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硕士学位论文

福建牡蛎铜和锌富集能力的遗传参数评估
及其与糖原含量关系初探

Estimation of the genetic parameters of Cu/Zn enrichment
capability in Fujian oyster, *Crassostrea angulata*, and its
relationship with glycogen content

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摘要

牡蛎是世界范围内重要的海产经济贝类,我国牡蛎的养殖产量长久以来居于世界首位。福建牡蛎(*Crassostrea angulata*)又称葡萄牙牡蛎,是我国南方地区(尤其是福建省)牡蛎养殖的主要品种。牡蛎对重金属(尤其是铜、锌和镉)具有强富集和强耐受性,其体内的铜锌富集水平往往高于同一生活环境中的其他贝类。近年来,工业化的发展使我国沿海地区的重金属污染日益加剧,并对牡蛎养殖海域造成了较大的影响。在一些重金属污染严重的海区甚至出现了软体呈蓝色或绿色的“蓝牡蛎”和“绿牡蛎”,这些牡蛎体内铜和锌的总含量高达软体干重的3%-7%。“蓝牡蛎”和“绿牡蛎”的出现无疑对我国的牡蛎养殖业和人类的食品安全造成了威胁,而短期内我国多数沿海的重金属污染情况并不能得到有效缓解。针对这一情况,本研究从牡蛎抗病性育种研究和实践中得到启发,结合牡蛎铜锌富集个体差异较大的现象,从遗传育种角度出发,对福建牡蛎铜锌富集能力和生长性状的遗传参数进行评估。同时利用构建的牡蛎家系初步探究福建牡蛎铜锌富集与其体内糖原含量的关系,以期探索牡蛎铜锌富集性状的遗传特性,为进行牡蛎金属富集能力的选育工作奠定理论基础。主要结果如下:

1. 福建牡蛎不同性腺发育阶段体内铜锌的组织分布

本研究对同一批次采集的福建牡蛎进行测定,以准确描述牡蛎对铜、锌的富集能力,确定在不同性腺发育阶段内均能代表牡蛎铜和锌富集水平的组织,消除性腺发育的影响。结果表明,不同性腺发育阶段内,福建牡蛎体内铜锌浓度最高的组织均为鳃,其次为外套膜;而外套膜和性腺中的铜锌总量在各阶段均达到了软体总含量的50%以上,其中外套膜中铜含量约占软体总含量的40%左右(除成熟期外)。不同性腺发育阶段内,与福建牡蛎全软体(除性腺外)铜锌浓度相关性最高的组织也都是外套膜。因此,福建牡蛎的外套膜中铜锌浓度可以代表全软体的铜锌富集水平,并且不受性腺发育的影响。

2. 福建牡蛎铜锌富集能力和生长性状的遗传参数评估

采用巢式设计法构建了60个福建牡蛎家系,并用其中9个半同胞家系和36个全同胞家系对牡蛎铜锌富集能力的遗传参数进行评估。结果显示,家系子代个体间铜浓度和锌浓度的变异系数分别为65.69%和47.34%,铜和锌富集能力的狭

义遗传力分别为 0.19 ± 0.07 和 0.32 ± 0.10 ，铜富集能力与锌富集能力之间的表型相关系数为 0.859，遗传相关系数为 0.796。可见，福建牡蛎的铜和锌富集具有很高的选育潜力，铜、锌富集能力均属于中等遗传力性状，铜和锌富集之间存在很高的表型相关和遗传相关，可以通过家系选育对二者进行联合选择。对福建牡蛎生长性状的遗传参数评估结果显示，子代个体间总重、软体干重和闭壳肌干重的变异系数分别为 40.28%、61.07% 和 48.19%，表现出较大的选育潜力；壳长和总重的狭义遗传力分别为 0.39 ± 0.11 和 0.43 ± 0.12 ，属于较高水平的遗传力；总重与壳长、壳宽、壳深之间存在很高的表型相关 ($r > 0.670$) 和遗传相关 ($r > 0.730$)，闭壳肌干重与壳宽、软体干重之间也存在较高程度的表型和遗传相关 ($r > 0.610$)，这些表明对福建牡蛎的生长性状进行选育可以获得良好的效果。另外，本研究中铜锌富集能力与生长性状间的表型相关 ($r \leq 0.316$) 和遗传相关 ($r \leq 0.436$) 都很低，相关性较差，因此无法通过生长性状对福建牡蛎的铜锌富集能力进行间接选育。但同时也表明，对铜锌富集能力的选育不会对生长性状产生不利影响。

