

其他成果证明材料 5——其他科研成果论文

唐洪玉除代表性成果中的论文外还以第一作者或通讯作者发表sci、CSCD及其核心期刊等论文 20 篇。具体涉及以下 4 个方面：

- 一、鱼类个体发育、生理生化及机制等相关研究（7篇）
- 二、稻渔综合种养水肥平衡、微生物群落和高值化稻渔种养模式研究（9 篇）
- 三、渔业资源相关研究（3 篇）
- 四、毒理学相关研究（1篇）

不同饲料蛋白水平对中华倒刺鲃幼鱼生长的影响

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摘要: 用 4 种不同蛋白水平 (26%、30%、34% 和 36%) 的等能半精制的浮性颗粒试验配合饲料, 对平均初始体重为 (25.0 ± 1.5) g 的中华倒刺鲃 (*Spinibarbus sinensis*) 幼鱼进行了为期 87 d 的培育试验, 研究了中华倒刺鲃对饲料中蛋白质的需求量。结果显示, 中华倒刺鲃的体重增重以 30% 蛋白组最高, 其次是 34%、36% 蛋白组, 最后是 26% 蛋白组; 饵料系数以 30% 蛋白组最低, 其次是 34%、36% 蛋白组, 最后是 26% 蛋白组; 绝对增重率以 30% 蛋白组最高, 其次是 34%、36% 蛋白组, 最后是 26% 蛋白组; 特定生长率以 30% 蛋白组最快, 其次是 34%、36% 蛋白组, 最后是 26% 蛋白组; 试验各蛋白组成活率均为 100%; 各试验组间的终体重、绝对增重率、特定生长率和饵料系数均达到显著性差异 ($P < 0.05$)。结果表明中华倒刺鲃饲料中蛋白水平以 30% 比较合适。

关键词: 中华倒刺鲃 (*Spinibarbus sinensis*); 幼鱼; 蛋白水平; 生长

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Effect of compound feed with different protein content on the growth of juvenile *Spinibarbus sinensis*

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Abstract: Juvenile *Spinibarbus sinensis* (initial weight 25 g) was bred by compound feed with four different protein content (26%, 30%, 34% and 36%) for 78 days. The test studied the protein demand of juvenile of *S. sinensis*. The results showed: the *S. sinensis* in 30% protein group had the highest body weight gain; 34% and 36% was the next; 26% was the lowest. For food coefficient, 30% group was the lowest, next was 34% and 36% group; 26% group was the highest. For absolute increase weight, 30% group was the highest, next was 34% and 36% group; 26% group was the lowest. For specific growth rate, 30% group was the highest, next was 34% and 36% group; 26% group was also the lowest. The survival rate of all group was 100%. The test indicated that 30% protein content suits the protein demands of juvenile *S. sinensis* better.

Key words: *Spinibarbus sinensis*; juvenile; protein content; growth

中华倒刺鲃 (*Spinibarbus sinensis*), 属鲤形目 (Cypriniformes) 鲤科 (Cyprinidae) 鲃亚科 (Bastinae), 是我国长江流域的名优水产养殖品种之一。近年来, 随着中华倒刺鲃人工繁育的成功, 养殖规模不断扩大, 经济效益良好, 深受养殖者和消费者的喜爱, 极具养殖前景。但目前国内外尚缺乏对中华倒刺鲃的营养与饲料研究。由于中华倒刺鲃没有

专门的配合饵料, 在生产上基本是养殖户自行搭配; 其配合饵料的蛋白质水平还没有一个基础指标, 易造成饵料使用不当、养殖效果欠佳、增长速度较慢等问题, 制约了中华倒刺鲃规模化健康养殖的进一步发展^[1-3, 6]。研究饲料中蛋白质适宜含量, 对优化饲料成本, 保持鱼类良好的生长和提高饲料蛋白利用率具有重要意义。为促进这一名优新品种

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在我国的推广养殖,本试验以中华倒刺鲃幼鱼为研究对象,根据其营养需求和生理生态特点,进行了中华倒刺鲃幼鱼阶段蛋白质需求量的生产性对比饲养试验,旨在了解中华倒刺鲃生长过程中饲料蛋白的适宜水平,以期中华倒刺鲃人工配合饲料的研制和养殖技术提供试验数据和实践指导。

1 材料与方法

1.1 试验幼鱼来源

本试验用中华倒刺鲃为重庆市东平水产养殖有限公司的基地静观鱼种场在2007年8月份人工繁殖的后代秋繁苗,经过池塘培育,初始平均体重(25.0 ± 1.5)g。试验前一周挑选规格基本一致、体格健壮、无病无伤的幼鱼2000尾,暂养于水泥池中驯养。

1.2 试验用配合饲料

试验用不同蛋白水平的饲料为重庆国雄股份有限公司协助制作的以鱼粉和酪蛋白为蛋白源的等能半精制的试验配合饲料,其饲料设计配方的原料组成和营养成分见表1。试验共分4组(即4个蛋白质梯度),其蛋白水平分别为:C1组为26%、C2组为30%、C3组为34%和C4组为36%。

表1 不同蛋白水平饲料的原料组成和成分分析

Tab.1 Formulation and proximate analysis of the test diets

项目	处理组			
	C1组	C2组	C3组	C4组
鱼粉	12.0	12.0	12.0	12.0
酪蛋白	18.5	23.0	26.5	29.5
糊精	39.5	35.0	31.5	28.5
α 淀粉	15.0	15.0	15.0	15.0
饲料原料	3.5	3.5	3.5	3.5
鱼油	3.5	3.5	3.5	3.5
豆油	3.5	3.5	3.5	3.5
纤维素	5.0	5.0	5.0	5.0
添加剂预混料	1.0	1.0	1.0	1.0
CaH_2PO_4	1.5	1.5	1.5	1.5
氯化胆碱	0.5	0.5	0.5	0.5
营养成分				
粗蛋白	25.91	30.37	34.02	36.80
粗脂肪	7.05	6.98	6.91	7.10
粗灰分	6.92	7.01	6.97	6.88

注:添加剂预混料由重庆国雄股份有限公司提供。

1.3 试验地点、时间和方法

在重庆市东平水产养殖有限公司静观鱼种场内选择12个条件一致的圆形水泥池进行试验,试验池面积 60 m^2 ,水深 1.3 m ,水源为抽提在蓄水池

的溪河水。试验期间水温 $26 \sim 33^\circ\text{C}$,水量充足,水质良好, $\text{pH } 6.8 \sim 7.6$,溶氧在 6 mg/L 以上,自然光照。

试验池和幼鱼的消毒均按常规池塘养殖消毒方法进行。试验开始前先将试验幼鱼在水泥池中驯养一周,正式试验于2008年7月4日开始,9月29日结束,共计87d。

本试验设计4个蛋白质水平,每个水平设3个重复,随机分配12个圆形水泥池,并将试验池分别编号为C1、C2、C3和C4组,分别投喂蛋白质含量为26%、30%、34%和36%的等能半精制的浮性颗粒配合饲料。每个水泥池随机放养中华倒刺鲃幼鱼150尾。

1.4 试验的日常管理

按“四定”投饵方法,每天投喂3次(7:00、12:00、17:00),投喂量为体重的3%~5%,根据体重及时调整投饵量;定期测定水中溶氧值,详细记录水温、实际投喂量等;每5~7d换水1次,换水量为 $1/3$ 。每天定时巡塘,及时清除污物,检查鱼的摄食等活动情况。

1.5 生长性能的测定

试验期间,定期抽样(每组每次20尾)检测各组试验鱼的体长和体重,按下式计算各评价指标:

成活率(%) = 终末尾数 / 初始尾数 $\times 100\%$;

$\text{FCR} = \text{饵料投喂量} / \text{鱼体净增重}$ 。

$\text{SGR} = [(\ln W_2 - \ln W_1) / (t_2 - t_1)] \times 100$;

鱼体增重率(%) = $100 \times (W_2 - W_1) / W_1$;

$\text{AGR} = (W_2 - W_1) / (t_2 - t_1)$ 。

式中: W_1 、 W_2 分别是时间为 t_1 与 t_2 时的体重(g); t_1 为试验开始时间(d), t_2 为试验结束时间(d)。FCR为饵料系数。SGR为特定生长率(%/d)。AGR为绝对增重率(g/d)。

1.6 统计分析

测得的试验数据均以平均值 $\pm$ 标准误(Mean $\pm$ SE)表示,采用SPSS 11.5软件对试验结果进行方差分析。

2 结果与分析

2.1 中华倒刺鲃幼鱼的生长

经过87d的养殖试验,各试验组幼鱼的生长情况见表2、图1。

由图1可以看出,随着饲养时间的延长,各蛋白水平组幼鱼的体重均显著增加。其中C2组的幼鱼体重增加速度最快,C3组次之,C1组最慢。

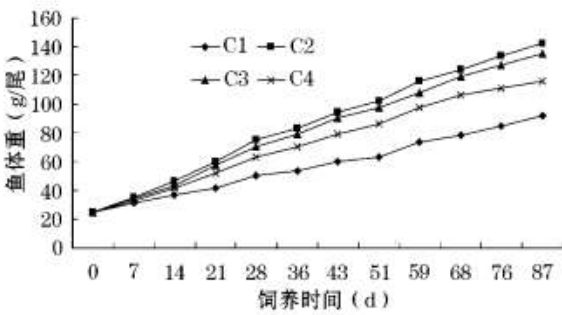


图 1 投喂不同蛋白水平饵料的中华倒刺鲃幼鱼的生长曲线

Fig.1 Comparison of growth performance of *S. sinensis* with different protein content feed

由表 2 可以看出，试验结束时，C2 组的鱼体平均体重达到了 142.05 g 显著高于 C3 组的 135.23 g、C4 组的 116.11 g 和 C1 组的 92.35 g ($P<0.05$)；同时 C2 组的平均体重增重也是最大，为 117.00 g 显著大于 C3 组的 110.20 g、C4 组的 91.04 g 和 C1 组的 67.25 g ($P<0.05$)。

表 2 饲料中不同蛋白水平对中华倒刺鲃幼鱼生长及饲料利用率的影响
Tab.2 Effect of dietary protein level on growth performance and dietary utilization of *S. sinensis*

生长指标	C1 组	C2 组	C3 组	C4 组
初始尾重 (g)	25.10±1.05	25.05±0.88	25.03±0.75	25.07±0.96
结束尾重 (g)	92.35±3.70 ^a	142.05±7.88 ^b	135.23±9.76 ^c	116.11±4.84 ^d
成活率 (%)	100	100	100	100
净增重 (g/尾)	67.25±1.35 ^a	117.00±2.74 ^b	110.20±2.45 ^c	91.04±2.36 ^d
绝对增重率 (g/d)	0.78±0.05 ^a	1.35±0.07 ^b	1.27±0.08 ^c	1.05±0.05 ^d
特定生长率 (%/d)	1.51±0.08 ^a	2.00±0.12 ^b	1.94±0.17 ^c	1.77±0.13 ^d
饲料系数 (FCR)	1.85±0.15 ^a	1.36±0.06 ^b	1.44±0.05 ^c	1.62±0.07 ^d

注：同行肩标字母不同者表示差异显著 ($P<0.05$)。

另外，4 个试验组的幼鱼成活率均为 100%，表明在饲养过程中，只要水质条件良好，同时加强日常管理，中华倒刺鲃幼鱼的病害就相对较少，基本不生病。但从试验池中水质的变化可以看出，饵料蛋白质含量高的试验组，池水质容易恶化，换水也较多。

由此可见，在本试验条件下，用蛋白质含量为 30% (C2 组) 的浮性颗粒配合饵料培育中华倒刺鲃幼鱼，可以获得较佳的生长效果。综合考虑以上各项指标，我们初步认为配合饵料蛋白质含量为 30%~34% 时，比较适合中华倒刺鲃幼鱼的生长需要。

由此可见，蛋白水平太高或太低都不利于中华倒刺鲃幼鱼的生长。

2.2 绝对增重率 (AGR)、特定生长率 (SGR) 和饵料系数 (FCR)

各试验组中华倒刺鲃幼鱼的绝对增重率、特定生长率、饵料系数和成活率等指标见表 2。从表 2 可以看出，随着饵料中蛋白质水平的增加，试验幼鱼的终体重、绝对增重率和特定生长率先是逐渐增大，之后又逐渐减小，其中，C2 组幼鱼的末体重、绝对增重率、特定生长率均显著大于 C3、C4 和 C1 组 ($P<0.05$)。

经数据分析，试验结束时中华倒刺鲃幼鱼的终体重、绝对增重率和特定生长率均表现为蛋白质 30% 组 (C2) > 蛋白质 34% 组 (C3) > 蛋白质 36% 组 (C4) > 蛋白质 26% 组 (C1)。从各试验组的饵料系数来看，C2 和 C3 组饵料系数显著小于 C4 和 C1 组 ($P<0.05$)，其中以 C2 组的饵料系数最低，为 1.36；C3 组饵料系数次之，为 1.44；C1 组饵料系数最高，为 1.85。

3 讨论

3.1 中华倒刺鲃的生长速度和条件

本试验结束时，C2 组的体重平均达到了 117 g 试验期间每天平均增重 1.35 g。据项目组试验期间的观测，在 7 月 20—8 月 24 日之间，每天平均增重近 2 g。这与几年前网箱养鲤的生长速度相近^[4]。试验结果表明中华倒刺鲃的生长速度较快。另外，项目组在饲养过程中还发现，中华倒刺鲃的抢食能力较强，具备快速生长的基础，其养殖潜力非常大。如果能完全根据中华倒刺鲃的营养需求，研制出中华倒刺鲃的最佳配合饵料，其生长速度应该还会提高。

根据项目组对水温、天气的记录情况可以看出,中华倒刺鲃在天气晴朗、水温 27~28℃ 时的生长速度最快,而水温高于 32℃ 时生长速度明显减慢。从本次试验开始到 7 月 20 日前,水温一直保持在 32~33℃,其生长速度相对较差。因此,为了保证中华倒刺鲃能快速生长,除要研制专用的中华倒刺鲃配合饵料外,还必须保证其在最适的环境条件中生长。如在水质良好,适宜的水温和高溶氧条件下,其食欲明显增强,生长速度也较快。

3.2 中华倒刺鲃的蛋白质需求

鱼类对饲料蛋白质的需求明显高于陆生动物,一般来说是陆生动物的 2~4 倍。蛋白质是维持鱼体生命活动所必需的营养成分,是构成鱼体的主要物质,也是能量的来源。中华倒刺鲃幼鱼对蛋白质的需求,国内还未见有报道。但在其他众多鱼类上的一系列试验都表明,蛋白质虽为生命的基础在生长和存活过程中必不可少,但是过高或过低都会带来一定的负面影响。

从试验结果来看,蛋白质含量 30% 和 34% 的幼鱼饵料较适合中华倒刺鲃幼鱼的生长,但中华倒刺鲃幼鱼饵料的最佳蛋白质含量需求,还有待进一步试验研究。因为本试验没有做梯度更多的对比试

验,饲养时间也不长;同时,在试验过程中,为了准确计算饵料系数,没有添加青饲料。中华倒刺鲃是偏植食性的杂食性鱼类,在养殖过程中需适当补充青饲料,项目组通过观察网箱养殖的中华倒刺鲃幼鱼时发现,如果饵料中长期不使用青饲料或添加维生素的量不够,则容易患溃烂病^[5,7]。因此中华倒刺鲃的最优饲料配方还有待进一步研制。

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饲养条件下不同食性鱼类肠上皮细胞 刷状缘膜酶活性研究

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摘要: 采用酶学分析法, 检测饲养条件下三种不同食性鱼类前、中、后肠上皮细胞刷状缘膜 (BBM) 上蔗糖酶、麦芽糖酶、碱性磷酸酶、 γ -谷氨酰转氨酶和琥珀酸脱氢酶的活力。结果表明: (1) 蔗糖酶和麦芽糖酶活力在三种鱼 BBM 上的分布表现出一致性, 加州鲈 (*Micropterus salmoides*) 和草鱼 (*Ctenopharyngodon idellus*) 为前肠 > 中肠 > 后肠, 湘云鲫 (*Carassius auratus*) 为中肠 > 前肠 > 后肠, 草鱼的蔗糖酶和麦芽糖酶比活力显著性高于湘云鲫和加州鲈 ($P < 0.05$); (2) 加州鲈和草鱼的 γ -谷氨酰转氨酶主要分布在前肠和中肠, 而湘云鲫主要分布在中肠和后肠, 碱性磷酸酶在三种鱼的肠道中比活力大小均为前肠 > 中肠 > 后肠, 加州鲈和湘云鲫 γ -谷氨酰转氨酶和碱性磷酸酶的比活力显著高于草鱼 ($P < 0.05$); (3) 加州鲈琥珀酸脱氢酶活力显著高于草鱼和湘云鲫 ($P < 0.05$), 但湘云鲫和草鱼三肠段之间差异不显著 ($P > 0.05$)。

关键词: 食性; 肠上皮细胞刷状缘膜; 酶活性

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Study on the enzymes activity of enterocytes brush border membrane of three cultured fishes with different feeding habits

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Abstract: The activities of five enzymes, sucrase, maltase, alkaline phosphatase, γ -glutamyltranspeptidase (γ -GT) and succinate dehydrogenase of the enterocytes brush border membrane (BBM) of *Ctenopharyngodon idellus*, *Micropterus salmoides* and triploid *Carassius auratus* was investigated by means of enzymes analyses. The results showed that the sucrase and maltase of the intestinal BBM of the foregut were higher than the midgut and the hindgut in the *M. salmoides* and *C. idellus*. as for triploid *C. auratus*, the activity of sucrase and maltase was midgut > foregut > hindgut, in addition, the sucrase and maltase activities of *C. idellus* were significantly higher than ($P < 0.05$) the triploid *C. auratus* and the *M. salmoides*. The γ -glutamyltranspeptidase (γ -GT) activity of the *M. salmoides* and the *C. idellus* were mainly distributed in foregut and midgut, while The γ -glutamyltranspeptidase (γ -GT) activity of the triploid *C. auratus* was mainly distributed in midgut and hindgut. The alkaline phosphatase activity was found to be the highest in the foregut of all species compared to other regions of the intestines. The γ -glutamyltranspeptidase (γ -GT) and alkaline phosphatase activities of the *M. salmoides* and triploid *C. auratus* were significantly higher ($P < 0.05$) than the *C. idellus*. The succinate dehydrogenase activity of the *M. salmoides* was significantly higher ($P < 0.05$) than the *C. idellus* and the triploid *C. auratus*, while the succinate dehydrogenase activity had no significant different in all regions of the intestines.

