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细指长臂虾的幼体发育

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提 要 1991年4月，从长江口采回细指长臂虾抱卵亲虾，至实验室进行幼体培育。孵化后的幼体，在水温22℃、盐度12、饵料充足的条件下，有规律的每2d蜕皮1次，经10个溞状幼体期，变态为仔虾。如在发育后期遇饵料不足等不良环境条件的影响，则幼体须经11次蜕皮后，始能发育为仔虾。

关键词 细指长臂虾，溞状幼体，仔虾，发育，蜕皮，变态

细指长臂虾 *Palaemon tenuidactylus* 是长江口到黄河口一带重要经济虾，每年3～4月间移至浅海或河口处繁殖。期间，我们从长江口采回抱卵亲虾，于实验室内进行幼体培育。经2个多月的饲养和培育，幼体完成变态，进入仔虾期。下面报道发育过程中产生的形态变化。

1 材料和方法

抱卵的亲虾于1991年4月捕自长江口，运回实验室后，在水温22℃、盐度12的条件下饲养。当幼体孵出后，即移至相同水温、盐度的培养缸中单独培养。为防止细菌和原生动物大量繁殖，定期更换新水。在第一、二期幼体期间，投喂褶皱臂尾轮虫，并加入适量的三角褐紫藻。到第三期幼体后，加入小量的卤虫无节幼体。到第五期幼体开始，全部单喂卤虫无节幼体。

2 结果

幼体孵出后，在盐度12‰、水温22℃、饵料充足的条件下，有规律的每2d蜕皮1次，经10个溞状幼体期，约20-22d后进入仔虾期。如在早期幼体期间单喂三角褐指藻为饵，则幼体均慢慢地先后死亡，未见有蜕皮进入第二期者。若在后期发育阶段遇到饵料不足等不良的环境条件，则幼体须经11个溞状幼体期，然后再发育进入仔虾期。

2.1 第一期幼体（图1）

体长3.01～3.21mm，额角发达，无齿。头胸甲前侧角（图1-a）具颊刺。眼柄短，与头胸甲前缘相联，不能转动。腹部6节，第3腹节背面具1突起，第6腹节与尾节相联。尾节扇状，后缘中部稍内凹，具7对刺毛，最外侧者外缘无毛。第一触角（图1-c）柄呈圆柱形，不分节，顶部具1短棒，末生1羽状毛、3鞭状感觉毛和1短刺毛，棒的基内侧具1长羽状毛。第二触角（图1-d）原肢2节；内肢棒状，顶端1羽状毛和1短刺；外肢扁平，顶端和内缘10羽状毛，外侧角1细刺。大颚（图1-e）切

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齿左右不对称，左侧4齿，右侧3齿；臼齿左右相似，具8-10小齿；在切臼齿间为1凹陷，内生2刺毛。第一小颚(图1-f)底节4刺和1侧刺，基节5刺；内肢顶端1刺。第二小颚(图1-g)底节基叶1、末叶2刺，基节基叶3、末叶4刺；内肢不分节，内侧为1缺刻，基部2刺，末端1毛；颚舟片边缘5羽状毛，后端的1根最粗壮。第一颚足(图1-h)底节内侧具1、基节为6刺；内肢3节，第2节内末角具1、末节末端具4毛；外肢2节，末节末端具6羽状毛。第二颚足(图1-i)基节内侧3毛；内肢4节，腕掌合节的内末角2刺，指节爪状，基部外侧和外末端各具1毛，内侧2刺毛；外肢2节，末节末端8羽状毛。第三颚足(图1-j)基节内侧3毛，座、长节内侧各1毛，掌节内末角2刺，指节爪状，外侧基部具1、末端具2等长的毛，内侧1刺；外肢末节末端8羽状毛。第一、二步足均双肢型，未分节。第一对(图1-k)稍大于第二对(图1-l)。

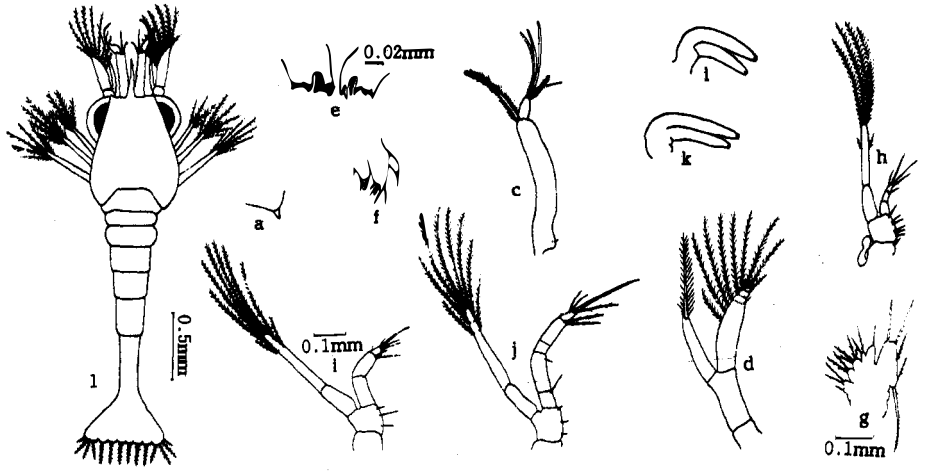

图1 第一期幼体

Fig. 1 The first zoeal stage

色素分布：额末端呈浅红色。头胸甲后端背面两侧具2块呈红略带黄的色素团，眼柄基部具红黑相间的色素团，触角基部为红的色素团，颚足基部为浅红的色素点。第三腹节的背面前端为紫红、后端为黄的色素团，第2-4腹节的腹甲边缘分布浅红色的色素点。尾节带黄色，凹陷处红色，肛门周围呈棕色。

2.2 第二期幼体(图2)

