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12 种麻醉剂对长蛸的麻醉效果

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摘要: 本研究旨在探讨硫酸镁、乙醇、氯化镁、丁香酚、MS-222、氯化锰、利多卡因、盐酸普鲁卡因、乙二醇苯醚、薄荷醇、苯佐卡因和三氯叔丁醇对长蛸(*Octopus minor*)成体的麻醉效果。结果表明, 在实验浓度下硫酸镁、丁香酚、MS-222、氯化锰、利多卡因、盐酸普鲁卡因、乙二醇苯醚、薄荷醇、苯佐卡因和三氯叔丁醇对长蛸无麻醉作用。在 0.2~0.6 mL/L 乙二醇苯醚、0.05 g/L 和 0.06 g/L 薄荷醇、0.5 g/L 苯佐卡因、40 μL/L 丁香酚和 50~500 mg/L MS-222 浓度的溶液中, 长蛸出现中毒现象。2 mL/L 乙醇和 2 g/L 氯化镁溶液对长蛸无麻醉作用; 6~40 mL/L 的乙醇和 6~35 g/L 的氯化镁溶液中长蛸出现麻醉现象。根据长蛸在 4~40 mL/L 乙醇和 4~40 g/L 氯化镁溶液中的麻醉和复苏过程将麻醉程度分为 5 个时期, 复苏过程分为 4 个时期。长蛸在 15~35 g/L 氯化镁和 10~40 mL/L 乙醇中能够达到第 4 期麻醉, 此时期适于观察和操作。在 4~35 mL/L 乙醇溶液中, 随着浓度的增加麻醉时间逐渐缩短, 复苏时间逐渐增加, 在 40 mL/L 时长蛸产生应激反应麻醉时间变长。3~35 g/L 氯化镁溶液中, 随着浓度的增加麻醉时间逐渐缩短, 复苏时间逐渐增加。10 mL/L 乙醇和 20 g/L 氯化镁对长蛸的麻醉和复苏总时间最短, 分别为 26 min 和 40 min。实验证明, 乙醇和氯化镁均可作为长蛸的麻醉剂, 在麻醉效率方面乙醇好于氯化镁。

关键词: 长蛸; 麻醉剂; 麻醉效果
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长蛸(*Octopus minor*)属于八腕目(Octopoda), 蛸科(Octopodidae), 蛸属, 分布于渤海、黄海、东海、南海和日本列岛水域, 肉质鲜嫩, 蛋白质丰富且营养均衡[1], 是中国沿海重要的经济蛸类[2]。在运输过程中, 长蛸在狭小空间里会强烈挣扎, 导致体表破损、自残(断腕)和喷墨, 死亡率较高。在实验操作中长蛸剧烈挣扎甚至喷墨, 导致实验无法顺利进行。同时, 欧盟要求在科学研究过程中需要考虑头足类的动物福利, 规定所有适用于脊椎动物的手术和实验操作也同样适用于头足类, 尽量减少疼痛和持久伤害[3-5]。根据这一规定, 头足类的实验操作应在麻醉和镇痛下进行, 当必须杀死实验样本时, 需要尽可能降低死亡过程的痛苦程度。因此, 筛选头足类适宜的麻醉剂, 并探明相应的麻醉复苏过程对于头足类的产业发展和科学研究具有重要意义。

在已有头足类麻醉剂研究中, 7.5%六水氯化镁溶液能够有效麻醉乌贼(*Sepia officinalis*)、福氏枪乌贼(*Loligo forbesii*)、锥异尾枪乌贼(*Alloteuthis subulata*)、真蛸(*Octopus vulgaris*)和尖盘爱尔斗蛸(*Eledone cirrhosa*)[6]。乙二醇苯醚麻醉爱尔斗蛸(*Eledone moschata*)的有效浓度为 1.2 mL/L、1.4 mL/L