Key words: feeding habits; enterocytes brush border membrane; enzymes activity

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随着水产养殖业的发展, 鱼类配合饵料越来越广泛使用, 研究和评价鱼类自身对营养物质的利用能力尤其重要。鱼类对营养物质消化和吸收的能力主要取决于肠道消化酶活性, 肠道中多种酶大量富集在肠道上皮细胞刷状缘膜(enterocytes brush border membrane, BBM)上, 这些酶的活性直接或间接决定着鱼体对于三大营养物质的消化吸收同化作用^[1-2]。其中, 麦芽糖酶和蔗糖酶活性可反应糖类在肠道的代谢情况, γ -谷氨酰转肽酶与蛋白质的消化有关, 碱性磷酸酶参与脂类、葡萄糖、钙和无机磷等营养的吸收和转运^[3], 琥珀酸脱氢酶则参与能量合成和分解。鱼类食性不同是影响其消化酶活性的重要因素^[4-6]。因此, 本试验选择饲养条件下三种不同食性同龄的淡水鱼-草鱼(*Ctenopharyngodon idellus*) (草食性)、湘云鲫(*Carassius auratus*) (杂食性) 和加州鲈(*Micropterus salmoides*) (肉食性), 研究其前、中、后肠上皮细胞刷状缘膜的以上5种消化酶活性, 以期了解不同食性鱼类对营养物质的消化吸收能力, 为不同食性鱼类人工配合饲料的研制提供理论依据。

1 材料与方法

1.1 试验材料

2013年7月, 从重庆吉冠水产养殖有限公司采购体质健壮加州鲈、草鱼和湘云鲫的二龄鱼种各30尾, 分别暂养于西南大学鱼类工厂化养殖车间的微流水玻璃缸中, 水温(26.0 ± 1.0) $^{\circ}\text{C}$ 。暂养期间, 分别投喂该公司提供的相应饲料, 暂养时间至少一周。取样前, 将鱼饥饿24 h, 测定其体长、肠长。草鱼体长平均(13.73 ± 0.50) cm, 肠长平均值(27.88 ± 3.82) cm; 湘云鲫体长平均(14.66 ± 3.40) cm, 肠长平均值(31.43 ± 9.60) cm; 加州鲈体长平均(23.00 ± 2.40) cm, 肠长平均值(11.77 ± 1.12) cm。

1.2 肠上皮细胞刷状缘膜的提取^[7]

1.2.1 初始肠粗酶液的制备

首先在冰盘上将鱼逐尾解剖, 迅速分离出肠道, 立即放入用生理盐水冻成的冰盘中, 用镊子和小剪刀剔除表面脂肪组织, 用4 $^{\circ}\text{C}$ 生理盐水冲洗, 将肠道分为前肠, 中肠和后肠, 然后剪开肠道, 先用4 $^{\circ}\text{C}$ 生理盐水, 再用4 $^{\circ}\text{C}$ 双蒸水将肠道冲洗干净, 滤纸吸干各肠段水分, 再将已分好的肠段平均剪成三小段, 取中间段分别代表整段肠道的前肠段、中肠段和后肠段。根据鱼体大小和肠道样品

量, 草鱼由每8尾鱼的同一肠段组成1个混合样品, 湘云鲫由每5尾鱼的同一肠段组成1个混合样品, 加州鲈由每4尾鱼的同一肠段组成1个混合样品, 每个样品均设3个平行组。取每个混合样品2.0 g, 溶于4 $^{\circ}\text{C}$ 、pH 7.0的50 mmol/L冰甘露醇(以2 mmol/L Tris-HCl配制)缓冲液20 mL中, 用组织匀浆机匀浆, 制得初始匀浆液。将初始匀浆液装入离心管内, 以3 000 r/min、4 $^{\circ}\text{C}$ 离心10 min, 弃沉淀, 取上清液, 即肠粗酶液。

1.2.2 钙离子沉淀法

准备数个离心管, 加入1.98 mL上清液, 再加入0.02 mL CaCl_2 (将其中加入1 mol/L的 CaCl_2 , 使最终浓度达到10 mmol/L为止), 然后进入4 $^{\circ}\text{C}$ 的水浴中振荡10 min, 再在4 $^{\circ}\text{C}$ 、3 000 r/min离心力的作用下离心10 min, 取上清液, 将上清液采用离心力16 000 r/min、4 $^{\circ}\text{C}$ 离心30 min后去掉上清液, 在沉淀中加入1.98 mL的甘露醇缓冲液, 使沉淀摇匀悬浮后, 再在16 000 r/min离心力作用下离心30 min, 去掉上清液, 得到白色沉淀, 加入50 mmol/L的冰甘露醇(以2 mmol/L Tris-HCl配制, pH 7.1) 0.5 mL, 使沉淀完全溶解^[8]。最后, 测定溶液中5种酶活性和蛋白质含量。

1.3 酶活性测定

鱼体肠道各段BBM上的蔗糖酶、麦芽糖酶、 γ -谷氨酰转肽酶、碱性磷酸酶和琥珀酸脱氢酶比活力的测定参照南京建成生物工程研究所的试剂盒使用说明进行, 用考马斯亮蓝法测定蛋白含量^[9]。

1.4 数据分析

试验数据用平均值 $\pm$ 标准差(Mean $\pm$ SD)表示, 并采用SPSS19.0统计软件进行所得数据进行多重比较和Duncan's检验, 显著性水平0.05。

2 结果

2.1 三种鱼肠道BBM蔗糖酶比活力

加州鲈、草鱼和湘云鲫BBM蔗糖酶比活力如表1所示, BBM蔗糖酶比活力加州鲈前肠>中肠>后肠, 其中, 前肠与中肠和后肠蔗糖酶比活力存在显著性差异($P < 0.05$); 草鱼前肠>中肠>后肠, 三段肠之间存在显著性差异($P < 0.05$); 湘云鲫中肠>前肠>后肠, 三肠段之间存在显著性差异($P < 0.05$)。三种鱼同肠道段的蔗糖酶比活力大小是: 草鱼前肠>加州鲈前肠>湘云鲫前肠, 草鱼前肠与加州鲈前肠和湘云鲫前肠BBM蔗糖酶比活力存在显著性差异($P < 0.05$); 草鱼中肠>湘云鲫中

Original Research Articles

Effects of short-time fasting and feeding frequencies within 24 hours on histology, cholecystokinin and trypsin enzyme activities of digestive organs in black bream, *Megalobrama pellegrini* (Tchang, 1930), juvenile

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Keywords: Black bream, Cholecystokinin, Fasting, Histology, Trypsin

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To study the regulation and feedback mechanism of cholecystokinin and trypsin in Black bream, *Megalobrama pellegrini* (Tchang, 1930) 60 days after hatching under 15 days short-term fasting and different feeding frequencies within 24 hours during the same period, Black bream (wet weight 183.75 ± 61.16 mg, total length 20.74 ± 4.08 mm) developed in a recirculating aquaculture system (RAS) were selected. In the short-term fasting trial, body weight, trypsin, and cholecystokinin (CCK) of the feeding control group (FCG) were higher than those of the fasting trial group (FTG). Trypsin and CCK in FTG reached the lowest value on day 9, and CCK content reached the highest value on day 11. In the 24-hour daily rhythm experiment, juvenile fish were randomly assigned to (A) once feeding, (B) twice feeding, (C) three times feeding, and (D) fasting. CCK showed a minimum at 1:00+ in group A, a peak at night in group B\C\D, and a maximum in group C, and a single satiety stimulus can lead to increased hunger. The four treatment groups had an apparent closed-loop regulation, while the control point of the fasting group (D) shifted forward to the next day. In this study, we found only negative feedback regulation of CCK and trypsin at the end of fasting, considering whether the secretory site or anti-inflammatory response caused the increase of CCK. The damage of epithelial cells in the villi of the foregut was greater than that in the hindgut and hepatopancreas, and the detachment of epithelial cells and the striatal margin was the main damage. Different feeding frequencies in a single day did not directly affect the long-term fluctuation of CCK and trypsin diurnal rhythm. Three meals per day may be more conducive to the long-term growth of juvenile Black bream. This study aimed to provide a reference for the feeding strategy of juvenile Black bream in the RAS.

INTRODUCTION

Megalobrama pellegrini (Tchang, 1930), the Black bream, is a type of fish found in the middle and upper reaches of the Yangtze River in China. It belongs to Cypriniformes, Cyprinidae, Culterinae, and *Meglobrama*, and it is economically important in the upper reaches of the Yangtze River. There is a lack of studies on the digestive physiology of juvenile Black bream in recirculating aquaculture systems (RAS), which hindered the development of high-quality, low-cost breeding processes. Therefore, it is essential to de-

velop intensive rearing methods to meet the increasing demand.¹

The cholecystokinin (CCK) gene was first discovered in the gastrointestinal tract of dogs² and has since been found in humans,³ *Oncorhynchus mykiss*, Rainbow trout,⁴ and *Salmo salar*, Atlantic salmon.⁵ Chaudhri⁶ confirmed that CCK is a typical postprandial satiety signaling factor. Murashita⁵ proposed that the expression pattern of CCK in different species is dynamic and changes with environmental factors. In Atlantic salmon, the expression of CCK in the brain significantly increased three hours after a meal. Liddle⁷ demonstrated that the concentration of CCK in hu-

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man plasma significantly increased within 15 minutes after a meal. Tartaglia⁸ reported that CCK can affect digestion and feeding in aquatic animals. The expression of CCK in the foregut decreased significantly after 72 hours of fasting in *Pelteobagrus fulvidraco*, Yellow catfish⁹ and *Megalobrama amblycephala*, Wuchang bream.¹⁰

According to Rojas-García, Morais, and Rønnestad,¹¹ CCK is involved in an individual's daily digestion, and its fluctuation rhythm is affected by the frequency of eating. The main biological effects of cholecystokinin (CCK) are to stimulate gallbladder contraction and bile secretion, promote the secretion and synthesis of trypsin, and regulate intestinal peristalsis. Additionally, it acts as a satiety factor to regulate food intake. CCK can release satiety signals, which are related to the secretion of trypsin. Then, CCK is released from the enteroendocrine cells of the enterocytes into body fluids and acts on target cells in the pancreas to induce the secretion of digestive enzyme precursors into the intestinal lumen. High intestinal trypsin activity acts as a negative feedback control on CCK release, suggesting a regulatory loop between these two factors.¹² This regulatory loop has also been reported in *Gadus morhua*, Atlantic cod larvae,¹³ Atlantic salmon.¹⁴ A negative feedback loop between CCK and trypsin was observed in the daily rhythm test of juvenile *Senegalese sole*, Sole fish.¹⁵ However, little is known about the changes of CCK and trypsin in juvenile *Black bream*. Few reports have focused on the relationship between CCK and trypsin in this species.

Increasing fasting duration can harm the digestive system and negatively impact CCK levels. Feeding frequency is a crucial aspect of aquaculture management that affects fish growth, feed utilization, and management costs. It also influences the metabolic state and body composition of the fish. In intensive aquaculture, feeding frequency can reduce size differences between individuals in the same batch and improve water quality. The effects of fasting and feeding frequency on fish digestion have been extensively studied. Our research group has also conducted significant research on fasting and re-feeding of Black bream. However, there are few studies on monitoring CCK content or feedback regulation through fasting and changes in feeding frequency, with most of them focusing on marine fishes. The short-term daily rhythms of Atlantic cod,¹³ Sole fish juvenile,¹⁵ and *Clupea harengus* L, Atlantic herring¹¹ had been reported after changing the feeding frequency and other influencing factors during individual development. The impact of fasting and refeeding on the growth and digestive enzyme activities of juvenile Black bream has been reported extensively. Additionally, research has reported the effects of refeeding on biochemical and non-specific immune parameters.

It has been documented that about 80 % of CCK in fish is produced by the central nervous system of the head, while only 6-10 % of CCK is secreted in the digestive tract.¹¹ To accurately observe the regulation mechanism of CCK and trypsin in the intestine and remove the influence of head hormones, we took special treatment dissection steps (removing the head, back tissue, and tail peduncle of the individuals) during sampling.

This experiment selected Black bream, which has just entered a relatively stable development stage, for fasting and short-term circadian rhythm experiments. The aim was to explore the effects of CCK and trypsin on feeding in this critical period and supplement the theoretical basis for culture in a recirculating aquaculture system (RAS).

MATERIALS AND METHODS

LARVAL REARING IN RAS

Black bream larvae were obtained from Dongping Aquaculture Co., Ltd, Chongqing, China. The recirculating aquaculture system (RAS) was established in a fish rearing room with a constant temperature of 28°C with air conditioning, and the water temperature was maintained at 28°C ± 0.5°C. On the fifth day after hatching (DAH5), fish larvae were randomly placed in the glass tanks of the RAS after being treated with 5000 ppm salt water for 15 minutes. Each tank had a volume of 40 L and a flow rate of 0.2 L/s. The RAS utilized LED tubes and was illuminated at a daily rate of 1200 lx from 7:00 to 19:00. The individuals were cultivated in tanks until DAH59 before the experiment, and the culture density was gradually reduced to 1.25 ind L⁻¹ (40 tails per glass tank) as the individuals grew. The fish were fed with Floating Fish Species Matching Feed (Shuangliu Zhengda Co., Ltd., Type I-F1). The main nutrient values were: crude protein ≥ 36%, crude fiber ≤ 8.0%, crude ash ≤ 16.0%, crude fat ≥ 3.0%, and moisture ≤ 12.5%. Individuals were fed three times a day until apparent satiation (at 9:00, 13:00, and 17:00). After each satiation, any remaining bait in the cylinder was removed. The dissolved oxygen and ammonia nitrogen in RAS were monitored (DO ≥ 4 mg/L, NH₄⁺ ≤ 0.1 mg/L, NO₂-N ≤ 0.02 mg/L). Dead fish were cleaned daily, and data were recorded.

SHORT-TERM FASTING TRIAL

For the short-term fasting trial on DAH59, six glass tanks with a water volume of 32 liters were selected. A total of 240 juvenile individuals with an initial body weight of 183.75 ± 61.16 mg and length of 20.74 ± 4.08 mm were randomly divided into the 15-day fasting test group (FTG) and the three times-a-day feeding control group (FCG), with three replicates for each group. The stocking density was 40 individuals in each tank. During the test period, growth data such as weight gain rate (WGR) and specific growth rate (SGR) were measured using the method described by He et al.¹⁶ The tanks were emptied after each feeding. In the fasting trial, three individuals were randomly sampled from every three tanks of the two experimental groups. Three individuals were collected from each group randomly in the morning on days 1, 3, 5, 7, 9, 11, 13, and 15 from DAH60 to DAH75. Three individuals were randomly sampled at each sampling point, frozen, and analyzed in triplicate.

DAILY RHYTHM EXPERIMENT

Four glass tanks with a water volume of 32 liters of recirculating aquaculture system (RAS) were selected to prepare for different feeding regimes within 24 hours. One hundred and sixty juvenile individuals (DAH62) were randomly divided into four groups (40 individuals in each tank) with an initial body weight of 238.71 ± 68.84 mg and a length of 22.81 ± 2.42 mm (mean and SD). After a one-day fast, four tanks were assigned to each of the following feeding regimes on DAH64: (A) feeding once at 9:00, (B) feeding twice at 9:00 and 17:00, (C) feeding three times at 9:00, 13:00, and 17:00, and (D) fasting. The fish were fed to apparent satiation at each feeding time, with the proportion of feed being approximately 5.6% of individual body weight. Sampling was conducted at seven different times throughout the day: 9:00, 13:00, 17:00, 21:00, $1:00^{+1} \cdot 9(p00)+1^{\wedge}$, and $9:00^{+1}$, to study the daily feeding rhythm within 24 hours. Three individuals were randomly sampled from each group at each sampling point, frozen, and analyzed in triplicate.

SAMPLING PREPARATION

The tests and procedures of Black bream in this study were approved by the Laboratory Animal Centre of Southwest University, and the qualification was obtained as a Laboratory Animal Practitioner (SWU_LAC-20210794).

Body weight and total length were measured at each sampling. Body weight was calculated by weighing a subsample of individuals using a millimeter analytical balance. The total length, measured from the tip of the maxilla to the end of the notochord in millimeters, was determined using a caliper. The subjects were anesthetized with tricaine MS-222 (120 mg/L) and subsequently dissected on ice, with only the abdominal viscera being retained. The head, dorsal muscle, and tail were frozen using liquid nitrogen and stored in 2 ml enzyme-free test tubes at -80°C until analysis. All samples were taken before feeding.

The study analyzed individual samples for CCK and trypsin enzyme activity using highly sensitive methods, as reported by Neda et al,¹⁵ which allowed for the resolution of both factors at the individual level. Previous research has indicated that a significant amount of CCK can be found in the head, primarily in the central nervous system, which may obscure changes in CCK in the gastrointestinal tract.¹¹ Thus, all analyses in the current study were conducted on juvenile dissections, excluding the head, back muscles, and tail.

ANALYSIS OF CCK AND TRYPSIN ENZYME ACTIVITY

The samples were homogenized in 0.05M PBS (pH 7.4) at a ratio of 1:9 using a tissue grinder. After centrifugation at 2500 rpm for 20 minutes at 4°C , the samples were immediately analyzed. All assays were performed in triplicate.

Trypsin activity was measured to detect total protein content using assay kits from Nanjing Jiancheng Bioengineering Institute. Prior to conducting the experiment, the diluted supernatant was further diluted tenfold with 0.05 M

PBS (pH 7.4). Enzyme activity was determined by measuring the change in absorbance.

The detection method used was based on the protocol developed by Neda et al.¹⁵ All groups were analyzed using the Fish Cholecystokinin (CCK) Elisa Assay Kit from Nanjing Jiancheng Bioengineering Institute, which employs a competition method to measure the CCK content.

HISTOLOGY

In the short-term fasting trial, Black bream underwent routine histology and hematoxylin-eosin (H-E) staining. The samples were fixed in 4% paraformaldehyde for 24 hours, washed, dehydrated, cleared, and embedded in paraffin. Sagittal sections ($5\text{ }\mu\text{m}$) were cut using a conventional microtome (RM2016, Shanghai Leica Instrument Co., Ltd.), placed on gelatin-coated slides, rehydrated, and stained. The tissue sections were analyzed using a Leica four-camera digital camera and ImageJ, after being observed under a light biomicroscope (B302, Chongqing Aote Optical Instrument Co., Ltd.).

