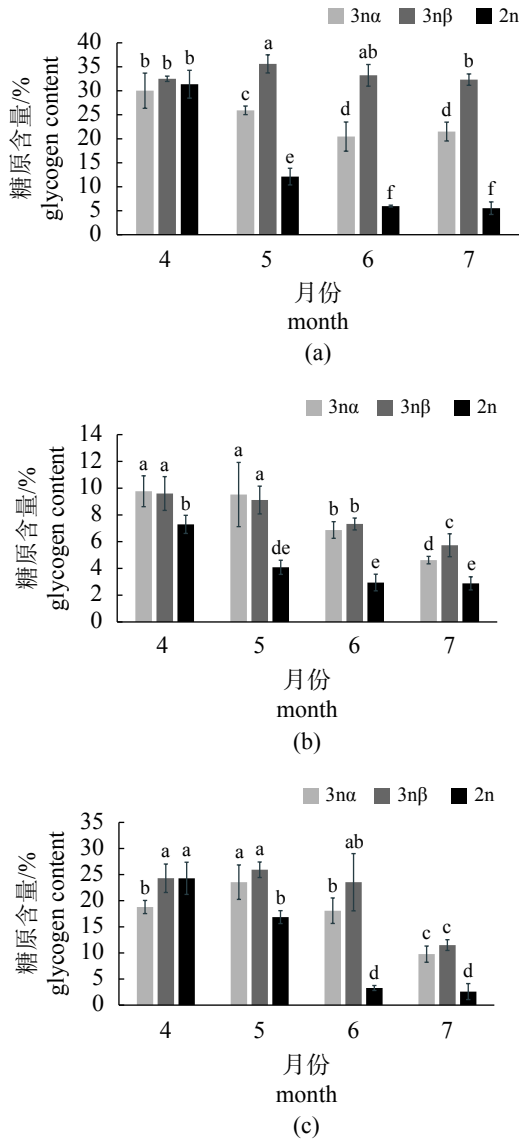


图 1 不同育性三倍体与二倍体长牡蛎 3 种组织中糖原含量(干重基础)变化

(a) 性腺—内脏团, (b) 闭壳肌, (c) 外套膜, 字母不同表示组间差异显著 ($P < 0.05$), 下同

Fig. 1 Variation in the glycogen content (dry weight basis) of three kinds of tissues from triploid and diploid *C. gigas*

(a) gonad-visceral mass, (b) adductor muscle, (c) mantle. Different letters denote significant difference between different groups ($P < 0.05$), the same below

