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# 生态学报

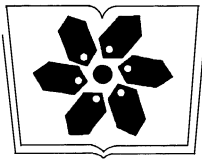
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# 生态学报

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## 目 次

黑龙江省大兴安岭林区火烧迹地森林更新及其影响因子.....	蔡文华, 杨 健, 刘志华, 等 (3303)
基于 B-IBI 指数的温榆河生态健康评价 .....	杨 柳, 李泳慧, 王俊才, 等 (3313)
川西亚高山暗针叶林不同恢复阶段红桦、岷江冷杉土壤种子损耗特征 ...	马姜明, 刘世荣, 史作民, 等 (3323)
老龄阔叶红松林下层木空间分布的生境关联分析.....	丁胜建, 张春雨, 夏富才, 等 (3334)
内蒙古高原荒漠区四种锦鸡儿属植物灌丛沙包形态和固沙能力比较.....	张媛媛, 马成仓, 韩 磊, 等 (3343)
角果藜的生长动态及其生殖配置.....	全杜娟, 魏 岩, 周晓青, 等 (3352)
基于 MODIS/NDVI 时间序列的森林灾害快速评估方法——以贵州省为例 .....	侍 昊, 王 笑, 薛建辉, 等 (3359)
祁连山西水林区土壤阳离子交换量及盐基离子的剖面分布.....	姜 林, 耿增超, 李珊珊, 等 (3368)
水分和温度对春玉米出苗速度和出苗率的影响.....	马树庆, 王 琪, 吕厚荃, 等 (3378)
施氮对水稻土 N <sub>2</sub> O 释放及反硝化功能基因 ( <i>narG/nosZ</i> ) 丰度的影响 .....	郑 燕, 侯海军, 秦红灵, 等 (3386)
中国西北潜在蒸散时空演变特征及其定量化成因 .....	曹 雯, 申双和, 段春峰 (3394)
基于植被降水利用效率和 NDVI 的黄河上游地区生态退化研究 .....	杜加强, 舒俭民, 张林波 (3404)
异速生长法计算秋茄红树林生物量.....	金 川, 王金旺, 郑 坚, 等 (3414)
乌兰布和沙漠沙蒿与油蒿群落的物种组成与数量特征.....	马全林, 郑庆中, 贾举杰, 等 (3423)
不同光强下单叶蔓荆的光合蒸腾与离子累积的关系.....	张 萍, 刘林德, 柏新富, 等 (3432)
浑善达克沙地沙地榆种子雨的扩散规律.....	谷 伟, 岳永杰, 李钢铁, 等 (3440)
咸水灌溉对沙土土壤盐分和胡杨生理生长的影响.....	何新林, 陈书飞, 王振华, 等 (3449)
外源 NO 对 NaHCO <sub>3</sub> 胁迫下黑麦草幼苗光合生理响应的调节 .....	刘建新, 王金成, 王 鑫, 等 (3460)
呼伦贝尔草地植物群落与土壤化学计量学特征沿经度梯度变化.....	丁小慧, 罗淑政, 刘金巍, 等 (3467)
海南稻田土壤硒与重金属的含量、分布及其安全性.....	耿建梅, 王文斌, 温翠萍, 等 (3477)
江苏省典型区农田土壤及小麦中重金属含量与评价.....	陈京都, 戴其根, 许学宏, 等 (3487)
应用稳定同位素研究广西东方洞食物网结构和营养级关系 .....	黎道洪, 苏晓梅 (3497)
利用细胞计数手段和 DGGE 技术分析松花江干流部分地区的细菌种群多样性 .....	屠 腾, 李 蕾, 毛冠男, 等 (3505)
中国主要入海河流河口集水区划分与分类.....	黄金良, 李青生, 黄 玲, 等 (3516)
基于 VGPM 模型和 MODIS 数据估算梅梁湾浮游植物初级生产力 .....	殷 燕, 张运林, 时志强, 等 (3528)
低温胁迫下虎纹蛙的生存力及免疫和抗氧化能力.....	王 娜, 邵 晨, 颜志刚, 等 (3538)
转 Bt 水稻土壤跳虫群落组成及其数量变化 .....	祝向钰, 李志毅, 常 亮, 等 (3546)
尼日利亚非洲蜂和安徽意大利蜜蜂及其杂交二代形态特征与微卫星 DNA 遗传多样性 .....	余林生, 解文飞, 巫厚长, 等 (3555)
北京城市公园湿地休憩功能的利用及其社会人口学因素 .....	李 芬, 孙然好, 陈利顶 (3565)
基于协整理论的经济增长与生态环境变化关系分析——以重庆市渝东南地区为例 .....	肖 强, 胡 翀, 肖 洋, 等 (3577)
感潮河网区环境合作博弈模型及实证 .....	刘红刚, 陈新庚, 彭晓春 (3586)
<b>专论与综述</b>	
国内外生态效率核算方法及其应用研究述评.....	尹 科, 王如松, 周传斌, 等 (3595)
全球变化背景下的现代生态学——第六届现代生态学讲座纪要.....	温 腾, 徐德琳, 徐 驰, 等 (3606)
<b>问题讨论</b>	
流域环境要素空间尺度特征及其与水生态分区尺度的关系——以辽河流域为例 .....	刘星才, 徐宗学, 张淑荣, 等 (3613)
<b>研究简报</b>	
不同光照强度对兴安落叶松几种主要防御蛋白活力的影响 .....	鲁艺芳, 石 蕾, 严善春 (3621)
木荷种源间光合作用参数分析.....	熊彩云, 曾 伟, 肖复明, 等 (3628)
基于能值分析的深圳市三个小型农业生态系统研究.....	杨卓翔, 高 阳, 赵志强, 等 (3635)