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和 1.6 mL/L[7]。1%~3%乙醇作为乌贼手术时的麻醉剂[8], 1.5%乙醇可以有效麻醉真蛸[9]。在使用氯化镁、苯佐卡因、乙醇和丁香油麻醉皮氏枪乌贼(*Doryteuthis pealeii*)时, 乙醇和氯化镁具有麻醉作用[10]。丁香油浓度为 200 mg/L 时适宜作为长蛸的麻醉剂[11]。使用乙二醇苯醚、MS-222、氯化镁、乙醇和丁香油麻醉乌贼时, 氯化镁、乙醇和丁香油能够麻醉乌贼,氯化镁溶液麻醉乌贼的适宜浓度为 27 g/L[12]。使用乙醇、氯化镁、硫酸镁、氯仿和水合氯醛麻醉拟乌贼(*Sepiotethis sepioidea*)时, 1%~3%乙醇、1.5%~2%氯化镁和 3%~4%硫酸镁具有麻醉效果[13]。氨基甲酸乙酯曾在真蛸和乌贼中用作麻醉剂[14-15], 但由于氨基甲酸乙酯属于致癌物质, 不再使用其作为麻醉剂[4, 16-17]。目前在头足类麻醉和复苏过程中对行为的描述[12, 18-19]相对较少, 且关于长蛸麻醉的研究较少。

综上所述, 本研究在参考前人研究工作的基础上, 挑选 12 种具有潜在麻醉作用的化学试剂对长蛸进行麻醉实验, 旨在筛选出适宜的长蛸麻醉试剂, 掌握长蛸麻醉和复苏的方法, 为实验操作和生产运输提供参考依据。

1 材料与方法

1.1 实验样本

长蛸来自于威海裕隆水产开发有限公司, 体质量范围为 138~151 g。

1.2 实验海水

经过砂滤的天然海水, 盐度为 28~30, 水温为(18±0.3) ℃, pH 为 8.0~8.2, DO>6 mg/L。

1.3 麻醉剂与麻醉方法

实验的麻醉剂有氯化锰、硫酸镁、乙二醇苯醚、乙醇、薄荷醇、利多卡因、氯化镁、MS-222、丁香酚、三氯叔丁醇、盐酸普鲁卡因和苯佐卡因。由于将长蛸放入带有麻醉剂的水体中及实验捞取、转移等操作, 会引起应激反应, 导致长蛸出现剧烈挣扎、四处游动、向水体外爬、喷墨等现象, 干扰实验准确性, 本研究采用向带有长蛸的水体中加入麻醉剂的方法开展实验。

1.4 实验器材

分析天平、塑料箱(47 cm×34 cm×32 cm)、电子温度计、1000 mL 烧杯、增氧泵、电子秤。

1.5 麻醉剂的配制

无水氯化镁易溶于水, 溶解过程中放出大量热, 用海水配制质量浓度为 320 g/L 的氯化镁溶液作为母液。MS-222、乙醇、氯化锰、盐酸普鲁卡因、硫酸镁和乙二醇苯醚易溶于水, 称取药品于烧杯中, 用海水溶解后按比例加入实验水体。丁香酚、利多卡因、苯佐卡因、三氯叔丁醇和薄荷醇微溶于水, 丁香酚使用时以 1∶5 的比例溶于无水乙醇; 利多卡因和苯佐卡因使用时以 1∶4 的比例溶于无水乙醇; 三氯叔丁醇 10 g 和薄荷醇晶体 0.5 g 分别溶解于 100 mL 无水乙醇中配制成母液。每种麻醉剂用于麻醉效果测定的浓度水平如表 1 所示。

1.6 麻醉效果测定

实验前选择体表无损伤、有活力的健康长蛸, 停食 24 h。每个浓度实验设置 3 个平行组, 每组长蛸 3 只, 实验水体 20 L。塑料箱中加正常海水, 放入长蛸稳定 10 min, 观察长蛸的活动状态。对长蛸活动稳定的组进行实验, 将配置好的麻醉剂按比例加入实验水体, 发现长蛸完全麻醉后, 立即将其转移到正常海水中进行复苏。记录在此过程中出现的各种行为特征及时间点。

1.7 麻醉与复苏分期测定

根据麻醉效果实验结果, 设计 4 mL/L、6 mL/L、8 mL/L、10 mL/L、15 mL/L、25 mL/L、35 mL/L、40 mL/L 8 个乙醇浓度梯度和 4 g/L、6 g/L、8 g/L、10 g/L、15 g/L、25 g/L、30 g/L、40 g/L 8 个氯化镁溶液梯度, 每个浓度长蛸 1 只, 平行重复 3 次以上, 进行观察记录。

