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大海马育苗池水华发生期间细菌动态及相关理化参数

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摘要：大海马(*Hippocampus kuda* Bleeker)育苗池为水泥池，规格为 4.0 m×6.0 m×1.6 m，分设正常池与水华发生池，各设 3 个平行池，每池养殖幼海马约 1 000 尾，测定水体水华发生时水体细菌数量和理化因子变化的规律。结果显示，当水温高于 24 ℃时，育苗池易发生水华，形成水华的优势藻为铜绿微囊藻(*Microcystic aeruginosa*)。正常育苗池水体中异养细菌总量平均比水华池的高出近 1 个数量级。正常池和水华池表层异养细菌变动范围分别为 2.50×10^{3}～7.23×10^{4} CFU/mL和 4.75×10^{2}～6.90×10^{3} CFU/mL，水华池比正常池减少了 60%～99%；底层异养细菌变动范围则分别为 4.75×10^{3}～7.53×10^{4} CFU/mL 和 6.25×10^{2}～1.50×10^{4} CFU/mL，水华池比正常池降低了 73%～97%。水华池与正常池底层异养细菌数量的差异极显著($P<0.01$)。两组池表层弧菌数量变动范围分别为 0.85×10^{2}～7.19×10^{3} CFU/mL和 0.33×10^{2}～8.92×10^{2} CFU/mL，水华池比正常池减少 5%～93%；底层弧菌数量变动范围则分别为 8.30×10^{2}～1.16×10^{4} CFU/mL 和 0.53×10^{2}～2.04×10^{3} CFU/mL，水华池比正常池降低 2%～97%。水华发生池的底层和表层的细菌数量与正常池的差异均显著($P<0.05$)。同时，水华池平均水温比正常池约低 1 ℃，溶解氧(DO)比正常池的降低了 22%～33%；而水华池中的氨氮含量则为正常育苗池的 1.41～2.34 倍。水华发生时，育苗池表层和底层水体中弧菌数量比正常池的分别减少了 61%～87%和 82%～93%。

关键词：水华；大海马；育苗池；环境因子
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“水华”(Water-bloom)指浮游藻类在水体中异常繁殖，并大面积覆盖于水面的现象[1]。目前，水华、赤潮的发生给水产养殖业带来影响方面的研究，多集中于养殖鲈鱼(Seriolinae)、鲷科鱼类(Sparidae)、鲀类(Tetrodontinae)、虾类(*Penaeus*)及鲍(*Haliotis*)等重要水生经济动物[2-3]，且多在形成水华(赤潮)的优势种群落生态、成因、毒性、危害机理及防治措施等角度报道[4-8]。有关海洋细菌与水华藻、赤潮藻的相互关系的研究，国外已有学者从群落水平上进行[9]，国内则相对薄弱[10]。研究表明，水体中菌与藻的关系密切，以微藻为基础的微小生物群落对大多数海水养殖生物体内外常见的弧菌菌群具有普遍排斥作用[11-12]。微生物生态是调控养殖水质的重要依据之一。但目前养殖用水水华的发生对水体中环境因子(细菌数量、理化因子)影响的报道尚少。本研究针对大海马(*Hippocampus kuda* Bleeker)育苗池水体在夏季常常发生藻类大量繁殖而形成水华，引起水质恶化的现象，分析当水华发生时，大海马养殖水体中细菌群落动态及相关理化参数的变化，以期掌握海水养殖水体中细菌群落在不同养殖条件下的变动规律，为养殖海马病害的生态防治及微藻资源开发提供理论依据。

1 材料与方法

1.1 取样地点与实验设计

取样地点位于粤东国内养殖规模最大的“广东中大亿达洲海马养殖基地”内的海马育苗池，水泥池规格：4.0 m×6.0 m×1.6 m，分 2 组，每组有 3 个平行池。调查期间充气、不换水，吸底污。并选养两类海马育苗池：第一组为正常育苗池(以下简称正常池)；第二组为水华发生池(以下简称水华池)。两组的 3 个平行池中各养幼海马约 1 000 尾。按照日常养殖管理技术，投喂适量鲜活桡足类饵料，每天 3 次，饵料的投喂量占体重的 5%～8%。时间从 2003

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年9月4日水华在育苗池表层发生时开始，至9月23日各水华池表面水华消退时结束。

1.2 水华池藻类优势种(简称水华藻)的鉴定

肉眼观察养殖池水色和水面藻类大量繁殖情况以及水华消亡状况。随机采集表层水样500 mL，加碘固定沉淀后，浓缩至50 mL，取混匀后的浓缩样1 mL，滴至洁净凹玻片中，置于Olympus BX50显微镜下观察，并进行种类鉴定[13]。