体长3.10～3.81mm，额角上缘基部生出1齿。头胸甲出现眼上刺，眼柄与头胸甲分离，能转动。第三腹节背突前方出现1弯钩，第五腹节后侧角呈刺状。尾节末端凹处的中央增生1对小刺。第一触角(图2-c)柄分3节，在第1、2、3节内末角各生1长羽状毛，第1节基部外侧具1突起，上生数毛，在第1、2节外末端各生数毛，第3节末端内侧具1丘状突，上生4毛，外鞭末端4感毛和1小刺毛。第二触角(图2-d)外肢13根羽状刚毛。大颚(图2-e)左侧凹处3刺毛，右侧与上期相同，未见增刺。第一小颚(图2-f)底、基节各增1刺。第二小颚(图2-g)颚舟片边缘羽状毛增至7根。第1、2步足(图2-k、l)分节，基节内侧2毛，座、长节内侧各生1毛，腕节外末角生1毛，掌节末端3刺，背1、腹2，指节爪状，外末角1细毛。第三、五步足芽生出，前者(图2-m)双肢型，后者(图2-

o)单肢型。尾肢的芽状突出现。

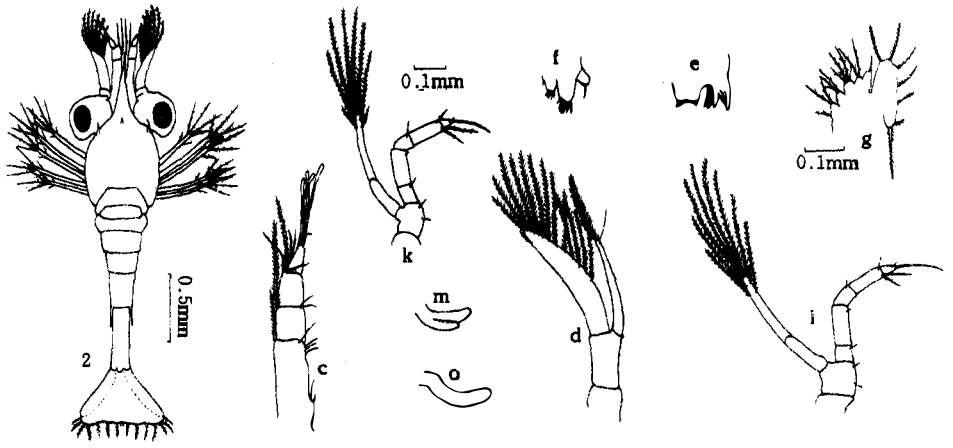

图2　第二期幼体

Fig. 2　The second zoeal stage

2.3　第三期幼体(图3)

体长3.65～4.12mm，额角上缘齿增至2个，头胸甲前侧角(图3-a)出现鳃甲刺。尾节与第6腹节分开。

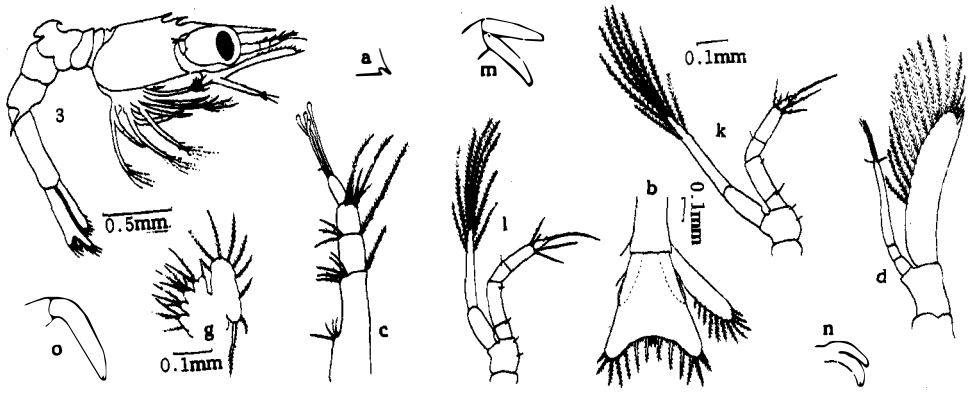

图3　第三期幼体

Fig. 3　The third zoeal stage

第一触角(图3-c)第1节基部外侧柄刺形成，平衡囊雏形产生，内、外肢的芽状突出现。第二触角(图3-d)内肢分3节；外肢外末角呈尖刺状，内缘16-18毛。第二小颚(图3-g)底节末叶增至3刺，颚舟片边缘9-11羽状毛。第一、二步足(图3-k、l)掌节背侧增生1刺。第四步足(图3-n)生出双肢型芽。尾肢(图3-b)与尾节分离，双肢型，内肢芽状，外肢11-12羽状毛。

2.4　第四期幼体(图4)

体长3.95～4.32mm，额角上缘3齿。尾节(图4-b)后端变窄，后缘刺5对，中央1对仍细小，

后侧角出现1对细刺。第一触角(图4-c)第3节末端4根长刚毛,内肢1节,末端1刺。大颚(图4-e)凹处小刺左4、右3。第一小颚(图4-f)基节增至8刺。第三颚足和第一、二步足(图4-j、k、l)外肢末端羽状毛均10根。第三、五步足分节,前者(图4-m)基节内侧2毛,座、长节内侧各1毛,腕节外末缘1毛,掌节末端3毛,背1、腹2,指节爪状,背末缘1细毛;外肢末端8羽状毛。后者(图4-o)基节内末角1毛,长节内侧基部和末端各1毛,腕节外末端1毛,掌节内末缘1毛,指节爪状,背末角1细毛。

