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生态学报

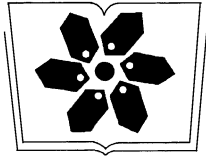
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生态学报

(SHENGTAI XUEBAO)

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彩图提供：陈建伟教授 北京林业大学 E-mail: cites.chenjw@163.com

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Hu Y F, Yan T, Cao W H, Li Z F, Peng Y, Cheng Z Q.Effects of K^+ and Cr^{6+} on larval development and survival rate of the acorn barnacle *Balanus reticulatus*.Acta Ecologica Sinica,2014,34(2):353-358.

K^+ 、 Cr^{6+} 对网纹藤壶幼虫发育和存活的影响

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摘要:以华南沿海污损生物群落优势种网纹藤壶(*Balanus reticulatus*)的幼虫为研究对象,探讨钾、铬 2 种金属离子对其幼虫存活及发育的影响。实验所用金属离子溶液是将 K^+ 和 Cr^{6+} 母液添加到过滤消毒海水中配制。将活泼健壮的网纹藤壶无节幼虫置于上述溶液中,以亚心形扁藻(*Platymonas subcordiformis*)为饵料于 30℃ 左右的黑暗环境中培养 5d,镜检观察记录幼虫发育状况并进行统计分析。结果表明, K^+ 对网纹藤壶幼虫发育和存活的影响均不明显;而 Cr^{6+} 对幼虫发育具有一定的毒杀和抑制作用,不仅幼虫存活率低于对照组,而且金星幼虫所占百分率也低于对照组。

关键词:网纹藤壶;幼虫;发育;存活; K^+ ; Cr^{6+}

Effects of K^+ and Cr^{6+} on larval development and survival rate of the acorn barnacle *Balanus reticulatus*

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Abstract: The acorn barnacle *Balanus reticulatus* plays an important role in certain marine ecological systems and is one of the most dominant fouling species in tropical and subtropical waters. Its larval development proceeds through six naupliar stages and a single cyprid stage. To understand the effects of K^+ and Cr^{6+} on the larvae of the barnacle *B. reticulatus*, the following study on different stage larvae and their survival rate to such free ions was conducted.

Adult *B. reticulatus* were collected from the aquaculture facilities in Dapengao of Daya Bay, Shenzhen, China and egg lamellae containing late stage embryos were removed from the mantle cavities by dissection. When such lamellae were placed in a 500mL glass beaker with filtered seawater, nauplii were released within a few minutes. The nauplii were collected and transferred to 100mL beakers with 50mL filtered seawater. Larval culture was conducted over five days at the initial density of 1 larvae/mL in complete darkness at 30℃ with the green algae *Platymonas subcordiformis* (2.5×10^5 — 3.0×10^5 cells/mL) added.

Stock metal solutions were made up from analytical grade potassium chloride (KCl) and chromium VI oxide (CrO_3) in distilled water (concentrations of stocks were 10g/L for K^+ and 1g/L for Cr^{6+} , respectively). Five test concentrations of each metal were prepared by diluting the stock solutions in filtered seawater. The concentrations of the K^+ solutions for testing were 0.1, 1, 10, 100 and 1000mg/L. For Cr^{6+} they were 0.01, 0.05, 0.1, 1 and 10mg/L, respectively. Five replicates

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were run for each concentration.

In the tests of K^+ treatments the survival rates of larvae were 57.4% in the Control, 62.6% in the group treated by K^+ at a concentration of 0.1mg/L, 58.9% at 1mg/L, 58.0% at 10mg/L, 58.4% at 100mg/L and 59.9% at 1000mg/L, respectively. There was no significant difference in the larval survival rates between the Control and treatment groups ($P>0.05$). In the Control group, Nauplii V accounted for 52.2% and Nauplii VI 5.2%. In the group treated by K^+ at a concentration of 0.1mg/L, Nauplii V was 58.6% and Nauplii VI 4.0%; at 1mg/L, Nauplii V 55.0% and Nauplii VI 3.9%; at 10mg/L, Nauplii V 54.8% and Nauplii VI 3.2%; at 100mg/L, Nauplii V 56.1% and Nauplii VI 2.3%; at 1000mg/L, Nauplii V 56.3% and Nauplii VI 3.6%. There was no significant difference in the larval survival percentages of Nauplii V and VI between the Control and treatment groups ($P>0.05$).