1.3 细菌样品的采集及培养计数

取样时间从2003年9月4日开始至9月23日，隔天定时(8:30～9:00 am)到各实验池取样。取样方法如下：分别取其表层多点混合水样及底层多点混合水样，立即带回实验室。作梯度稀释处理后，接种于异养细菌2216E培养基和弧菌专用TCBS培养基上，于28 ℃恒温培养箱中培养48 h后，进行菌落计数[14-15]。

1.4 理化因子的测定及数据统计与分析

在采集水样的同时，测定其理化因子。用海水水温计测定养殖池水温；日产5－10E折光仪测定养殖池盐度。养殖用水取样后，立即带回实验室，利用意大利哈纳C200多参数离子计测定DO、氨氮。采用Excel软件作出细菌数量、理化因子变化曲线图，依《生物统计学》[16]进行方差分析。

2 结果与分析

2.1 水华的发生对育苗池水体中异养细菌数量的影响

正常池及水华池水体中异养细菌变动状况见图1。结果显示，正常池中水体表层、底层异养细菌数量平均值分别为2.44×10^4 CFU/mL和3.29×10^4 CFU/mL，均比水华池水体表、底层异养细菌数量平均值2.22×10^3 CFU/mL和4.05×10^3 CFU/mL高1个数量级。在整个调查期间，两组池表层异养细菌数量变动范围分别为$2.50\times10^3\sim7.23\times10^4$ CFU/mL与$4.75\times10^2\sim6.90\times10^3$ CFU/mL，水华池比正常池少60%～99%；底层分别为$4.75\times10^3\sim7.53\times10^4$ CFU/mL与$6.25\times10^2\sim1.50\times10^4$ CFU/mL，水华池比正常池少73%～97%。水华发生初期，水华池与正常池底层异养细菌数量的差异极显著($P<0.01$)，这种情况维持到调查结束。在正常池水体中，其表层、底层异养细菌数量呈不规则波动，变动情况如图1所示。而水华池异养菌的数量除在水华刚形成时出现较大波动外，直到调查后期相对正常池处于较低的水平上。

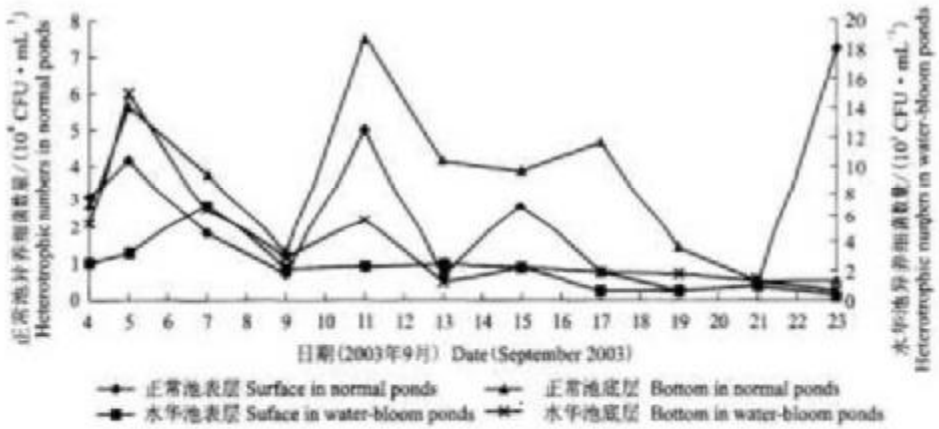

图1 正常池、水华池养殖水体中异养细菌动态变化

Fig.1 Dynamic changes of heterotrophic numbers in culture water of normal ponds and water-bloom ponds

2.2 水华的发生对育苗池水体中弧菌数量的影响

正常池及水华池水体中弧菌数量变动状况见图2。结果显示，正常池中水体表层、底层弧菌数量平均值分别为1.74×10^3 CFU/mL和3.79×10^3 CFU/mL，比水华池水体表层、底层弧菌数量平均值2.32×10^2 CFU/mL与6.48×10^2 CFU/mL高1个数量级。两组池表层弧菌数量变动范围分别为$0.85\times10^2\sim7.19\times10^3$ CFU/mL与$0.33\times10^2\sim8.92\times10^2$ CFU/mL，水华池比正常池少5%～93%；底层分别为$8.30\times10^2\sim1.16\times10^4$ CFU/mL与$0.53\times$

10^2～2.04×10^3 CFU/mL,水华池比正常池少2%～97%。在水华发生初期,两者即出现较大的差异。差异显著性分析结果表明,两组池表层弧菌数量差异显著($P<0.05$),底层弧菌数量差异极显著($P<0.01$)。