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**封面图说:** 爬升樟木沟的暖湿气流——樟木沟是中国境内横切喜马拉雅山脉南坡的几条著名大沟之一,它位于我国西藏聂拉木县境内的希夏邦马峰东南侧,延绵 5400km 的 318 国道在此沟中到达其最西头。从聂拉木县城到樟木口岸短短的 30km 中,海拔从 4000m 急降至 2000m。在大气环流作用下,来自印度洋的暖湿气流沿樟木沟不断费力地往上爬升,给该沟谷留下了大量的降水。尤其是在雨季到来时,山间到处是流水及悬垂崖头的瀑布,翠峰直插云霄,森林茂密苍郁,溪流碧澄清澈,奇花异葩繁多,风景美如画卷,气势壮丽非凡。

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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## 低温胁迫下虎纹蛙的生存力及免疫和抗氧化能力

王 娜,邵 晨,颜志刚\*,凌云,程东海

(浙江师范大学化学与生命科学学院生态研究所,金华 321004)

**摘要:** 全球气候变化是造成世界范围内两栖类种群衰退和灭绝的重要因素之一。随着极端天气出现变得日趋频繁,非季节性的、短期且剧烈的气温变化可能会严重干扰两栖类动物的生存与种群稳定。监测了浙江省金华市南山野生虎纹蛙 (*Hoplobatrachus rugulosus*) 分布区冬季的环境气温,并参考监测数据在实验室条件下研究了虎纹蛙在短期梯度降温(2 °C/24 h)和急性冷暴露(即冷休克)(2 °C)下的生存力及冷休克对机体免疫功能和抗氧化能力的影响。结果表明,虎纹蛙在冬季(2009-12-01—2010-03-31)经历的温度范围普遍在 0—14 °C 之间,主要遭遇的低温区间在 0—4 °C,主要高温区间在 10—14 °C。通过梯度降温实验,发现温度降至 12 °C 累积死亡率约为 28.1%,10 °C 为 87.5%,8 °C 为 100%。在一定温度范围内,虎纹蛙死亡率与环境温度呈显著负相关(Pearson test,  $r = -0.952$ ,  $P < 0.05$ )。经曲线拟合,回归方程计算可得半数致死温度为 11.5 °C。虎纹蛙在冷休克处理下,在第 6 h 累积死亡率为 45%,12 h 为 80%,24 h 达到 100%。虎纹蛙死亡率与冷休克时间呈显著正相关(Pearson test,  $r = 0.91$ ,  $P < 0.05$ ),半数致死时间为 7.6 h。此外,冷休克(2 °C, 6 h)显著抑制了虎纹蛙脾脏巨噬细胞呼吸爆发强度( $t = 3.827$ ,  $df = 6$ ,  $P < 0.05$ )、全血吞噬活性( $t = 5.388$ ,  $df = 3.037$ ,  $P < 0.05$ )及胃溶菌酶活力( $t = 6.37$ ,  $df = 6$ ,  $P < 0.05$ );肝脏( $t = 0.773$ ,  $df = 8$ ,  $P > 0.05$ )和肾脏( $t = 0.164$ ,  $df = 4.542$ ,  $P > 0.05$ )组织脂质过氧化物产物丙二醛(MDA)的含量虽无明显变化,但肝脏( $t = -2.817$ ,  $df = 6$ ,  $P < 0.05$ )和肾脏( $t = -11.302$ ,  $df = 6$ ,  $P < 0.05$ )组织抗氧化物谷胱甘肽(GSH)含量及肝脏( $t = -3.3$ ,  $df = 6$ ,  $P < 0.05$ )超氧化物歧化酶(SOD)活性均显著升高。上述结果表明虎纹蛙对低温较为敏感,冷休克能够诱导机体的免疫抑制,并导致机体对抗氧化物质需求的增加。可以推测,当遭遇极端低温天气时,低温胁迫可能会严重干扰虎纹蛙生理机能,加大种群的生存压力和疾病感染的风险。

**关键词:** 虎纹蛙;低温胁迫;冷休克;免疫力;抗氧化物

## Viability and changes of physiological functions in the tiger frog (*Hoplobatrachus rugulosus*) exposed to cold stress

WANG Na, SHAO Chen, XIE Zhigang\*, LING Yun, CHENG Donghai

Institute of Ecology, College of Chemistry and Life Science, Zhejiang Normal University, Jinhua 321004, China

**Abstract:** Global climate change has been considered to be one of the key factors leading to declines and losses of amphibian populations. Though global warming is one of the acknowledged causes of fluctuations of amphibian population worldwide, unseasonal sharp fall in air temperature as result of global climate change may also affect the survival and stability of amphibian population severely. As amphibians are typical poikilothermic animals, environmental temperature can influence their physiological functions and enhance their susceptibility to infectious diseases. The immunological competence of amphibians may, therefore, make them vulnerable to parasites and disease particularly after discontinuous and extreme events (e. g. winter rainfall, late spring coldness). In this article, we have investigated how cold stress affected the physiology and viability of tiger frogs (*Hoplobatrachus rugulosus*). The winter temperatures experienced by wild