外套膜的脂肪含量一直高于 3nα 型和二倍体牡蛎。

2.4 蛋白质含量变化

性腺—内脏团的总蛋白质含量变化情况: 4 月, 三倍体和二倍体相差不明显; 5—7 月, 二倍体的总蛋白质含量较 4 月显著上升, 且显著高

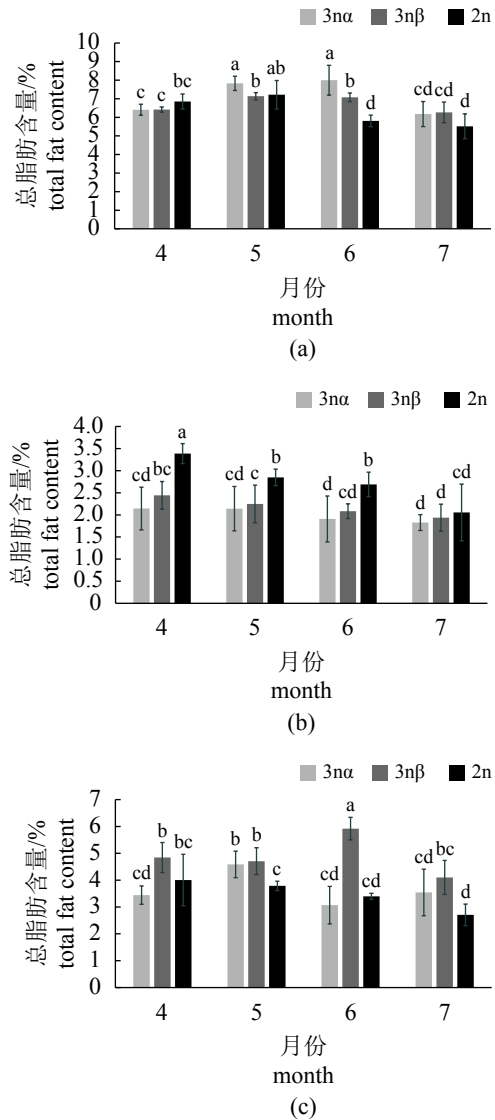


图 2 不同育性三倍体与二倍体长牡蛎 3 种组织中总脂肪含量(干重基础)变化

Fig. 2 Variation in the total fat content (dry weight basis) of three kinds of tissues from triploid and diploid *C. gigas*

于 3nα 和 3nβ 型三倍体, 6 月达到最高值, 比 4 月上升了 49.54%; 3nα 型变化趋势与二倍体类似, 随着配子发育总蛋白质含量逐渐上升, 6 月达到最高值, 比 4 月升高了 13.73%; 3nβ 型的总蛋白质含量从 4 月至 7 月, 总体变化幅度不大。3 种类型长牡蛎闭壳肌的总蛋白质含量在 4—7 月均呈增加趋势, 其中二倍体闭壳肌总蛋白质含量一直高于同时期的三倍体。3 种类型长牡蛎外套膜中的总蛋白质含量在 4—7 月总体上变化不大, 没有出现大幅上升或下降现象 (图 3)。

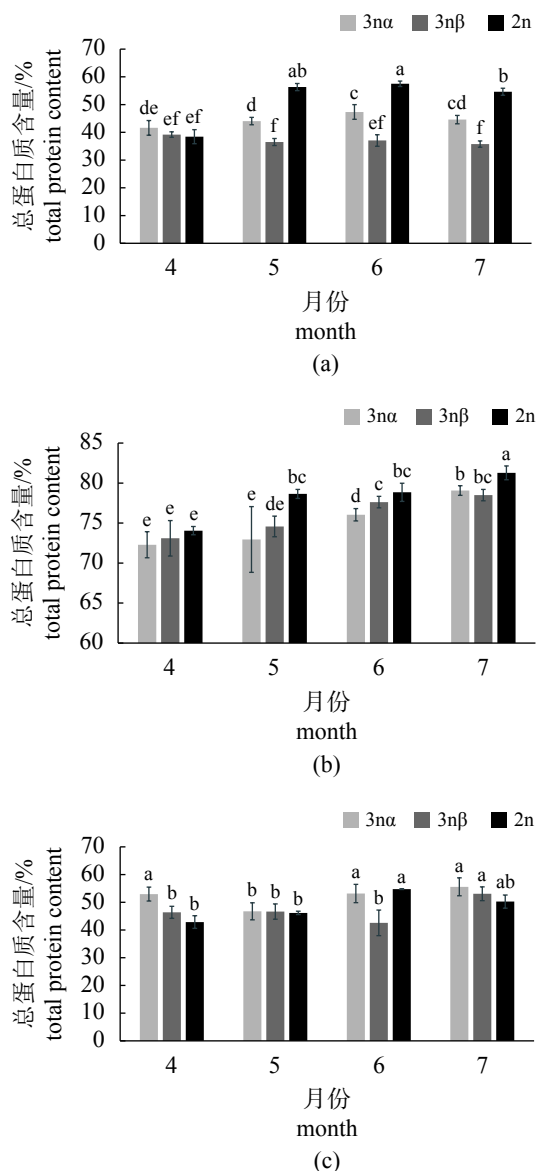


图3 不同育性三倍体与二倍体长牡蛎3种组织中总蛋白质含量(干重基础)变化

Fig. 3 Variation in the total protein content (dry weight basis) of three kinds of tissues from triploid and diploid *C. gigas*

3 讨论

3.1 不同育性三倍体长牡蛎性腺发育差异

一般来说,春、夏季是长牡蛎的繁殖季节,包括配子发生和产卵。通过对三倍体长牡蛎的性腺进行组织学观察,发现三倍体长牡蛎存在一定比例的可育型(3nα),这类三倍体的性腺组织在繁殖季节会产生大量成熟的精卵,但是与二倍体相比,3nα型性腺的滤泡数目、大小及成