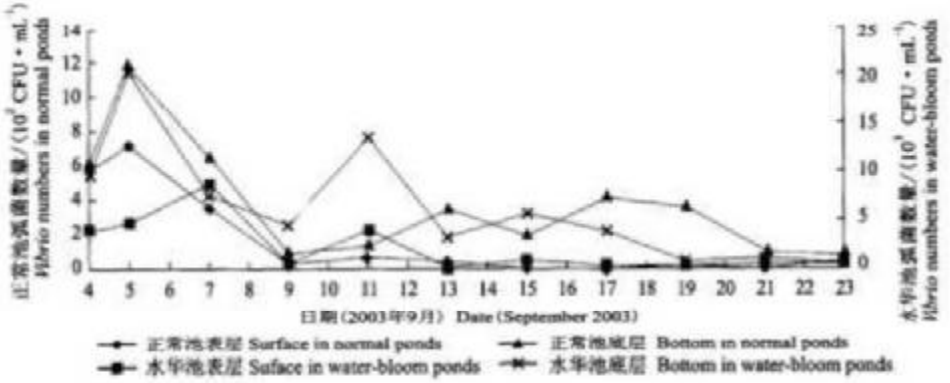

图2　正常池、水华池养殖水体中弧菌动态变化

Fig.2　Dynamic changes of *Vibrio* numbers in culture water of normal ponds and water-bloom ponds

2.3　发生水华与无水华育苗池水体理化因子的变动

两组池理化因子变动状况见图3、4。结果显示,正常池与水华池表层水温波动范围分别为24.5～29.0 ℃和25.8～29.0 ℃;底层水温波动范围则为27.5～32.0 ℃和27.0～29.5 ℃,正常池水温高于水华池。两组池盐度前期相差不大,而在实验后期(9月17日后),由于天气降水比较多,正常池出现盐度下降现象,而水华池的盐度范围较正常池稳定。

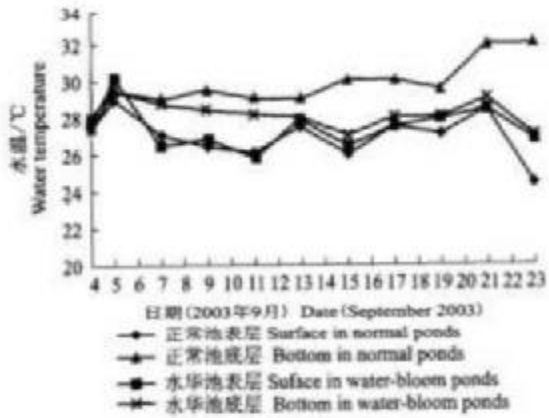

图3　正常池与水华池水温变化动态

Fig.3　Dynamic changes of water temperature in culture water of normal ponds and water-bloom ponds

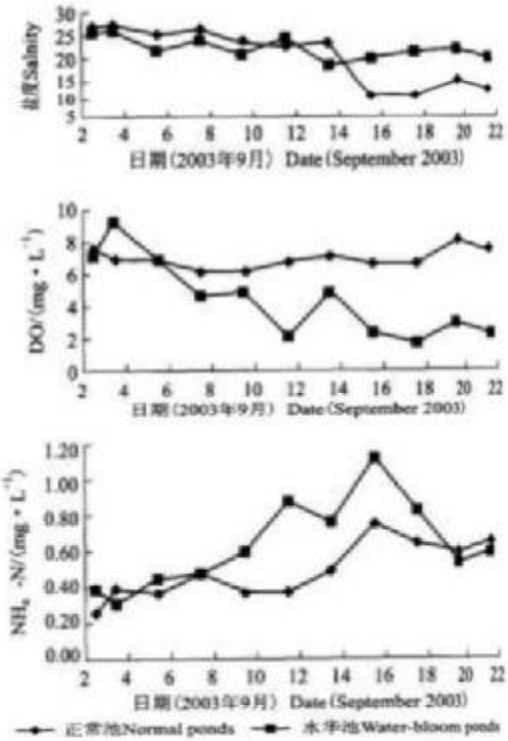

图4　正常池与水华池盐度、溶氧和氨氮水平变化动态

Fig.4　Fluctuations of salinity, DO and NH_3－N levels in normal ponds and water-bloom ponds

大部分时间里，水华池DO水平比正常池的低，最低时仅为正常池的33%，其变动范围分别为1.83～9.29 mg/L与6.14～8.13 mg/L。其中，水华池DO从9月9日始开始明显低于正常池，并于9月19日出现最低值1.83 mg/L，同期观察到池中幼海马常在水面游动，且不集群。氨氮检测结果显示，水华池氨氮变动范围为0.31～1.12 mg/L，在大部分养殖期间里比正常池氨氮含量(0.26～0.76 mg/L)高，最高时为正常池的2.34倍。但在调查后期，两组池氨氮含量基本一致。