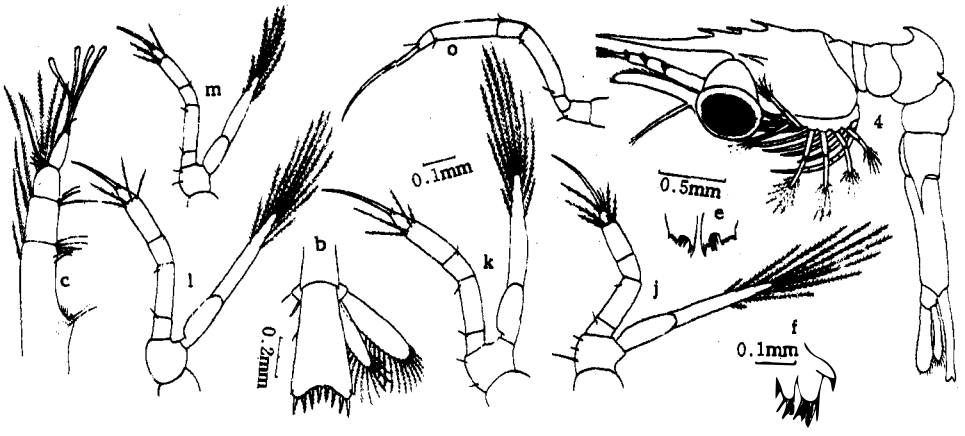

图4 第四期幼体

Fig. 4 The fourth zoeal stage

2.5 第五期幼体(图5)

体长4.12～4.90mm,尾节(图5-b)基部与末端约等宽。第一触角(图5-c)末节末端具5根长刚毛(此后各期均未见再增加)。第二触角(图5-d)内肢5节。第一小颚(图5-f)底节6刺。第二小颚颚舟片18羽状毛。第一颚足(图5-h)基节内侧7毛,内肢第2节内末角2毛;外肢基部外侧1毛。第一、二步足(图5-k、l)腕节腹侧增生1毛。第三步足(图5-m)长节外侧末端和腕节内侧末端各增生1毛,掌节背缘增生1毛。第四步足(图5-n)分节,基节内侧2毛,长节内侧基部和末端各具1毛,掌节末端3刺,背1、腹2,指节爪状,末端背侧1细毛;外肢末端6羽状毛。第五步足(图5-o)腕节腹缘末端1毛,掌节背、腹缘各增1毛,指节腹缘基部增生1粗刺。

2.6 第六期幼体(图6)

体长4.78～5.65mm,尾节末端趋窄,变成基部宽末端窄。第一触角(图6-c)外鞭的感觉毛末端4根,亚末端2根,内鞭延长,末端具1或2毛。第一小颚(图6-f)底节刺增至7根。第二小颚的颚舟片边缘21根羽状毛。第一颚足(图6-h)外肢第1节外侧增生1毛。第一、二步足(图6-k、l)长节外末缘生出1毛,第二步足掌节前腹缘向前突出,形成不动指雏形。第五步足(图6-o)长节外末缘生出1毛。腹肢(图6)出现乳头状突起。

2.7 第七期幼体(图7)

体长5.62～6.35mm,尾节(图7-b)末端外侧大刺基部增生1对小刺,中央的一对小刺消失。第二小颚(图7-g)基节的基叶增至4刺,颚舟片边缘22-23羽状毛。第三颚足(图7-j)腕节内末缘生出1毛。第一、二步足(图7-k、l)指节腹缘末端向前突出,形成不动指。第三、四步足(图7-m、n)长节外末角生出1毛,外肢末端增生2羽状毛。第五步足(图7-o)掌节腹缘增至3毛。腹肢

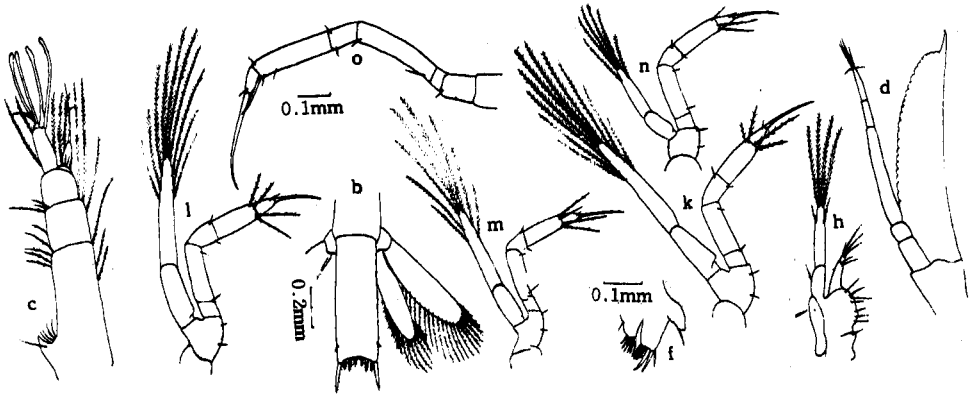

图5　第五期幼体

Fig. 5　The fifth zoeal stage

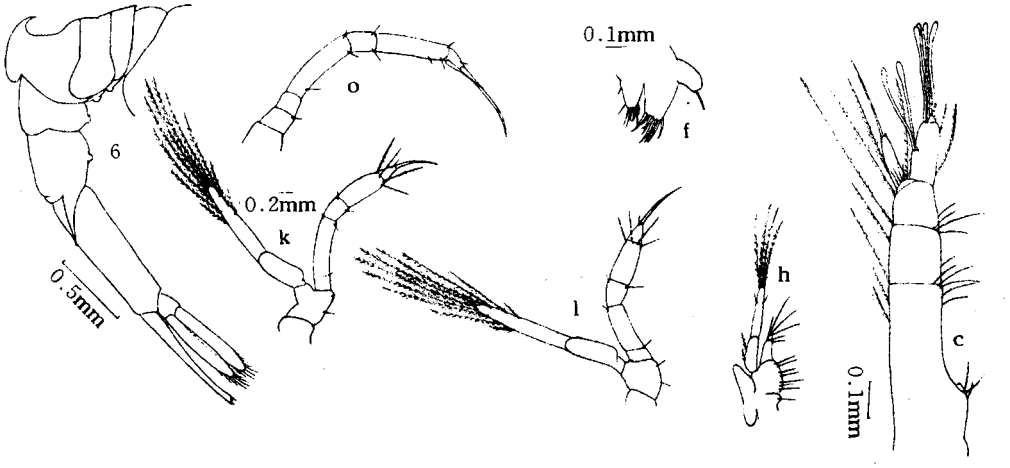

图6　第六期幼体

Fig. 6　The sixth zoeal stage

(图7-p-t)延长，呈双肢型。

2.8　第八期幼体(图8)