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\* 通讯作者 Corresponding author. E-mail: xiezhigang@zjnu.cn



tiger frogs in natural winter in the Southern Mountain of Jinhua in Zhejiang Province was monitored, and then replicated in subsequent experiments. The aim of the experiments was to determine how gradient cooling ( $2\text{ }^{\circ}\text{C}/24\text{ h}$ ) and cold shock ( $2\text{ }^{\circ}\text{C}$ ) affected the mortality rate of tiger frogs, and affected various aspects of their immune function and oxidation resistance. The results showed that the survival temperature range of wild overwintering was between  $0\text{--}14\text{ }^{\circ}\text{C}$  in 12/1/2009—3/31/2010, and minimum and maximum temperature ranged between  $0\text{--}4\text{ }^{\circ}\text{C}$  and  $10\text{--}14\text{ }^{\circ}\text{C}$  respectively. During the process of gradient cooling, we found that the accumulated mortality rate was 28.1% at  $12\text{ }^{\circ}\text{C}$ , 87.5% at  $10\text{ }^{\circ}\text{C}$  and 100% at  $8\text{ }^{\circ}\text{C}$ . A significant negative correction was found between environmental temperature and frog mortality within a certain temperature range (Pearson test,  $r=-0.952$ ,  $P<0.05$ ). And lethal temperature of 50% occurred at  $11.2\text{ }^{\circ}\text{C}$  based on a fitted curve regression equation. Under cold shock, 45% mortality was observed after being directly stimulated at  $2\text{ }^{\circ}\text{C}$  for 6 h, 80% mortality appeared at  $2\text{ }^{\circ}\text{C}$  for 12 h, and increased to 100% after 24 h. There appeared to be positive correction between cold shock time and mortality (Pearson test,  $r=0.91$ ,  $P<0.05$ ), and lethal time of 50% is 7.6 h at  $2\text{ }^{\circ}\text{C}$  according to fitted curve regression equation. Moreover, cold shock significantly inhibited the respiratory burst of spleen macrophages ( $t=3.827$ ,  $df=6$ ,  $P<0.05$ ), peripheral blood phagocytic activity ( $t=5.388$ ,  $df=3.037$ ,  $P<0.05$ ) and gastric lysozyme activity ( $t=6.37$ ,  $df=6$ ,  $P<0.05$ ) at  $2\text{ }^{\circ}\text{C}$  for (6 h). Although there was no significant change in malonaldehyde (MDA) content, the glutathione (GSH) content of the liver ( $t=-2.817$ ,  $df=6$ ,  $P<0.05$ ) and kidney ( $t=-11.302$ ,  $df=6$ ,  $P<0.05$ ) increased. The activity of superoxide dismutase (SOD) also increased significantly in the liver ( $t=-3.3$ ,  $df=6$ ,  $P<0.05$ ), while there was no apparent change within the kidney. The results indicate that tiger frogs are sensitive to low temperatures and that cold shock might lead to greater oxidative stress, more requirements for antioxidants and produce immunosuppression in tiger frogs. Consequently, low temperature stress will stimulate organism endocrine disorder and subject the frogs to a more intensive disturbance of physiological function, increase the survival pressure and susceptibility to infectious disease, which may affect development and dynamics of amphibian populations.

**Key Words:** *Hoplobatrachus rugulosus*; cold stress; cold shock; immunity; antioxidant

全球气候变化是当今人类公认的导致世界范围内两栖类动物种群衰退和灭绝的重要因素之一<sup>[1-3]</sup>。两栖类作为典型的、由水生向陆生过渡的变温动物类群,环境中稳定和有规律的气温和降水条件对其生存起着至关重要的作用<sup>[4]</sup>。由于气候异常使得极端天气的出现变得日趋频繁,非季节性的、短期且剧烈的气温变化可能会严重影响两栖类动物机体的生理机能,并能从多种途径增加其感染疾病的风险,最终影响种群的生存与稳定<sup>[5-6]</sup>。

由于生境破坏和人类的滥捕等因素,中国虎纹蛙(*Hoplobatrachus rugulosus*)野生种群濒临灭绝,已被列入中国濒危动物红皮书,是我国二级保护动物。虎纹蛙分布于热带和亚热带地区,该物种通常选择温暖潮湿的生境,对温度变化较为敏感。近年来我国南方已经开展虎纹蛙的人工驯养,但在养殖实践中发现该蛙耐寒性较差,尤其在气温波动幅度较大的季节交替期,患病率和死亡率均较高,严重制约了该产业的发展,急需相关的技术资料。为此,本研究监测了浙江省金华市南山野生虎纹蛙分布区冬季的环境气温,在实验室条件下研究了虎纹蛙在短期梯度降温 and 急性冷暴露(即冷休克)下的生存力及冷休克对机体免疫功能和抗氧化能力的影响。研究结果可为探讨全球气候变化导致两栖类种群衰退的机制提供理论依据,并为该蛙的人工养殖提供技术支持。

## 1 材料与方法

### 1.1 越冬期生境温度监测

利用温度记录仪(RC-20, 上海精创公司)于2009年12月1日至2010年3月31日对浙江金华南山虎纹蛙分布区进行环境温度监测。通过程序设置,将温度记录仪监测的时间间隔设为1次/h。温度探头置于较浅的洞穴内(距洞口约10 cm处),以免阳光直射和雨水浸泡。

## 1.2 动物与驯化

由于虎纹蛙属于国家二级保护动物,本研究所用虎纹蛙均选自浙江省金华市某虎纹蛙养殖场。将蛙饲养于水族箱(90 cm × 40 cm × 40 cm, 20 只/缸)内,提供较充足的水陆环境和遮蔽物,控制水温(22±1) °C,光周期为 12 L:12 D,每日以黄粉虫(*Tenebrio molitor*)活体作为饵料投食 1 次,隔日用经曝气脱氯的自来水换水 1/3,驯化时间为 1 周。