3 讨论

3.1 大海马育苗池水华原因藻与优势种的观察

在水温超过24℃时，大海马育苗池常发生水华现象。此时的池水呈腥味，水质轻度黏性，手触摸后易过敏。所形成的水华呈黄绿色或浅褐色，有气泡，在水面上形成膜状薄层。藻类取样后经显微镜观察，优势藻形态构造简单，单细胞，球形或长形，以无结构的胶质结合形成球形或椭圆形、不规则形穿孔的群体。根据其形态特点应属于蓝藻门(Cyanophyta)，蓝球藻目(Chroococcales)，微囊藻属(*Microcystis* Lemin,1907)，铜绿微囊藻(*Microcystis aeruginosa* Kuza 1933)[17]。该水华确定为铜绿微囊藻水华，江浙一带又称"潮腥"[18]。

当铜绿微囊藻在海马育苗池藻群中成为优势种时，水华发生。铜绿微囊藻约占藻群的90%以上，远超出了吕军仪等[13]对未经有益微生物处理的无水华大海马池中铜绿微囊藻的百分比。吕军仪等报道的大海马池中藻群(总丰度为23.67×10^2/mL)组成为绿藻 *Chlorophyta*(13.13%)，硅藻 *Bacillariophyta*(9%)、甲藻 *Pyrrophyta*(22.35%)、蓝藻 *Cyanophyta*(35.28%)、隐藻 *Cryptophyta*(19.65%)。

3.2 水华生、消过程中水环境理化参数变化的分析

在大海马育苗池中，发生水华的时间主要集中在从晚春至初秋，其最适的温度范围为23～25℃，此外浮游藻类的结构、水体营养化程度也是水华发生的重要条件[2]。在陆丰乌坎湾的大海马育苗池，在每年水温低于24℃的季节时，少有水华发生；当在高温季节(24℃)时，育苗池中常出现水华现象。而且水华出现时间往往在晴天的午后12:30～15:00，这时的气温、水温正逐步上升，当达到了水华发生的有利温度范围后，水华有可能发生。用黑帆布对养殖池遮光，泼洒适量淡水、加冰等措施能有效抑制水温过高。盐度与水华的发生亦有一定的相关性，水华池中由于存在大量的海水藻等中、高盐度海洋生物，在一定程度上缓和了养殖水体的盐度变动消长情况，如图4所示；低盐度(<20)的海马育苗池水体未见有水华出现。实验的同时还发现，水华在育苗池中的出现由于受温度等条件的影响而具有一定的时效性，在调查过程中以9月12日为水华发生的高峰期，后因降雨气温变凉等天气条件的变化，改良了环境，缓解了水华的发生程度。从9月14日起水华逐步减轻，至9月24日完全消退。

在养殖水体中，有机质很难得到及时有效的控制而极易增强水体的富营养化程度。而投喂的桡足类、轮虫饵料只有部分在养殖中被幼海马摄食，残饵在水中残留至死亡腐败，释放出大量溶解性有机质，造成与微囊藻生长、繁殖有很大关系的有机N、P等元素含量的增多，并使水体呈碱性状态[6,18]。藻类作为海马养殖中培饵和造水的重要物种而在养殖中广泛应用，藻群内不乏水华藻的存在，为水华的发生提供了潜在的可能。一旦管理不当致使水温、营养化程度等诸条件适宜水华藻的大量繁殖时，水华便大规模暴发，成团成块在育苗池水面形成一层浅褐色的藻层。相反，如果大海马育苗池的管理妥当，那么即使在适温范围内也不会出现大规模的水华现象。

水华的发生主导着水体中的DO、氨氮等因子的变动。卢敏等[19]对胶州湾东部赤潮发生海区调查发现其DO值比未发生时提高将近1倍。作者调查发现，水华池水体中DO含量下降，到后期池水缺氧，相反氨氮含量却有所增加，这种变化趋势一直持续到水华消长的后期。DO、氨氮水平是多种因素综合作用的结果，但在水华大量发生的海马育苗池中，DO、氨氮水平会因水华发生的程度的不同而不同。一般来说，当发生水华时，在白天阳光下，虽然产生氧气，却在夜晚的时候被大量消耗掉，导致水体缺氧；另外，水华的消长会影响水体中某些藻类数量的变动，而这些藻类在一定程度上可以产生大量的氧气而引起养殖水体中DO和氨氮水平的波动。实验表明DO、氨氮水平的昼夜变动以及CO_2含量在光合作用下的不断变化，都是造成水华池水质不断恶变的原因。