体长6.15～6.55mm，额角上缘第1齿基部前方生出1毛。第一触角(图8-c)外鞭2节，副鞭感觉毛的排列为4:2。第二触角(图8-d)内肢7节。大颚(图8-e)凹处刺为左5右4。第二小颚(图8-g)基节末叶5刺，颚舟片边缘27-28羽状毛。第一颚足(图8-h)基节内缘8-10刺毛；外肢第1节外侧羽状毛增至5-6根。第二颚足(图8-i)外肢末端的羽状毛10根。第三颚足(图8-j)长节腹缘和长、腕节外末缘各生出1毛；外肢末端12羽状毛。第一、二步足(图8-k、l)长节内侧3毛，不动指突出更显著；外肢末端12羽状毛。第三、四步足(图8-m、n)长节内缘增生1毛。第五步足(图8-o)掌节腹缘增生1毛。腹肢延长，分节完全。

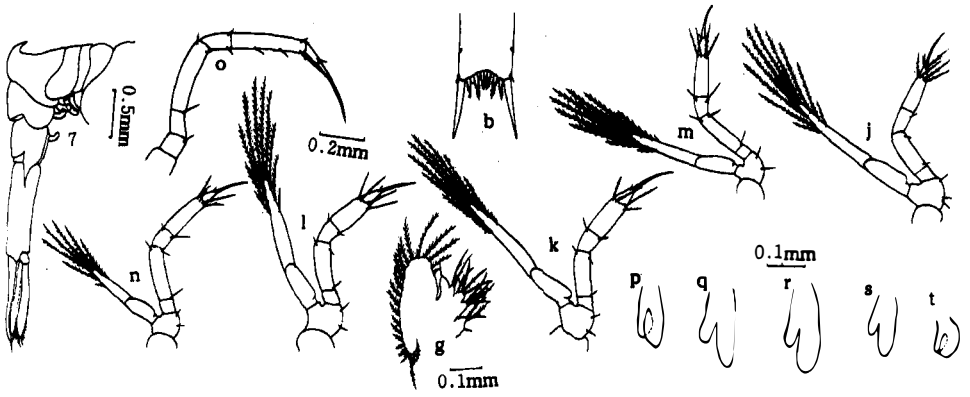

图7 第七期幼体

Fig. 7 The seventh zoeal stage

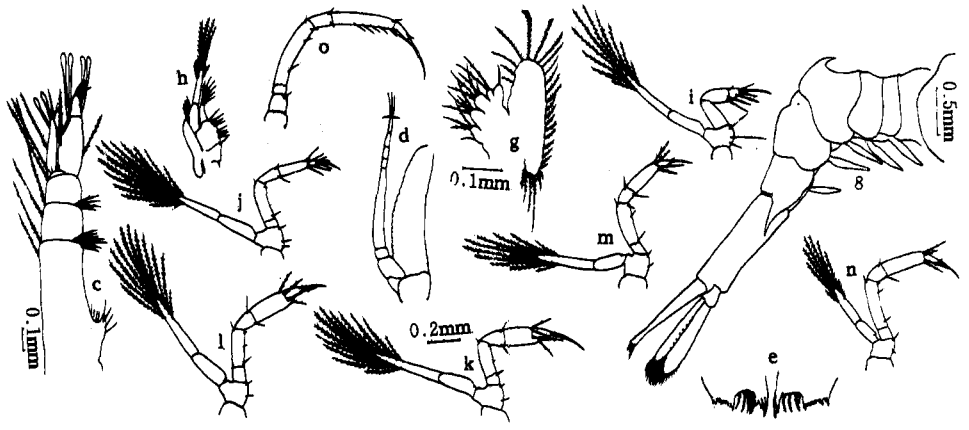

图8 第八期幼体

Fig. 8 The eighth zoeal stage

2.9 第九期幼体(图9)

体长6.55～7.89mm，额角上缘第1齿基部前缘具2毛。尾节(图9-b)2对背侧刺和2对后侧刺，末端变凸，两内刺间3对刺毛。第一触角(图9-c)内鞭分3节，外鞭4～5节，副鞭感觉毛的排列为4:3(2):2。第二触角(图9-d)内肢13节。第一小颚(图9-f)底、基节各具9刺。第二小颚(图9-g)基节的基叶5，末叶7根刺毛。第一颚足(图9-h)基节内侧14根刺毛；外肢第1节外侧5-7根羽状毛。第三颚足(图9-j)长节内缘和腕节末端各增至3毛；外肢末端的羽状毛12根。前四对步足(图9-k、l、m、n)腕末各增至3毛。第五步足(图9-o)长节内侧增至3毛，腕节外侧增至2毛，掌节腹缘增至5毛。腹肢(图9-p-t)延长并出现内附肢。

2.10 第十期幼体(图10)

体长7.85～8.51mm，额角上缘第一齿基部前方具4毛。尾节(图10-b)末端外侧刺增大。第一触角(图10-c)内鞭4节，外鞭5—6节，副鞭感觉毛的排列为4:3:3:2。第二触角内肢18-19节。

大颚(图10-e)凹处的刺毛增至左6、右5。第二小颚(图10-g)基节基叶和末叶各为8刺毛。第一颚足(图10-h)底节增至2毛，基节内侧18-23根；外肢基部外侧7-8羽状毛。第一、二步足(图10-k、l)外肢末端14羽状毛。第四步足(图10-n)掌节背侧增2毛；外肢末端的羽状毛增至12根。第五步足(图10-o)腕节末端增至3毛，掌节背、腹缘各增1毛。