1.8 最终麻醉程度测定

依据麻醉分期结果, 设计 4 mL/L、6 mL/L、8 mL/L、10 mL/L、15 mL/L、20 mL/L、25 mL/L、35 mL/L、40 mL/L 9 个乙醇浓度梯度和 3 g/L、5 g/L、8 g/L、10 g/L、15 g/L、20 g/L、25 g/L、30 g/L、35 g/L 9 个氯化镁溶液梯度, 每个浓度长蛸 1 只, 平行重复 3 次以上, 测定 24 h 内长蛸达到的最终麻醉程度。

1.9 麻醉及复苏时间测定

根据麻醉与复苏分期结果, 设计 4 mL/L、

表 1　麻醉剂用于麻醉效果测定的浓度水平

Tab.1　Levels of anesthetic concentration for the anesthesia effect test

麻醉剂 anesthetic	浓度 concentration					
硫酸镁/(g/L) magnesium sulphate	5	10	25	30	40	50
丁香酚/(μL/L) eugenol	1	3	10	20	30	40
乙二醇苯醚/(mL/L) ethelene glycol monophenyl ether	0.1	0.2	0.3	0.4	0.5	0.6
氯化镁/(g/L) magnesium chloride	2	6	10	20	30	35
乙醇/(mL/L) ethanol	2	6	10	25	35	40
薄荷醇/(g/L) L-menthol	0.01	0.02	0.03	0.04	0.05	0.06
氯化锰/(g/L) manganese(II) chloride	2	3	4	6	8	10
利多卡因/(mL/L) lidocaine	1	2	4	6	8	10
苯佐卡因/(g/L) benzocaine	0.01	0.04	0.08	0.1	0.3	0.5
盐酸普鲁卡因/(g/L) procaine hydrochloride	0.1	0.2	0.3	0.4	0.5	0.6
三氯叔丁醇/(g/L) 2-trichloromethyl-2-propanol	0.1	0.2	0.3	0.4	0.5	0.6
MS-222/(mL/L)	5	15	25	50	100	500

6 mL/L、8 mL/L、10 mL/L、25 mL/L、35 mL/L、40 mL/L 7 个乙醇浓度梯度和 3 g/L、5 g/L、8 g/L、10 g/L、20 g/L、30 g/L、35 g/L 7 个氯化镁溶液梯度, 每个浓度长蛸 1 只, 平行重复 3 次以上, 分别测定长蛸达到最终麻醉和转入正常海水到复苏的时间。

2　结果与分析

2.1　麻醉剂对长蛸的麻醉效果

实验结果(表 2)表明, 硫酸镁、丁香酚、MS-222、氯化锰、利多卡因、盐酸普鲁卡因、乙二醇苯醚、薄荷醇、苯佐卡因和三氯叔丁醇溶液在实验浓度

表 2　12 种麻醉剂对长蛸麻醉效果

Tab. 2　The anesthesia effect of 12 anesthetics on *Octopus minor*

麻醉剂 anesthetic	麻醉效果 anesthesia effect
硫酸镁 magnesium sulphate	6 个浓度下未麻醉, 40 g/L 和 50 g/L 浓度时体表失水出现褶皱. No anesthesia was observed under six concentrations, and the body surface of specimens wrinkled at 40 g/L and 50 g/L due to dehydration.
丁香酚 eugenol	6 个浓度下未麻醉, 40 μL/L 浓度时出现全身僵直和喷墨现象. No anesthesia was observed under six concentrations, and specimens stiffened and ink-jet appeared at 40 g/L.
乙二醇苯醚 ethelene glycol monophenyl ether	6 个浓度下未麻醉, 0.2 mL/L 浓度出现抽搐, 0.3 mL/L 浓度出现喷墨, 0.4 mL/L、0.5 mL/L 和 0.6 mL/L 浓度出现全身僵直和喷墨现象. No anesthesia was observed under six concentrations, and convulsions occurred at 0.2 mL/L, ink-jet appeared at 0.3 mL/L, stiffness and ink-jet appeared at 0.4 mL/L, 0.5 mL/L and 0.6 mL /L.
氯化镁 magnesium chloride	2 g/L 浓度无麻醉现象, 6–35 g/L 浓度出现体色逐渐变白、呼吸频率逐渐降低、活动逐渐减少和对外界刺激的反应逐渐丧失等麻醉现象. No anesthesia was detected at 2 g/L. Anesthesia characteristics such as gradual whitening of body color, decrease of respiratory rate, reduced activity and loss of response to external stimuli were observed at 6–35 g/L.
乙醇 ethanol	2 mL/L 浓度无麻醉现象, 6 mL/L 浓度以上出现麻醉现象, 40 mL/L 浓度剧烈挣扎后麻醉. No anesthesia was detected at 2 mL/L, and anesthesia occurred above 6 mL/L, anesthesia was detected at 40 mL/L after intense struggle.
薄荷醇 L-menthol	6 个浓度下未麻醉, 0.05 g/L 和 0.06 g/L 浓度出现全身僵直和喷墨现象. No anesthesia was observed under six concentrations, specimens stiffened and ink-jet appeared at 0.05 g/L and 0.06 g/L.
氯化锰 manganese(II) chloride	6 个浓度下未麻醉, 3 g/L 浓度出现剧烈挣扎现象, 8 g/L 和 10 g/L 体表失水出现褶皱. No anesthesia was observed under six concentrations, violent struggle occurred at 3 g/L, and body surface wrinkled at 8 g/L and 10 g/L due to dehydration.
利多卡因 lidocaine	6 个浓度下未麻醉, 10 mg/L 浓度出现眼睛下陷, 紧闭漏斗现象. No anesthesia was observed under six concentrations, the eyes were sunken and the funnel was closed at 10 mg/L.