3.3 大海马育苗池水华发生水体中菌—藻关系的分析

构成大海马育苗池水生微生态系统的藻群、菌

群是影响系统平衡的重要因素。随着水华藻铜绿微囊藻在池水中大量繁殖及藻细胞密度逐步增大,对异养细菌和弧菌都有明显的抑制作用,导致养殖水体中无论是异养细菌总数还是弧菌数量均比正常池约低 1 个数量级,数量差异显著($P<0.01$),且水华的暴发程度直接影响了细菌数量的变动,该趋势一直持续到水华在养殖水面上消亡。这与林伟等[20]指出的在饵料微藻系统中,当藻培养处于指数生长后期至静止期,藻细胞密度达到高峰时,抑制弧菌能力最强的结论相近。

藻菌之间存在着拮抗作用,在藻细胞周围形成的藻际(Phycosphere)微环境的细菌群落中存在着抑制藻细胞生长的细菌[11]。徐金森等[21]在实验条件下发现细菌滤液对赤潮藻细胞生物量的影响与细菌处理浓度有关,高浓度的细菌滤液对藻细胞生长具抑制作用。反过来藻类对细菌的生长也有抑制作用,如某些藻类能够产生抑制细菌生长的类抗生素物质[11,20],水体中相当多的硅藻如根管藻、盒形藻、骨条藻等对水生微生物有抑制作用,影响其生长繁殖[22]。更多针对赤潮的研究认为,某些种类的微生物是赤潮的诱导因素,它可以促进赤潮的大量繁殖,赤潮水域中细菌量和有机质含量与浮游生物量呈正相关,其原因可能是细菌在分解有机质的过程中,产生了对其中的部分藻类生长起抑制作用的有毒物质,而使另一些藻类生长旺盛,成为优势种,并最终导致这些藻类的爆发性增殖而形成赤潮[12,23]。曾活水等[24]对厦门港赤潮发生区进行微生物调查时发现在赤潮发生的海域细菌数量比正常时期高出一倍。该结论与本文报道的养殖池中水华的发生抑制了细菌数量增长的结果不同。作者认为,出现差异的原因可能是在本调查铜绿微囊藻形成水华的大海马育苗池中,藻际微环境对细菌的抑制作用得到了发挥,细菌增殖受到了抑制,数量出现下降;且下降的程度与水华藻与细菌之间相互作用的种属特异性及强度有关,但究竟其种属特异性如何作用、程度有多大,有待进一步的研究来证明。

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Effects of water-bloom on environmental factors in breeding water for juvenile seahorse, *Hippocamppus kuda* Bleeker

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Abstract: Water-bloom usually takes place in breeding water for juvenile seahorse, *Hippocampus kuda* Bleeker in summer and autumn in South China Sea. This study was conducted in Seahorse Culture Base of Lufeng City, Guangdong Province, China. Two groups of ponds were chosen: the first group containing 3 normal ponds without water-bloom and the second containing 3 ponds with water-bloom. The experiment period was 20 d. The results showed that water-bloom easily took place easily when water temperature was upper to 24 ℃, and the dominant algae in the water-bloom ponds was *Microcystic aeruginosa*. The amount of heterotrophic bacteria in the first group was more than that in the second group averagely. In the surface of breeding water for the two groups, the variance of heterotrophic bacteria was from 2.50×10^3 CFU/mL to 7.23×10^4 CFU/mL and from 4.75×10^2 CFU/mL to 6.90×10^3 CFU/mL respectively; the variable ranges of *Vibrio* numbers were from 0.85×10^2 CFU/mL to 7.19×10^3 CFU/mL and from 0.33×10^2 CFU/mL to 8.92×10^2 CFU/mL respectively. Meanwhile, at the bottom of culture water, the average variable ranges of heterotrophic bacteria were from 4.75×10^3 CFU/mL to 7.53×10^4 CFU/mL and from 6.25×10^2 CFU/mL to 1.50×10^4 CFU/mL respectively; the average variable ranges of *Vibrio* numbers were from 8.30×10^2 CFU/mL to 1.16×10^4 CFU/mL and from 0.53×10^2 CFU/mL to 2.04×10^3 CFU/mL, respectively. The difference of bacteria numbers between the two groups was significant ($P<0.05$). Average water temperature in the water-bloom ponds was lower by about 1 ℃ than that in the normal ponds and dissolved oxygen in the water-bloom ponds decreased by 22% − 33%. But ammonium content in the water-bloom ponds was 1.41 to 2.34 times of that in the normal ponds. The amounts of *Vibrio* in the surface water and at the bottom of breeding ponds decreased by 61% − 87% and 82% − 93% respectively when water-bloom was took place.