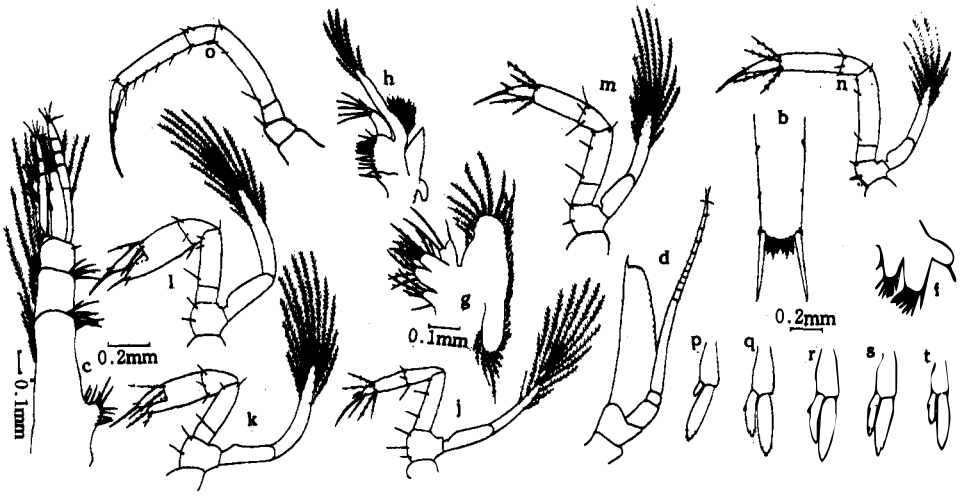

图9　第九期幼体

Fig. 9　The ninth zoeal stage

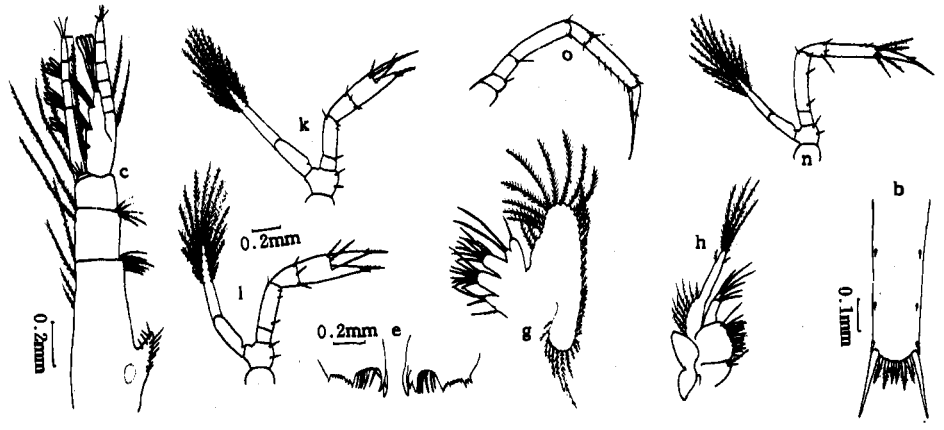

图10　第十期幼体

Fig. 10　The tenth zoeal stage

2.11　仔虾(图11)

体长9.22～10.48mm，额角伸至超出第一触角柄的末端，上缘10-12齿，下缘1-2齿。头胸甲具触角刺及鳃甲刺，颊刺消失。尾节(图11-b)具2对背侧刺。末端呈尖刺状，后侧角的2对刺，内

长、外短，在2内刺间具1对长刚毛。第一触角(图11-c)柄刺发达，基节的外末角1尖刺，末节末端的丘状突上3-4细毛。内、外鞭细长，副鞭5节，感觉毛的排列为4:3:3:2:2。第二触角鞭已分节完全。大颚(图11-e)切、臼齿分裂完全，切齿左4，右3，内肢具芽状突。第一小颚(图11-f)底、基节上各生许多刺毛，内肢双叶状，刺毛消失。第二小颚(图11-g)底、基节上各生许多刺毛，内肢叶状，外侧具细毛；颚舟片边缘46羽状毛。第一颚足(图11-h)底节3长刚毛及许多刺毛，基节具许多刺毛；内肢狭长，内末角1长刚毛；外肢基部外侧突出，上生10羽状毛，末端生6羽状毛。第二颚足(图11-i)腕节外末角1毛，掌节宽于指节，掌、指末缘具许多刺毛；外肢末端10羽状毛。第三颚足(图11-j)内肢4节，各节上均生粗刚毛；外肢发达，2节，末端的羽状毛消失。步足前2对(图11-k、l)钳状，第二对显著粗长。后三对(图11-m、n、o)爪状。前4对具发达的外肢，由前向后依次变短。腹肢(图11-p-t)内、外肢均发达，上生羽状毛，除第一对外均具发达的内附肢，末端生出数个小钩。尾肢内、外肢上均具发达的羽状毛。