(待续 to be continued)

(续表 2 tab. 2 continued)

麻醉剂 anesthetic	麻醉效果 anesthesia effect
苯佐卡因 benzocaine	6 个浓度下未麻醉, 0.1 g/L 浓度出现眼睛下陷, 紧闭漏斗现象, 0.5 g/L 出现全身僵直和喷墨现象. No anesthesia was observed under six concentrations, the eyes sank and the funnel was closed at 0.1 g/L, and stiffness and ink-jet appeared at 0.5 g/L.
盐酸普鲁卡因 procaine hydrochloride	6 个浓度下未麻醉, 0.5 g/L 和 0.6 g/L 浓度出现眼睛下陷, 紧闭漏斗现象. No anesthesia was observed under six concentrations, the eyes sank and the funnel was closed at 0.5 g/L and 0.6 g/L.
三氯叔丁醇 2-trichloromethyl-2-propanol	6 个浓度下未麻醉, 0.3 g/L 浓度出现眼睛下陷, 0.5 g/L 和 0.6 g/L 浓度出现剧烈挣扎现象. No anesthesia was observed under six concentrations, the eyes sank at 0.3 g/L and violent struggle was observed at 0.5 g/L and 0.6 g/L.
MS-222	6 个浓度下未麻醉, 50 mg/L、100 mg/L 和 500 mg/L 浓度出现抽搐和喷墨现象. No anesthesia was observed under six concentrations, convulsions and ink-jet appeared at 50 mg/L, 100 mg/L and 500 mg/L.

下对长蛸无麻醉作用, 稍高浓度中长蛸出现高盐胁迫反应或中毒现象。长蛸在 6~40 mL/L 浓度乙醇和 6~35 g/L 浓度氯化镁溶液中出现麻醉现象。

2.2 麻醉程度及复苏过程的分期

根据长蛸的体表颜色变化, 根据游泳活力强弱、呼吸频率变化、腕部活动能力强弱、吸盘吸力强弱、腕部对玻璃棒刺激的反应和将头部翻转眼睛向下是否能够恢复正常这 6 个指标, 将其区分为 5 个麻醉时期(表 3)和 4 个复苏时期(表 4)。

2.3 长蛸在乙醇和氯化镁溶液中最终麻醉程度

随着乙醇和氯化镁浓度的增加, 长蛸达到的最终麻醉程度增大(图 1, 图 2)。10~40 mL/L 乙醇溶液和 15~35 g/L 氯化镁溶液对长蛸麻醉均能够达到第 4 期麻醉, 此时期能够有效麻醉长蛸, 进行观察和测量等操作而不伤害长蛸。长蛸在 40 mL/L 乙醇和 35 g/L 氯化镁中达到 5 期麻醉, 表现为呼吸停止, 应及时转移进行复苏否则死亡, 表明过高浓度乙醇和氯化镁导致长蛸死亡。