Key words: water-bloom; *Hippocampus kuda* Bleeker; juvenile cultural pond; environmental factor

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·Research Note·

Morphologic characters and sequence analysis of insulin-like growth factor-Ⅰ gene in triangular bream *Megalobrama terminalis*

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Abstract: The morphologic characters were studied and the IGF-Ⅰ cDNA and gene of triangular bream *Megalobrama terminalis* from its liver were cloned by RT-PCR for the first time. The traditional appearance data in triangular bream is similar to the data in bluntnose bream. Sequence analysis indicated that the IGF-Ⅰ cDNA of triangular bream consisted of 486 nucleotides encoding 161 amino acids which spanned the complete signal peptide and domains B, C, A, D and E. Compared with bluntnose bream (*Megalobrama amblycephala*), another member of *Megalobrama*, triangular bream IGF-Ⅰ shared 99.8% and 99.4% identity in cDNA sequence and predicted amino acid sequence, respectively. Thus, it can be concluded that triangular bream and bluntnose bream are very similar.

Key words: *Megalobrama terminalis*; morphologic characters; IGF-Ⅰ; species differences

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Triangular bream *Megalobrama terminalis* is distributed in most fresh water areas in China. Triangular bream has been studied a lot on artificial culture and breeding technique. However, only a few reports[1] can be found on the genetic characterization of triangular bream. The insulin-like growth factor-Ⅰ (IGF-Ⅰ) of fish, which consists of 70 amino acids and serves as a mediator for cell division, differentiation, embryonic development, growth regulation, cell death restraint and osmosis pressure regulation etc, plays an important role in the growth and generation of fishes[2]. In this experiment, the morphologic characters of triangular bream were studied and the IGF-Ⅰ cDNA and gene were cloned (GenBank Accession No. AY247412) and the nucleotides sequence were determined, which provided basic data for the research on its heredity, genetic identification and genetic resources protection.

1 Materials and methods

1.1 Samples

Triangular bream were supplied by Institute of Aquatic Science Research of Hangzhou Academy of Agriculture Science Research. The livers of triangular bream were rapidly separated and grounded in liquid nitrogen.

1.2 Morphologic characters analyzing

According to the method of Li Sifa[3] and Chen Makang[4], the data of triangular bream morphologic characters were measured and analyzed.

1.3 Total RNA extraction

Total RNA was extracted from 50 mg liver tissues with RNA Extraction Kit (Promega). Total RNA quality was examined on formaldehyde denatured electrophoresis.

1.4 cDNA Cloning and sequencing

Due to the high conservation of IGF-Ⅰ gene, a

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pair of primers were designed and synthesized based on the conserved region of bluntnose bream IGF-Ⅰ ORF (Opening reading frame), which was closely related to triangular bream: 3'-primer (5' CGCGGATCCTTACTAAATGCGATAGTTTC3') and 5'-primer (5' CGGAATTCATGTCTAGCGGACATTTC3'). A 5'-primer was expected to extend from the first amino acid code ATG of IGF-Ⅰ signal peptide; a 3'-primer was expected to extend reversely from the stop code TAG of IGF-Ⅰ signal peptide. A *Bam*H Ⅰ restriction site and an *Eco*R Ⅰ restriction site were inserted into 3'-primer and 5'-primer, respectively, to facilitate cloning of PCR products. The first strand of cDNA was synthesized from 10μg of total RNA with 3'-primer using MMLV RT (Promega) at 42 ℃ for 1 h. The PCR amplification program was as follows: 10 cycles with denaturing at 94 ℃ for 2 min, denaturing at 94 ℃ for 45 s; annealing at 50 ℃ for 45 s and extension at 68 ℃ for 1 min; 25 cycles with denaturing at 94 ℃ for 45 s, annealing at 55 ℃ for 45 s and extension at 68 ℃ for 1 min followed by a final extension of 10 min at 72 ℃ then stored at 4 ℃. RT-PCR products were analyzed by electrophoresis on 1.0% agarose gel. After being separated and purified, RT-PCR products were ligated with pMD 18-T vectors (Takara). *E. coli* TG1 was transformed with the recombinant DNA by $CaCl_2$ method to obtain transformants. Two positive clones were screened out by gel electrophoresis primarily, and analyzed by digesting with restriction enzyme, then sequenced by Beijing Genomics Institute (Hangzhou), Genomics and Bioinformatics Center Chinese Academy of Sciece.

2 Results and analysis

2.1 Morphologic characters of triangular bream

Table 1 presents the observed numbers of triangular bream. Table 2 presents the average values and standard deviation of 12 items of measurable parameters in two-, three-and four-year-old triangular bream.

2.2 RT-PCR

Total RNA of triangular bream liver was expanded by RT-PCR. RT-PCR product was a specific fragment about 500 bp by gel electrophoresis (Fig. 1).

RT-PCR products were reclaimed in low melting temperature agarose gels and ligated with vectors. *E. coli* TG1 was transformed with the recombinant DNA by $CaCl_2$ method to get transformants. Two positive clones were screened out by gel electrophoresis primarily and analyzed by digesting with *Eco*R Ⅰ (Fig. 2).