3. 福建牡蛎铜锌富集水平与糖原含量的关系初探

采集同一批次的福建牡蛎，用以探究在不同性腺发育阶段均能代表牡蛎体内糖原含量的组织，消除性腺发育的影响。结果显示，不同性腺发育阶段内，福建牡蛎体内糖原浓度和含量最高的组织几乎均为外套膜，与全软体糖原浓度（除性腺外）相关性最高组织也是外套膜。表明福建牡蛎外套膜中的糖原浓度可以代表全软体的糖原浓度，并且不受性腺发育的影响。在此基础上，从构建的牡蛎家系中挑出 6 个存在铜锌富集差异的家系，分别测定其外套膜的铜锌和糖原浓度。结果显示，福建牡蛎体内的铜锌富集水平与其糖原含量之间没有一定的规律，二者之间的相关性也很低（L2 家系除外）。因此，生活在洁净海域的福建牡蛎体内的铜锌富集水平与其糖原含量之间没有明显的关联。

关键词：福建牡蛎；铜锌富集；遗传力；遗传相关；糖原含量

Abstract

Oyster is an important marine economic shellfish in the world. In recent years, the production of oysters in China has always been the first in the world. Fujian oyster (*Crassostrea angulata*), also known as the Portuguese oyster, is the main cultured oyster species in southern China, especially in Fujian Province. However, with the rapid development of industrialization, the heavy metal pollution in coastal areas of China is increasing day by day. Oysters are strong accumulator of heavy metals, especially for copper, zinc and cadmium, and they can still be alive with extremely high body metal concentration. In recent years, “blue-colored” and “green-colored” oysters have always been found in some contaminated oyster farms in China, and the total content of Cu and Zn in their body can reach up to 3%-7% of the whole-body tissue dry weight! The appearance of such “blue-” and “green-colored” oysters has undoubtedly posed great threat to the oyster industry and human seafood safety. However, it's impossible to alleviate the heavy metal contamination effectively in a short term in China. In view of this, based on the characteristics of Cu/Zn accumulation in oysters, as well as inspired by the researches about disease resistance of oysters, we focused on the genetic breeding of Cu and Zn enrichment capability, estimating the genetic parameters of Cu/Zn enrichment capability and growth traits, and carrying out a preliminary study on the relationship between Cu/Zn enrichment capability and glycogen content with our constructed *C. angulata* families. The main results are as follows:

1. Study on the tissue distribution of Cu and Zn at different gonadal development stages of *C. angulata*

In order to find a tissue which can represent the Cu and Zn enrichment level of oysters during different gonadal development stages, Fujian oysters were collected at a same batch and dissected into mantle, gill, adductor, visceral mass and gonad after identifying the gonadal development stages, and then the Cu and Zn concentrations were determined separately. The results showed that gill has the highest Cu/Zn

concentration which was followed by mantle, and the highest Cu/Zn content occurred in mantle (except for Cu content at gonadal maturation stage). Correlation analysis showed that Cu/Zn concentrations of mantle had the highest correlation coefficient with the concentrations of whole-body tissue (except for gonad). The results indicated that the Cu/Zn concentration of mantle can represent the whole-body Cu/Zn enrichment level of *C. angulata*, and is not affected by gonadal development.