熟精卵的数量都低于二倍体,说明可育型三倍体长牡蛎的性腺发育受到一定程度的抑制。绝大部分的三倍体长牡蛎为不可育型(3nβ),组织学观察结果显示,在4—7月,性腺基本不发育,虽然性腺中出现少量滤泡,但一直到7月,滤泡腔中极少出现成熟的精卵,这说明3nβ型长牡蛎确实不育。

3.2 不同育性三倍体和二倍体长牡蛎3种主要组织中营养成分的差异

研究表明,海洋贝类营养成分随着性腺发育而发生明显的变化,在食物充足的条件下,贝类将调节体内储存的糖原、蛋白质和脂肪等营养物质为性腺发育和配子发生供能^[18-19]。糖原是海洋贝类的主要能量载体,是配子发生的主要能量来源,这在魁蚶(*Scapharca broughtonii*)、四角蛤蜊(*Maetra veneriformis*)、褶牡蛎(*C. plicatula*)、紫石房蛤(*Saxidomus purpurata*)、江珧(*Atrina pectinata*)等多种贝类中都有所报道^[20-24]。此外,糖原可以转化为脂肪和甘油三酯,甘油三酯是卵母细胞中卵黄合成的重要结构物质,Gabbott^[25]认为双壳贝类通过由糖原转化为脂肪这一过程来满足卵黄蛋白合成的能量和物质需求。本研究结果显示,二倍体牡蛎3个主要组织(性腺—内脏团、闭壳肌和外套膜)的糖原含量都随着配子发育而急剧下降,进一步证实了糖原是牡蛎配子发育的重要能量来源。3nβ型三倍体牡蛎由于其性腺不发育,不能或仅产生极少数配子,闭壳肌、外套膜和性腺—内脏团中的糖原含量在4—6月并没有显著下降;而可育型(3nα型)三倍体牡蛎的性腺能够产生大量配子,闭壳肌、外套膜和性腺—内脏团的糖原含量在4—6月显著下降,但是仍高于同时期的二倍体糖原含量,这可能与3nα型牡蛎的配子数量少于同时期二倍体的配子数量有关。此外,研究结果显示无论是二倍体长牡蛎还是三倍体长牡蛎,性腺—内脏团和外套膜的糖原含量均显著高于闭壳肌,暗示性腺—内脏团和外套膜可能是储存糖原的主要器官,与邓传敏等^[26]对二倍体长牡蛎选育群体的研究结果一致。

脂肪对动物的性腺发育有重要影响,是维持性腺发育的能量物质^[27],是卵母细胞中的重要营养成分,脂肪的贮存与利用和贝类的繁殖活动密切相关。在本研究中,不同倍性的长牡蛎

性腺—内脏团中的脂肪含量均高于闭壳肌和外套膜, 表明性腺—内脏团可能是储存脂肪的主要器官。二倍体长牡蛎性腺—内脏团中的糖原含量从 5 月开始急剧下降, 而总脂肪含量略微增加; 3n α 型长牡蛎性腺—内脏团的糖原和脂肪含量在 5—6 月中存在明显负相关现象, 这可能是由于糖原可以转化成脂肪(甘油三酯)供性腺发育^[25, 28-29]。二倍体与 3n α 型长牡蛎的这种现象, 说明长牡蛎性腺—内脏团中的糖原与脂类可能存在转化关系, 为性腺发育后期提供营养和物质基础。Barber 等^[30]对海湾扇贝的研究也表明甘油三酯是卵细胞生成的必需成分, 另外, 3 种组织中性腺—内脏团的脂肪含量最高, 说明脂肪对配子发育可能发挥重要作用。

蛋白质是维持个体生物合成和酶合成的基本组成成分, 在生物生长和繁殖过程中具有重要作用, 其在海洋双壳贝类的配子形成和成熟过程中扮演着重要角色, 既是雌性卵黄的重要成分, 也是雄性性腺发育的能量来源^[31-33]。在饥饿或食物缺乏的条件下, 蛋白质是牡蛎生长发育的主要能量来源^[34]。在本研究中, 二倍体和 3n α 型长牡蛎性腺—内脏团中的蛋白质含量在 5—7 月都出现上升趋势, 说明性腺—内脏团中的蛋白质并未为配子的发生和发育提供能量, 而是随着性腺发育逐渐积累, 3n α 型的蛋白质含量低于二倍体, 这可能与其配子数量低于二倍体有关。与 3n α 型和二倍体不同的是, 3n β 型长牡蛎性腺—内脏团中的蛋白质含量在繁殖季节中几乎没有明显变化。