STATISTICAL ANALYSIS

The mean $\pm$ SD expression of CCK content and trypsin enzyme activities in the abdominal extracts of Black bream were evaluated. Homogeneity of variance and normality tests were conducted. The specific activity of CCK was assessed using one-way ANOVA. Multiple comparisons of enzymatic activity over time were performed using the Duncan test. The analysis of variance between groups was conducted using an independent samples t-test. Histological sections were analyzed with Image J (National Institutes of Health). The statistical analysis was conducted using IBM® SPSS® Statistics26 (IBM, International Business Machines Corporation), and the graphs were created using Origin 2021 (OriginLab Corporation). A significance level of $P < 0.05$ was utilized.

RESULTS

SHORT-TERM FASTING TRIAL

TRYPSIN ENZYME ACTIVITY AND CCK CONTENT

All groups exhibited an increasing trend in body weight of Black bream from DAH60 to DAH75 (Fig. 1), with a significant difference between groups ($P < 0.05$). The weight gain of both groups did not significantly increase during the first three days. The weight gain of the FCG was significantly higher than that of the FTG ($P < 0.01$). There was no significant difference in the weight gain of FTG ($P > 0.05$), and the SGR of FTG was 0.49%. The SGR of FCG reached 8.68 %.

There were no extreme values of trypsin activity in FCG, and the final trypsin activity level was higher than the initial value (Figs. 2,5). FCG exhibited a higher level of trypsin activity compared to FTG (Fig. 4). The trypsin activity of FTG showed a decreasing trend on the first day of fasting and reached its lowest value of 279.43 U/mgprot on the ninth day of fasting (DAH72), which was significantly dif-

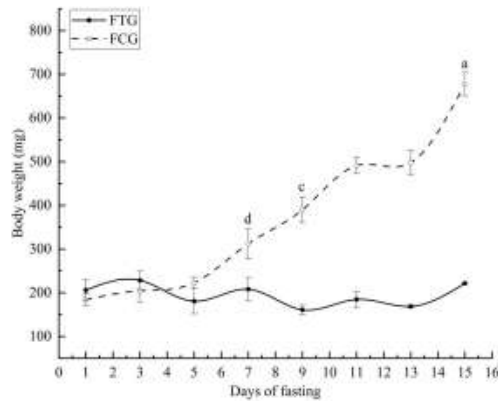


Figure 1. Body weight (mg) of FTG and FCG within 15 days of the fasting trial.

Feeding in FCG was performed as usual at 9:00, 13:00, and 17:00 three times a day. The dotted line represented FCG, and the solid line represented FTG. Data were presented as mean $\pm$ standard deviation ($n = 3$ individuals, all groups $n = 3$ tanks). Lowercase letters represent intra-group significance, and $P < 0.05$ is used. The growth rate and specific growth rate during the experiment were as follows ("T" denotes FTG, "C" denotes FCG):

WGR (%) = (FBW-IBW) / W0

SGR(%) = $-(\ln \text{FBW} - \ln \text{IBW}) / t \times 100\%$

WGR-T-7.61%

SGR-T-0.49%

WGR-C-267.63%

SGR-C-8.68%

Including FBW is the final weight of the Black bream. IBW is the initial weight and 't' is the time.

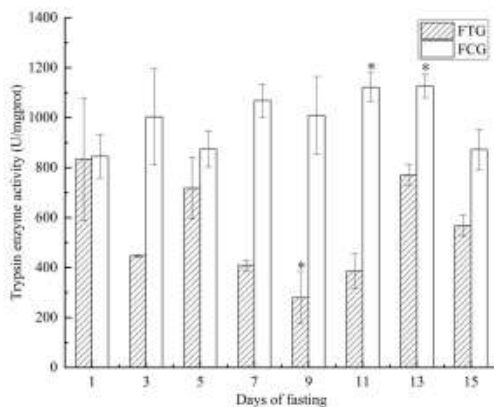


Figure 2. The trypsin activity (U/mgprot) of FTG and FCG of the fasting study.

The grey stripe indicates the experimental group and the white stripe indicates the control group (feeding at 9:00, 13:00, and 17:00, three times a day as usual). Data are presented as mean $\pm$ standard deviation ($n = 3$ individuals, all feeding treatments $n = 3$ tanks) and $P < 0.05$ is used, "***" represents the significant difference within a group.

ferent from other time points ($P < 0.05$). After a slight increase, the trypsin activity fell to a level lower than the initial activity on the fifteenth day of fasting. There was a significant difference in trypsin enzyme activity between FTG and FCG ($P < 0.05$). The values of each point in FCG were greater than those in FTG (Fig. 2).

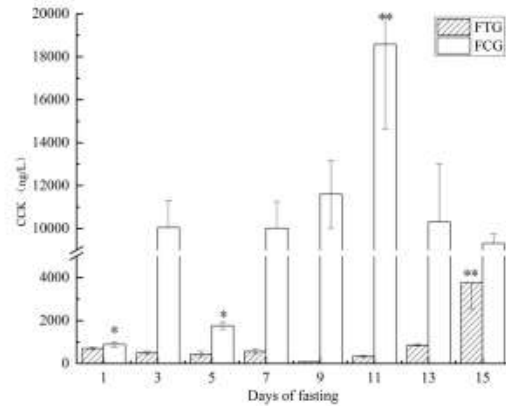


Figure 3. CCK content in FTG and FCG of the fasting trial.

The grey strip indicated FTG and the white strip indicated FCG (feeding at 9:00, 13:00, and 17:00, three times a day as usual). Y-axis coordinates were processed with break-points between 5000 and 9000 scales. Data are presented as mean $\pm$ standard ($n = 3$ individuals, all feeding treatments $n = 3$ tanks), and $P < 0.05$ is used, "***" represents the significant difference within a group, "*" indicates an extremely significant difference.

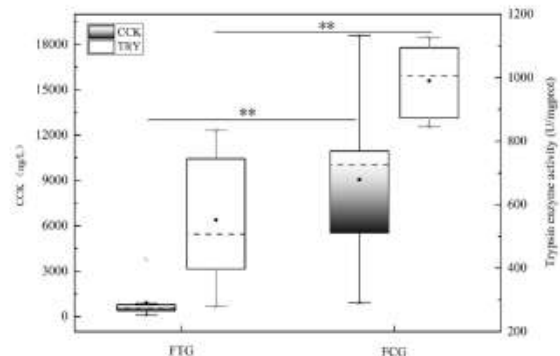


Figure 4. CCK content and trypsin activity of FTG and FCG are statistically analyzed between groups.

Dark grey represents CCK content, white box line represents trypsin (TRY). Data are presented as mean $\pm$ standard ($n = 3$ individuals, all feeding treatments $n = 3$ tanks) and $P < 0.05$ is used, "***" indicates an extremely significant difference.

The trypsin enzyme activity test revealed significantly higher levels in FCG on days 11 and 13, with peaks exceeding adjacent points (Fig. 5). The enzyme activity levels of all sampling points in FCG were higher than the baseline value. FTG exhibited two downward trends, reaching a low of 279.43 U/mgprot on the ninth day of fasting (Fig. 6). The enzyme activity level of FTG was lower than the baseline value on the fifteenth day of fasting (Fig. 6). The enzyme activity level of FCG was consistently higher than that of FTG, with a significant difference between the two groups (Fig. 4).

The CCK content in FCG exhibited two upward fluctuations, with the second peak occurring at DAH70, reaching

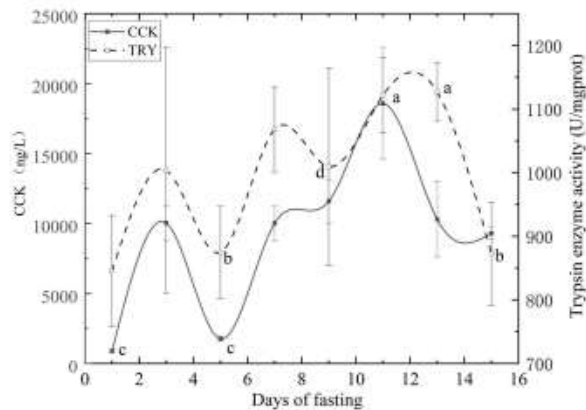


Figure 5. The feedback regulation curve between CCK and TRY in FCG. The solid line represents the value of CCK, and the dotted line represents the value of TRY.

Data were presented as mean $\pm$ standard deviation ($n = 3$ individuals, all groups $n = 3$ tanks). Lowercase letters represent intra-group significance, and $P < 0.05$ is used.

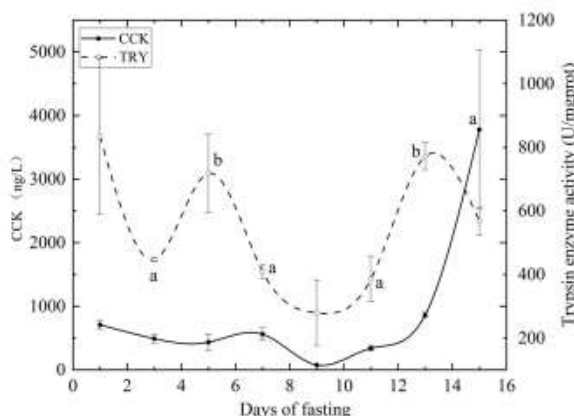


Figure 6. The feedback regulation curve between CCK and TRY in FTG. The solid line represents the value of CCK, and the dotted line represents the value of TRY.

Data were presented as mean $\pm$ standard deviation ($n = 3$ individuals, all groups $n = 3$ tanks). Lowercase letters represent intra-group significance, and $P < 0.05$ is used.

the highest value of 18583.85 ng/L (Figs. 3,5). In FTG, a continuous upward trend was observed after the lowest value of 77.41 ng/L on day 9. During the first 14 days, there was no significant fluctuation of CCK in FTG (Fig. 6). However, on the 15th day, the CCK content reached its highest value of 3777.26 ng/L, which was still significantly lower than that in FCG. When compared with the mean values (Fig. 4), the CCK content in FCG remained significantly higher than that in FTG. The final values of both groups were significantly higher than the initial values.

During the test, the control group experienced multiple negative feedback fluctuations, although these were not stable due to factors such as developmental stage. The fasting group, on the other hand, only showed a reverse trend

on the 15th day. Additionally, the CCK content did not change with the variation of trypsin in the early stage of the experiment.

HISTOLOGY

On the first day of fasting, intact intestinal chorion and a single layer of enterocytes were observed (Fig. 7a). The intestinal tissue structure appeared healthy, with goblet cells distributed among the intestinal epithelial cells. The muscular mucosae and basement membrane were closely arranged, and the connective tissue in the lamina propria was dense. On the ninth day of fasting (Fig. 7b), there was evacuation between the monolayer of enterocytes of Black bream. The number of goblet cells significantly decreased compared to the first day of fasting, and the muscular mucosa was loosely arranged within the chorion. On the 13th day of fasting, partial detachment of the striated border was noted (Fig. 7c). The degree of villus shrinkage increased, and the villus height decreased. The intestinal mucosa exhibited necrotic enterocytes that sloughed off, edematous lamina propria, and a looser shape than on the 9th day of fasting (Fig. 7b). On the 15th day of fasting, complete goblet cells were difficult to observe (Fig. 7d), although the area of a single goblet cell did not significantly change (Table 1). On day 15, the intestinal chorion had partially detached, the monolayer of columnar epithelial cells showed signs of vacuolation and degeneration, and the loss of the striated border was more pronounced than on the 13th day of fasting (Fig. 7c). The lamina propria's connective tissue was loose, and the mucous membrane and basement membrane were somewhat damaged. The mucosal muscle was loose and deformed, and the gap between the mucosal muscle and the lamina propria increased.

The Black bream lacks an independent liver and pancreas, instead having a combined hepatopancreas in its digestive system. After one day of fasting, the hepatocytes contained large lipid vacuoles, causing the nuclei to be displaced and the cells to become compact (Fig. 8a). By the third day, the liver cells displayed noticeable cord-like connections, with the dense pancreas embedded within them (Fig. 8b). On day 9, the volume of liver cells significantly decreased, intracellular fat decreased, intercellular spaces became loose, cells became irregularly arranged, cell borders blurred, and the alveolar cells of the pancreas shrunk (Fig. 8c). The degree of vacuolar degeneration of liver cells increased, pancreatic cells gradually shrank and became loose, and the nucleoli of pancreatic cells deformed and displaced on day 11 (Fig. 8d). After 13 days of fasting, the liver cell vacuoles were observed to have degenerated, the cell borders were blurred (Fig. 8e), the nucleoli were severely displaced, and the zymogen particles were reduced (Fig. 8f). On the last day of fasting, the hepatocytes had almost no cytoplasm, the intercellular space of liver cells increased, and the vacuoles were almost damaged and ruptured (Fig. 8h). Additionally, the pancreatic acinar cells gradually shrank (Fig. 8g).

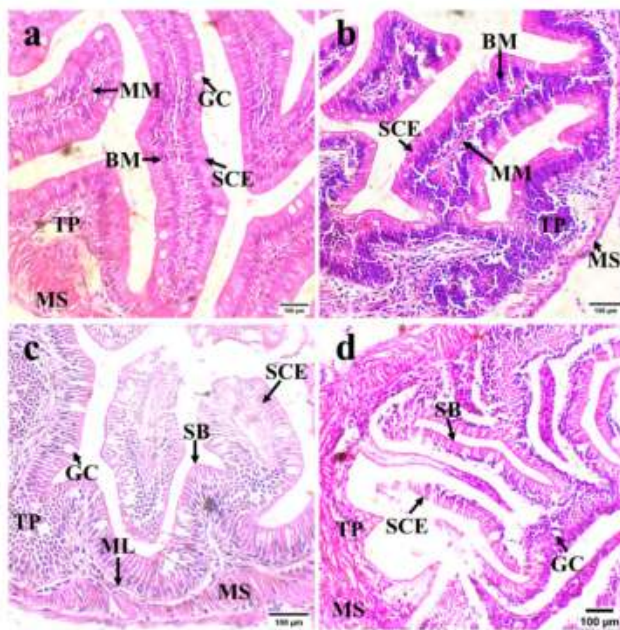


Figure 7. The first day of the fasting trial intestinal (middle and anterior intestinal tract) corresponds to figure a, figure b is the ninth day of fasting intestinal slices, figure c is the 13th day of fasting intestinal slices, and the 15th day of fasting intestinal slices was shown in figure d. HE staining, microscope eyepiece ($\times 40$) observation.

MS: muscular, SCE: simple columnar epithelium (enterocytes), ML: mucous layer, BM: basement membrane, TP: tunica propria, SB: striated border, MM: muscularis mucosae, GC: goblet cell

DAILY RHYTHM EXPERIMENT

There were no significant differences in body length or weight among the four groups in the daily rhythm experiment, and this trend continued throughout the experiment.

The CCK content of the first group (Fig. 9A) peaked at 17:00, which was the only peak at this time among the four groups. It then showed a downward trend until an upward trend began after the lowest level appeared at 1:00⁺¹ the next day. The CCK content at 1:00⁺¹ and 5:00⁺¹ the next day was significantly different from other nodes. The trypsin activity of group A tended to increase after the first feeding and to decrease after 17:00, but there was no significant difference between the trypsin activities in this group.

The CCK content of the second group exhibited a single peak at 21:00 (Fig. 9B), which was significantly different

from the other three groups. After 21:00, a significant decreasing trend was observed, which was also significantly different from the trypsin activity at 5:00⁺¹.

In group C, two peaks of CCK were observed at 13:00 and 1:00⁺¹ (Fig. 9C), with the CCK content at 1:00⁺¹ being significantly increased. Trypsin activity peaked at 13:00 and showed a continuous downward trend until 1:00⁺¹. Group C exhibited higher trypsin activity levels in the first 12 hours compared to the other groups.

The CCK content of the fasting group (Fig. 9D) reached its maximum at 1:00⁺¹ and continued to decrease to a minimum at 9:00. At 13:00, the CCK content of this group was significantly lower than that of the third group. The CCK content at 9:00 AM was 236.01 ng/L, which was significantly lower than that of the second and third groups. In the fasting group, trypsin activity exhibited two peaks at 17:00 and 1:00⁺¹. The peak at 1:00⁺¹ was significantly higher than the levels before and after, while a significantly low level was observed at 5:00⁺¹. The change in CCK content and baseline at 5:00⁺¹ was less than that at 9:00⁺¹.

DISCUSSION

The objective of this study was to examine the feedback-regulatory effects of short-term fasting and different feeding frequencies in a single day on the secretion of CCK and trypsin in juvenile Black bream. The juveniles had an initial body weight of 183.75 ± 61.16 mg. Although this was not strictly a growth test, the FCG still exhibited a better growth trend. This may suggest that the experimental individuals were still in the early stages of ontogeny.

SHORT-TERM FASTING TRIAL

Extended fasting can cause damage to the epithelium of the digestive tract and the hepatopancreas. The degree of damage to the digestive system is directly proportional to the duration of fasting. While there are numerous reports on fasting experiments, there are relatively few studies on the effects of fasting during the early stages of individual development. Hunger is a significant obstacle to individual development and a major negative factor, assuming the same commodity feed. To obtain more reasonable experimental data, this study selected Black bream in its juvenile stage as the test subject. The results of WGR and SGR indicate that FTG was significantly lower than FCG, suggesting that during the juvenile stage of Black bream, the individual was in the rapid development stage of nutrient accumulation. In

Table 1. Effects of short-term fasting on intestinal epithelial cells.