## 1.3 梯度降温实验

选择 100 只体重相近、体表无伤的健康虎纹蛙(75.77±1.66) g 随机分配至 4 个玻璃槽内(30 cm×20 cm×15 cm,保持 1 cm 水位),然后置入可精确控温的低温恒温培养箱(MIR-253, SANYO, Japan)内。培养箱起始温度设定为(22±0.5) °C,通过编程设置降温速率为 2 °C/24 h,每 24 h 记录死亡个体数,计算累积死亡率。

## 1.4 冷休克实验

将 60 只虎纹蛙随机分配至 3 个玻璃槽内,置入低温恒温培养箱,箱内温度预降至(2±0.5) °C,其它条件同上。在第 2、4、6、12、24 小时记录存活情况,计算累积死亡率。

另取 30 只虎纹蛙随机分为两组,分别置于两台低温恒温培养箱内。其中,对照组设为 22 °C,处理组设为 2 °C。冷休克 6 h(接近半数致死时间)后,每组取 6 只蛙用于免疫和抗氧化指标分析。

## 1.5 免疫指标的测定

### 1.5.1 血细胞吞噬

蛙经毁髓后,断头取血,用 EDTA-2Na 抗凝,采用荧光标记法测定蛙全血吞噬能力,吞噬原为经荧光标记酵母(*Saccaromyces cerevisiae*),参考 Miliukienė 等<sup>[7]</sup>方法。

### 1.5.2 脾巨噬细胞呼吸爆发

采用氮蓝四唑(Bitroblue tetrazolium, NBT)还原法测定蛙脾脏巨噬细胞的呼吸爆发,参照 Couso 等<sup>[8]</sup>建立的方法。

### 1.5.3 胃溶菌酶活力

采用比浊法测定蛙胃溶菌酶活力,以溶壁微球菌(*Micrococcus lysodeikticus*)为底物,参考 Shugar<sup>[9]</sup>的方法进行。

## 1.6 氧化指标的测定

蛙肝脏和肾脏用于测定脂质过氧化产物丙二醛(Malonaldehyde, MDA)、抗氧化物谷胱甘肽(Glutathione, GSH)含量以及超氧化物歧化酶(Superoxide dismutase, SOD)活力,测定方法详见试剂盒说明书(南京建成生物技术研究所)。

## 1.7 数据分析

实验数据采用统计软件 SPSS17.0 进行统计分析。免疫和抗氧化指标相关数据进行 Kolmogorov-Smirnov 正态分布检验,符合正态进行独立样本 *t* 检验(Independent-sample *T*-test);死亡率与环境温度、冷休克时间相关分析采用 Pearson 分析,回归分析采用 Curve Estimation 分析。实验结果以平均值±标准误(Mean±SE)表示,当  $P<0.05$  时表示组间差异显著,当  $P<0.01$  时表示差异极显著。

## 2 结果与分析

### 2.1 越冬期环境温度监测

监测数据分析显示,浙江省金华市南山虎纹蛙自然分布区内冬季温度范围普遍在 0—14 °C 之间波动,偶尔会降至 0 °C 以下,其越冬期间的主要低温区间在 0—4 °C,主要高温区间在 10—14 °C(图 1)。

### 2.2 梯度降温与低温耐受力

当温度降至 14 °C 时未发现有虎纹蛙死亡,降至 12 °C 以下时虎纹蛙死亡率急剧上升。12 °C 累积死亡率约为 28.1%,10 °C 为 87.5%,8 °C 为 100%。在一定温度范围内,虎纹蛙死亡率与环境温度呈显著负相关(Pearson test,  $r=-0.952$ ,  $P<0.05$ ),经曲线拟合,通过回归方程计算可得半数致死温度为 11.5 °C(图 2)。

2.3 冷休克与低温耐受力

在 2℃冷休克 4 h 内无死亡现象,但 4 h 以后死亡率急剧增加,6 h 时死亡率为 45%,12 h 为 80%,至 24 h 时虎纹蛙全部死亡。虎纹蛙死亡率与冷休克时间呈显著正相关(Pearson test,  $r = 0.91$ ,  $P < 0.05$ ),经曲线拟合,通过回归方程计算可得半数致死时间为 7.6 h(图 3)。

2.4 冷休克对非特异性免疫指标的影响

如表 1 所示,处理组(2℃, 6 h)脾巨噬细胞呼吸爆发(NBT 反应)强度( $t = 3.827$ ,  $df = 6$ ,  $P < 0.05$ )、胃溶菌酶活力( $t = 6.37$ ,  $df = 6$ ,  $P < 0.05$ )、血细胞吞噬能力( $t = 5.388$ ,  $df = 3.037$ ,  $P < 0.05$ )均显著低于对照组(表 1),说明冷休克导致机体的免疫抑制。

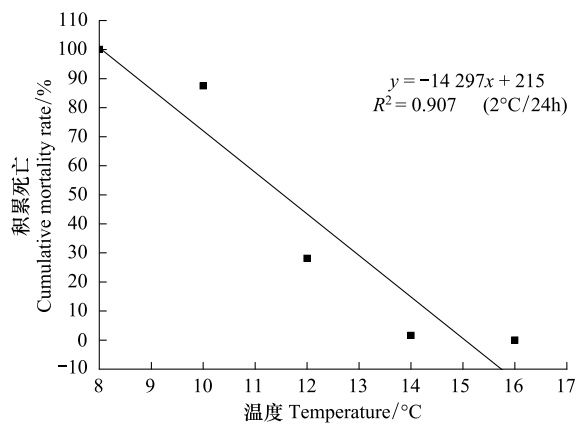


图 2 梯度降温过程中虎纹蛙的累积死亡率  
Fig. 2 Cumulative mortality rate in *Hoplobatrachus rugulosus* during gradient cooling