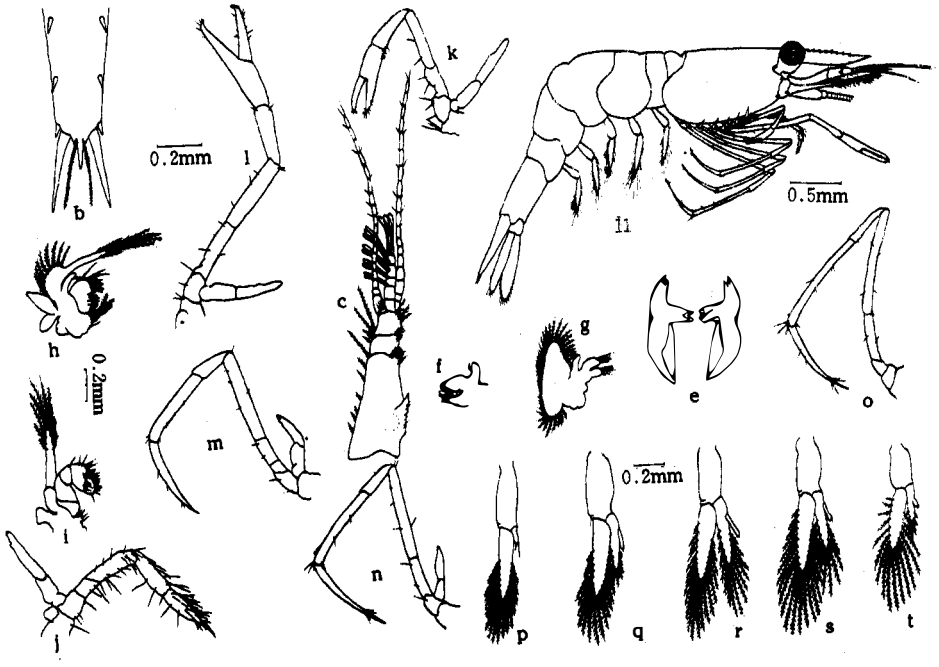

图11 仔虾

Fig. 11 The postlarval

3 讨 论

细指长臂虾的幼体，在培养的过程中出现蜕皮次数有10次或11次的不恒定现象，这种现象Broad[1957]在培养 *Palaemonetes pugio* 时就曾发现。作者认为细指长臂虾的蜕皮次数不恒定，与培养过程中投饵不足等不适合的环境因子有关。

从长臂虾类幼体培育的结果中看到，步足和腹肢的发育早迟与卵型的大小密切相关。在大

卵型的安氏白虾，在第一期各附肢不但已全部出现，且各对均已分节完全。而中卵型的脊尾白虾，其第一期只生出第1、2和5三对，到第二期再生出第4、5对步足芽，直到第四期才分节完全。然而，在小卵型的细指长臂虾和葛氏长臂虾中，第一期仅生出第1、2两对，第二期始生出第3、5对，第三期再生出第4对步足芽，直到第五期才分节完全。腹肢的出现则延得更迟。在大卵型中，不但已全部生出，且已分节完全；中卵型要到第三期才生出芽突，第五期才分节完全。然而，在小卵型，要等到第六期始见芽状突起，第八期才分节完全，第九期生出内附肢。

细指长臂虾与葛氏长臂虾成体非常相似，刚孵出的第一期 溞状幼体，除体色略有不同外，无论是外形或各附肢上的刚毛数均完全相同，两者无法相区别。但随着蜕皮次数的增加，其各附肢上的毛数逐渐出现差异。现就主要不同之点，比较如下表：

特　征	细指长臂虾	葛氏长臂虾
第一期幼体		
色素分布	头胸甲后侧具红带黄的色素团	头胸甲后侧仅具分散的色素点
第二期幼体		
大颚凹处刺数	3刺 以后各期变化无规律	2刺 每蜕皮2次增加1刺
第二小颚底节末叶刺数	2刺	3刺
第三期幼体		
尾肢外肢刚毛数	11根	13根
第三颚足掌节外末缘毛数	无刺	1刺
第四期幼体		
第二触角内肢节数	3节	4节
第三颚足、第一、二步足外肢末端毛数	10根	8根
第三步足外肢末端毛数	8根	6根
第五期幼体		
第三颚足外肢末端毛数	10根	8根
第六期幼体		
第四步足外肢末端毛数	6根	8根
第七期幼体		
第一触角副鞭感觉毛的排列	4，2	4，3，2
尾节末端中央小刺	消失	存在
外侧角小刺	出现	未见
第四步足外肢末端毛数	8根	10根
第八期幼体		
第二颚足外肢末端毛数	10根	8根
第九期幼体		
第一触角副鞭感觉毛的排列	4，2，2	4，2，2，1
第一、二步足外肢末端毛数	12根	14根
第十期幼体		
第一触角副鞭感觉毛的排列	4，3，3，2	4，2，2，2
第一颚足外肢末端毛数	6根	8根
仔虾期		
第一颚足外肢末端毛数	6根	8根
第二颚足外肢末端毛数	10根	12根

参加本试验的还有陆建辉、康梅同志，原川沙县水产局姜德荣、金其林等同志协助提供亲虾，张道南同志提供活饵料，均此一并致谢。

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THE LARVAL DEVELOPMENT OF *PALAEMON TENUIDACTYLUS*

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ABSTRACT *Palaemon tenuidactylus* Liu *et al.* is an important commerial shrimp, which lives in brackish estuaries of China. It shows small egg type of developmental pattern. The buds of the first and second pereiopods appear at the first zoeal stage, but the third and fifth at the second zoeal stage, and finally, the fourth at the third zoeal stage. Segmented pereiopods were completed in the fifth zoeal stage. Department of the pleopods in small egg type is delayed too. The buds of the pleopods appear in the sixth zoeal stage. The appendix interna appears at the ninth zoeal stage. *Palaemon tenuidactylus* and *Palaemon gravieri* live in the brackish estuaries. The larvae of these are also alike. For the sake of convenience, major different characteristics of the two species are fully given in the present paper.

KEYWORDS *Palaemon tenuidactylus*, zoea, postlarvae, development, moult, metamorphosis

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A PRELIMINARY STUDY ON FISH INTERFERON SYSTEM

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ABSTRACT According to the conservative regions in mammalian β-interferon (IFN) genes, two pairs of degenerate primers were synthesized. A fragment with expected molecular size (about 278 bp) was amplified from grass carp (*Ctenopharygodon idellus*) genomic DNA by PCR using degenerate primers 5'TCCTG(C/T)TGTG(C/T)TTCTCCACN3' and 5'GTCTCA(T/G)(T/A)CCA(G/C)CCAGTGCN3'. Southern hybridization results indicated that it was homologous to a 197 bp Hinf I fragment located between (but not containing) the two primer sequences of human β-IFN gene.