2. Estimation of the genetic parameters of Cu and Zn enrichment capability and growth traits in *C. angulata*

A nest design was carried out with 12 sires and 60 dams to produce 12 half-sib families and 60 full-sib families, of which 9 half-sib and 36 full-sib families were used to estimate the genetic parameters after one year. Results showed that, the CVs of Cu and Zn concentrations among different *C. angulata* individuals were 65.69% and 47.34% respectively, the values of narrow-sense heritability were 0.19 ± 0.07 for Cu and 0.32 ± 0.10 for Zn enrichment capability, the phenotypic and genetic correlation coefficient between Cu and Zn enrichment were 0.859 and 0.796 respectively. These indicated that the Cu and Zn enrichment capability in *C. angulata* has a high breeding potential, and the narrow-sense heritability for Cu enrichment is moderate while for Zn is medium-to-high, both the phenotypic and genetic correlation between Cu and Zn enrichment were positive and high, so they can be both effectively selected by pedigree breeding at the same time. The estimation for growth traits of *C. angulata* showed that, the CVs of live weight, dry meat weight and dry adductor weight were 40.28%, 61.07% and 48.19% respectively, meaning high breeding potential. The narrow-sense heritability for live weight and shell length were 0.39 ± 0.11 and 0.43 ± 0.12 , which were higher than Cu and Zn. Live weight showed high phenotypic and genetic correlation with shell length, shell width and shell depth ($r > 0.670$). And both the phenotypic and genetic correlation among dry adductor weight, shell width and dry meat weight were high, too ($r > 0.610$). Genetic parameters suggested that most of the growth traits of *C. angulata* can be improved by genetic breeding. The phenotypic and genetic correlation coefficients between Cu/Zn enrichment capability and growth traits were all below 0.436, means low correlation. Therefore, it is not

possible to indirectly select the Cu/Zn enrichment capability of *C. angulata* by growth traits, but the selection for Cu/Zn enrichment capability will not affect these growth traits.

3. Preliminary study on the relationship between Cu/Zn enrichment capability and glycogen content of *C. angulata*

The same materials of *C. angulata* as in chapter 2 were used to find a tissue which can best represent the glycogen content of oysters during different gonadal development stages. The results showed that mantle has both the highest glycogen concentration (content) and the highest correlation coefficient with the concentrations of whole-body tissue (except for gonad), indicating that the glycogen concentration of mantle can represent the whole-body glycogen level during different gonadal development stages. Based on this, six *C. angulata* families with differential Cu/Zn enrichment capability were selected to investigate the relationship between Cu/Zn enrichment and glycogen content. The results showed there was no specific regularity between Cu/Zn enrichment and glycogen content, and the correlation between them was low (except for family L2). As a result, we concluded that there is no significant relationship between Cu/Zn enrichment capability and glycogen content in *C. angulata* living in clean seawater.

Key words: *Crassostrea angulata*; Cu and Zn enrichment; heritability; genetic correlation; glycogen content

第一章 绪论

1.1 我国牡蛎养殖产业现状

牡蛎隶属软体动物门、双壳纲、珍珠贝目、牡蛎科，具有肉味鲜美、营养价值高等特点，素有“海洋牛奶”之称，是一种重要的海洋生物资源。牡蛎的地理分布十分广泛，在世界沿海国家几乎都有分布。作为一种滤食性贝类，牡蛎的食物链短、生长快、产量高，具有良好的经济效益，是我国乃至世界海水养殖的重要品种。

我国牡蛎养殖历史悠久，目前牡蛎已成为我国四大海水养殖经济贝类之一。我国沿海的牡蛎有 20 余种，常见的有长牡蛎 (*Crassostrea gigas*)、福建牡蛎 (*Crassostrea angulata*) 和香港巨牡蛎 (*Crassostrea hongkongensis*) 等。2007 年以来，我国牡蛎的养殖规模和产量都居世界首位，近 15 年来我国每年的牡蛎产量更是达到 300 万吨以上，在世界牡蛎养殖产业中占有重要地位 (FAO,2016)。据统计，2015 年我国沿海各省牡蛎养殖的总产量达 457 万吨，占到海产贝类总产量的 36%，具有重要的经济地位 (中国渔业统计年鉴，2016)。