2.4 麻醉及复苏时间测定

图 3 表明, 以 4~35 mL/L 浓度的乙醇作为长蛸麻醉剂, 随着浓度增加, 麻醉时间从 128 min 到 8 min 逐渐缩短, 复苏时间从 5 min 到 26 min 逐渐增加。在 40 mL/L 乙醇中出现剧烈挣扎, 麻醉时间明显增加, 可见乙醇浓度过高容易导致长蛸产生应激反应。图 4 表明, 以 3~35 g/L 的氯化镁溶液作为长蛸麻醉剂, 随着浓度增加, 麻醉时间从 176 min 到 13 min 逐渐缩短, 复苏时间从 6 min 到 116 min 逐渐增加。

表 3 长蛸麻醉程度分期及行为特征

Tab. 3 Stages and behavior characteristics of *Octopus minor* under anesthesia

麻醉程度分期 stage of anesthesia	行为特征 behavior characteristic						
	体色 body color	呼吸频率/(次/min) respiratory rate	游动活力 swimming activity	腕部活动 wrist activity	吸盘吸力 sucker suction	腕部对刺激反应 wrist response to stimuli	将头部翻转眼部向下后反应 turn the whole body upside down
正常 normal	浅棕色 light brown	18–22	游动自如 swimming freely	频繁 frequent	强 strong	敏感 sensitive	迅速恢复 recover quickly
第 1 期 stage1	浅棕色 light brown	14–17	不游动 no swimming	减弱 reduced	强 strong	敏感 sensitive	迅速恢复 recover quickly
第 2 期 stage 2	浅棕色变淡 light brown	10–13	不游动 no swimming	无活动 adiaphoria	弱 weak	迟缓 insensitive	挣扎后恢复 recover after struggle
第 3 期 stage 3	苍白 white	6–9	不游动 no swimming	无活动 adiaphoria	弱 weak	无反应 adiaphoria	挣扎后恢复 recover after struggle
第 4 期 stage 4	苍白 white	4–6	不游动 no swimming	无活动 adiaphoria	无 no suction	无反应 adiaphoria	不能恢复 irreparability
第 5 期 stage 5	苍白 white	0	不游动 no swimming	无活动 adiaphoria	无 no suction	无反应 adiaphoria	不能恢复 irreparability

表 4　长蛸复苏分期及行为特征

Tab. 4　Recovery stages and behavior characteristics of *Octopus minor*

复苏分期 stages of recovery	行为特征 behavior characteristic					
	体色 body color	呼吸频率/(次/min) respiratory rate	腕部活动 wrist activity	吸盘吸力 sucker suction	腕部对刺激反应 wrist response to stimuli	将头部翻转眼部向下后反应 turn the whole body upside down
第 1 期 stage1	苍白 white	4–7	无 adiaphoria	无 no suction	无 adiaphoria	无 adiaphoria
第 2 期 stage 2	苍白 white	8–11	少量 a little	弱 weak	迟缓 insensitive	无 adiaphoria
第 3 期 stage 3	浅棕色加深 light brown deepening	12–17	增强 enhanced activity	强 strong	敏感 sensitive	挣扎后恢复 recover after struggle
第 4 期 stage 4	浅棕色 light brown	18–22	频繁 frequent	强 strong	敏感 sensitive	迅速恢复 recover quickly

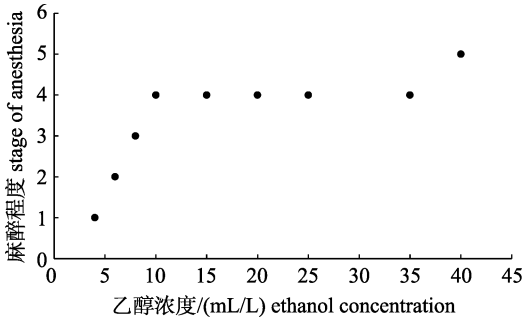

图 1　长蛸在 9 种乙醇浓度中达到的最终麻醉程度

Fig 1　Final anesthetic stages of *Octopus minor* under nine ethanol concentrations