参考文献 (References):

- [1] 王如才, 王昭萍. 海水贝类养殖学 [M]. 青岛: 中国海洋大学出版社, 2008: 116-170.
Wang R C, Wang Z P. Science of marine shellfish culture [M]. Qingdao: China Ocean University Press, 2008: 116-170 (in Chinese).
- [2] Guo X M, DeBrosse G A, Allen J S K. All-triploid Pacific oysters (*Crassostrea gigas* Thunberg) produced by mating tetraploids and diploids [J]. *Aquaculture*, 1996, 142(3-4): 149-161.
- [3] 田传远, 王如才. 6-DMAP 诱导太平洋牡蛎三倍体 [J]. 青岛海洋大学学报(自然科学版), 1998, 28(4): 560-566.
Tian C Y, Wang R C. Triploid pacific oyster (*Crassostrea*
- gigas*) induced with 6-DMAP [J]. *Journal of Ocean University of Qingdao (Natural Science Edition)*, 1998, 28(4): 560-566 (in Chinese).
- [4] Guo X M, Allen Jr S K. Viable tetraploid Pacific oyster (*Crassostrea gigas* Thunberg) produced by inhibiting polar body I in eggs of triploids [J]. *Molecular Marine Biology and Biotechnology*, 1994, 3(1): 42-50.
- [5] 何毛贤, 林岳光, 姜卫国. 三倍体合浦珠母贝不育性研究 [J]. 热带海洋, 1996, 15(2): 17-21.
He M X, Lin Y G, Jiang W G. Studies on the sterility of triploid pearl oyster, *Pinctada martensii* (D.) [J]. *Tropic Oceanology*, 1996, 15(2): 17-21 (in Chinese).
- [6] Tabarini C L. Induced triploidy in the bay scallop, *Argopecten irradians*, and its effects on growth and gametogenesis [J]. *Aquaculture*, 1984, 42(2): 151-160.
- [7] Cal R M, Vidal S, Gómez C, et al. Growth and gonadal development in diploid and triploid turbot (*Scophthalmus maximus*) [J]. *Aquaculture*, 2006, 251(1): 99-108.
- [8] Wolters W R, Libey G S, Chrisman C L. Effect of triploidy on growth and gonad development of channel catfish [J]. *Transactions of the American Fisheries Society*, 1982, 111(1): 102-105.
- [9] Benfey T J, Sutterlin A M. Growth and gonadal development in triploid landlocked Atlantic salmon (*Salmo salar*) [J]. *Canadian Journal of Fisheries and Aquatic Sciences*, 1984, 41(9): 1387-1392.
- [10] Jouaux A, Heude-Berthelin C, Sourdain P, et al. Gametogenic stages in triploid oysters *Crassostrea gigas*: irregular locking of gonial proliferation and subsequent reproductive effort [J]. *Journal of Experimental Marine Biology and Ecology*, 2010, 395(1-2): 162-170.
- [11] Dheilly N M, Jouaux A, Boudry P, et al. Transcriptomic profiling of gametogenesis in triploid Pacific oysters *Crassostrea gigas*: towards an understanding of partial sterility associated with triploidy [J]. *PLoS One*, 2014, 9(11): e112094.
- [12] Suquet M, Malo F, Quere C, et al. Gamete quality in triploid Pacific oyster (*Crassostrea gigas*) [J]. *Aquaculture*, 2016, 451: 11-15.
- [13] 刘文广, 李琪, 高凤祥, 等. 长牡蛎繁殖周期、生化成分的季节变化与环境因子的关系 [J]. 热带海洋学报, 2011, 30(3): 88-93.
Liu W G, Li Q, Gao F X, et al. Seasonal variation in