KEYWORDS fish, interferon

1 INTRODUCTION

Viral diseases often cause severe losses in aquaculture. Control of these diseases is very difficult due to diversity of pathogens and lack of effective drugs. Interferons (IFN) can inhibit the propagation of a wide range of virus and have been used to treat human viral diseases. With the development of transgenic technology [Zhu, 1989], it is possible to produce viral-resistant fish by transfer of recombinant interferon genes. Interferon-like activities have been observed in fish, but they have not been well characterized on molecular level [De Kinkelin and Dorson, 1973]. In human, interferons can be classified into three groups, that is, α, β and γ [Stewart *et al.*, 1980]. The α and β families are believed to evolve from an ancestral β-IFN-like gene by gene duplication just before the mammals-birds/reptiles divergence [Miyata *et al.*, 1985]. This means that fish has the ancestral IFN gene. So far, non-mammalian IFN genes have not been cloned. Due to lack of non-mammalian sequence information of α- or β-IFN genes, cloning of fish interferon gene is technically difficult. By comparison of six mammalian β-IFN nucleotide sequences, two conservative regions were identified. Using degenerate primers corresponding to these regions, a DNA fragment homologous to human β-IFN gene was amplified.

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2 MATERIALS AND METHODS

2.1 MATERIALS

Restriction endonucleases, Proteinase K and Prime - A - Gene kit were purchased from Promega. PCR kit was obtained from Fudan University. α -^{32}P - dATP (10mCi/ml) was purchased from Amersham corporation.

2.2 METHODS

DNA was extracted from liver of grass carp according to the methods described by Taggart *et al.* [1992] and Sambrook *et al.* [1989]. Two PCR buffer systems were used: 5 X Buffer A (125 mM Tris - HCL, pH 8.2 at 25 ℃, 10 mM $Mgcl_2$, 0.5 mg/ml gelatin, 125 mM $(NH_4)_2SO_4$, 25% formamide) and 10 X Buffer B (100 mM Tris - HCl, ph 8.3 at 25 ℃, 15 mM $Mgcl_2$, 500 mM KCl, 0.1% gelatin). The PCR product was analyzed by electrophoresis on 1.2-1.5% agarose gels containing 0.5 μg/ml ethidium bromide. The buffer system for electrophoresis was 1 X TBE.

For southern hybridization, PCR product was separated on 1% agarose gel in 1 X TBE, transferred to nitrocellulose filter (pore size 0.22 μm) in 10 X SSC. The hybridization procedures were generally similar to the methods described by Sambrook *et al.* [1989], except that pre - hybridization and hybridization were performed in 3 X SSC at 60℃ and the filters were washed with 3 X SSC at 64℃ or 67℃.

The probe used in hybridization was a 197 bp *Hinf* I fragment of human β - IFN gene (Fig. 1). The probe was radiolabelled with α -^{32}p - dATP using Promega's Prime - A - Gene kit.

To search conservative regions in mammalian β - IFN genes, six mammalian β - IFN genes were aligned using the software CLUSATAL (1.0) [Higgins and Sharp, 1988]. Two conservative regions corresponding to positions 32 - 50 and 289 - 309 of human β - IFN (numbering from translational initiator ATG) were found (Fig. 1). Two pairs of degenerate primers according to these regions were synthesized.

3 RESULTS

3.1 PCR AMPLIFICATION USING PRIMER 1 (P1) AND PRIMER 2 (P2)

PCR reaction was performed in a 0.5 ml eppendorf tube containing 10 μl dNTP (2mM), 20 μl Buffer A (5X), 3 μl primer 1 (25 pmol/μl), 3 μl primer 2 (25 pmol/μl), 10 μl grass carp DNA (0.167 μg/μl), 2 μl (4 units) FD polymerase and 52 μl H_2O. The reaction conditions are: 92.5℃ 5 min, then 92.5℃ 1 min, 58℃ 1 min, 70℃ 1 min for 45-50 cycles. The PCR product was analyzed by agarose gel electrophoresis. Two fragments with expected molecular weight was detected (Fig. 2). Similar results were obtained using Buffer B (data not shown).

(a)

```
human     5' TCCTGTTGTGCTTCTCCAC----GCACTGGCTGGAATGAGAC 3'
horse     5' TCCTGCTGTGTTTCTCCAC----GCACTGGCTGGAATGAGAC 3'
bovine 1  5' TCCTGCTGTGTTTCTCCAC----GCACTGGCTGGTCTGAGAC 3'
bovine 2  5' TCCTGCTGTGTTTCTCCAC----GCACTGGCTGGTCTGAGAC 3'
bovine 3  5' TCCTGCTGTGTTTCTCCAC----GCACTGGCTGGTCTGAGAC 3'
mouse     5' TCCTGCTGTGCTTCTCCAC----GCACTGGGTGGAATGAGAC 3'
              *****  ****  ********       *******  ***   ******
```

(b)

```
p1          5' TCCTG(C/T)TGTG(C/T)TTCTCCACN 3'
p3    5' GG GGATCCTG(C/T)TGTG(C/T)TTCTCCAC 3'
            BamH I
p2            5' GTCTCA(T/G)(T/A)CCA(G/C)CCAGTGCN 3'
p4    5' GG GGATCCGTCTCA(T/G)(T/A)CCA(G/C)CCAGTGC 3'
            BamH I
```

(c)

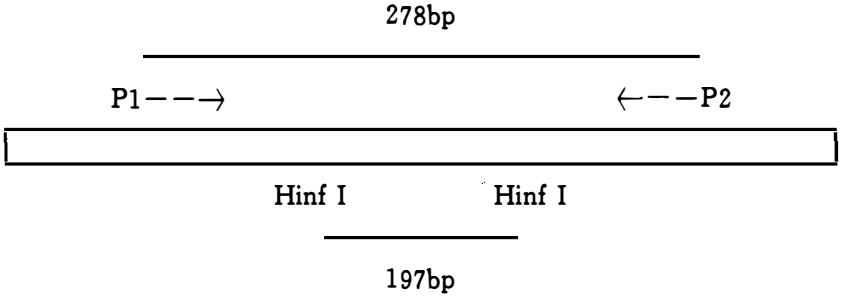


Fig. 1 Conservative regions in mammalian β-IFN genes

(a) Alignment of six mammalian β-IFN gene sequences, highly conservative positions indicated by (b)Primer sequences. N represents A, T, C or G; (c) Restriction map of human β-IFN gene.