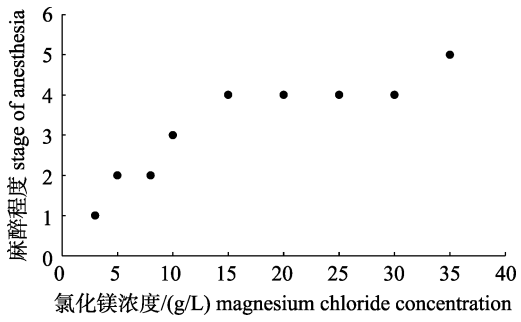

图 2　长蛸在 9 种氯化镁浓度中达到的最终麻醉程度

Fig. 2　Final anesthetic stages of *Octopus minor* under nine magnesium chloride concentrations

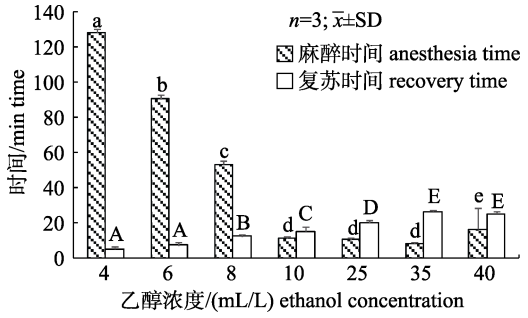

图 3　长蛸在 7 个乙醇浓度梯度下的麻醉与复苏时间
不同小写字母表示不同乙醇浓度下麻醉时间差异显著(P<0.05), 不同大写字母表示不同乙醇浓度下复苏时间差异显著(P<0.05).

Fig. 3　Anesthesia time and recovery time of *Octopus minor* under 7 ethanol concentrations
Different small letters indicate significant differences on anesthesia time (P<0.05) and capital letter indicate significant differences on recovery time (P<0.05).

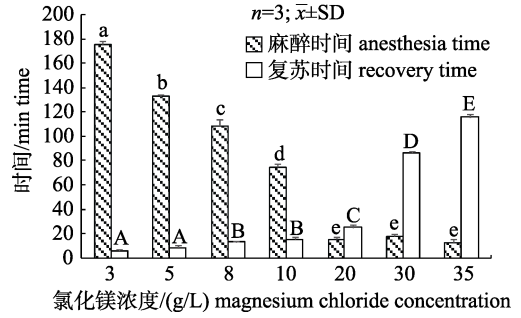

图 4　长蛸在 7 个氯化镁浓度梯度下的麻醉和复苏时间
不同小写字母表示不同乙醇浓度下麻醉时间差异显著(P<0.05), 不同大写字母表示不同乙醇浓度下复苏时间差异显著(P<0.05).

Fig. 4　Anesthesia time and recovery time of *Octopus minor* under 7 magnesium chloride concentrations
Different small letters indicate significant differences on anesthesia time (P<0.05) and capital letter indicate significant differences on recovery time (P<0.05).

3　讨论

3.1　麻醉剂的选择

本研究中, 麻醉效果的判定基于长蛸的体色、活动量、呼吸频率和对外界刺激反应变化, 以及麻醉过程中是否产生高盐胁迫或中毒现象。基于上述标准, 发现乙醇和氯化镁对长蛸有麻醉作用, 综合比较长蛸在不同浓度作用下的最终麻醉

状态、麻醉及复苏时间, 得出乙醇和氯化镁麻醉的有效浓度为 10~40 mL/L 和 15~35 g/L。已有研究表明, 乙醇和氯化镁能够有效麻醉真蛸[20]、皮氏枪乌贼[10]、乌贼[12]和拟乌贼[13]。本研究中乙醇和氯化镁能够有效麻醉长蛸, 说明乙醇和氯化镁可作为头足类潜在的通用麻醉剂, 其余 10 种麻醉剂对其他头足类具有麻醉作用, 因物种、生活方式与实验方法的不同, 有待进一步进行探究。