- reproductive cycle and biochemical composition in relation to environmental factors in the Pacific oyster *Crassostrea gigas*[J]. *Journal of Tropical Oceanography*, 2011, 30(3): 88-93(in Chinese).
- [14] Allen J S K, Downing S L. Performance of triploid Pacific oysters, *Crassostrea gigas* (Thunberg). I. Survival, growth, glycogen content, and sexual maturation in yearlings[J]. *Journal of Experimental Marine Biology and Ecology*, 1986, 102(2-3): 197-208.
- [15] 曾志南, 林琪, 吴建绍, 等. 二倍体和三倍体太平洋牡蛎(*Crassostrea gigas*)肉重和生化成分的周年变化[J]. *海洋科学*, 1999(5): 54-56.
- Zeng Z N, Lin Q, Wu J S, *et al.* A annual changes of meat weight and biochemical composition between diploids and triploids in the Pacific oyster, *Crassostrea gigas*[J]. *Marine Sciences*, 1999(5): 54-56(in Chinese).
- [16] 孔令锋, 王昭萍, 于瑞海, 等. 二倍体和三倍体太平洋牡蛎(*Crassostrea gigas*)繁殖前后生化成分及氨基酸组成的比较研究[J]. *海洋湖沼通报*, 2001(4): 44-49.
- Kong L F, Wang Z P, Yu R H, *et al.* Comparison of biochemical composition and amino acid contents between diploid and triploid Pacific oyster, *Crassostrea gigas*, pre-and-post spawning[J]. *Transactions of Oceanology and Limnology*, 2001(4): 44-49(in Chinese).
- [17] Wang W J, Yang J M, Li Q, *et al.* Development of calibration models for rapid determination of chemical composition of Pacific oyster (*Crassostrea gigas*) by near infrared reflectance spectroscopy[J]. *Journal of Shellfish Research*, 2015, 34(2): 303-309.
- [18] Pérez Camacho A, Delgado M, Fernández-Reiriz M J, *et al.* Energy balance, gonad development and biochemical composition in the clam *Ruditapes decussatus*[J]. *Marine Ecology Progress Series*, 2003, 258: 133-145.
- [19] Joaquim S, Matias D, Lopes B, *et al.* The reproductive cycle of white clam *Spisula solida* (L.) (Mollusca: Bivalvia): implications for aquaculture and wild stock management[J]. *Aquaculture*, 2008, 281(1-4): 43-48.
- [20] 阮飞腾, 高森, 李莉, 等. 山东沿海魁蚶繁殖周期与生化成分的周年变化[J]. *水产学报*, 2014, 38(1): 47-55.
- Ruan F T, Gao S, Li L, *et al.* Gonad development and biochemical composition in the ark shell *Scapharca broughtonii* from coast of Shandong Peninsula[J]. *Journal of Fisheries of China*, 2014, 38(1): 47-55(in Chinese).
- [21] Ke Q Z, Li Q. Annual dynamics of glycogen, lipids, and proteins during the reproductive cycle of the surf clam *Macra veneriformis* from the north coast of Shandong Peninsular, China[J]. *Invertebrate Reproduction & Development*, 2013, 57(1): 49-60.
- [22] Li Q, Liu W G, Shirasu K, *et al.* Reproductive cycle and biochemical composition of the Zhe oyster *Crassostrea plicatula* Gmelin in an eastern coastal bay of China[J]. *Aquaculture*, 2006, 261(2): 752-759.
- [23] Wang C B, Li Q, Xu C X, *et al.* Seasonal changes of reproductive activity and biochemical composition of pen shell *Atrina pectinata* Linnaeus, 1767 in Bohai Sea, China[J]. *Journal of Ocean University of China*, 2017, 16(3): 479-489.
- [24] Bi J H, Li Q, Zhang X J, *et al.* Seasonal variation of biochemical components in clam (*Saxidomus purpuratus* Sowerby 1852) in relation to its reproductive cycle and the environmental condition of Sanggou Bay, China[J]. *Journal of Ocean University of China*, 2016, 15(2): 341-350.
- [25] Gabbott P A. Storage cycles in marine bivalve molluscs: a hypothesis concerning the relationship between glycogen metabolism and gametogenesis[M]//Barnes H B. Ninth European marine biology symposium. Aberdeen, UK: Aberdeen University Press, 1975: 191-211.
- [26] 邓传敏, 孔令锋, 于瑞海, 等. 长牡蛎壳金选育群体性腺发育与营养成分的周年变化[J]. *中国水产科学*, 2017, 24(1): 40-49.
- Deng C M, Kong L F, Yu R H, *et al.* Seasonal variation in gonadal development and nutritive components in the golden shell colored strain of Pacific oyster (*Crassostrea gigas*)[J]. *Journal of Fishery Sciences of China*, 2017, 24(1): 40-49(in Chinese).
- [27] 黄晓春, 刘慧慧, 苏秀榕, 等. 7种经济贝类生殖腺脂肪酸含量的研究[J]. *水产科学*, 2005, 24(8): 20-22.
- Huang X C, Liu H H, Su X R, *et al.* Fatty acid levels in 7 economic shellfishes[J]. *Fisheries Science*, 2005, 24(8): 20-22(in Chinese).
- [28] Dridi S, Romdhane M S, Elcafsi M. Seasonal variation in weight and biochemical composition of the Pacific oyster, *Crassostrea gigas* in relation to the gametogenic cycle and environmental conditions of the Bizert lagoon, China[J]. *Journal of Fisheries of China*, 2014, 38(1): 47-55(in Chinese).