Concerning the tests using Cr^{6+} treatments the larval survival rates were 55.4% in Control, 49.0% treated by Cr^{6+} at a concentration of 0.01mg/L, 45.9% at 0.05mg/L, 44.2% at 0.1mg/L, 42.4% at 1mg/L and 0% at 10mg/L, respectively. There was a significant difference between the Control and treatment groups ($P<0.05$). Of them, the survival percentage of naupliar V was 8.3%, naupliar VI 11.2%, and cyprid 35.9% in the Control. Treated by Cr^{6+} at 0.01mg/L, the survival percentage of naupliar V was 8.2%, naupliar VI 9.0%, and cyprid 31.8%; at 0.05mg/L, the survival percentage of naupliar V was 13.4%, naupliar VI 15.0%, and cyprid 17.4%; at 0.1mg/L, naupliar V was 13.4%, naupliar VI 15.0%, and cyprid 15.7%; at 1mg/L, naupliar V was 14.0%, naupliar VI 14.0%, and cyprid 14.4%, respectively. No larvae survived the 10mg/L Cr^{6+} treatment. The cyprid percentages in the groups treated by Cr^{6+} were significantly less than the Control ($P<0.05$). However, compared with the Control, more larvae at the stage of the naupliar VI occurred in the treatment groups under the influence of Cr^{6+} in the range from 0.05mg/L to 1mg/L ($P<0.05$).

According to the results gained it can be concluded that the effect of K^+ on *B. reticulatus* nauplii survival rate and development was neutral, whereas there was a marked negative effect with the Cr^{6+} treatments, which presented a marked inhibitory effect on larval development of the acorn barnacle *B. reticulatus*.

Key Words: *Balanus reticulatus*; nauplii; development; survival; K^+ ; Cr^{6+}

海洋环境中富含多种金属元素,会对海洋生物的生长发育产生影响,其中过量的钾离子会对部分种类的贻贝和苔藓虫等无脊椎动物幼虫的附着变态产生诱导作用^[1-3],但却对纹藤壶(*Balanus amphitrite*)幼虫表现出抑制作用^[4]。铬虽是海洋生物必需的微量元素之一,但也是毒性较高、造成海洋环境污染的重金属元素^[5-6]。近年来,海水中金属离子对海洋生物的影响受到广泛关注,许多生物(尤其幼体)成为生态毒理学重要的研究对象^[7-8]。

藤壶属甲壳动物蔓足亚纲,是一类常见的海洋底栖生物,在海洋生态环境中占据重要地位,并且还是海洋污损生物群落中的重要组成部分^[9]。在热带、亚热带海域,占优势地位的网纹藤壶(*Balanus reticulatus*)常附着于船舰或浮标等物体上,是引发生物污损危害最为严重的种类,其幼虫发育过程要经过6期无节幼虫和1期金星幼虫两个阶段,且对外界环境十分敏感,可作为海洋环境监测的良好

对象^[10-11]。

目前有关金属离子对藤壶幼虫的影响多着眼于短期急性实验^[12-14],尚未见对其生长发育影响的报道。本研究选取了网纹藤壶幼虫作为研究对象,探讨两种常见的金属离子(K^+ 和 Cr^{6+})对其存活和发育的影响,以期为进一步研究金属元素在海洋生态系统中的迁移转化过程及其影响提供基础资料,并为近岸海域生态环境保护提供参考和依据。

1 材料与方法

1.1 样品采集

在位于大亚湾西南侧大鹏澳的海洋水产养殖设施上采集网纹藤壶成熟个体,仔细挑选外观完整且体积较大的个体,稍用海水冲洗清除表面杂质后带回实验室。解剖取出其中的成熟受精卵块,放入装有过滤消毒海水的500mL烧杯中,数分钟后无节幼虫即可孵化出来。收集活泼健壮的王节幼虫备用。

1.2 K^+ 、 Cr^{6+} 对无节幼虫发育和存活的影响

实验容器为 100mL 烧杯,实验水体 50mL,药品 KCl 、 CrO_3 均为分析纯试剂。以蒸馏水配制金属离子溶液母液(其中 K^+ 的母液浓度为 10g/L, Cr^{6+} 的母液浓度为 1g/L),并根据预实验结果,通过添加上述母液到过过滤消毒海水中,按表 1 所示的浓度梯度配制所需的 K^+ 和 Cr^{6+} 溶液。另设置不添加金属离子的对照组。对照组和各实验处理组均分别设 5 个平行样。