Days	Villus height (μm)	Villus width (μm)	Goblet cells area (μm)	Muscle layer thickness (μm)
1	932.47 ± 190.76^a	264.56 ± 21.28^a	672.45 ± 161.81^{ab}	167.42 ± 65.87^a
3	762.42 ± 68.75^b	256.93 ± 38.17^{ab}	993.00 ± 398.69^a	72.39 ± 18.06^b
9	663.48 ± 126.43^b	259.12 ± 50.46^{ab}	298.17 ± 108.76^b	52.16 ± 14.45^b
13	514.05 ± 49.97^c	221.91 ± 39.68^b	950.20 ± 529.88^a	75.30 ± 21.63^b
15	448.41 ± 87.25^c	141.26 ± 20.42^c	552.35 ± 336.93^b	137.44 ± 22.78^a

野生与养殖大鳍鲩肠刷状缘膜消化酶活性的比较

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摘要: 采用酶学分析法, 检测野生与养殖条件下大鳍鲩 (*Mystus macropterus*) 前、中、后肠刷状缘膜 (BBM) 上蔗糖酶、麦芽糖酶、碱性磷酸酶、 γ -谷氨酰转肽酶和 $\text{Na}^+ - \text{K}^+ - \text{ATP}$ 酶活性。结果显示: (1) $\text{Na}^+ - \text{K}^+ - \text{ATP}$ 酶在两种条件下大鳍鲩各肠段刷状缘膜活性大小不存在显著性差异, 且 $\text{Na}^+ - \text{K}^+ - \text{ATP}$ 酶的富集系数均大于 1.0; (2) 蔗糖酶、麦芽糖酶、 γ -谷氨酰转肽酶和碱性磷酸酶在两种条件下大鳍鲩各肠道刷状缘膜上分布规律相同, 其中, 肠段刷状缘膜蔗糖酶、麦芽糖酶和碱性磷酸酶比活力在各肠段分布均为前肠 > 中肠 > 后肠, 而 γ -谷氨酰转肽酶比活力分布为中肠 > 后肠 > 前肠; (3) 同种酶在不同条件下大鳍鲩同肠段的活性均为养殖大鳍鲩大于野生大鳍鲩, 仅碱性磷酸酶比活力在野生大鳍鲩中肠略大于养殖大鳍鲩中肠。

关键词: 大鳍鲩 (*Mystus macropterus*); 野生; 养殖; 肠上皮细胞刷状缘膜; 酶活性

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Study on the difference of enzymes activities of enterocytes brush border membrane of wild and farmed *Mystus macropterus*

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Abstract: The activities of five enzymes, sucrase, maltase, alkaline phosphatase, γ -glutamyltranspeptidase (γ -GT) and $\text{Na}^+ - \text{K}^+ - \text{ATP}$ of the enterocytes brush border membrane (BBM) of wild and farmed *Mystus macropterus* was investigated by means of enzymes analyses. The results showed that: (1) The activity of $\text{Na}^+ - \text{K}^+ - \text{ATP}$ in wild and that in farmed *M. macropterus* was not significant different from each other in all regions of the intestine, and both of the enrichment factors of $\text{Na}^+ - \text{K}^+ - \text{ATP}$ were higher than 1.0; (2) The distribution characteristics of sucrase, maltase, alkaline phosphatase and γ -glutamyltranspeptidase (γ -GT) of the enterocytes brush border membrane (BBM) were the same, as well as, the activities of sucrase, maltase and alkaline phosphatase were foregut > midgut > hindgut, meanwhile activity of γ -glutamyltranspeptidase (γ -GT) was midgut > hindgut > foregut. (3) The data rules of activities of the same enzymes in different regions of the intestine in these two fishes were that activities of the farmed *M. macropterus*'s enzymes were higher than the wild *M. macropterus*'s, except that the wild *M. macropterus*'s alkaline phosphatase activity in the midgut was higher than farmed *M. macropterus*'s.

Key words: *Mystus macropterus*; wild; farmed; enterocytes brush border membrane; enzymes activity

大鳍鲩 (*Mystus macropterus*) 又叫石扁头、江鼠等, 为鲇形目鲇科鲇属鱼类, 广泛分布于嘉陵江、金沙江、长江干流及其支流等。其具有肉质细嫩, 味道鲜美, 无肌间刺, 可食部分大, 不易死亡, 适温范围较广的特点。近年来大鳍鲩资源急剧下降,

为了保护天然资源和满足市场需求, 其人工繁育、养殖技术的研究已日益受到人们的重视^[1]。然而, 大鳍鲩繁殖习性制约了其人工繁殖苗种规模的快速扩大, 而提高大鳍鲩苗种成活率和生长率非常重要。因此, 研究不同生长条件下其酶活力变化特

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征, 配制出合适的大鳍鲩苗种生长饵料是当务之急。动物肠刷状缘膜(enterocytes brush border membrane, BBM)高度富集多种水解酶, 即肠刷状缘膜消化酶, 在食物的消化过程中起着关键性的作用^[2-3]。本试验选择生活在不同环境中的大鳍鲩, 通过对其肠道蔗糖酶、麦芽糖酶、 γ -谷氨酰转肽酶、碱性磷酸酶和 Na^+/K^+ -ATP酶等消化酶的研究, 了解野生与养殖条件下大鳍鲩酶活力变化规律, 为研究其消化生理及饲料研制等提供科学依据。

1 材料与方法

1.1 试验鱼

养殖条件下大鳍鲩: 2014年7月, 从重庆市东平水产养殖有限公司采购养殖条件下2龄大鳍鲩40尾, 放置西南大学鱼类工厂化养殖车间的微流水玻璃缸中, 水温(26.0 ± 1) $^{\circ}\text{C}$, 将鱼停食暂养24 h, 体长(26.08 ± 3.29)cm, 体重(130.56 ± 38.07)g。

野生条件下大鳍鲩: 2014年9月, 从嘉陵江合川段收集2龄大鳍鲩40尾, 相同条件处理, 体长(17.55 ± 4.59)cm, 体重(49.85 ± 32.70)g。

1.2 样品制备

1.2.1 初始肠粗酶液的制备

首先在冰盘上将鱼逐尾解剖, 迅速分离出肠道, 立即放入用生理盐水冻成的冰盘中, 用镊子和小剪刀剔除表面脂肪组织, 用4 $^{\circ}\text{C}$ 冰生理盐水冲洗, 将肠道分为前肠、中肠和后肠, 然后剪开肠道, 再次用4 $^{\circ}\text{C}$ 冰生理盐水冲洗后, 用4 $^{\circ}\text{C}$ 的双蒸水将肠道冲洗干净, 滤纸吸干各肠段水分, 再将已分好的肠段平均剪成三小段, 取中间段分别代表整段肠道的前肠段、中肠段和后肠段。根据鱼体大小和肠道样品量, 由13尾鱼的同一肠段组成一个混合样品, 每个样品均设3个平行组。取每个混合样品4.0 g, 溶于4 $^{\circ}\text{C}$ 、pH 7.0的50 mmol/L冰冷甘露醇(以2 mmol/L Tris-HCl配制)缓冲液40 mL中, 用组织匀浆机匀浆, 制得初始匀浆液。将初始匀浆液装入离心管内, 以3000 r/min 4 $^{\circ}\text{C}$ 离心10 min, 弃沉淀, 取上清液, 即肠粗酶液^[4]。取适量肠粗酶液保存于-80 $^{\circ}\text{C}$ 冰箱中, 用于酶活力和蛋白质含量测定。

1.2.2 肠刷状缘膜制备

准备数个1.0 mL离心管, 加入0.99 mL肠粗酶液上清液, 再加入0.01 mL CaCl_2 , 然后进入

4 $^{\circ}\text{C}$ 的水浴中振荡10 min, 再在4 $^{\circ}\text{C}$ 、3000 r/min离心10 min, 取上清液, 将上清液采用14000 r/min、4 $^{\circ}\text{C}$ 离心30 min后去掉上清液, 在沉淀中加入0.99 mL的甘露醇缓冲液, 使沉淀摇匀悬浮后, 再在14000 r/min离心力作用下离心30 min, 去掉上清液, 得到白色沉淀, 加入50 mmol/L的冰甘露醇(以2 mmol/L Tris-HCl配制, pH 7.1)0.25 mL, 使沉淀完全溶解, 即为肠刷状缘膜, 并保存于-80 $^{\circ}\text{C}$ 冰箱中, 用于酶活力和蛋白质含量测定^[5-6]。

1.3 酶活性测定

鱼体肠道各段的蔗糖酶、麦芽糖酶、 γ -谷氨酰转肽酶、碱性磷酸酶和 Na^+/K^+ -ATP酶比活力的测定参照南京建成生物工程研究所的试剂盒使用说明书进行, 用考马斯亮蓝法测定蛋白含量。蔗糖酶和麦芽糖酶活力单位定义为在37 $^{\circ}\text{C}$ 、pH 6.0的条件下, 每毫克蛋白组织每分钟水解1 nmol相应物质, 即U/mg prot。 γ -谷氨酰转肽酶活力单位定义为每克组织蛋白与基质在37 $^{\circ}\text{C}$ 作用10 min释放对硝基苯胺1 μmol , 即U/g prot。碱性磷酸酶活力单位为金氏单位/g prot, 一个金氏单位定义为每克组织蛋白在37 $^{\circ}\text{C}$ 与基质作用15 min产生1 mg酚。 Na^+/K^+ -ATP酶活力单位规定为每小时每毫克组织蛋白的组织中ATP酶分解ATP产生1 μmol 无机磷的量, 即微摩尔分子磷/毫克蛋白/小时($\mu\text{molPi}/\text{mg prot}/\text{hour}$)。

1.4 数据分析

试验数据用平均值 $\pm$ 标准差(Mean $\pm$ SD)表示, 并采用SPSS19.0统计软件进行所得数据多重比较、Duncan's检验和独立样本 T 检验, 显著性水平0.05。

2 结果与分析

2.1 野生与养殖大鳍鲩肠粗酶液消化酶活性比较

由表1, 野生大鳍鲩肠粗酶液前肠和中肠蔗糖酶比活力之间不存在显著性差异, 与后肠差异性显著, 养殖大鳍鲩肠粗酶液蔗糖酶比活力分布规律为前肠>中肠>后肠, 三段肠之间均具有显著性差异。两种条件下大鳍鲩三段肠粗酶液麦芽糖酶比活力同为前肠>中肠>后肠, 三段肠之间均具有显著性差异。野生大鳍鲩 γ -谷氨酰转肽酶比活力在三段肠之间无显著性差异, 养殖大鳍鲩前肠显著性大于中肠和后肠, 中肠和后肠之间不存在显著性差异。野生大鳍鲩三段肠粗酶液碱性磷酸酶之间均具

循环流水槽养殖草鱼与池塘精养草鱼营养品质比较

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摘 要: 为了解循环流水槽养殖草鱼与池塘精养草鱼营养品质的差异,选取相同饲料源的循环流水槽养殖草鱼[平均体重:(723.89±71.54) g,平均体长:(340.86±8.88) mm]和池塘精养草鱼[平均体重:(680.35±155.13) g,平均体长:(334.00±21.40) mm]各 12 尾,测定形态指标与肌肉剪切力、质构特性、色泽以及常规营养成分、氨基酸、脂肪酸和矿物元素含量。结果显示:1) 循环流水槽养殖草鱼的硬度、黏性、咀嚼性、回复性和剪切力均显著高于池塘精养草鱼($P<0.05$)。2) 循环流水槽养殖草鱼的肌肉水分含量显著低于池塘精养草鱼($P<0.05$),肌肉粗脂肪含量低于池塘精养草鱼,但差异不显著($P>0.05$),肌肉粗蛋白质和粗灰分含量均高于池塘精养草鱼,但差异不显著($P>0.05$)。3) 循环流水槽养殖草鱼的肌肉氨基酸总量(TAA)、必需氨基酸总量(TEAA)和呈味氨基酸总量(TDAA)高于池塘精养草鱼,但差异不显著($P>0.05$)。循环流水槽养殖草鱼和池塘精养草鱼肌肉中 TAA 分别为 77.11%和 76.61%,TEAA 和 TDAA 分别占 TAA 的 40.87%和 40.43%、46.10%和 46.06%。4) 循环流水槽养殖草鱼肌肉中不饱和脂肪酸(UFA)占脂肪酸总量的百分比(69.68%)高于池塘精养草鱼(68.44%),但差异不显著($P>0.05$)。循环流水槽养殖草鱼和池塘精养草鱼肌肉中饱和脂肪酸(SFA)占脂肪酸总量的百分比分别为 21.78%和 18.58%,两者差异显著($P<0.05$)。循环流水槽养殖草鱼肌肉中未检测到二十碳五烯酸(EPA),二十二碳六烯酸(DHA)含量为 0.44%;池塘精养草鱼肌肉中 EPA 含量为 0.10%,DHA 含量为 0.55%。5) 循环流水槽养殖草鱼肌肉中钾、镁、铁、铜含量显著高于池塘精养草鱼($P<0.05$),钠、锌显著低于池塘精养草鱼($P<0.05$)。由此得出,循环流水槽养殖草鱼的营养品质总体上优于池塘精养草鱼。

关键词: 循环流水槽养殖;草鱼;肌肉品质;质构特性;氨基酸;脂肪酸

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草鱼(*Ctenopharyngodon idellus*)属硬骨鱼纲,鲤形目,鲤科,雅罗鱼亚科,草鱼属,和青鱼、鲢鱼、鳙鱼并称为“四大家鱼”,栖息于平原地区的江河湖泊^[1]。目前草鱼养殖有较多模式,不同的养殖模式会影响到草鱼的产量和肌肉品质^[2]。池塘内循环流水槽养殖模式(又称“跑道鱼”)是在传统池塘精养模式上发展而来的,该模式于 2012 年从美

国引进,并在国内大力推广^[3],养殖面积和范围越来越大。2018 年 8 月全国水产技术推广总站发布了“跑道鱼”养殖模式示范工作的通知^[4],进一步规范了其养殖技术。

目前对池塘内循环流水槽养殖模式的研究主要涉及养殖技术^[5]、养殖规模、经济效益^[6]等宏观方面,对肌肉品质研究的报道较少。朱春艳等^[7]

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研究显示流水槽养殖鳊鱼腹腔脂肪量明显偏少,且品质优于常规池塘养殖鳊鱼,而有关流水槽养殖草鱼的品质情况未见报道。鉴于此,本试验拟对循环流水槽养殖草鱼与池塘精养草鱼的肌肉品质和质构特性等指标进行分析与评价,比较循环流水槽养殖草鱼与池塘精养草鱼营养品质的差异,以便进一步了解不同养殖模式对水产品品质的影响,并为养殖生产中草鱼品质的改善提供一定的理论依据。

1 材料与方法

1.1 试验材料

1.1.1 试验样品鱼来源

试验样品鱼均来自于重庆市潼南区生态渔业健康养殖技术集成试验示范基地,所选个体为2

龄鱼,健壮无病害,2种养殖模式草鱼苗种均来自于潼南本地。选取相同饲料源的循环流水槽养殖草鱼[平均体重:(723.89 ± 71.54) g,平均体长:(340.86 ± 8.88) mm]和池塘精养草鱼[平均体重:(680.35 ± 155.13) g,平均体长:(334.00 ± 21.40) mm]各12尾,其中6尾用于测定含肉率,6尾用于测定其他指标。养殖期间2种养殖模式的试验鱼均投喂通威草鱼专用膨化配合饲料,饲料的粗蛋白质含量 $\geq 30.0\%$,粗纤维含量 $\leq 12.0\%$,粗脂肪含量 $\geq 3.0\%$,粗灰分含量 $\leq 15.0\%$ 。每天07:00—08:00、11:00—12:00和17:00—18:00各投喂1次饲料,每次投喂以1 h吃完为准。从2018年8月初开始,每14 d测定1次水质,共监测8次。2种养殖模式下养殖池主要水质指标见表1。

表1 养殖过程中的养殖池主要水质指标

Table 1 Principal water quality parameters of culture ponds during aquaculture period

项目 Items	溶解氧含量 DO content/ (mg/L)	pH	透明度 Diaphaneity/ cm	总氮含量 TN content/ (mg/L)	氨氮含量 NH ₃ -N content/ (mg/L)	亚硝酸盐氮含量 NO ₂ -N content/ (mg/L)	化学需氧量 COD/ (mg/L)
循环流水槽 Circulating flume	5.83 ± 0.38	7.53 ± 0.21	28.76 ± 3.39	3.80 ± 0.16	1.17 ± 0.20	0.05 ± 0.01	9.11 ± 0.81
精养池塘 Intensive culture pond	4.74 ± 0.52	7.31 ± 0.19	30.06 ± 3.27	4.56 ± 0.43	1.41 ± 0.25	0.08 ± 0.02	10.55 ± 0.38

1.1.2 养殖环境条件

循环流水槽养殖系统位于基地内,养殖面积40亩(1亩 ≈ 666.67 m²),有砖混钢筋结构循环流水槽6条(25 m $\times$ 5 m),单条面积125 m²,水流速度为0.1~0.3 m/s。流水槽上游安装有气推增氧装置,每套设备由罗茨鼓风机与曝气管相连接组成,为底部增加氧气^[8]。精养池塘养殖面积8亩,为传统土池养殖,安装有2台功率为3 kW的叶轮增氧机。

1.1.3 样品处理

以钝物击打头部将试验鱼击晕,测量体长、体重,解剖,去除内脏后称空壳重。2种养殖模式草鱼各取6尾放于-18℃冰箱保存,用于含肉率的测定,另各取6尾剔除脊椎两侧的肌肉,用于其他指标测定。每次取样后将肌肉放置于-18℃冰箱保存,用于后续指标测定。

1.2 测定仪器

FA1004A电子天平(精度为0.000 1 g),上海精天电子仪器有限公司;DHG-9240A电热恒温鼓风干燥箱,上海齐欣科学仪器有限公司;KDN-812定氮仪,上海纤检仪器有限公司;XL-1200马弗炉,苏州江东精密仪器有限公司;TA.XT Plus型物性测试仪,英国Stable Micro Systems公司;UltraScan PRO色差仪,美国HunterLab公司;配有WBS(warner-bratzler shear)刀具的TX-700剪切力物性测定仪,嘉盛(香港)科技有限公司;安捷伦GC7890气相色谱仪,美国安捷伦科技公司;日立L-8900氨基酸自动分析仪,日本日立公司;iCE 3300 AAS原子吸收光谱仪,赛默飞世尔科技(中国)有限公司。