- Tunisia[J]. *Aquaculture*, 2007, 263(1-4): 238-248.
- [29] Ojea J, Pazos A J, Martínez D, *et al.* Seasonal variation in weight and biochemical composition of the tissues of *Ruditapes decussatus* in relation to the gametogenic cycle[J]. *Aquaculture*, 2004, 238(1-4): 451-468.
- [30] Barber B J, Blake N J. Intra-organ biochemical transformations associated with oogenesis in the bay scallop, *Argopecten irradians concentricus* (Say), as indicated by ^{14}C incorporation[J]. *Biological Bulletin*, 1985, 168(1): 39-49.
- [31] Qin Y P, Zhang Y H, Ma H T, *et al.* Comparison of the biochemical composition and nutritional quality between diploid and triploid Hong Kong oysters, *Crassostrea hongkongensis*[J]. *Frontiers in Physiology*, 2018, 9: 1674.
- [32] Ren J S, Marsden I D, Ross A H, *et al.* Seasonal variation in the reproductive activity and biochemical composition of the Pacific oyster (*Crassostrea gigas*) from the Marlborough Sounds, New Zealand[J]. *New Zealand Journal of Marine and Freshwater Research*, 2003, 37(1): 171-182.
- [33] Ruiz C, Martínez D, Mosquera G, *et al.* Seasonal variations in condition, reproductive activity and biochemical composition of the flat oyster, *Ostrea edulis*, from San Cibrán (Galicia, Spain)[J]. *Marine Biology*, 1992, 112(1): 67-74.
- [34] Whyte J N C, Englar J R, Carswell B L. Biochemical composition and energy reserves in *Crassostrea gigas* exposed to different levels of nutrition[J]. *Aquaculture*, 1990, 90(2): 157-172.

Comparison of nutritional components of different fertility triploid Pacific oyster (*Crassostrea gigas*) during gonadal development

WANG Shuo¹, XUE Mingyuan¹, YANG Qiong¹, YU Hong^{1,2*}, LI Qi^{1,2}

(1. Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China;

2. Laboratory for Marine Fisheries Science and Food Production Processes,

Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China)