表 1 两种金属离子的浓度梯度
Table 1 Concentrations of K^+ and Cr^{6+}

	浓度 Concentration/(mg/L)
K^+	0、0.1、1、10、100、1000
Cr^{6+}	0、0.01、0.05、0.1、1、10

采集活泼健壮的网纹藤壶无节幼虫,转移到分别盛有 50mL 不同浓度金属离子溶液的 100mL 烧杯中,幼虫密度为 1 个/mL。以亚心形扁藻 (*Platymonas subcordiformis*) 作为饵料(投放量约 2.5×10^5 — 3.0×10^5 个细胞/mL),置于 30℃ 左右的恒温培养箱中于黑暗环境下培养。每天于 9:00 和 17:00 前后将烧杯取出置于阳光下 0.5—1h^[15]。培养 5d 后,镜检观察幼虫发育状况,记录各发育时期幼虫数目。采用最小显著差数法进行差异显著性分析。

2 实验结果

2.1 K^+ 对网纹藤壶幼虫发育的影响

图 1 和图 2 显示了在不同浓度 K^+ 作用下,5d 后网纹藤壶幼虫的发育状况。结果表明,对照组 V 期幼虫约占 52.2%,各实验处理组则分别为 58.6%、55.0%、54.8%、56.1%、56.3%,经 LSD 检验,与对照组均无显著性差异 ($P>0.05$);至于 VI 期幼虫,对照组约 5.2%,各实验处理组则分别为 4.0%、3.9%、3.2%、2.3%、3.6%,与对照组无显著性差异 ($P>0.05$);在幼虫存活率方面,对照组为 57.7%,而各实验处理组分别为 62.6%、58.9%、58.0%、58.4%、59.9%,与对照组之间也无显著性差异 ($P>0.05$)。

2.2 Cr^{6+} 对网纹藤壶幼虫发育的影响

图 3—图 5 显示了在不同浓度 Cr^{6+} 作用下,培养 5d 后网纹藤壶幼虫的发育状况。结果表明,对照组 V 期幼虫约 8.3%,各实验处理组分别为 8.2%、13.4%、13.4%、14.0%、0,除浓度为 0.01mg/L 的实验处理组

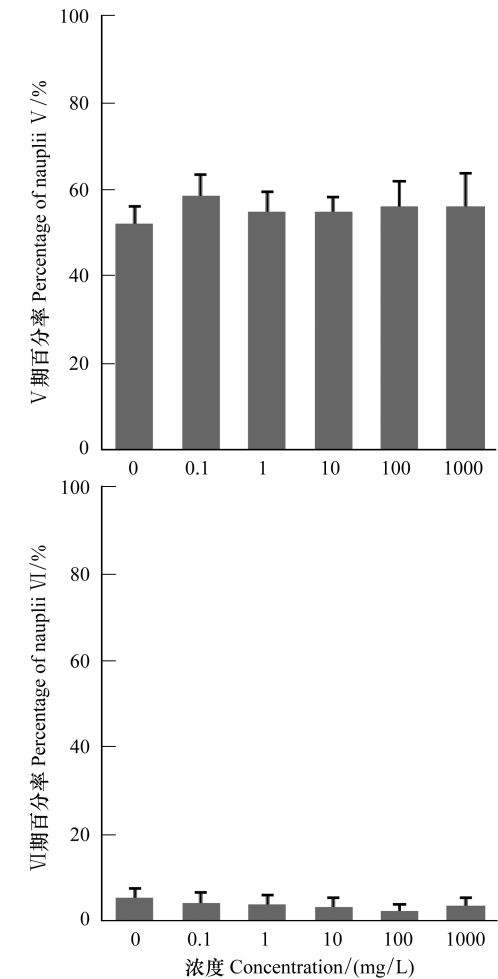


图 1 K^+ 作用下 V 期和 VI 期无节幼虫所占百分率
Fig.1 The percentages of Nauplii V and VI after exposure to K^+