1.3 测定指标与方法

1.3.1 形态指标测定

体长使用直尺直接测量;体重、空壳重使用电

续表 6

氨基酸 Amino acids	循环流水槽养殖草鱼 Grass carp cultured in circulating flume	池塘精养草鱼 Grass carp cultured in intensive culture pond
丙氨酸 Ala ^{★●}	4.98±0.15	5.01±0.12
半胱氨酸 Cys [●]	0.28±0.01	0.28±0.01
缬氨酸 Val [☆]	4.10±0.11	4.10±0.08
甲硫氨酸 Met [☆]	2.39±0.06	2.40±0.06
异亮氨酸 Ile [☆]	3.83±0.13	3.81±0.09
亮氨酸 Leu [☆]	6.77±0.22	6.77±0.15
酪氨酸 Tyr ^{★●}	2.95±0.12 ^a	2.92±0.06 ^b
苯丙氨酸 Phe ^{☆☆}	3.35±0.27 ^a	3.19±0.06 ^b
赖氨酸 Lys [☆]	7.49±0.30 ^a	7.21±0.21 ^b
组氨酸 His [●]	2.28±0.11 ^a	2.17±0.03 ^b
精氨酸 Arg [●]	5.07±0.13	5.05±0.14
脯氨酸 Pro [●]	2.57±0.19	2.59±0.16
氨基酸总量 TAA	77.11±2.46	76.61±1.84
呈味氨基酸总量 TDAA	35.65±1.36	35.41±1.69
必需氨基酸总量 TEAA	31.52±1.44	31.10±1.34
半必需氨基酸总量 THEAA	2.95±0.12	2.92±0.06
非必需氨基酸总量 TNEAA	45.59±1.57	45.77±1.82
呈味氨基酸总量占氨基酸总量百分比 TDAA/TAA	46.10±0.21	46.06±0.10
必需氨基酸总量占氨基酸总量百分比 TEAA/TAA	40.87±0.29	40.43±0.15
半必需氨基酸总量占氨基酸总量百分比 THEAA/TAA	3.82±0.04	3.80±0.02
非必需氨基酸总量占氨基酸总量百分比 TNEAA/TAA	59.13±0.29	59.53±0.15

☆;必需氨基酸 essential amino acids; ★;呈味氨基酸 delicious amino acids; ●;非必需氨基酸 non-essential amino acids。

2.5 肌肉脂肪酸组成

如表 7 所示,在循环流水槽养殖草鱼肌肉中共检测到 10 种脂肪酸,其中饱和脂肪酸(SFA)3 种,占脂肪酸总量的 21.78%,不饱和脂肪酸(UFA)7 种,占脂肪酸总量的 69.68%,UFA 中单不饱和脂肪酸(MUFA)有 2 种,占脂肪酸总量的

37.32%,多不饱和脂肪酸(PUFA)占脂肪酸总量的 32.36%;在池塘精养草鱼肌肉中共检测到 11 种脂肪酸,其中 SFA 3 种,占脂肪酸总量的 18.58%,UFA 8 种,占脂肪酸总量的 68.44%,UFA 中 MUFA 有 2 种,占脂肪酸总量的 31.19%,PUFA 占脂肪酸总量的 37.25%。

表 7 循环流水槽养殖草鱼和池塘精养草鱼肌肉脂肪酸组成的比较

Table 7 Comparison of muscle fatty acid composition between grass carp cultured in circulating flume and intensive culture pond (n=6)

脂肪酸 Fatty acids	循环流水槽养殖草鱼 Grass carp cultured in circulating flume	池塘精养草鱼 Grass carp cultured in intensive culture pond
豆蔻酸 C14:0	0.88±0.16 ^a	0.74±0.13 ^b
棕榈酸 C16:0	17.10±0.26 ^a	14.33±0.60 ^b
棕榈烯酸 C16:1	3.05±0.28	2.35±0.41
硬脂酸 C18:0	3.84±0.02	3.50±0.24
油酸 C18:1n-9	34.33±0.71 ^a	28.83±1.72 ^b

Analysis on Nutritional Quality between Grass Carp Cultured in Circulating Flume and Intensive Culture Pond

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Abstract: This experiment was conducted to analyze the difference of nutritional quality between grass carp cultured in circulating flume and intensive culture pond. Twelve grass carp cultured in circulating flume [average body weight: (723.89 ± 71.54) g, average body length: (340.86 ± 8.88) mm] and intensive culture pond [average body weight: (680.35 ± 155.13) g, average body length: (334.00 ± 21.40) mm] and fed the same feed source were selected, respectively, which were used to analyze morphological indices, and muscle texture characteristics, color, shearing force, the contents of common nutrients, amino acids, fatty acids and mineral elements. The results were as follows: 1) the hardness, gumminess, chewiness, resilience and shearing force of grass carp cultured in circulating flume were significantly higher than those of grass carp cultured in pond intensive ($P < 0.05$). 2) The muscle moisture content of grass carp cultured in circulating flume was significantly lower than that of grass carp cultured in pond intensive grass carp ($P < 0.05$), the muscle crude fat was lower than that of grass carp cultured in pond intensive grass carp, and the muscle crude protein and ash contents were higher than those of grass carp cultured in pond intensive grass carp, but no significant difference in muscle crude fat, crude protein and ash contents ($P > 0.05$). 3) The total amount of amino acids (TAA), the total amount of essential amino acids (TEAA) and the total amount of delicious amino acids (TDAA) in muscle of grass carp cultured in circulating flume were higher than those of grass carp cultured in pond intensive grass carp, but the differences were not significant ($P > 0.05$). The TAA of grass carp cultured in circulating flume and intensive culture pond was 77.11% and 76.61%, and the TEAA and the TDAA accounted for were 40.87% and 40.43%, 46.10% and 46.06%, respectively, of the TAA. 4) The proportion of unsaturated fatty acids (UFA) in total fatty acids (69.68%) in muscle of grass carp cultured in circulating flume was higher than that of grass carp cultured in intensive culture pond (68.44%), but no significant difference ($P > 0.05$). The proportion of saturated fatty acids (SFA) in total fatty acids in muscle of grass carp cultured in circulating flume and intensive culture pond was 21.78% and 18.58%, respectively, and the difference was significant ($P < 0.05$). Eicosapentaenoic acid (EPA) was not detected and the content of docosahexaenoic acid (DHA) was 0.44% in muscle of grass carp cultured in circulating flume, and the contents of EPA and DHA in muscle of grass carp cultured in intensive culture pond were 0.10% and 0.55%, respectively. 5) The contents of potassium (K), magnesium (Mg), iron (Fe) and copper (Cu) in muscle of grass carp cultured in circulating flume were significantly higher than those of grass carp cultured in intensive culture pond ($P < 0.05$), while the contents of sodium (Na) and zinc (Zn) in muscle were significantly lower than those of grass carp cultured in intensive culture pond ($P < 0.05$). To sum up, the nutritional quality of grass carp cultured in circulating flume is slightly better than that of grass carp cultured in intensive culture pond. [Chinese Journal of Animal Nutrition, 2020, 32(2):948-958]

Key words: cultured in circulating flume; grass carp; muscle quality; texture characteristics; amino acids; fatty acids

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(责任编辑 管景颖)

灰裂腹鱼肌肉营养分析与评价

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摘要: 采用常规生化分析方法对灰裂腹鱼(*Schizothorax griseus*)肌肉进行分析并评价。分析的主要指标: 常规营养成分、氨基酸、脂肪酸和矿物质元素。灰裂腹鱼的含肉率为71.22%, 肌肉鲜样的粗蛋白含量高达22.98%, 粗脂肪含量低, 为1.35%。其干样含有18种氨基酸, 氨基酸总量(TAA)为77.02%, 包括8种人体必需氨基酸(EAA), 总量为31.69%, 必需氨基酸的构成比例符合FAO/WHO的标准。灰裂腹鱼的第一限制性氨基酸是色氨酸(Trp), 赖氨酸(Lys)的氨基酸评分(AAS)和化学评分(CS)都最高, 分别为2.28和1.76, 呈味氨基酸的总量为34.73%。肌肉干样的饱和脂肪酸(SFA)、单不饱和脂肪酸(MUFA)、多不饱和脂肪酸(PUFA)含量分别为24.4%、41.3%、25.6%; 一共检测到8种矿物质元素铁、锰、锌、铜、钙、镁、钾、钠, 其比例合理。结论: 灰裂腹鱼味道鲜美, 具有较高的营养价值和巨大的开发潜能。

关键词: 灰裂腹鱼(*Schizothorax griseus*); 常规营养成分; 氨基酸; 脂肪酸; 矿物质元素

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Analysis and evaluation for the nutrition components of *Schizothorax griseus* muscles

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Abstract: Conventional biochemical analysis methods were adopted to analyze and evaluate the content of nutrients, amino acids, fatty acids and minerals in *Schizothorax griseus* muscles. The analysis results showed that the flesh content was 71.22%, the rough protein in wet samples of muscle reached 22.98%, with the fat was only 1.35%. There were 18 amino acids in the dry samples of muscles, the total content of amino acid contained 77.02%, in which essential amino acid took up 31.69%. The EAA composition met the Food and Agriculture Organization/World Health Organization standard. According to the nutritional evaluation on acid score (AAS) and chemical score (CS), the first limited amino acid was tryptophan (Trp), with the lysine (Lys) having the highest score of 2.28 and 1.76, respectively. The content of flavor amino acids was 34.73%. The content of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) was 24.4%, 41.3% and 25.6%, respectively. Total 8 minerals (Fe, Mn, Zn, Cu, Ca, Mg, K, Na) were tested in this study. The proportion of these minerals were reasonable. According to the results, *S. griseus* is nutritious and delicious in taste, it has high nutritional value and great potential to develop.

Key words: *Schizothorax griseus*; routine nutrients composition; amino acids; fatty acids; minerals

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灰裂腹鱼(*Schizothorax griseus*)属硬骨鱼纲鲤形目裂腹鱼属,是一种特产于亚洲高原地区的鱼类,主要分布于澜沧江、南盘江、北盘江、乌江、金沙江、龙川江和大盈江等湍急的支流中^[1]。灰裂腹鱼味道鲜美,具有较高的经济价值,近几年由于水利工程的兴建导致生境改变,过度捕捞和环境污染等问题,野生灰裂腹鱼资源遭到严重破坏,2016年被列入了中国脊椎动物红色名录的濒危种^[2],保护该种质资源刻不容缓。科研人员克服重重困难,于2015年3月云南省渔业科学研究院对灰裂腹鱼的人工繁殖工作才得以突破。目前,国内外对灰裂腹鱼的研究较少,仅限于分类学和形态学^[3,4],尚未见有关其营养成分的研究报道,本研究采用生化分析方法对灰裂腹鱼的肌肉营养成分进行分析评价,为其人工养殖和开发提供一定的理论依据。

裂腹鱼属的常见种类齐口裂腹鱼(*Schizothorax prenanti*)和重口裂腹鱼(*Schizothorax davidi*)是我国重要的经济鱼类,其人工养殖技术已经比较成熟,对两者的分类学、组织学^[5]、遗传学^[6]和营养学^[7]研究都比较深入。研究表明,齐口裂腹鱼和重口裂腹鱼都是肉质细嫩、味道鲜美、营养丰富的优质食用鱼类。目前,灰裂腹鱼的人工养殖技术不及齐口裂腹鱼和重口裂腹鱼成熟,还有待研究和提高。

1 材料与方法

1.1 试验材料

本试验所用的灰裂腹鱼均为野生成鱼,2016年8月,由云南省大理白族自治州鱼种站的工作人员在金沙江捕获,经充氧、加冰包装处理运回西南大学渔业资源与保护学实验室。9尾灰裂腹鱼平均体重(56.60 ± 9.54) g,平均体长(17.21 ± 3.09) cm。

1.2 试验仪器

日立L-8900氨基酸自动分析仪(日本日立公司);安捷伦气相色谱仪(美国安捷伦科技公司);定氮仪(上海昕瑞仪器仪表有限公司);TAS-990原子吸收分光光度计(北京普析通用仪器有限责任公司);LGJ-10真空冷冻干燥机(北京松源华兴科技发展有限公司);电子天平($d=0.0001$ g,上海舜宇恒平科学仪器有限公司)。

1.3 试验方法

随机选取7尾试验用鱼,麻醉致死,去皮,取

脊椎两侧的全部肌肉,用去离子水洗净,用滤纸吸干后切成2-3 cm肉片,在小型绞肉机中捣碎,将样品分为3份,分别用于测定常规营养成分、氨基酸和脂肪酸和矿物质含量^[8]。

1.3.1 含肉率的测定

擦干鱼体表水分,测定全长、体长和鱼体质量。按常规方法解剖分离性腺、内脏、鳃、鳍、(鳞片+皮肤)和骨骼等非肉质部分后称量净重,之后放在蒸锅内隔水蒸至肉与骨骼能完全分离,取出冷却至室温后去除肌肉等可食部分,洗净骨骼和鳍条,滤纸吸干后称重。分别计算含肉率以及内脏、鳃、骨骼、鳍条、鳞片和皮肤的占有率。鱼体肉质部分重量占鱼体总质量的百分比称为含肉率;鱼体内脏,鳃,骨骼,鳍条,鳞片和皮肤的质量占鱼体总质量的百分比分别称为内脏占有率,鳃占有率,骨骼占有率,鳍条占有率,鳞片和皮肤占有率^[9]。

灰裂腹鱼含肉率的计算公式:

$$\text{含肉率} = (\text{空壳质量} - \text{鳃质量} - (\text{鳞片} + \text{皮肤}) \text{质量} - \text{骨骼质量} - \text{鳍条质量}) / \text{总质量} \times 100\%$$

1.3.2 常规营养成分的测定

水分的测定采用直接干燥法(GB 5009.3-2010);粗蛋白的测定采用凯氏定氮法(GB 5009.5-2010);粗脂肪的测定采用索氏抽提法(GB 5009.6-2003)。

氨基酸的测定使用日立L-8900氨基酸自动分析仪(日本日立公司)依据《食品中氨基酸的测定》(GB 5009.124-2003)进行测定。

脂肪酸测定采用GB/T22223-2008《食品中总脂肪酸、饱和脂肪(酸)、不饱和脂肪(酸)的测定》水解提取-气相色谱仪法,结果用相对比值表示。

钠、钾元素含量的测定采用火焰发射光谱法,参考GB/T5009.91-2003《食品中钾、钠的测定》;铁、镁、锰元素含量的测定采用原子吸收分光光度法,参考GB/T5009.90-2003《食品中铁、镁、锰的测定》;钙元素含量的测定采用原子吸收分光光度法,参考GB/T5009.92-2003《食品中钙的测定》;铜元素含量的测定采用原子吸收光谱法,参考GB/T5009.13-2003《食品中铜的测定》;锌元素含量的测定采用原子吸收光谱法,参考GB/T5009.14-2003《食品中锌的测定》。

1.4 数据处理及评价标准

使用EXCEL和SPSS 20.0对实验数据进行分

盐酸环丙沙星对厚颌鲂幼鱼 GOT 和 GPT 活性的影响

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摘要: 采用一次性胸腔注射法, 研究了盐酸环丙沙星对(30±2.3) g/尾的厚颌鲂(*Megalobrama pellegrini*) 幼鱼谷草转氨酶(GOT)和谷丙转氨酶(GPT)活性的影响。结果显示: 随着给药时间的延长, 厚颌鲂的肝脏、肌肉和鳃组织 GOT 活性呈现不规则变化; 随着给药浓度的增加, 则出现先升后降, 然后趋于稳定的趋势; 随着给药时间的延长, 试验前期肝脏 GPT 活性基本保持不变, 试验中期以后则出现先升后降, 然后趋于稳定的趋势, 肌肉和鳃组织 GPT 活性呈现先降后升再降的趋势; 随着给药浓度的增加, 总体则基本呈现不规则变化; 盐酸环丙沙星对厚颌鲂幼鱼各组织 GOT 和 GPT 活性大小依次为肝脏>鳃>肌肉; 通过 GOT 活性变化可判断, 肝脏、肌肉和鳃组织的休药期分别为 29、27 和 32 d; 通过 GPT 活性变化可判断, 肝脏、肌肉和鳃组织的休药期分别为 37、28 和 34 d。

关键词: 盐酸环丙沙星; 厚颌鲂(*Megalobrama pellegrini*); GOT; GPT; 休药期

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The effects of ciprofloxacin hydrochloride on GPT and GOT activity in juveniles of *Megalobrama pellegrini*

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Abstract: The effects of ciprofloxacin hydrochloride on GPT and GOT activity in juveniles of *Megalobrama pellegrini* (30±2.3g) were studied by the method of disposable pleural injection, and the withdrawal period. The results showed that, during the trial period, GOT activity in liver, muscle and gill tissue were changed irregularly with the increment of time; With the increment of the concentration of administration, it decreased after increased firstly, and then tended stabilizing; With the increment of time, in the early test, GPT activity in liver were remained no changes, in the medium test, it increased firstly and then decreased, and stabilized, GPT activity in muscle and gill tissue increased after decreased firstly, and then decreased; With the increment of the concentration of administration, the base irregular changed, the order of, GOT and GPT activity were liver > gill tissue > muscle; according to the changes of GOT activity,

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the withdrawal period of liver, muscle, gill tissue were 24 d, 23 d, 27 d respectively; according to the changes of GPT activity, the withdrawal period of liver, muscle, gill tissue were 29 d, 27 d, 30 d respectively.

Key words: Ciprofloxacin Hydrochloride; *Megalobrama pellegrini*; GOT; GPT; Withdrawal period.