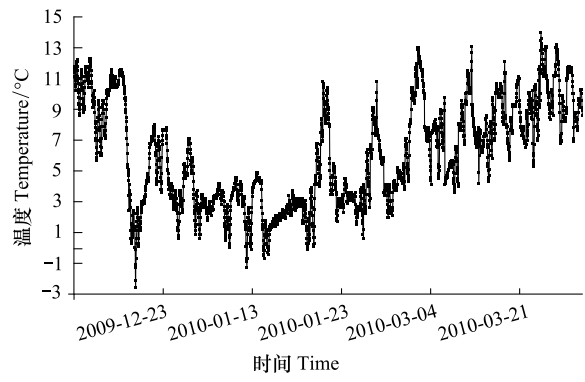


图 1 浙江金华市南山野生虎纹蛙(*Hoplobatrachus rugulosus*)分布区越冬期环境温度变化  
Fig. 1 Changes of surviving environmental temperature of *Hoplobatrachus rugulosus* during overwintering period in the Southern Mountain of Jinhua in Zhejiang Province

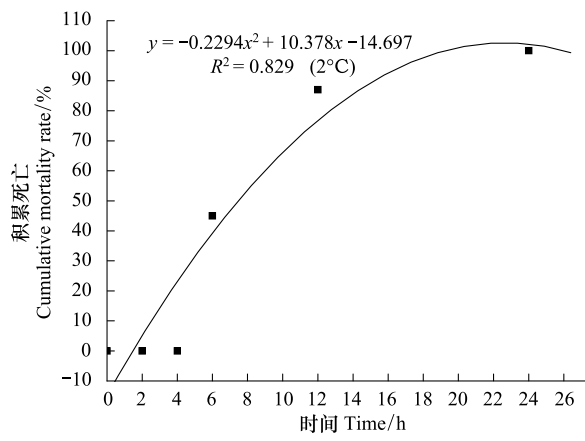


图 3 冷休克期间虎纹蛙的累积死亡率  
Fig. 3 Cumulative mortality rate in *Hoplobatrachus rugulosus* during cold shock

表 1 冷休克对虎纹蛙(*Hoplobatrachus rugulosus*)非特异性免疫的影响  
Table 1 The effect of cold shock on non-specific immunity in *Hoplobatrachus rugulosus*

组别 Groups	NBT 反应强度 NBT response intensity/OD <sub>600nm</sub>	吞噬能力 Phagocytic activity/a. u.	溶菌酶活力 Lysozyme activity/(μg/g)
对照组 Control	0.956 ± 0.024	14.898 ± 2.31	43.270 ± 0.901
处理组 Treatment	0.623 ± 0.084 **	2.415 ± 0.316 **	34.576 ± 1.025 **

平均值以 Mean±SE 表示, \* \* 表示显著水平为 0.01, n=4—6

2.5 冷休克对氧化压力参数及抗氧化指标的影响

冷休克 6 h 后虎纹蛙肝脏( $t = 0.773$ ,  $df = 8$ ,  $P > 0.05$ )和肾脏( $t = 0.164$ ,  $df = 4.542$ ,  $P > 0.05$ )组织脂质过氧化物 MDA 含量与对照组无明显差异(图 4);而肝脏中抗氧化酶 SOD 的活性显著升高( $t = -3.3$ ,  $df = 6$ ,  $P < 0.05$ ),但肾脏中 SOD 活力无明显变化( $t = 1.708$ ,  $df = 8$ ,  $P > 0.05$ )(图 5);肝脏( $t = -2.817$ ,  $df = 6$ ,  $P < 0.05$ )和肾脏( $t = -11.302$ ,  $df = 6$ ,  $P < 0.05$ )抗氧化物质 GSH 含量均显著增加(图 6)。结果表明冷休克(2℃, 6 h)能够诱导机体对抗氧化物质需求的增加。

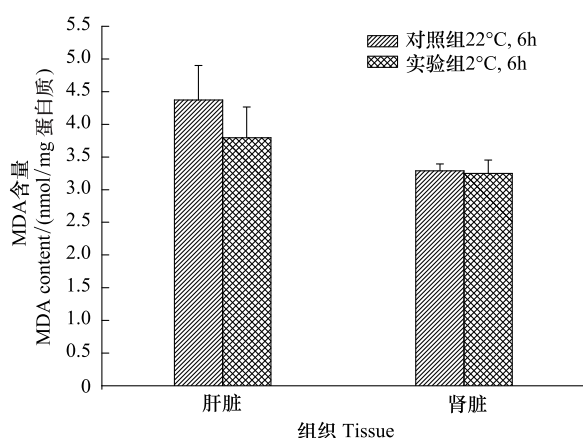


图4 冷休克对虎纹蛙肝脏和肾脏丙二醛(MDA)含量的影响

Fig. 4 The effect of cold shock on malonaldehyde (MDA) content in liver and kidney of *Hoplobatrachus rugulosus*