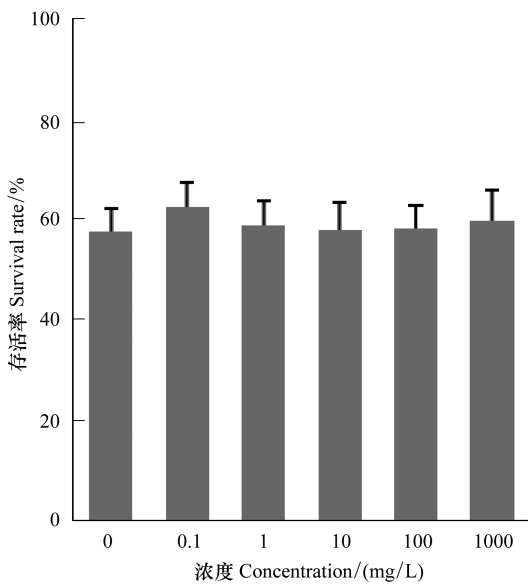


图 2 K^+ 对幼虫存活率的影响
Fig.2 The larval survival rate after exposure to K^+

外,其余 4 个实验处理组均与对照组之间存在显著性差异($P<0.05$)。对照组Ⅵ期幼虫约占 11.2%,各实验处理组分别为 9.0%、15.0%、15.0%、14.0%、0,均与对照组差异显著($P<0.05$)。至于金星幼虫所占百分率,对照组为 35.9%,各实验处理组分别为 31.8%、17.4%、15.7%、14.4%、0,也均与对照组差异显著($P<0.05$)。在幼虫存活率方面,对照组约 55.4%,各实验处理组分别为 49.0%、45.9%、44.2%、42.4%、0,且与对照组之间差异显著($P<0.05$)。

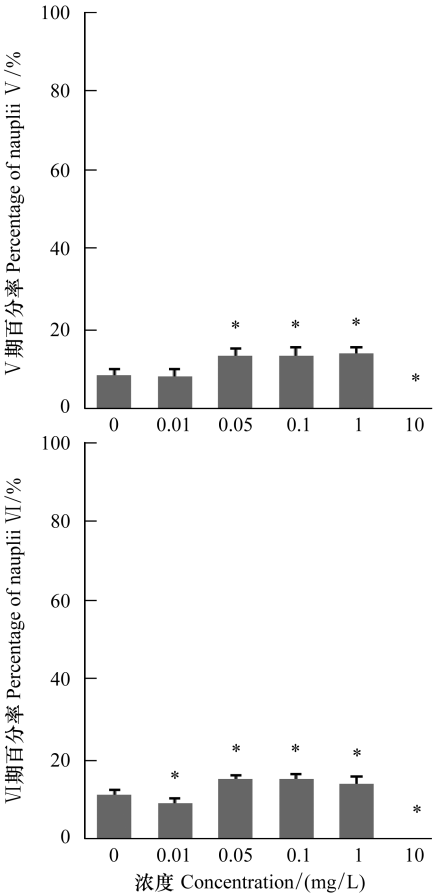


图 3 Cr^{6+} 作用下 V 期和 VI 期无节幼虫所占百分率
Fig.3 The percentages of Nauplii V and VI after exposure to Cr^{6+}
* 该实验处理组与对照组差异显著 ($P<0.05$)

3 讨论

从上述实验结果可以看出,在 K^{+} 浓度为 0.1—1000mg/L 的作用下,幼虫存活率及各期幼虫所占百分率均与对照组之间无显著性差异($P>0.05$),由此看来该浓度范围的 K^{+} 不会对网纹藤壶幼虫发育产生显著影响。 Cr^{6+} 浓度为 0.01mg/L 时即可对幼虫产生明显毒杀作用,高浓度 (10mg/L) 处理导致其全部

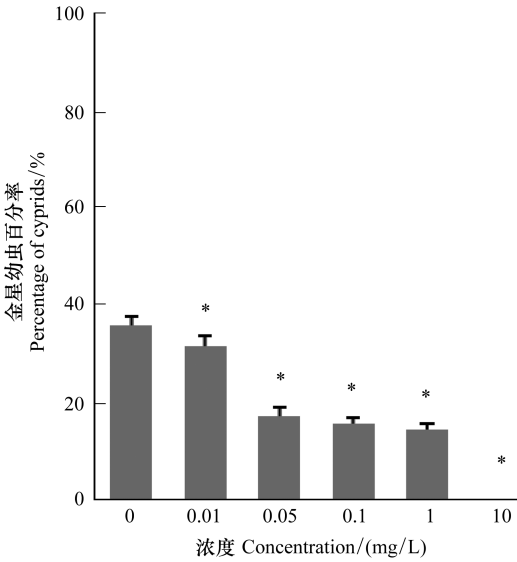


图 4 Cr^{6+} 作用下金星幼虫所占百分率
Fig.4 The percentage of crpyids after exposure to Cr^{6+}
* 表示该实验处理组与对照组差异显著 ($P<0.05$)