盐酸环丙沙星(Ciprofloxacin hydrochloride)为第三代喹诺酮类抗菌药品,具有抗菌谱广、抗菌活性强、耐药性低、药效较强等特点,对大部分革兰氏阴性菌有明显的抑制作用,但是近年来随着在临床上的广泛应用,关于其不良反应报道也随之增多^[1-3]。为了解盐酸环丙沙星对鱼类的影响,试验以厚颌鲂(*Megalobrama pellegrini*)幼鱼为研究对象,探讨了盐酸环丙沙星对厚颌鲂幼鱼肝脏、肌肉和鳃组织中的谷草转氨酶(GOT)和谷丙转氨酶(GPT)活性的影响,从而为喹诺酮类药物的安全用药、毒理学研究提供基础资料和科学依据。

1 材料与方法

1.1 试验对象

健康厚颌鲂幼鱼购自重庆东平水产养殖公司,平均质量为 (30 ± 2.3) g。试验前,采用1.5%食盐消毒5 min,消毒后,放于水箱中暂养7 d,暂养期间正常喂食,选择正常健康、无病无伤、规格一致的个体进行正式试验。

1.2 试验材料

水缸(圆柱形,规格为 $0.192\ 9\ \text{m}^3$)、玻璃匀浆器、电子天平($0.0001\ \text{g}$)、高速冷冻离心机、紫外可见分光光度计、冰箱、解剖工具、注射器。

生理盐水(0.86%),鱼血安(盐酸环丙沙星,净含量200 g,规格10%,休药期为 $500\ ^\circ\text{C} \cdot \text{d}$)购自北京大北农动物保健科技有限责任公司,考马斯亮蓝(G250)、GOT和GPT试剂盒均购自南京建成生物科技研究所。

1.3 试验方法

将盐酸环丙沙星粉剂溶解于去离子双蒸水中,制成混悬液,然后根据需要的浓度配置。经过预实验,设置6个浓度组,浓度分别为0、50、150、250、350和450 mg/kg鱼体重,0 mg/kg即为对照组,每组2个重复,每个重复20尾,2 d换水1次。试验水温为 $(25 \pm 1.7)\ ^\circ\text{C}$ 。试验期间,每天早晚各投喂1次,试验时采用一次性胸腔注射,注射剂量为0.2 mL/尾,对照组注射0.2 mL的生理盐

水。

1.4 样品采集与处理

在给药后的7、14、21、28和35 d采样,分别从每组取出2尾厚颌鲂幼鱼进行解剖;解剖时,取出鱼的鳃、肌肉和肝脏组织,滴加生理盐水,整个解剖过程在冰盘上进行以保持样品的鲜活;称重时,用预冷的去离子双蒸水冲洗干净后,用滤纸吸干再称重。

1.5 酶活性的测定

GOT、GPT酶活性严格按照南京建成试剂盒说明书的操作方法进行测定,组织蛋白质含量采用考马斯亮蓝法测定。

1.6 休药期的判定依据

本试验休药期的判定依据为,当其他浓度组的酶活性含量与对照组酶活性含量的平均值刚好达到同一水平时,即判定为此浓度时的休药期。

1.7 数据处理

数据处理采用Excel2003软件,单因素方差分析采用SPSS19.0统计软件,显著水平为 $P < 0.05$,符号不同时,表示差异显著;相同时表示差异不显著。

2 结果与分析

2.1 盐酸环丙沙星对厚颌鲂幼鱼肝脏组织酶活性的影响

盐酸环丙沙星对厚颌鲂幼鱼肝脏GOT活性的影响见表1。从表1中可看出,在35 d的试验期内,随着时间的延长,各浓度组基本呈现先降低后升高再降低,然后趋于稳定的趋势,150 mg/kg和250 mg/kg组,最大值出现在第7天($P < 0.05$),其他组则均出现在第21天($P < 0.05$);在0~450 mg/kg范围内,随着浓度的增加,第14、28和35天时,酶活性变化不明显,最大值均与对照组差异不显著($P > 0.05$);第7天,酶活性出现先升后降的趋势,最大值出现在150 mg/kg组($P < 0.05$);第21d时,则出现先升后降再升的趋势,最大值出现在450 mg/kg组($P < 0.05$)。

RESEARCH ARTICLE

Effects of integrated rice-frog farming on soil bacterial community composition

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ABSTRACT

Rice-fish integrated farming model has become one of the main directions for sustainable agricultural development due to its good ecological and environmental effects and ability to produce green rice (*Oryza sativa* L.) that meets the market's food safety needs. Its study characterizes soil bacterial community structure in this model for the improvement of planting and breeding technology and realization of ecological regulation. The community structure of soil microorganisms was compared between two models of rice-frog (black-spotted pond frog; *Pelophylax nigromaculatus* Hallowell, 1861) co-cropping (DW) and rice monoculture (DD) at tillering stage, full heading stage and maturity stage, to accumulate data for research on ecology of rice-frog integrated farming and provide a theoretical basis for optimization of this production technology. Main bacterial phyla occurring in rhizosphere soil of rice fields in both models under three stages were *Proteobacteria*, *Chloroflexi*, *Acidobacteriota*, *Bacteroidota*, *Firmicutes*, *Actinobacteriota*, *Nitrospirota* and *Desulfobacterota*. Compared to DD, DW group had more *Actinobacteriota* in the dominant phyla at maturity and less *Desulfobacterota* throughout the growth period. The main dominant genera were *norank_f Anaerolineaceae*, *norank_f norank_o norank_c Thermodesulfovibrionia*, *norank_f norank_o SBR1031*, *norank_f norank_o Vicinamibacterales*, *unclassified_k norank_d Bacteria* and *Thiobacillus*. There were five categories with significant difference, *norank_f Anaerolineaceae*, *norank_f norank_o SBR1031*, *norank_f norank_o Vicinamibacterales*, *Thiobacillus* and *unclassified_k norank_d Bacteria*; all were higher in relative abundance in DW than in DD group. It indicates that rice-frog crop model promotes the growth of these five types of bacteria, which have a boosting effect on rice growth. The bacterial Chao index and Shannon index of rhizosphere soil of rice field increased after introduction of frog in DW group. The two models showed less variation in soil bacterial community structure in root system from full heading to maturity stage, and co-cropping model showed less variation compared to rice monoculture.

Key words: *Oryza sativa*, *Pelophylax nigromaculatus*, relative abundance, rice-frog farming, soil bacterial, tiller stage.

INTRODUCTION

Soil microorganisms, as the main participants of the ecosystem processes, actively participate in all aspects of the C and N cycle, such as C and N mineralization, nitrification, denitrification and other processes (Edwards et al., 2015). And through its own metabolism and turnover, it promotes nutrient cycling and plant effectiveness, becoming an important source of effective soil nutrients. Soil microbial diversity and function are the most sensitive indicators of soil quality (Zak et al., 1994). For the study of soil microbial community structure, the high-throughput sequencing technology used obtains a large number of gene sequences and can detect non-culturable and trace microorganisms, which has become an important tool for studying the

diversity of microbial communities (Han et al., 2016; Zhang et al., 2016). Rhizosphere is the direct interface of material and energy conversion between plant roots and soil (Prashar et al., 2014; Zhang et al., 2018), rhizosphere microorganisms are closely related to plant health, pest and disease defense and yield (Li et al., 2007). Some studies have shown that each plant has a specific rhizosphere microbiome (Zhang et al., 2017a), and the rhizosphere soil microbial diversity of different varieties of oleander differed (Zhang et al., 2017b). The rice (*Oryza sativa* L.) variety used in this experiment is giant rice, and it is important to study the composition and diversity of the rhizosphere soil microbial community of giant rice to ensure the production of giant rice and promote the development of giant rice industry.

Frog is a native amphibian in rice field, and introducing frog into rice field is a more ecological and natural rice planting mode. Many studies have shown that the introduction of frogs can effectively reduce the populations of rice pests such as rice lice, and improve the nutrient status of the soil (Chang et al., 2021). To a certain extent, it increases soil microbial biomass and soil dehydrogenase, peroxidase, acid phosphatase and other soil enzymes, thus promoting rice growth and seed yield of rice. In addition, the rice-frog crop model also has the potential to reduce greenhouse gas CH₄ emissions. Studies have shown that a variety of paddy ecological farming models have the ability to influence the structure of soil microbial communities, the soil microbial population and microbial biomass C in the rice-duck crop model were significantly increased (Dexin et al., 2005). And the integrated rice-loach farming has certain influence on soil microbial community structure (Zhao et al., 2021). Little research has been reported on the changes in soil microbial community structure of giant rice paddies with rice growth. This experiment compared the diversity characteristics of soil bacteria in the rhizosphere under two modes of rice-frog crop and paddy monoculture, and explored the dynamic changes of soil microbial community structure and composition in the rhizosphere of rice under the two cropping modes at different periods of rice growth, in order to reveal the internal relationship among frog metabolism, rice growth and soil microbial community structure.

MATERIALS AND METHODS

Experimental field description

The experimental site is located in Renshou City (29°51'15.58" N, 104°12'41.56" E), Sichuan Province, China. This area belongs to the central subtropical humid monsoon climate zone with an average annual temperature of 16.8 °C, an average annual rainfall of 1153.7 mm, an average annual number of rainfall days of 159 d, and an annual frost-free period of about 310 d.

The experimental field is the first year of implemented rice (*Oryza sativa* L.)-frog integrated farming. The experiment was designed with two treatments, the rice-frog integrated farming group (DW) and the rice monoculture group (DD), with three replicates of each treatment (six fields), each with an area of 667 m² and a 1.5 m wide ridge between the fields. Each rice-frog integrated field is surrounded by 1 m wide food table, of which the inner side is a 1 m wide and 50 cm deep ring ditch, and the central rice planting area of about 450 m². Each field is surrounded by an anti-escape net, which covers food table with one side 30 cm below the ring ditch and the other side 1 m above the food table. The rice, giant rice 'Jufeng No. 5', was transplanted on 20 May with a plant spacing of 30 cm × 30 cm. Rice harvesting was carried out on 29 September.

The breed of frog is the black-spotted pond frog (*Pelophylax nigromaculatus* Hallowell, 1861). About 100 000 tadpole fries were placed in each field on 5 April, and 2.5 ± 0.5 kg powdered forage was fed to each field every day, and the survival rate of tadpoles was about 30%. Tadpoles begin to metamorphose in mid to late May. During the metamorphosis period, tadpoles rely on the absorption of the tail as a source of nutrition and do not need to feed. After metamorphosis into juvenile frogs, feed small pellets with 40% animal protein according to 2%-3% of the frog's body weight. When they grow into adult frogs after 40 d, large pellets were fed with 38% animal protein according to 2%-3% of their body weight in the morning and evening. Fertilizer was not applied to rice-frog integrated fields, while rice monoculture fields were fertilized with base fertilizer and topdressing. The first time before rice transplanting, 450 kg ha⁻¹ potassium

sulfate type compound fertilizer (N:P:K = 24%:9%:18%) was applied, and the second time 40 d after rice transplanting, 90 kg ha⁻¹ were applied.

Soil sampling and measurements

Soil samples were collected on 1st April (before planting and raising), 27 June (tiller stage), 27 August (full heading stage) and 27 September (maturity stage) in 2021 respectively. In the tiller stage, DW group is recorded as aa1 and DD group is recorded as aa2. In the full heading stage, DW group is recorded as aa3 and DD group is aa4. In the maturity stage, DW group is recorded as aa5 and DD group is aa6. The five-point sampling method was used to select five points in the four corners and center of each paddy field, taking soil around the root system at 10-20 cm depth, then mixing the soil from the five points into one sample, with sample numbers DW1, DW2, DW3 and DD1, DD2, DD3. Samples were taken back to the laboratory in 5 mL centrifuge tubes and stored at -80 °C for microbial diversity analysis. In addition, take five points of soil from 10 to 20 cm and mix well, remove plant and animal residues, weeds and stone impurities and then use for soil physical and chemical property testing. Soil physical and chemical property, including soil pH, organic matter (OM), total N (TN), total P (TP), total K (TK), available P (AP), available K (AK) and alkali hydrolyzed N (AN), were measured with experimental methods according to Bao (2000).

Soil DNA extraction, PCR amplification and sequencing

The soil samples were weighed (0.3 g), extracted with the Rapid Soil Genomic DNA Extraction Kit (Sangon Biotech, Shanghai, China) and detected by 1% agarose gel electrophoresis, then stored at -20 °C in a refrigerator. The common primers 515F (5'-GTGCCAGCMGC-CGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') were used to amplify the high variant region V3 ~ V4 of 16S rRNA gene. Each sample was repeated three times, after which the PCR products were recovered by gel cutting using the AxyPrep DNA Gel Recovery Kit (Axygen Scientific, Union City, California, USA), eluted with Tris_HCl; detected by 2% agarose electrophoresis. The PCR products were detected and quantified by QuantiFluor, -ST Blue Fluorescence Quantification System (Promega Corporation, Madison, Wisconsin, USA), after the concentrations of each sample were normalized, mixed and sequenced. The sequencing platform was the MiSeq PE300 platform from Illumina (San Diego, California, USA).

Data processing and analysis

After 16S rRNA MiSeq sequencing, the quality of reads was quality-controlled filtered by using the software Trimmomatic and FLASH, and data filtering was completed after the operation process of removing low-quality bases and splice contamination sequences to obtain high-quality target sequences that can be used for subsequent analysis. The follow-up bioinformatics operations were done using USEARCH and mothur softwares, the source of the comparison data was Silva's bacterial database, and the data statistics and graphing were done using R language. The differences in soil physicochemical property data, microbial diversity index data and relative abundance of major genera in the paddy fields under the two models were analyzed by one-way ANOVA and Duncan method using SPSS 16.0 software (IBM, Armonk, New York, USA), and $P < 0.05$ indicated significant differences.

RESULTS AND DISCUSSION

Physical and chemical properties

There was nonsignificant difference ($P > 0.05$) in the physical and chemical properties of the soil between the two modes before the start of the experiment (Table 1). Compared with the DD group, the DW group had significantly higher soil total N (TN) and alkaline N (AN) content throughout the growth period, higher soil total P (TP) and available P (AP) content at the full heading and maturity stages, and higher soil available (AK) content at the tiller stage. From the tillering stage to the full heading stage, soil pH, organic matter (OM), total N (TN) and total P (TP) increased significantly in both groups, and from the full heading stage to the maturity stage, soil TN and TP, alkaline N content decreased significantly in both groups.

Table 1. Soil properties of paddy field in two models. The same lowercase letters mean nonsignificant differences ($P > 0.05$) in this indicator between the two modes during the same period, while different lowercase letters mean significant differences ($P < 0.05$) between two modes. The same uppercase letters mean nonsignificant differences ($P > 0.05$) in the same mode at different times, while different uppercase letters mean significant differences ($P < 0.05$) in the same mode at different times. OM: Organic matter; TN: total N; TP: total P; TK: total K; AN: alkali hydrolyzed N; AP: available P; AK: available K; DW: rice-frog co-cropping; DD: rice monoculture; aa1, aa3, aa5: average values of DW group of three fields for tillering, full heading and maturity stages, respectively; aa2, aa4, aa6: average values of DD group of three fields for tillering, full heading and maturity stages, respectively.

Period	Treatments	pH	OM	TN	TP	TK	AN	AP	AK
			g kg ⁻¹				mg kg ⁻¹		
Before planting and raising	DW	8.01 ± 0.05 ^{aB}	11.61 ± 0.24 ^{aC}	0.60 ± 0.03 ^{aD}	0.38 ± 0.02 ^{aC}	16.60 ± 0.93 ^{aB}	66.87 ± 5.48 ^{aC}	6.80 ± 0.40 ^{aC}	77.00 ± 0.64 ^{aC}
	DD	8.03 ± 0.10 ^{aA}	11.40 ± 0.05 ^{aC}	0.66 ± 0.08 ^{aC}	0.36 ± 0.04 ^{aB}	16.93 ± 0.67 ^{aA}	67.67 ± 6.25 ^{aD}	7.03 ± 0.35 ^{aC}	79.87 ± 1.51 ^{aC}
Tiller stage	aa1	7.47 ± 0.09 ^{aC}	23.13 ± 0.50 ^{aB}	1.46 ± 0.07 ^{aC}	0.42 ± 0.01 ^{aBC}	16.17 ± 1.01 ^{aB}	166.67 ± 2.40 ^{aA}	12.87 ± 1.42 ^{aB}	142.67 ± 5.36 ^{aA}
	aa2	7.50 ± 0.06 ^{aB}	18.57 ± 0.43 ^{bBC}	1.10 ± 0.04 ^{bB}	0.40 ± 0.02 ^{aB}	14.47 ± 0.09 ^{bB}	114.67 ± 1.45 ^{bB}	12.10 ± 0.64 ^{aB}	114.00 ± 4.62 ^{bB}
Full heading stage	aa3	7.87 ± 0.09 ^{aB}	31.37 ± 0.35 ^{aA}	2.33 ± 0.10 ^{aA}	0.78 ± 0.13 ^{aA}	17.73 ± 0.48 ^{aA}	184.33 ± 6.39 ^{aA}	52.03 ± 4.94 ^{aA}	146.00 ± 2.52 ^{aA}
	aa4	7.87 ± 0.15 ^{aA}	27.10 ± 2.44 ^{bA}	1.59 ± 0.12 ^{bbA}	0.52 ± 0.04 ^{bA}	16.97 ± 0.85 ^{aA}	142.00 ± 2.65 ^{bA}	17.60 ± 1.96 ^{bA}	143.67 ± 4.10 ^{aA}
Maturity stage	aa5	8.17 ± 0.07 ^{aA}	26.33 ± 5.34 ^{aAB}	1.86 ± 0.22 ^{aB}	0.54 ± 0.02 ^{aB}	17.80 ± 0.32 ^{aA}	130.67 ± 18.41 ^{aB}	48.67 ± 1.14 ^{aA}	128.00 ± 8.89 ^{aB}
	aa6	7.37 ± 0.03 ^{bB}	14.63 ± 0.52 ^{bC}	0.81 ± 0.03 ^{bC}	0.39 ± 0.05 ^{bB}	15.40 ± 0.67 ^{bB}	85.93 ± 8.43 ^{bC}	11.27 ± 0.80 ^{bB}	113.40 ± 11.66 ^{bB}

Overview of MiSeq sequencing and diversity analysis

The average total sequence numbers obtained from MiSeq sequencing of rice-frog co-cropping and rice monoculture root soils were $4.21 \times 10^5 \pm 6.27 \times 10^3$ and $3.66 \times 10^5 \pm 1.04 \times 10^4$ at tiller stage, $2.44 \times 10^5 \pm 1.84 \times 10^4$ and $2.21 \times 10^5 \pm 9.32 \times 10^3$ at full heading stage, $2.62 \times 10^5 \pm 2.44 \times 10^4$ and $2.00 \times 10^5 \pm 1.41 \times 10^4$ at maturity stage. After removing the primers, ambiguous bases and sequences with chimeras, the total effective sequence number finally obtained was 5 938 318, with sequence lengths ranging from 360 to 400 and average fragment length of 376 bp. At the operational taxonomic unit (OTU) classification level of 97% similarity, the sequencing coverage reached about 98%, reflecting that the sequencing results were sufficiently representative of the real situation of the microorganisms in the samples. In addition, to ensure the same sequencing depth of the samples used, we selected 172 155 (i.e., the one with the lowest number of valid sequences among all samples) valid sequences from each sample for normalization and used them for the next data analysis.

The results of soil microbial diversity analysis of rice roots under two modes showed that the Chao index and Shannon index of soil bacteria in the root system of the rice-frog co-cropping mode were significantly higher than the rice monoculture mode in all periods ($P < 0.05$) (Figure 1). The Chao index and Shannon index of soil bacteria in both models were highest at the tiller stage and significantly higher than those at the full heading stage and maturity stage ($P < 0.05$), and there was nonsignificant change in the two groups from the full heading stage to maturity stage. It indicates that farmed frogs can increase the diversity and richness of soil microorganisms in paddy fields, and the changes of soil microorganisms in paddy fields are more obvious at the tiller stage.