Tab. 1 Observed numbers of triangular bream

表 1 三角鲂可数性状

Dorsal fin formula	Anal fin formula	Lather line scales	Gill rakes
D·3,7－7	A·3,24－32	50－60	16－22

Tab.2 Average value and standard deviation of measurable parameters of triangular bream

表 2 三角鲂可测量性状参数 $\bar{X}\pm SD$

Item	Age/a		
	1 (1⁺) (*n*=124)	3(2⁺) (*n*=130)	4(3⁺) (*n*=121)
Total length/cm	30.94±2.79	40.49±3.30	49.54±2.61
Standard length/cm	26.12±2.54	34.74±2.85	42.36±2.24
Body weight/g	379.3±109.6	1 048.2±293.2	1 797.4±298.6
BL/BW	2.21±0.15	2.25±0.12	2.36±0.10
BL/HL	4.44±0.29	4.92±0.35	5.12±0.13
HL/SL	3.08±0.30	3.20±0.24	3.31±0.25
HL/ED	3.85±0.42	4.31±0.46	4.56±0.27
HL/Interorbital space	2.39±0.44	2.22±0.21	2.18±0.27
HL/CPL	9.97±1.53	11.27±1.43	11.09±1.78
HL/CPW	8.38±0.78	8.54±0.55	8.48±0.44
CPL/CPW	1.13±0.23	1.07±0.21	1.07±0.19
Dorsal fin-rays length/HL	0.95±0.11	0.99±0.17	1.06±0.15

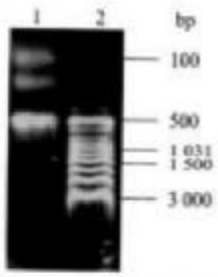

Fig.1 Electrophoresis result of RT-PCR products
1: Triangular bream; 2: Marker
图 1 三角鲂肝脏总 RNA RT-PCR 结果
1:三角鲂; 2:Marker

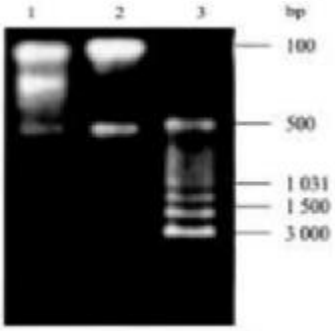

Fig. 2 Identification of PCR products of positive clones
1,2: Positive clones; 3: Marker
图 2 阳性克隆的 PCR 鉴定结果
1,2: 三角鲂; 3:Marker

2.3 cDNA sequence of triangular bream IGF-Ⅰ

Sequence analysis showed that triangular bream IGF-Ⅰ consisted of a complete ORF of 486 bp (submitted to GenBank, Accession No. AY247412). Triangular bream IGF-Ⅰ preprotein contained 161 amino acids, which spanned the complete signal peptide, mature IGF-Ⅰ and E-domain. Signal peptide contained 132 nucleotides encoding 44 amino acids; mature IGF-Ⅰ contained 210 nucleotides encoding 70 amino acids and E-domain consisted of 141 nucleotides encoding 47 amino acids. Analysis of E domain indicated that cloned triangular bream IGF-Ⅰ lacked Ea-2 structure and belonged to IGF-Ⅰ Ea-2 subtype (Fig. 3).