本研究表明, 10 mL/L 的乙醇对长蛸产生快速的麻醉作用, 没有明显的痛苦迹象。结果与乙醇麻醉乌贼、真蛸、皮氏枪乌贼和拟乌贼效果相似, 使用乙醇做麻醉剂无不良反应[9-10, 13, 19, 21]。实验通过麻醉与复苏最短总时间对乙醇和氯化镁的麻醉效果进行比较, 乙醇的最短总时间为 26 min (浓度 10 mL/L), 氯化镁为 40 min (20 g/L), 在麻醉和复苏的效率上, 乙醇好于氯化镁。根据上述结果, 当长蛸的麻醉操作时间短于 15 min 时, 使用乙醇作为麻醉剂, 而 $MgCl_2$ 溶液更适合超过 15 min 的麻醉操作。图 3 和图 4 结果表明, 在一定浓度范围内, 随着麻醉剂浓度的增加, 长蛸麻醉所需时间不断缩短, 同时复苏时间不断增加, 这与圆斑星鲽(*Verasper variegates*)[22]、许氏平鲉(*Sebastes schlegeli*)[23]、日本对虾(*Penaeus japonicus*)[24]和大口黑鲈(*Micropterus salmoides*)[25]等的研究结果一致。

丁香油作为一种麻醉化合物在长乌贼(*Sepia elongata*)、皮氏枪乌贼和长蛸中已经得到了应用, 麻醉后死亡率极低, 麻醉和复苏时间短[10-11, 26]。然而, 部分学者在丁香油中添加乙醇以促进其在海水中的溶解[11, 26], 由于乙醇对长蛸具有麻醉作用, 乙醇的添加干扰了对丁香油是否具有麻醉作用的判定。Mooney 等[10]在使用不添加乙醇的丁香油麻醉皮氏枪乌贼时, 出现了喷墨、体色剧烈变化和死亡的现象。Estefanell 等[9]和 Escánez 等[20]在使用丁香油麻醉真蛸时, 真蛸未麻醉并出现喷墨等应激反应。本研究中, 长蛸在低浓度丁香酚中无麻醉作用, 在稍高浓度丁香酚中出现全身僵直和喷墨现象, 结论为丁香酚不能作为长蛸的麻醉剂。

3.2 麻醉与复苏过程

Butler-Struben 等[18]和 Winlow 等[27]总结了在头足类麻醉研究中常用的麻醉状态判定参考依据, 包括呼吸频率变化、体色变化和对按压刺激有无反应。Andrews 等[4]对头足类麻醉状态判定标准进行了探讨, 包括呼吸频率、体色变淡、腕部活动、吸盘吸力、身体平衡的失去与恢复和对有害刺激的反应。本研究在参考前人工作的基础上, 选择长蛸的体色变化、游泳活力强弱、呼吸频率高低变化、腕部活力大小、吸盘吸力强弱、腕部对玻璃棒刺激的反应和将头部翻转眼睛向下的反应作为长蛸麻醉和复苏程度的判定标准。

长蛸麻醉初期四处爬行和游动, 随后吸附于容器的一处, 腕部活动频繁。用玻璃棒刺激腕部, 腕部反应迅速, 将长蛸头部翻转眼部向下能够迅速恢复至原状态。随着麻醉时间延长, 体色由浅棕色逐渐变白, 呼吸频率逐渐降低, 腕部活动频率由有到无, 腕部对玻璃棒刺激的反应逐渐迟钝直至无反应, 吸盘吸力不断减弱直至无吸力, 长蛸头部翻转眼部向下的反应从迅速恢复到挣扎后恢复到最后不能恢复。复苏时, 外套膜的收缩舒张幅度由大变小, 呼吸频率逐渐变快后稳定, 腕部开始活动并支撑身体平衡, 体色逐渐恢复为浅棕色。

3.3 最终麻醉状态

长蛸的最终麻醉状态是确定运输过程适宜麻醉剂量的重要参考标准。在鱼类麻醉研究中, 视觉与触觉丧失, 呼吸频率略减, 并且能够维持自身平衡是适宜运输的最佳状态[28]。长蛸达到第 2 期麻醉时, 腕部对外界刺激反应迟缓, 呼吸频率降低, 能够保持身体平衡, 可作为运输的最适麻醉阶段。若麻醉程度达到第 4、5 期, 长蛸将失去平衡, 沉到容器底部, 易造成个体间的挤压, 增加死亡率。本研究初步测得 24 h 内长蛸达到的最终麻醉时期: 在乙醇浓度为 6 mL/L 和氯化镁浓度为 5 g/L、8 g/L 时为 2 期, 乙醇浓度为 8 mL/L 和氯化镁浓度为 10 g/L 时为 3 期, 可以作为长蛸运输过程中麻醉剂使用的参考标准。