Among the 22 086 OTUs obtained at 97% similarity, 4612 OTUs were shared by six groups, accounting for 20.88% of the total OTUs, indicating that there were some differences in the composition of microbial species among the groups (Figure 2). The OTU species specific to the DW group were higher than those in the DD group in all three periods, indicating that frog farming can increase soil microbial richness in rice fields. Both the DW group and the DD group had the most unique OTU species at the tiller stage, accounting for 13.15% and 8.35%, respectively. The number of unique OTU species in both groups decreased sharply at the full heading stage, and did not change much at the maturity stage.

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稻蛙综合种养模式对水肥平衡的影响研究

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摘要:为探究稻蛙综合种养模式对稻田水质、土壤理化性质以及巨型稻产量的影响,采集分蘖期、齐穗期、成熟期、青蛙越冬前期、越冬中期和越冬后期水样和土壤样品,通过内梅罗综合指数法评价土壤肥力。结果显示:与巨型稻单作相比,稻-蛙综合种养模式在水稻生长期水体溶氧量较低,在齐穗期达到最低为 (3.54 ± 0.18) mg/L, pH 在成熟期显著升高 ($P < 0.05$), 水体中总氮、总磷和硝态氮含量在齐穗期及以后各期均显著升高 ($P < 0.05$)。稻蛙综合种养模式下土壤 pH 和有机质在成熟期及以后各期均显著高于水稻单作模式 ($P < 0.05$)。水稻单作田在成熟期及越冬期土壤 pH 显著降低,说明水稻单作模式可能导致土壤 pH 降低,在越冬中期 pH 为 6.9, 而引入黑斑蛙后 pH 能较好地维持在 7.9。稻蛙共作模式土壤全氮、全钾和碱解氮在整个时期均显著高于水稻单作模式 ($P < 0.05$)。对土壤肥力进行综合评价结果显示:稻蛙共作田在齐穗期时土壤肥力指数最高,为Ⅱ级,说明此时土壤肥沃;其余时期稻蛙共作田与水稻单作田土壤肥力指数均为Ⅲ级,属一般肥力水平,且稻蛙共作田的肥力指数均高于水稻单作田。稻蛙共作模式下水稻的千粒重和有效穗粒数均显著高于稻田单作模式 ($P < 0.05$), 而水稻结实率无明显差异。综上所述,稻蛙共作模式土壤肥力可以满足水稻生长需要,且对水稻生长有促进作用,水稻单作模式土壤全磷含量缺乏,应结合当地实际,稳定氮肥,增施磷肥。

关键词:内梅罗肥力指数; 土壤肥力; 水稻品质

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Effects of Integrated Rice-frog Farming Model on Water and Fertilizer Balance

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Abstract: In order to explore the effects of integrated rice-frog farming on the water quality, soil physicochemical properties, and yield of giant rice in paddy fields, this experiment collected water and soil samples from the tillering stage, full heading stage, maturity stage, early overwintering stage, mid overwintering stage, and late overwintering stage of frogs, and evaluated soil fertility using the Nemero comprehensive index method. The results showed that compared with monoculture of giant rice, the integrated rice-frog farming had a lower dissolved oxygen content in the water during the rice growth period, reaching the lowest level at (3.54 ± 0.18) mg/L during the full heading period. The pH significantly increased during the maturity period ($P < 0.05$), and the total nitrogen, total phosphorus, and nitrate nitrogen content in the water significantly increased during and after the full heading period ($P < 0.05$). The soil pH and organic matter under the integrated rice-frog farming were significantly higher than those under the single cropping mode of rice ($P < 0.05$) during and after the maturity stage. The soil pH in rice monoculture fields significantly decreases during the mature and overwintering stages, indicating that the rice monoculture model may lead to a decrease in soil pH. The pH is 6.9 in the mid-winter stage, while the introduction of the black spotted frog can maintain a pH of 7.9, which can effectively prevent a decrease in soil pH. The total nitrogen, total potassium, and alkaline nitrogen in the soil of the rice frog intercropping model were significantly higher than those of the rice monoculture model throughout the entire period ($P < 0.05$). A comprehensive evaluation of soil fertility was conducted, and the soil fertility index of the integrated rice-frog farming was the highest at the full heading stage, reaching level II, indicating that the soil was fertile at this time. In the remaining periods, the soil fertility index of rice-frog co-culture and rice monoculture fields were both at level

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III, belonging to the general fertility level, and the fertility index of rice-frog co-culture fields was higher than that of rice monoculture fields. The thousand-grain weight and effective number of grains per panicle of rice under the rice frog co-culture mode were significantly higher than those under the rice field monoculture mode ($P < 0.05$), while there was no significant difference in rice setting rate. From this, it can be seen that the soil fertility of the integrated rice-frog farming can meet the needs of rice growth and promote rice growth. However, the soil total phosphorus content in the rice monoculture mode is insufficient. Therefore, it is necessary to combine local conditions, stabilize nitrogen fertilizer, and increase phosphorus fertilizer application.

Key words: nemero fertility index; soil fertility; rice quality

巨型稻,由中国科学院历经十余年创制的超高产优质水稻新种质,是在现有优异水稻种源的基础上,运用突变体筛选、籼粳亚种间及野生稻远缘杂交等一系列生物遗传育种技术,获得的有较大生物量的水稻新种质材料,使我国水稻产量上升一个新的台阶,对我国粮食安全有重大意义^[12]。

稻田综合种养模式是一种生态的养殖模式,养殖的经济物种可以为水稻提供所需的营养物质。罗衡等^[3]研究表明,稻鳖综合种养能够提升土壤中总氮、总磷、铵态氮和硝态氮的含量。倡国涵等^[4]研究表明,长期稻虾轮作显著提高了水稻土壤速效钾、总磷、速效氮、有机碳、全氮和硝态氮含量。目前关于稻田综合种养土壤肥力随水稻生长的变化趋势及其评价的研究较少,致使难以科学地评估不施肥情况下经济物种粪便所提供的营养物质能否满足水稻生长的需要及某一最小因子的限制因素。因此,对稻田综合养种的土壤肥力进行评价分析,具有重要的科学价值和现实意义。

本文以稻蛙综合种养模式为例,对稻蛙共作及水稻单作水质和土壤理化性质进行测定,根据内梅罗肥力指数评估土壤综合肥力状况,并对水稻品质进行测定,旨在揭示稻蛙综合种养模式及水稻单作模式土壤肥力状况和对水稻的影响,为我国稻田综合种养模式的可持续发展提供依据和指导。

1 材料与方法

1.1 试验设计

试验场地位于中国四川省仁寿县(29° 51' 15.58" N, 104° 12' 41.56" E),属于中亚热带湿润季风气候区,年平均气温 16.8 °C,年平均降雨量 1 153.7 mm,年平均降雨日数为 159 d,全年无霜期大约 310 d。

试验田为第一年实行稻蛙综合种养的田块,对稻田本地土壤肥力指标进行检测(表 1)。在本底调查基础上,选择各田块之间的水质、土壤状况无显

著性差异($P>0.05$)、受人类活动影响小及地势平坦的田块作为试验田和对照田。

试验设计稻蛙共作组(DW)和水稻单作组(DD) 2 个处理,每个处理各 3 个重复,共 6 块田,每块田的面积为 667 m²,田块之间有 1.5 m 宽的田埂。每块稻蛙共作田的四周为 1 m 宽的食台,食台内侧是宽 1m、深 50 cm 的环沟,中心是水稻种植区,面积约为 450 m²,田块示意图如图 1 所示。每块共作田四周设置防逃网。

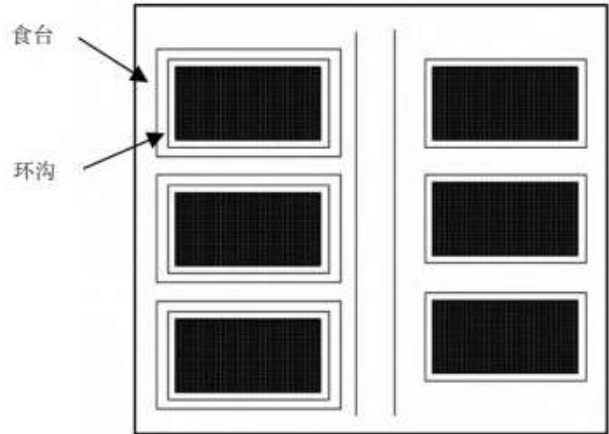


图 1 稻蛙综合种养模式田块示意图
Fig. 1 Field schematic diagram of integrated rice-frog farming

表 1 试验田本底数据
Tab. 1 Background data of experimental fields

土壤指标 soil indicators	检测结果 detection result
pH	8.01~8.13
有机质 OM /(g·kg ⁻¹)	11.61~12.40
全氮 TN /(g·kg ⁻¹)	0.60~0.76
全磷 TP /(g·kg ⁻¹)	0.36~0.40
全钾 TK /(g·kg ⁻¹)	15.67~17.60
碱解氮 AN /(mg·kg ⁻¹)	61.39~73.92
有效磷 AP /(mg·kg ⁻¹)	6.40~8.38
速效钾 AK /(mg·kg ⁻¹)	77.00~82.87

评价,在水稻种植前,总氮和总磷含量为低水平,有机质、碱解氮、有效磷和速效钾含量均为中下水平,说明土壤养分供给能力弱,需要施基肥。而稻蛙共作组稻田土壤全氮、全磷、全钾、有效磷、速效钾和碱解氮含量均高于水稻单作组,说明单作稻田即使施肥后,土壤养分供给能力仍低于稻蛙共作田。这与禹盛苗等^[21]研究发现稻鸭种养模式内土壤有机质、全氮、有效磷和速效钾含量水平显著高于不养鸭种稻;喻记新等^[22]发现稻虾综合种养田面土壤的TN和TP含量较水稻单作稻田呈现了升高趋势;余经纬等^[23]发现稻田生态综合种养模式稻田中土壤的全氮和碱解氮含量均显著高于单季水稻种植模式,速效钾含量显著高于单季和双季水稻种植模式等的研究结果相同。

稻蛙共作田土壤pH在成熟期显著大于水稻单作田($P < 0.05$),原因可能是单作田中肥料分解产生的碳酸根等酸性离子在土壤中累积使土壤pH降低,而稻蛙共作田中蛙的粪便中含无机盐,可以中和一些酸性离子,防止土壤酸化^[24]。两种模式下土壤有机质含量总体上属中上水平,在分蘖期和齐穗期,两种模式土壤有机质含量并无显著差异($P > 0.05$),但在成熟期稻蛙共作田土壤有机质含量显著高于水稻单作田。土壤有机质在分解转化的过程中,产生的有机酸和腐殖质对土壤矿物部分有一定溶解能力,可以促进矿物分化,有利于土壤养分的有效化^[25]。其中产生的腐殖质是一种胶体,具有巨大的比表面和表面能,具有极强的保水保肥能力^[26]。因此,稻蛙综合种养模式对于土壤保水保肥,来年的水稻生长有促进作用。稻蛙共作中土壤全磷含量水平为中上,而水稻单作土壤中全磷含量为中下,两种模式下土壤有效磷含量均为中上。因为水稻种植前,土壤磷含量较低,为了满足水稻生长需要,所施肥料中多为可溶性磷,即有效磷。土壤中磷元素与矿物元素之间的化学作用使得磷元素易与土壤中的金属元素结合,形成难以被植物利用的难溶性磷^[27]。因此,为了提高肥料中磷的利用率,建议将磷肥与有机肥混合施用,减少磷肥与土壤接触面。

稻蛙共作田中土壤全氮的丰瘠水平为高,碱解氮为很高,水稻单作稻田土壤全氮和碱解氮丰瘠水平均为中上。蛙排泄物中的氮素大多为铵态氮和易水解的蛋白质,均为碱解氮,因此共作田土壤碱解氮达到很高水平^[28]。两种模式稻田土壤氮素均在齐

穗期含量最高,其原因可能是水稻根系吸收养分的方法有扩散、质流与截取等^[29]。在水稻生命周期中由于蒸腾量比较大,因此,通过质流方式运输到根表的养分量也比较多。而水稻的蒸腾量与光照、温度等有关,在其成熟期温度较齐穗期降低,因此通过质流方式运输到根表的养分量也降低。当质流养分的速度大于根系的吸收速度时,养分可在根表面积累;反之,则根表面会形成养分的亏缺区;而当二者速度相等时,根表面的养分浓度可以保持不变。试验组在越冬期土壤全氮和碱解氮含量均显著升高,而对照组土壤全氮和碱解氮含量均较稳定,说明稻蛙综合种养模式对于土壤保氮能力更强,对第二年作物栽种提供足够氮源。从水稻生长期看,全钾与速效钾含量的变化也不大。原因是土壤中的钾主要分为矿物钾、固定态钾、交换性钾和水溶性钾4种形态^[30],而水溶性钾所占比例最少,约为1%^[31],因此,能被植物直接利用的钾也最少,钾的含量变化也不大。土壤全氮、全磷、有机质、碱解氮和速效磷含量均呈现先升高后降低的趋势,原因可能是随着青蛙生长,食量变大,代谢废物增多,因此土壤营养素含量先增加,到成熟期时,气温变低,青蛙打洞准备越冬场所,活动减少,所产生的代谢废物也减少,因此土壤肥力降低^[32,33]。水稻单作田在施追肥之后,由于水稻生长消耗土壤中养分,没有养分补充,呈现先升高后降低的趋势。

土壤肥力是反映养分效应及稻田生态系统生产力的综合指标,通过内梅罗指数法计算的土壤肥力指数,既反映了限制植物生长最小因子定律,又综合考虑了各指标。只有在齐穗期,稻蛙共作田块的肥力指数为Ⅱ级肥沃,其余均为Ⅲ级一般。全磷在水稻单作田中的土壤肥力指数最低,表明土壤全磷含量高低是水稻单作田土壤肥力的主要影响因素,稻蛙共作田土壤肥力指数因子均相对较高。

3.3 结论

(1) 稻蛙综合种养模式能够提高水体pH,防止水体pH降低。

(2) 稻蛙综合种养模式能够提高土壤肥力,为水稻生长提供养分,提高水稻品质。

(3) 水稻单作稻田在施肥时应根据土壤中磷素与钾素的缺乏状况,结合当地实际,稳定氮肥,增施磷肥和钾肥。

生态养殖

三种稻渔生态种养对稻田土壤化学性质和酶活性的影响

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[摘 要] 稻渔生态种养是中国“十四五全国渔业发展规划”鼓励发展的一种绿色生态的养殖模式, 为探究不同时期不同生态种养模式稻田土壤理化性质和酶活性的变化特征, 丰富稻渔生态种养的基础理论, 该研究在检测水稻不同生长期 12 种与土壤肥力和健康状况相关指标的基础上, 分析评价重庆地区 3 种稻田生态种养对稻田土壤肥力的影响。结果显示: 3 种稻田生态种养模式在水稻不同生长期土壤 pH 显著高于对照组 ($P < 0.05$), 但土壤有机质、全氮、碱解氮、速效钾等营养元素以及土壤碱性磷酸酶活性在大部分时期均显著低于对照组 ($P < 0.05$)。进一步分析显示, 3 种稻田生态种养存在土壤肥力和养分等级下降的问题, 其中稻蟹鸭组土壤肥力和养分等级下降最为严重, 其次是稻蟹组, 稻虾组相对较好。说明养殖动物密度低投喂量少的稻田生态种养存在不同程度的土壤肥力下降趋势, 建议适当提高动物养殖密度和投饵量, 并根据水稻不同生长期对营养成分的需要, 适当补充有机肥, 有利于稻田水肥平衡。

[关键词] 稻渔生态种养模式; 稻田; 土壤化学性质; 酶活性

[中图分类号] S811.5; S153; S964.2

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稻渔综合种养是利用稻田生态关系, 通过共生或轮作方式, 融合渔业生产、畜禽养殖等多产业协同的一种增效、生态、环保的循环生态农业发展模式^[1], 也是中国十三五、十四五渔业规划鼓励发展的生态渔业模式^[2-3]。2024 年, 中国稻渔综合种养面积 3.07×10^6 hm^2 , 产出各类水产品 4.43×10^6 t ^[4], 是中国除池塘外的第 2 大淡水养殖水域类型。为满足有机产品生产标准, 进一步提高稻田产值和生产效益, “少施肥”、“不打农药”、主要利用稻渔互利共作关系的稻渔生态种养模式^[5-7]越来越受从业者的欢迎。然而, 生产实践中发现, 部分稻渔生态种养从业者在生产管理过程中, 片面追求“少施肥、少投饵”, 从而忽视了对作物生长和产量至关重要的稻田土壤肥力和健康状况^[8-9]。长此以往, 必然导致水稻产量下降, 甚至会影响中国粮食安全。因此, 急需对目前常见的主要稻渔生态种养模式的土壤肥力和健康状况进行较为全面的评价, 以便为稻渔生态种养模式的健康发展奠定良好的基础。

在土壤质量的分析和评价指标中, 除了土壤 pH、有机质以及氮磷钾等常见营养素的含量外, 主要由微生物、植物根系分泌物和动植物残体分解产生的土壤酶^[10]也参与土壤养分循环、有机质分解和能量流动^[11], 从而影响土壤肥力和健康状况。为此, 本研究通过测定水稻不同生长期土壤全氮、碱解氮、全磷、有效磷、全钾、速效钾、pH 和有机质等土壤化学因子, 以及碱性磷酸酶、碱性蛋白酶、蔗糖酶和过氧化氢酶等土壤酶活性等 12 种与土壤肥力和健康状况相关的指标, 并采用主成分分析法对稻蟹鸭、稻蟹和稻虾等 3 种重庆地区常见的稻田生态种养对土壤肥力进行了对比分析和评价, 以期对稻渔生态种养模式的健康发展提供基础资料。

1 材料与方法

1.1 试验稻田及条件

试验稻田位于重庆市潼南区重庆稻梦空间农业

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发展有限公司的养殖基地(105°38'56"N, 30°7'31"E), 海拔约 300 m, 属亚热带湿润季风气候, 气候温和, 热量丰富、雨量充沛、日照充足、四季宜耕。基地稻田总面积约为 $3.00 \times 10^5 \text{ m}^2$, 土壤类型为川渝地区常见的石灰性紫色土, 分类属遂宁组(J3sn), 种植水稻品种为籼型 3 系杂交水稻-宜香优 2115。本研究所用试验稻田每块面积约为 $3.80 \times 10^4 \text{ m}^2$, 给排水系统完善, 稻田内设环沟, 环沟内保持全年有水。试验稻田形态和结构如图 1 所示。