平均值以 Mean±SE 表示,  $n=4-6$

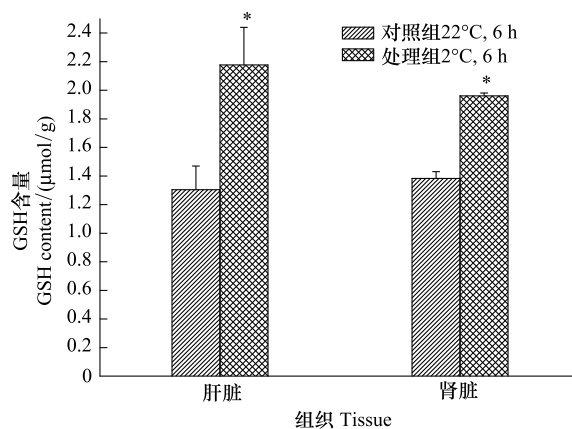


图5 冷休克对虎纹蛙肝脏和肾脏谷胱甘肽(GSH)含量的影响

Fig. 5 The effect of cold shock on glutathione (GSH) content in liver and kidney of *Hoplobatrachus rugulosus*

平均值以 Mean±SE 表示, \* 表示显著水平为 0.05,  $n=4-6$

### 3 讨论

全球气候变化导致极端天气的出现变得日趋频繁,非季节性的、短期且剧烈的气温变化如入冬前环境温度大幅波动可能会严重干扰两栖类动物种群的生存与稳定<sup>[1-6]</sup>。然而,国外相关研究主要集中于野外种群调查和病原生物调查<sup>[10-11]</sup>,并结合气候数据进行模型预测和分析<sup>[12]</sup>,从而得出气候异常与两栖类生存现状的相关规律和假说,而缺乏在实验室条件下模拟气候变化导致的气温极端变化对两栖类生理影响的相关证据。本研究为了解野生虎纹蛙在自然条件下可能遭遇的冷害程度,通过对浙江金华地区虎纹蛙分布区环境温度的监测(图1),可以看出在监测期内冬季高温区间在10—14℃,低温区间主要在0—4℃。根据有关文献的初步报道,虎纹蛙成蛙的适宜生长温度范围为22—28℃,温度低于12℃时停止摄食开始冬眠,温度低于4℃易引起死亡<sup>[13]</sup>。本研究通过梯度降温实验发现,在一定温度

范围内,虎纹蛙死亡率与环境温度呈显著负相关,表明环境温度变化会严重影响虎纹蛙存活率。通过回归方程计算,11.5℃是虎纹蛙半数致死温度,可推测该温度可能是虎纹蛙在非冬眠状态下的低温耐受临界点。本研究在野生虎纹蛙可能遭遇的低温区间内选择了2℃进行冷休克实验,发现死亡率随冷休克时间延长而增加,在第7.6h虎纹蛙出现半数致死。这些结果说明虎纹蛙对环境温度较为敏感,反映出热带和亚热带物种抗寒性较差的特征,但缺乏其它两栖类相关研究结果与之比较。入冬前环境温度大幅下降可能会严重降低虎纹蛙的存活率。

低等脊椎动物的非特异性免疫在免疫防御方面的作用比特异性免疫更为广泛和重要<sup>[14-15]</sup>,尤其在低温环境下非特异性免疫占有主导地位<sup>[16]</sup>,然而非特异性免疫同样具有明显的温度依赖性<sup>[17-19]</sup>,如虹鳟(*Oncorhynchus mykiss* L.)白细胞数目和头肾吞噬细胞呼吸爆发强度有显著的季节性变化,在冬季均降至最低<sup>[17]</sup>。Nikoskelainen等<sup>[20]</sup>也发现低温能够降低虹鳟全血呼吸爆发强度。罗非鱼(*Oreochromis aureus*)在遭受

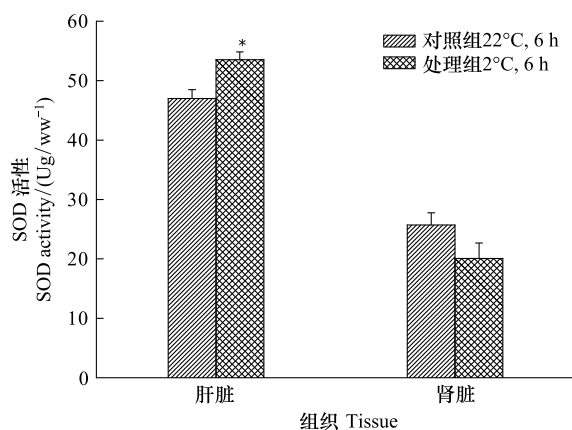


图6 冷休克对虎纹蛙肝脏和肾脏超氧化物歧化酶(SOD)活性的影响

Fig. 6 The effect of cold shock on Superoxide dismutase (SOD) content in liver and kidney of *Hoplobatrachus rugulosus*

平均值以 Mean±SE 表示, \* 表示显著水平为 0.05,  $n=4-6$



冷刺激(12℃)后,其白细胞吞噬能力显著下降<sup>[21]</sup>。中国林蛙(*Rana temporaria*)全血吞噬能力会随环境温度降低而降低<sup>[22]</sup>。豹蛙(*Rana pipiens*)在低温暴露(5℃)后其淋巴细胞和白细胞数目显著减少<sup>[23]</sup>。本研究发现虎纹蛙在冷休克后呼吸爆发强度和全血吞噬能力也均下降。溶菌酶作为一种广谱抗菌作用的酶类,其活力具有明显的温度依赖特征,如凡纳滨对虾(*Litopenaeus vannamei*)、虹鳟、大西洋大比目鱼(*Hippoglossus hippoglossus* L.)溶菌酶活力在低温下均受到抑制,本研究结果与这些研究报道一致<sup>[17, 24-25]</sup>。此外,低温胁迫能够诱导动物体神经内分泌系统产生应激反应<sup>[26]</sup>,动物体通过丘脑-垂体-肾间组织轴(Hypothalamus-pituitary-interrenal axis, HPI)分泌一系列应激激素包括儿茶酚胺、皮质类固醇等,进而抑制机体免疫和代谢等生理功能<sup>[21, 27-30]</sup>。因此,可以推测非季节性低温胁迫可能能够破坏虎纹蛙机体的内稳态并诱导免疫抑制,导致机体对病原生物敏感性的增加,加大种群的生存压力。