3 Discussion

The appearance of triangular bream is beautiful, which is similar to that of bluntnose bream. The significant differences in their appearance are that the second hard ray length of dorsal fin in triangular bream is longer than its head length; and its lip is thicker and hornier. The cDNA sequence of triangular bream IGF-Ⅰ was very similar to that of bluntnose bream, another member of the same genus (GenBank No. AF332865)[5]. According to this study, triangular bream ORF nucleotides shared 99.8% sequence identity with that of bluntnose bream, in which 100% sequence identity in mature IGF-Ⅰ and E-domain while 99.2% sequence identity in signal peptide. The predicted amino acid sequence of triangular bream ORF shared 99.4% sequence identity with that of bluntnose bream and there was difference only in one out of the 161 amino acids (Fig.4). In this study, we also found that triangular bream IGF-Ⅰ E domain was highly conservative[6-7]. It also resulted in high homology of nucleotide sequences and amino acid sequences between triangular bream and bluntnose bream. Thus, it can be concluded that triangular bream and bluntnose bream are very similar to each other. Similar result was also reported by Li in 2002[11].

```
ATGTCTAGCGGACATTTCTTCAGGGGCATTGGTGTGATGTCTTTAAGTGTACCATGCGCTGTCTCTGTGCACCCACACCCTCTCACTG   90
 M  S  S  G  H  F  F  Q  G  H  W  C  D  V  F  K  C  T  M  R  C  L  S  C  T  H  T  L  S  L   30
GTGCTGTGCGTCCTCGCGTTGACTCCCCGGACACTGGAGGCGGGTCCGGAGACCCTGTGCGGGGCGGAGCTTGTAGACACGCTGCAGTTT  180
 V  L  C  V  L  A  L  T  P  A  T  L  E  A  G  P  E  T  L  C  G  A  E  L  V  D  T  L  Q  F   60
GTGTGTGGAGACAGGGGCTTTTATTTCAGCAAACCAACAGGATATGGGCCTAGTTCGAGACGGTCACACAACCGCGGCATTGTGGACGAA  270
 V  C  G  D  R  G  F  Y  F  S  K  P  T  G  Y  G  P  S  S  R  R  S  H  N  R  G  I  V  D  E   90
TGCTGCTTTCAAAGCTGCGAACTGCGGGGCCTCGAGATGTACTGTGCACCTGTGAAAACCGGCAAAACTCCACGATCCCTACGAGCGCAA  360
 C  C  F  Q  S  C  E  L  R  R  L  E  M  Y  C  A  P  V  K  T  G  K  T  P  R  S  L  R  A  Q  120
CGGCACACAGATATCACCAGGACAGCAAAGAAACCTATATCTGGACATAGCCACTCTTCCTGTAAGGAGGTTCATCAGAAGAACTCAAGC  450
 R  H  T  D  I  T  R  T  A  K  K  P  I  S  G  H  S  H  S  S  C  K  E  V  H  Q  K  N  S  S  150
CGAGGAAACACAGGGGGCAGAAACTATCGCATTTAG                                                        486
 R  G  N  T  G  G  R  N  Y  R  I                                                            161
```

Fig.3 Triangular bream IGF-Ⅰ cDNA sequence and predicted amino acid sequence
图 3 三角鲂 IGF-Ⅰ的 cDNA 序列及其推测的氨基酸序列

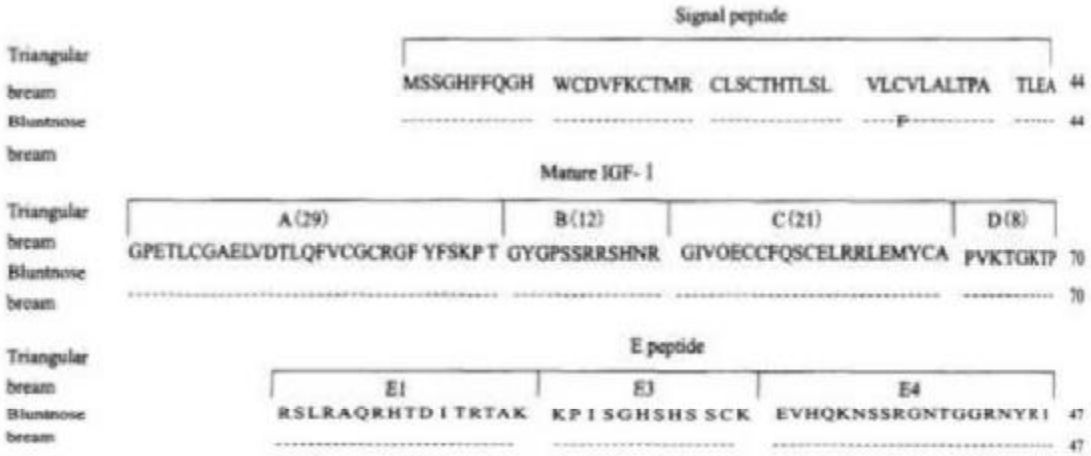

Fig.4 Amino acid sequences of preprotein in IGF-Ⅰ of triangular bream and bluntnose bream

图 4 三角鲂、团头鲂 IGF-Ⅰ cDNA 及其前蛋白原氨基酸序列

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三角鲂形态特征和胰岛素样生长因子-Ⅰ基因的序列分析

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摘要:对三角鲂(*Megalobrama terminalis*)形态学特征进行研究,用 RT-PCR 的方法从三角鲂肝脏克隆了三角鲂胰岛素样生长因子-Ⅰ(IGF-Ⅰ)基因的 cDNA 序列。三角鲂传统的形态学数据与团头鲂相似。序列分析表明,三角鲂 IGF-Ⅰ cDNA 由 486 个核苷酸构成,编码 161 个氨基酸,包含整个信号肽、B、C、A、D 和 E 区,与鲂属团头鲂比较,三角鲂与团头鲂 IGF-Ⅰ cDNA 序列同源性为 99.8%,氨基酸序列同源性为 99.4%。由此可见三角鲂的遗传学特征与团头鲂非常相似。

关键词:三角鲂;形态特征;IGF-Ⅰ;种间差异