3.2 PCR AMPLICATION WITH PRIMER 3 (P3) AND PRIMER 4 (P4)

Using primer 3 and primer 4 and Buffer B, a DNA fragment about 278 bp was amplified, but when Buffer A was used, the fragment was not detected (Fig. 3).

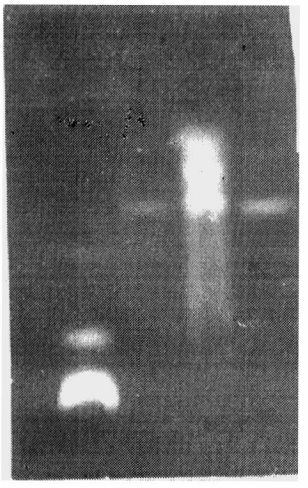
3.3 SOUTHERN HYBRIDIZATION

The 278 bp fragment amplified from human β-IFN gene with P3 and P4 was digested with *Hinf* I to remove the primer-containing sequences. The resulting 197 bp *Hinf* I fragment was recovered from low-melting-temperature agarose gel, extracted with phenol, and dissolved in H_2O. Before hybridization, the DNA was labelled with α-^{32}P-dATP using Promega Prime-A-Gene kit.

The hybridization result was shown in Fig. 4. A fragment about 278 bp was detected in P1 and P2 amplified products. No signal was detected in P3 and P4 amplified PCR products.

1 2 3 4

Fig. 2 PCR amplification with P1 and P2
Lane 1: λ DNA/Hind III; Lane 2 and Lane 4: 278 bp DNA fragment amplified from pBR13 (a plasmid containing human β - IFN gene) with P1 and P2 and Buffer B; Lane 3: PCR product of grass carp genomic DNA. Two fragments about 278 bp amplified.



1 2 3 4

Fig. 3 PCR amplification with P3 and P4
Lane 1: λ/Hind III; Lane 2 and Lane 4: Fragment amplilfied from human β - IFN gene using P3 and P4; Lane 3: PCR product of grass carp genomic DNA.

4 DISCUSSIONS

4.1 PRIMER DESIGN

Because there are currently no sequence information of nonmammalian IFN genes, the primers were designed according to six reported mammalian sequences using the following guidelines: a) the sequence between two primers should neither too long (PCR amplificable) nor too short (informative), and b) at least six bases at 3' ends of primers should be highly conservative.

To keep maximum homology between the primers and fish IFN sequences, we did not incorporate restrict sites into P1 and P2. A random nucleotide was added to 3' ends of both primers according to the observations of Batzer *et al.* [1991]. To facilitate subsequent cloning of PCR product, a *BamH* I site was added at 5' ends of P3 and P4.

In P1 and P2 directed PCR, it is very important to add the polymerase before denaturing

of DNA, or several non - specific bands will be produced. In P3 and P4 directed PCR, a band about 278 bp could be easily amplified using Buffer B, but not when using Buffer A. This is probably caused by the presence of formamide, a reagent which reduces non - specific priming, in the Buffer A. The additional sequences at 5' ends seem to have adverse effects on the annealing specialty in amplification of complex genomes, but did not prevent correct annealing in amplification of pBR13(a kind gift from Dinter).

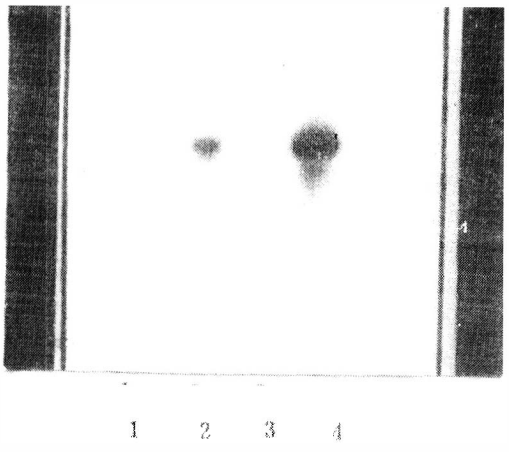


Fig. 4 Southern hybridization result
Lane 1: PCR product of grass carp genomic DNA with P3 and P4; Lane 2: PCR product of grass carp genomic DNA with P1 and P2; Lane 3: P3 and P4 (control); Lane 4: human β - IFN fragment amplified with P3 and P4.

4.2 ON THE RESULT OF SOUTHERN HYBRIDIZATION

A PCR fragment of known sequence can be easily identified by hybridization using an internal probe. Because the fish sequence is unknown, we chose to use an internal fragment of human β - IFN PCR product as probe (Fig. 1). Hybridizations were performed under relatively low stringent conditions (3 X SSC, 60℃). A band was detected in the P1 and P2 directed PCR product when the filters were washed at either 64℃ or 67℃. No signal was detected in the P3 and P4 PCR product. The absence of the band in the lane of P3 plus P4 exclude the possible contamination of primer sequences in the probe (Fig. 4). Contamination of human sequences is also highly impossible, because negative controls in PCR did not produce any bands. The postulation is also supported by the absence of hybridization signal in the P3 and P4 PCR product. Therefore, we believed that the detected signals represented the authentic fish IFN gene fragment. This is consistent with the observations of Wilson *et al.* [1983].

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