低温胁迫能够诱导机体产生过多的活性氧(Reactive oxygen species, ROS),改变细胞的氧化还原状态,从而打破机体抗氧化系统的平衡。有研究表明,应激激素能够增加肝脏细胞色素 P450 依赖复合功能氧化酶系统的活力<sup>[31]</sup>,而该系统是 ROS 产生的主要来源。在哺乳类研究中发现,大鼠遭受低温刺激后组织脂质过氧化产物 MDA 的显著升高<sup>[32]</sup>,小白鼠在低水温环境游泳会导致肝脏 MDA 含量显著增加<sup>[33]</sup>。在低等脊椎动物中,北海绵鳎(*Zoarces viviparus*)在 5℃ 下冷刺激 2 h 后其 MDA 含量也有所增加<sup>[34]</sup>。然而,树蛙(*Rana sylvatica*)经过冷冻后组织 MDA 含量却无明显变化<sup>[35]</sup>,本研究的结果与此类似,说明不同低温胁迫方式可能会导致不同的结果,或不同物种间存在反应差异。另外,低温胁迫能够诱导皮质类固醇的升高,该类激素会刺激抗氧化物质的产生<sup>[36]</sup>。如大鼠受到冷刺激后肾脏和肝脏 SOD 活性明显增强<sup>[32]</sup>。Katja 等<sup>[34]</sup>对北海绵鳎进行 1℃ 急性冷刺激也发现其肝脏抗氧化酶 SOD 活性显著增加。在本研究中,虎纹蛙肝脏 SOD 活力在冷休克后显著增加,而肾脏却无显著变化。这可能是由于肝脏含有大量内质网,而内质网微粒体上的 NADPH-细胞色素 P450 酶系统是细胞脂质过氧化物质产生的主要来源<sup>[37]</sup>,因此肝脏需要产生足够 SOD 用于抗氧化。GSH 是清除氧自由基重要的非酶抗氧化物质,本研究表明冷休克增加了虎纹蛙肝脏和肾脏 GSH 含量,这与北海绵鳎<sup>[34]</sup>的研究相一致。但是,哺乳类如多纹黄鼠(*Spermophilus tridecemlineatus*)在冬眠期间肠粘膜 GSH 含量降低,且 GSH 还原酶活性降低了 50%<sup>[38]</sup>;又如大鼠在遭受冷胁迫后其肝脏 GSH 总含量也明显降低<sup>[32, 39]</sup>。这些结果说明鱼类和两栖类等低等脊椎动物存在区别于哺乳动物的应对低温胁迫的应答调节机制,或拥有更具低温适应性的非酶抗氧化系统来保护细胞或组织免受低温损伤<sup>[40]</sup>,有待进一步研究。

综上所述,虎纹蛙对温度变化较为敏感,耐寒能力差,低温胁迫能诱导机体产生免疫抑制并加大对抗氧化物质的需求。因而,全球气候变化引起的局部天气条件的剧烈变动可能会增加其生存压力和疾病感染的风险,最终可能影响虎纹蛙野生种群的发展与稳定。另外,虎纹蛙人工养殖在温度剧烈变化期需注意防寒,保持环境清洁,以防止疾病爆发。

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# ACTA ECOLOGICA SINICA Vol. 32, No. 11 June, 2012 (Semimonthly)