Abstract: Oysters represent a significant molluscan taxon that is widely distributed in world oceans and is the leading molluscan species by quantity produced. By now, oysters have been cultured on all continents, excluding Antarctica. Among all oysters, *Crassostrea gigas*, also known as the Pacific oyster, is the most economically important in aquaculture around the world. It is native to China, Japan and Korea, but has been introduced to many countries of the world, because of its potential for rapid growth and tolerance of a wide range of environment conditions. The global aquaculture production of *C. gigas* continues to expand year by year. In China, *C. gigas* is also one of the dominant species of marine culture. Like many other oysters, diploid *C. gigas* have an inferior taste and low meat quality during the reproductive seasons (spring and summer) each year, which can be attributed to the sharp decrease of glycogen content during gametogenesis. At the same time, it will also be accompanied by decreased stress resistance and slower growth rate during spawning. Therefore, reproductive control has been an important research field in genetic breeding of oysters. At present, artificial induction of triploid oysters is the main way to control the fecundity of *C. gigas*. There are two primary methods to produce triploid *C. gigas*: by inhibiting polar body formation after fertilization through chemical induction and crossing tetraploid oysters with diploid ones. Chemical induction is not reliable in producing a hundred percent triploids, while crossing tetraploids with diploids can reach very close to pure triploids. Furthermore, to avoid chemical toxicity, triploid *C. gigas* have been mostly produced by crossing tetraploid males and diploid females in recent years. Triploid oysters have advantage in terms of fast growth, delicious meat, and high survival rate during reproductive seasons, due to the poorly developed gonad, which has improved marketability relative to diploid in the reproductive seasons. Triploid *C. gigas* has entered into commercial farming in many countries, including China and America. Although triploid *C. gigas* has generally been considered to be sterile, some triploid *C. gigas* exhibit the same fecundity as diploids. Two types of gametogenic pattern have been reported in triploid *C. gigas*: fertile and sterile types. Most triploid oysters cannot produce or produce a very small number of gametes, which are referred to as being sterile ($3n\beta$) and as genetically confined. Nevertheless, about 25% of triploid oysters can produce a significant number of mature gametes, referred to as non-sterile or fertile triploids ($3n\alpha$). It has been reported that gametes of triploid oysters can reach functional maturity and can produce some viable progeny. The existence of triploid oysters with different fertility provides important materials for researchers to carry out studies on the gonadal development of polyploid molluscs. The development of gonad in molluscs is closely related to the changes in the biochemical composition of the edible part. Under the condition of sufficient food, molluscs can regulate the stored glycogen, protein and fat to provide energy for gametogenesis. The gametogenesis of oysters requires stored glycogen to provide energy. Though the contents of biochemical components of triploid *C. gigas* have been reported in the previous studies, the authors did not specifically distinguish between fertile and infertile triploid oysters. Whether there are differences in the content of biochemical components during gonadal development of triploid oysters with different fertility has not been reported yet. In addition, glycogen content is an important indicator of the high quality of triploid

oysters and the main molecular contributor to flavor quality in oysters, and whether it changes with fertility is also an important issue for researchers. Therefore, in the present study, in order to clarify the relationship between gonadal development and the changes of nutritional components in different fertility triploid *C. gigas*, we analyzed the main nutritional components (glycogen, total protein and total fat content) in the gonadal development of sterile and fertile triploid *C. gigas*, and compared them with diploid *C. gigas*. According to the near-infrared reflectance spectroscopy (NIR) analysis model, Fourier transform NIR spectrometer was used to analyze the optical density of the transmitted or reflected light of the sample. The content of different substances has a linear relationship with the absorption peaks of multiple different wavelength points in the near-infrared region. According to this theory, the glycogen content, total protein content and total fat content of each sample was tested. The results showed that the content of glycogen in gonad-visceral mass, adductor muscle and mantle of triploid *C. gigas* was significantly higher than that of diploid *C. gigas* in the same period, and the content of total protein in gonad-visceral mass and adductor muscle of triploid *C. gigas* was significantly lower than that of diploid *C. gigas* in the same period. During gonadal development, the glycogen content in gonad-visceral mass of fertile triploid *C. gigas* decreased by 31.88%, that of diploids decreased by 82.41%, and that of sterile triploid *C. gigas* decreased by 0.55%, which was closely related to the energy supply of glycogen for gametogenesis. In addition, the content of glycogen, total protein and total fat in gonad-visceral mass of sterile triploid *C. gigas* did not fluctuate significantly in the reproductive seasons, while the trend of nutrient content of fertile triploid *C. gigas* was similar to that of diploid because of the development of gonad to a certain extent. The results showed that there were significant differences in nutritional components between sterile and fertile triploid *C. gigas* during the reproductive seasons, and the glycogen quality of sterile triploids was better than that of fertile triploids. It will be interesting to address the questions why there are two different types of gametogenic pattern in triploid *C. gigas* and what controls the changes of biochemical components in future. To our knowledge, this is the first demonstration of nutritional components in two different types of triploid *C. gigas*. The findings in the present study not only are important for promoting the application of triploid *C. gigas* in the industry, but also provide important information for reproductive control in the breeding of *C. gigas*.

Key words: *Crassostrea gigas*; triploid; different fertility; nutritional components

Corresponding author: YU Hong. E-mail: hongyu@ouc.edu.cn

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