我国海岸线绵延数千公里，从南到北均有牡蛎分布，主要的牡蛎养殖区集中在广东、广西、福建和山东，其中福建省天然的地理位置优势使得其牡蛎养殖面积和产量均居全国之首，其养殖产量几乎占到了全国牡蛎养殖总产量的 50% 左右 (曾志南和宁岳，2011)，而福建牡蛎是福建省最主要的牡蛎养殖品种。

随着我国牡蛎养殖业的迅速发展，养殖规模不断扩大、养殖产量大幅提高，但养成的牡蛎品质却呈现下降趋势，并且养殖过程中出现生长慢、出肉率低、抗病性差和存活率低等现象，这些都严重制约着我国牡蛎养殖产业的可持续发展。造成这些现象的原因主要有三方面：一是种质资源的退化，目前我国牡蛎种质来源主要是人工育苗和自然海区半人工采苗，长期人工育苗中不合理的繁育方式所造成的近交衰退、自然采苗的苗种质量参差不齐等都会对牡蛎的种质资源产生不良影响，进而影响牡蛎的养殖产量和质量；二是传统养殖方式的局限性，传统养殖往往具有养殖品种单一且密度高的特点，海区牡蛎的超负荷养殖不仅导致海区生产力下降、牡蛎生长缓慢、死亡率高，同时牡蛎排放的代谢废物也造成海区底

质环境的污染；三是养殖水域环境污染恶化，伴随着沿海经济的快速发展，人工废弃物越来越多地进入海洋，并已成为影响养殖水体的主要外源污染源（曾志和宁岳，2011；阙华勇和张国范，2016；Forrest et al., 2009）。

针对牡蛎种质资源退化的问题，过去的几年里，国内一些学者通过遗传育种技术相继选育出了“海大1号”、“华南1号”和“金蛎1号”3个牡蛎新品种(系)，这些新品种(系)都具有生长速度快的特点（丛日浩，2014；喻子牛等，2017；曾志南，2015），在一定程度上缓解了牡蛎养殖过程中种质资源退化的现象。针对传统牡蛎养殖方式局限性的问题，近年来兴起的多营养层次综合养殖模式为贝类养殖环境所面临的多重压力提供了一条有效的解决途径（Chopin et al., 2001）。多营养层次综合养殖是指：由不同营养等级的生物组成综合养殖系统，在这个系统中，一些生物的排泄物可作为其他生物的营养来源，从而实现养殖系统中营养物质的循环利用、提高养殖产量、降低养殖成本的目的，同时还可控制养殖所造成的污染（Neori et al., 2004；唐启升等，2013）。未来这一养殖模式有望应用到牡蛎养殖产业，达到既满足饵料需求、又能控制污染的目的。

牡蛎养殖水域污染恶化的现象集中表现在水体重金属、细菌和贝类毒素超标。目前我国海产贝类养殖所面临的污染主要有工业污染、微生物污染和生物毒素污染三种（吕浩，2007）。随着工业废弃物进入近岸海区的污染物种类繁多，其中对贝类养殖产业危害较大的有重金属、石油烃和农药等；微生物污染主要源自城市生活污水的排放，一些致病菌随之进入近岸水体、造成贝类疾病，影响贝类的生长和存活；生物毒素污染则主要是由赤潮等藻类大爆发引起的，包括贝类毒素、河豚毒素、血卡毒素等。

在众多类型的污染中，海洋重金属污染一直是困扰人们的问题之一。由于重金属不像有机污染物那样可以在海洋生物化学过程中被降解为毒性较小的化合物，重金属污染会对生物和环境产生巨大而持久的影响。有毒的重金属被海洋生物吸收后会进入食物链并传入上层营养级，最终通过食物链传递到人体，并在体内积累，对人体产生危害（Wang et al., 2002；Bryan et al., 1979）。而目前针对我国牡蛎养殖水域污染恶化的问题，除了预防污染和加强养殖牡蛎质量安全控制外，并无其他更有效的措施。

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