## CONTENTS

Controls of post-fire tree recruitment in Great Xing'an Mountains in Heilongjiang Province .....	CAI Wenhua, YANG Jian, LIU Zhihua, et al (3303)
The assessment of river health using Benthic-Index of biotic integrity for Wenyu River .....	YANG Liu, LI Yonghui, WANG Juncai, et al (3313)
Consume of soil seeds of <i>Betula albo-sinensis</i> and <i>Abies faxoniana</i> in different natural successional stages of subalpine dark coniferous forest in western Sichuan, China .....	MA Jiangming, LIU Shirong, SHI Zuomin, et al (3323)
Habitat associations of understory species spatial distribution in old growth broad-leaved Korean pine ( <i>Pinus koraiensis</i> ) forest .....	DING Shengjian, ZHANG Chunyu, XIA Fucui, et al (3334)
Nabkha morphology and sand-fixing capability of four dominant <i>Caragana</i> species in the desert region of the Inner Mongolia Plateau .....	ZHANG Yuanyuan, MA Chengchang, HAN Lei, et al (3343)
Growth dynamics, biomass allocation and ecological adaptation in <i>Ceratocarpus arenarius</i> L. ....	QUAN Dujuan, WEI Yan, ZHOU Xiaoqing, et al (3352)
A rapid assessment method for forest disaster based on MODIS/NDVI time series; a case study from Guizhou Province .....	SHI Hao, WANG Xiao, XUE Jianhui, et al (3359)
Soil cation exchange capacity and exchangeable base cation content in the profiles of four typical soils in the Xi-Shui Forest Zone of the Qilian Mountains .....	JIANG Lin, GENG Zengchao, LI Shanshan, et al (3368)
Impact of water and temperature on spring maize emergence speed and emergence rate .....	MA Shuqing, WANG Qi, LÜ Houquan, et al (3378)
Effect of N application on the abundance of denitrifying genes ( <i>narG/nosZ</i> ) and N <sub>2</sub> O emission in paddy soil .....	ZHENG Yan, HOU Haijun, QIN Hongling, et al (3386)
Temporal-spatial variations of potential evapotranspiration and quantification of the causes in Northwest China .....	CAO Wen, SHEN Shuanghe, DUAN Chunfeng (3394)
Analysis of ecosystem degradation and recovery using precipitation use efficiency and NDVI in the headwater catchment of the Yellow River basin .....	DU Jiaqiang, SHU Jianmin, ZHANG Linbo (3404)
An assessment method of <i>Kandelia obovata</i> population biomass .....	JIN Chuan, WANG Jinwang, ZHENG Jian, et al (3414)
Quantitative characteristics and species composition of <i>Artemisia sphaerocephala</i> and <i>A. ordosica</i> communities in the Ulanbuh Desert ...	MA Quanlin, ZHENG Qingzhong, JIA Jujie, et al (3423)
Photosynthesis and transpiration in relation to ion accumulation in <i>Vitex trifolia</i> under varied light intensity .....	ZHANG Ping, LIU Linde, BAI Xinfu, et al (3432)
Diffusion of elm seed rain in Otindag Sand Land .....	GU Wei, YUE Yongjie, LI Gangtie, et al (3440)
Effect of saline water irrigation on sand soil salt and the physiology and growth of <i>Populus euphratica</i> Oliv. ....	HE Xinlin, CHEN Shufei, WANG Zhenhua, et al (3449)
Regulation of exogenous nitric oxide on photosynthetic physiological response of <i>Lolium perenne</i> seedlings under NaHCO <sub>3</sub> Stress .....	LIU Jianxin, WANG Jincheng, WANG Xin, et al (3460)
Longitude gradient changes on plant community and soil stoichiometry characteristics of grassland in Hulunbeir .....	DING Xiaohui, LUO Shuzheng, LIU Jinwei, et al (3467)
Concentrations and distributions of selenium and heavy metals in Hainan paddy soil and assessment of ecological security .....	GENG Jianmei, WANG Wenbin, WEN Cuiping, et al (3477)
Heavy metal contents and evaluation of farmland soil and wheat in typical area of Jiangsu Province .....	CHEN Jingdu, DAI Qigen, XU Xuehong, et al (3487)
The studies on the food web structures and trophic relationships in Guangxi Dongfang Cave by means of stable carbon and nitro- gen isotopes .....	LI Daohong, SU Xiaomei (3497)
Analysis of bacterial diversity in the Songhua River based on nested PCR and DGGE .....	TU Teng, LI Lei, MAO Guannan, et al (3505)

Preliminary delineation and classification of estuarine drainage areas for major coastal rivers in China .....	HUANG Jinliang, LI Qingsheng, HUANG Ling, et al (3516)
Estimation of spatial and seasonal changes in phytoplankton primary production in Meiliang Bay, Lake Taihu, based on the Vertically Generalized Production Model and MODIS data .....	YIN Yan, ZHANG Yunlin, SHI Zhiqiang, et al (3528)
Viability and changes of physiological functions in the tiger frog ( <i>Hoplobatrachus rugulosus</i> ) exposed to cold stress .....	WANG Na, SHAO Chen, XIE Zhigang, et al (3538)
Community structure and abundance dynamics of soil collembolans in transgenic Bt rice paddyfields .....	ZHU Xiangyu, LI Zhiyi, CHANG Liang, et al (3546)
Morphological characteristics and microsatellite DNA genetic diversity of Nigeria African honey bee, Anhui <i>Apis mellifera</i> and theirs hybrid generation II .....	YU Linsheng, XIE Wenfei, WU Houchang, et al (3555)
Effects of social-demographic factors on the recreational service of park wetlands in Beijing .....	LI Fen, SUN Ranhao, CHEN Liding (3565)
Co-integration theory-based analysis on relationships between economic growth and eco-environmental changes: taking the south-east district in Chongqing city as an example .....	XIAO Qiang, HU Dan, XIAO Yang, et al (3577)
The cooperative environmental game model in the Tidal River Network Regions and its empirical research .....	LIU Honggang, CHEN Xingeng, PENG Xiaochun (3586)

#### **Review and Monograph**

Review of eco-efficiency accounting method and its applications .....	YIN Ke, WANG Rusong, ZHOU Chuanbin, et al (3595)
Overview on the 6th international symposium on modern ecology series of 2011 .....	WEN Teng, XU Delin, XU Chi, et al (3606)

#### **Discussion**

Scale analysis of environmental factors and their relationship with the size of hierarchical aquatic ecoregion: a case study in the Liao River basin .....	LIU Xingcai, XU Zongxue, ZHANG Shurong, et al (3613)
--	--

#### **Scientific Note**

Effects of different light intensities on activities of the primary defense proteins in needles of <i>Larix gmelinii</i> .....	LU Yifang, SHI Lei, YAN Shanchun (3621)
An analysis of photosynthetic parameters among <i>Schima superba</i> provenances .....	XIONG Caiyun, ZENG Wei, XIAO Fuming, et al (3628)
Research on three small-scale agricultural ecological-economic systems in Shenzhen City based on emergy analysis .....	YANG Zhuoxiang, GAO Yang, ZHAO Zhiqiang, et al (3635)



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