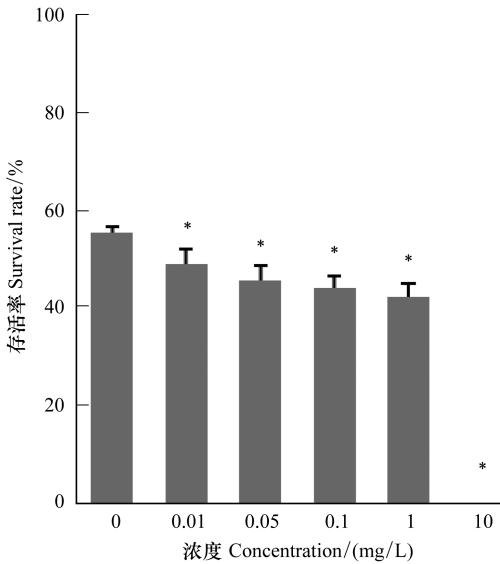


图 5 Cr^{6+} 对幼虫存活率的影响
Fig.5 The larval survival rate after exposure to Cr^{6+}
* 该实验处理组与对照组差异显著 ($P<0.05$)

死亡;另外,各实验处理组金星幼虫所占百分率均低于对照组 ($P<0.05$),表明相关浓度的 Cr^{6+} 会对网纹藤壶幼虫的生长发育产生一定抑制作用。

钾是生物体生长发育必不可少的元素^[16],但长期摄食过量的钾则可能产生不利影响^[17]。至于本实验中 K^{+} 对网纹藤壶幼虫发育和存活的影响不明显,可能与海水中 K^{+} 的含量较为丰富、幼虫可通过一定方式维持自身的渗透压及电解质平衡有关。另

外,过量 K⁺会对某些海洋无脊椎动物幼虫的附着和变态产生诱导作用^[4,18-21],但其对网纹藤壶金星幼虫附着和变态行为产生何种影响则需进一步探讨。

铬是重金属元素,在海水中常以 Cr³⁺和 Cr⁶⁺的形式存在。由于藤壶吸收的 Cr³⁺约 82%可通过净化作用排出,不易在其体内富集^[22-23],故本研究特意选择 Cr⁶⁺开展测试工作,以探讨其对藤壶幼虫生长发育的影响。上述结果表明,随着 Cr⁶⁺浓度的增大,其对网纹藤壶幼虫的毒杀效果越发明显。基于 Cr⁶⁺的毒性受阳离子、盐度和温度的影响较大^[24],实验过程中应严格控制实验条件,确保结果的科学性和可重复性。

金星幼虫是藤壶幼虫发育、营浮游生活的最后一个阶段,其数量多寡可反映在特定培养条件下幼虫的发育状况。从实验结果可以看出,在 Cr⁶⁺的作用下,金星幼虫所占百分比均低于对照组($P<0.05$),显示其幼虫发育过程有所滞后。在贝类幼虫生长发育方面,Cr⁶⁺对海湾扇贝(*Argopecten irradians*)幼虫也显示出明显的抑制作用^[25];类似现象在玻璃海鞘(*Ciona intestinalis*)的早期发育阶段也可观测到^[26]。由此看来,Cr⁶⁺对海洋生物幼体的影响主要表现出毒杀和抑制作用,其具体作用机理尚需进一步探讨。

在 K⁺对网纹藤壶幼虫生长发育影响的实验结果中未见金星幼虫,很可能与外界刺激导致幼虫提前孵化和设定的幼虫培养时间较短有关。在自然环境中,藤壶繁殖行为取决于光照、水温、潮汐、饵料和季节等多种环境因素的综合作用,故赴野外采集样品时每次藤壶个体的成熟程度可能有所不同,而当解剖摘取成熟卵块时,那些尚未充分发育的胚胎可能会因受刺激而提前孵化,从而导致从无节幼虫发育至金星幼虫的阶段所需时间延长,故在上述设定的培养时间内无金星幼虫出现。

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