3.4 展望

头足类的年龄、性别、生长阶段、体重、生

理与健康状况之间的差异对麻醉剂有效性的影响, 以及麻醉剂与环境参数之间的相互作用, 仍有待探索。乙醇产生镇静效果似乎与水温有关: 在较低温度下乙醇麻醉枪乌贼(*Loligo pealii*)的效果会下降[29], 在 19~26 ℃下麻醉乌贼、皮氏枪乌贼、福氏枪乌贼和真蛸的效果稳定[19]。在整个麻醉过程中, 需要控制溶液的盐度变化, 因为添加 $MgCl_2$ 会引起盐度改变, 盐度改变可能影响头足类的生理与健康状态, 从而影响麻醉效果。目前头足类已筛选出多种药物或试剂作为麻醉剂(如氯化镁和乙醇), 麻醉剂对头足类的生理影响[18, 30-31]和麻醉作用的机制有待进一步探究。

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Anesthetic effects of several anesthetics on *Octopus minor*

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Abstract: Anesthetics have been used in aquaculture to minimize the stress and damage of *Octopus minor* during harvesting, grading, transportation, spawning induction, and handling. In this study, the effects of several anesthetics, including magnesium sulphate, magnesium chloride, ethanol, eugenol, MS-222, manganese(II) chloride, lidocaine, procaine hydrochloride, ethylene glycol phenyl ether, L-menthol, benzocaine, and 2-trichloromethyl-2-propanol, on adult *O. minor* were tested at a temperature of (18±0.3) ℃. The body weight range of adult octopuses was 138–151 g. Six concentration gradients were designed for each reagent. The results showed that magnesium sulfate, eugenol, MS-222, manganese(II) chloride, lidocaine, procaine hydrochloride, ethylene glycol phenyl ether, l-menthol, benzocaine, and 2-trichloromethyl-2-propanol had no anesthetic effects on *O. minor*. In solutions of 0.2–0.6 mL/L ethylene glycol phenyl ether, 0.05 g/L and 0.06 g/L L-menthol, 0.5 g/L benzocaine, 40 μL/L eugenol, and 50–500 mg/L MS-222, *O. minor* presented convulsions, rigidity, and ink-jets, which indicate toxic effects. Moreover, solutions of 2 mL/L ethanol and 2 g/L magnesium chloride had no anesthetic effects on *O. minor*. In solutions of 4–40 mL/L ethanol and 4–40 g/L magnesium chloride, *O. minor* was found to show anesthetic responses, such as gradually turning white in color, a decreased respiratory rate, weak swimming vitality, decreased wrist movement, and lack of response to external stimuli. Based on the different body color changes, swimming dynamic strengths, breathing rates, wrist activity strengths, sucker suction strengths, wrist responses to stimuli, and whether an upside-down individual can return to normal, the level of anesthesia was divided into five periods, whilst the recovery process was divided into four periods. Individuals in 15–35 g/L magnesium chloride and 10–40 mL/L ethanol can achieve phase 4 anesthesia, this stage is most suitable for the observations and measurements of *O. minor*. Individuals in phase 5 can recover quickly after being transferred into normal environment without an anesthetic. As the concentration of ethanol solution increased from 4 mL/L to 35 mL/L, the anesthesia time decreased from 128 to 8 minutes, while the recovery time increased from 5 to 26 minutes, indicating a positive correlation between anesthesia time and concentration of ethanol, and a negative correlation between recovery time and concentration of ethanol. At 40 mL/L, the anesthesia time increased due to the stress reaction of *O. minor*. As the concentration of magnesium chloride solution increased from 3 g/L to 35 g/L, the anesthesia time presented a gradually decreasing trend from 176 to 13 minutes, and the recovery time presented a gradually increasing trend from 6 to 116 minutes, indicating results similar to those of ethanol. Individuals in 40 mL/L of ethanol and 35 g/L of magnesium chloride could reach phase 5 anesthesia, which is characterized by a lack of breathing. *O. minor* in these conditions would die if not transferred in a timely manner, thus high concentrations of ethanol and magnesium chloride are lethal to *O. minor*. The anesthesia and recovery times for 10 mL/L of ethanol and 20g/L of magnesium chloride were the shortest, which were 26 and 40 minutes, respectively. The present study demonstrated that ethanol and magnesium chloride solutions are effective anesthetic agents for *O. minor*.

Key words: *Octopus minor*; anesthesia; anesthetic effect

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