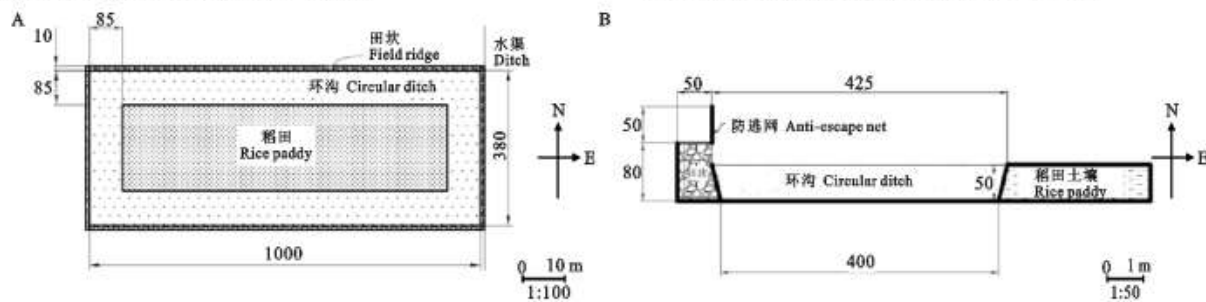


图 1 试验稻田平面(A)和结构图(B)

Fig.1 Plan (A) and structural diagrams (B) of the experimental rice paddy

表 1 不同养殖对象放养和收获情况一览表

Table 2 Stocking and harvesting details for different co-culture species

种类 Species	组别 Group	放养时间 Time	放养密度 Density	放养规格 Specifications	收获时间 Harvest time	备注 Note
麻鸭 <i>Tadorna</i>	RTD	2020 年 7 月	25 只/667 m ²	25~30 日龄	2021 年 2 月	主要以田里的螺蛳、杂草以及收割时散落的稻谷为食, 视情况投喂玉米等农作物作为补充饲料投喂量约为 5 kg/667 m ²
克氏原螯虾 <i>Procambarus clarkii</i>	RS	2020 年 5 月	20 kg/667 m ²	300 尾/kg	2020 年 9 月	前期主要以稻田水体的饵料生物为食, 后期加投部分人工饵料如饼粕、谷粉等, 每天投喂一次, 以 2 h 内吃完为准, 未被收获留作次年亲本
中华鳖 <i>Trionyx sinensis</i>	RT	2019 年 11 月	100 只/667 m ²	150 g/只	未收获	鱼苗作为中华鳖饲料, 种类包括鲤、鲫、鲢、鳙, 在 2019 年开始放养, 次年 5 月和 8 月持续少量补充, 在 2020 年底开始少量收获。中华鳖养殖周期为 3 a, 试验结束时仍未收获
鱼苗 Fish larvae	RTD	2019 年 11 月 2020 年 5 月 2020 年 8 月	20 kg/667 m ² 10 kg/667 m ² 5 kg/667 m ²	3~5 cm 3~5 cm 3~5 cm	2020 年 2020 年 2020 年	

1.3 田间管理

试验组及对照组均在 2020 年 5 月份采用直播方式播种水稻, 且只施基肥不施追肥。稻田育秧前保持稻田土壤湿润, 待水稻萌发到 3 叶后开始蓄水。之后依据水稻生长特点调控水位, 水稻成熟期(2020 年 8 月)将稻田水位降低到田面以下, 整个水稻生长期不使用农药和除草剂。水稻于 2020 年 9 月 28 日用机器收割, 收割的秸秆直接全部粉碎还田; 再生稻于 10 月 20 日号收割, 收割后秸秆同样粉碎还田。

1.4 样品采集

试验期间分别于 2020 年 7 月 15 日(水稻抽穗期, S1)、2020 年 8 月 20 日(水稻成熟期, S2)、2020 年 11 月 8 日(水稻秸秆还田后, S3)、2020 年 12 月 15 日(再生稻收割秸秆再次还田后, S4)、2021 年 5 月 20 日(水稻育秧前期, S5), 按照《土壤养分循环实

1.2 试验设计

选择远离公路, 受人类活动影响小、地势平坦, 且水质、土壤状况无显著性差异($P > 0.05$)的田块作为试验田。试验共分 1 个对照组 3 个稻渔生态综合种养组, 对照组(CK)为传统的水稻单作, 试验组分别为稻-鳖-鸭模式组(rice-turtle-duck, RTD)、稻-鳖模式组(rice-turtle, RT)、稻-虾模式组(rice-shrimp, RS)。对照组和试验组均设置 3 个重复。各组种苗放养及收获详情如表 1 所示。

地采样调查方法^[12]进行土壤样品采集。样品采集时选择稻田土面平整无污染、水稻生长良好无病害、环境稳定无干扰的区域作为采样点, 采样时在每种模式每个田内随机设置 3 个采样区域, 每个区域内采用 5 点法取表层 0~20 cm 的土壤, 并将 3 个采样区域的样品混匀后收集 2 kg 土壤样品装于密封袋, 避光保存, 运回实验室。采集的样品先在试验室条件下阴干, 然后挑出石粒、杂草等杂质后对样品进行研磨, 最后用 40 目与 60 目筛网分筛后用于各项指标检测。

1.5 样品测定

预处理后的土壤样品送往西南大学资源与环境学院进行检测, 检测指标包括 pH、全氮(total nitrogen, TN)、碱解氮(alkali-hydrolyzable nitrogen, AN)、全磷(total phosphorus, TP)、有效磷(available phosphorus, AP)。

ble phosphorus, AP)、全钾(total potassium, TK)、速效钾(available potassium, AK)和有机质(soil organic matter, SOM)。土样土壤酶活测定采购自南京建成生物工程研究所土壤酶活性试剂盒进行测定,检测指标包括土壤碱性磷酸酶(soil alkaline phosphatase, S-ALP)、土壤碱性蛋白酶(soil alkaline protease, S-ALPT)、土壤蔗糖酶(soil sucrase, S-SC)和土壤过氧化氢酶(soil catalase, S-CAT)。

1.6 主成分分析

首先对不同生态种养模式下的12个土壤特征指标进行相关性分析,然后对相关系数绝对值大于0.3的指标进行KMO和巴特利特球形度检验,之后对KMO值大于0.6,巴特利特球形度检验的相伴概率 $P < 0.01$ 的土壤指标采用SPSS软件进行主成分分析,并计算各主成分特征值及方差贡献率,依此按以下公式计算各模式的 F 值。

$$F = \frac{\lambda_1 f_1 + \lambda_2 f_2 + \lambda_3 f_3}{\lambda_1 + \lambda_2 + \lambda_3}$$

式中: $\lambda_1, \lambda_2, \lambda_3, \dots, \lambda_n$ 分别为对应各主成分的贡献率, $f_1, f_2, f_3, \dots, f_i$ 分别为各主成分的特征值。

1.7 数据处理与分析

试验获得的各项数据采用Microsoft Excel 2016进行处理,并使用Origin 2021制作图表,运用IBM SPSS 23采用单因素(One-way ANOVA)方差分析,所得结果采用平均数±标准差($\bar{x} \pm s$)表示,以 $P < 0.05$ 作为显著差异标准。

2 结果与分析

2.1 不同模式对稻田土壤理化性质的影响

不同模式、不同时期稻田土壤的理化指标检测结果见表2。

2.1.1 不同模式对稻田土壤全氮和碱解氮含量的影响 由表2可见:对照组土壤全氮和碱解氮含量在大部分时期均显著高于3种稻渔生态种养模式($P < 0.05$)。而在3种稻渔生态种养模式中,稻鳖鸭组土壤全氮和碱解氮最低,且呈现先下降后上升的平缓变化趋势;稻鳖组土壤全氮和碱解氮总体呈下降趋势;稻虾组全氮和碱解氮总体呈上升趋势。

2.1.2 不同模式对稻田土壤全磷和有效磷含量的影响 由表2可见:不同模式土壤总磷和有效磷的变化规律和趋势不一致,土壤全磷总体呈上升趋势,土壤有效磷在S1~S4基本保持平稳,S5则显著上升($P < 0.05$)。

2.1.3 不同模式对稻田土壤全钾和速效钾含量的影响 由表2可见:对照组土壤全钾大部分时期均显著高于3种稻渔生态种养模式($P < 0.05$)。在3种稻渔生态种养模式中,稻鳖鸭组土壤全钾和速效钾呈下降趋势;稻鳖组全钾为各组最低,且在S1~S2迅速下降,其后基本保持稳定,速效钾总体呈下降趋势;稻虾组全钾总体呈下降趋势,但速效钾总体呈上升趋势。

2.1.4 不同稻渔生态种养模式对稻田土壤有机质含量的影响 由表2可见:对照组土壤有机质多数时候显著高于3种稻渔生态种养模式($P < 0.05$),且总体呈上升趋势。在3种稻渔生态种养模式中,稻鳖鸭组土壤有机质为各组最低,且呈现先下降(S1~S2)后平稳(S2~S4)再上升(S4~S5)的变化趋势;稻鳖组有机质各时期差异不显著($P > 0.05$);稻虾有机质总体呈上升趋势。

2.1.5 不同稻渔生态种养模式对稻田pH的影响

由表2可见:对照组土壤pH在各时期均明显低于3种稻渔生态种养模式,且除S3和S4期略高于7外,其余各期土壤pH均低于7。3种稻渔生态种养模式在不同时期土壤pH均在7以上。其中稻虾组土壤pH在各个时期均在8.0以上,介于8.0~8.3之间,呈较强碱性。

2.2 稻渔生态种养模式对不同时期稻田土壤酶活性的影响

不同模式、不同时期稻田土壤酶活性检测结果见表3。

2.2.1 不同稻渔生态种养模式对稻田土壤S-ALP活性的影响 由表3可见:对照组S-ALP活性在除S1之外的其他各期均显著高于3种稻渔生态种养模式($P < 0.05$),且呈先上升(S1~S3)后下降(S3~S5)的变化趋势。3种稻渔生态种养模式S-ALP活性变化趋势各不相同,其中稻鳖鸭组在各时期均为最低,且S1至S5呈上下波动的变化态势;稻鳖组总体呈下降趋势,但在S2~S4基本保持稳定;稻虾组S-ALP活性总体呈先上升(S1~S2)后下降(S2~S5)的趋势。

2.2.2 不同稻渔生态种养模式对稻田土壤S-ALPT活性的影响 由表3可见:对照组S-ALPT活性呈明显的先上升(S1~S2)后下降(S2~S5)的趋势,并在S1显著高于稻鳖组和稻虾组($P < 0.05$),极显著高于稻鳖鸭组($P < 0.01$),在S2仅显著高于稻鳖组和稻鳖鸭组($P < 0.05$),在S3、S4和3个稻渔生态种养模式基本一致($P > 0.05$),在S5仅显著高于稻

3 讨论

3.1 不同稻渔生态种养模式对稻田土壤化学性质的影响

土壤的化学性质直接影响土壤肥力、污染物固定能力和植物生长环境,因而土壤 pH、有机质、全氮、全磷、全钾、碱解氮、有效磷、速效钾等指标是评价耕作土壤肥力的主要化学因子^[13]。

目前的研究表明,土壤酸碱性及其动态变化关系着土壤中微生物活动、养分分解与转化、营养元素在土壤与作物之间的迁移,从而影响作物生长发育^[14-15],因而保持较高的土壤 pH 有利于作物生长。本研究结果显示,3 种稻渔生态种养模式的土壤 pH 在各时期均呈碱性,且都高于或显著高于对照组稻田,表明 3 种稻渔生态种养模式均可改善稻田土壤酸碱条件,有利于水稻生长,这可能和稻田引入养殖动物有关。相关研究表明,养殖动物在田间的活动可降低土壤容重,提高孔隙度或土壤团聚化程度,增加土壤透气性,从而改善土壤结构^[16],并利于水稻光合作用,加速 CO₂ 的利用和释放到空气中,从而提高稻田综合种养土壤 pH^[17]。

众多研究均显示,稻鱼^[18]、稻蟹^[19]、稻虾^[20-22]、稻蛙^[23]、稻鳖^[24]、稻鸭^[25-26]等综合种养模式可通过养殖动物的粪便来增加稻田土壤有机质和其他土壤养分含量,并通过养殖动物活动改善土壤结构,加速物质循环,提高土壤肥力^[16]。但本研究结果显示,3 种稻渔生态种养模式的土壤全氮、碱解氮、总磷、全钾、速效钾、有机质等大部分指标在水稻生长的大部分时期均显著低于对照组稻田。造成这种差异的主要原因可能和田间管理有关,一般稻渔综合种养模式为提高养殖动物的产量,会尽可能地增加动物投放密度和饵料投喂量,从而使得养殖动物的排泄物较多。而本研究中的 3 种生态综合种养模式为满足有机产品生产标准,不但不施追肥,而且动物养殖量和投饵量也较少,这可能是导致土壤有机物等主要肥分降低的主要因素。

3.2 稻渔生态种养模式下不同时期稻田土壤酶活性变化

土壤酶主要由植物根系、微生物代谢及动植物残体分解产生,其活性可反映土壤生化过程强度,故可作为衡量土壤肥力的指标^[27-30]。土壤酶种类很多,已发现有 50~60 种,其中土壤碱性磷酸酶(S-ALP)、碱性蛋白酶(S-ALPT)、蔗糖酶(S-SC)、过氧化氢酶(S-CAT)是参与土壤碳、氮、磷代谢,维持土

壤健康的主要酶类。

有关稻渔综合种养对土壤酶活性影响的研究较多,但结果不尽相同。部分研究结果认为,稻渔综合种养可显著提高土壤酶活性^[31-34],而李成芳等^[35]的研究发现,稻鸭与稻渔共作虽然显著地提高了土壤脲酶、脱氢酶和蛋白酶活性,但对过氧化氢酶活性影响不大;甚至倡国涵^[36]的研究认为稻虾共作模式下 0~40 cm 土层中的土壤蔗糖酶、酸性磷酸酶和脲酶活性呈降低趋势,脲酶活性在 10~20 cm 土层显著降低。本研究结果显示,3 种稻渔生态种养模式对土壤蔗糖酶和过氧化氢酶活性的影响不大,但会降低或显著碱性磷酸酶和碱性蛋白酶的活性,尤其是稻鳖鸭组。其中 3 种稻渔生态种养模式对过氧化氢酶活性影响李成芳等^[35]结果一致,对土壤蔗糖酶活性影响与倡国涵^[36]较为接近,而对碱性磷酸酶和碱性蛋白酶活性影响和以上研究均不同。造成不同研究者研究结果不同甚至相反的主要原因,可能和不同研究者采用的模式以及稻田的土壤特性不尽相同有关。有研究表明,土壤理化性质^[10,37]和耕作方式^[38-39]都对土壤酶的活性及稳定性有很大影响。如土壤碱性磷酸酶(S-ALP)同土壤有机质、有机磷、无机磷和土壤有效氮含量有很强的相关性^[40],土壤蛋白酶活性受到土壤理化性质、CO₂ 浓度、土壤污染物和耕作方式等影响^[41],土壤蔗糖酶产物同土壤有机质、氮、磷含量,微生物数量及土壤呼吸强度密切相关,土壤过氧化氢酶(S-CAT)与土壤全碳、全氮呈负相关关系^[42]。

3.3 不同生态种养模式对土壤肥力影响综合评价

土壤肥力是农作物生长和粮食丰收的基础,受多种因素影响,特别是耕作方式。稻渔综合种养因养殖动物的引入和更复杂的田间管理而对土壤的理化性质及土壤肥力影响更大。本研究在系统测定不同时期稻田土壤主要化学因子和土壤酶活力的基础上,进一步采用在土壤质量定量评价中应用最为广泛的主成分分析法(PCA)^[43-45]对重庆地区 3 种稻渔生态种养模式对土壤肥力的影响进行了评价。结果显示,虽然 3 种稻渔生态种养模式均可改善稻田土壤 pH,但有机质、总氮、碱解氮、总磷、有效磷、全钾、速效钾在大多数时期均不高于或显著低于对照组,主成分分析综合排序结果也显示,3 种稻渔生态种养模式 F 值在各个时期均远低于对照组。对照全国第二次土壤普查养分分级标准发现,对照组土壤有机质含量在各期比 3 种稻渔生态种养模式高 1~4 个等级,土壤全氮和碱解氮含量在各期比 3 种

稻鳖鸭综合种养对稻田杂草影响

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摘要:调查了水稻单作和稻鳖及稻鳖鸭共作田间杂草种类、数量及盖度变化。结果显示, 稻鳖鸭及稻鳖共作的杂草种类、数量与盖度均显著低于水稻单作, 稻鳖鸭及稻鳖共作杂草种类相同, 但稻鳖鸭共作其杂草数量与盖度均显著低于稻鳖共作, 说明稻鳖共作对田间杂草有抑制作用, 田间增加养鸭控制杂草效果更显著。

关键词:杂草多样性; 稻田; 综合种养

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水稻是我国主要的粮食作物之一, 全国约有60%的人以大米为食^[1]。杂草是水稻种植中的常见问题, 是造成水稻减产的重要原因之一。因为杂草具有抗不良环境能力强、生长快、繁殖能力强等特点, 经常与水稻争夺营养、阳光、生存空间、水分等, 而且很容易占据主动地位^[2]。

研究表明, 稻田综合种养模式是可持续发展模式, 是保障粮食安全, 增加种养殖户收入的良好途径^[3]。在原本的生态系统中引入养殖动物后, 增加了生态系统的复杂性, 达到了稳量增渔, 提质增效的效果。养殖动物的活动、摄食、代谢等都会对生态系统的能量流动和物质循环产生影响^[4]。而同处于生态系统中的杂草必然会受到一定影响。目前, 有关稻田综合种养对于杂草生长、分布等影响的研究较少。现通过对比水稻单作、稻鳖共作与稻鳖鸭共作稻田杂草的种类和数量, 分析稻田中鳖和鸭的引入对杂草的影响, 以为稻田杂草的生态防治提供一定的依据。

1 材料与方法

1.1 取样地点

取样地点位于重庆市潼南区稻梦空间公司。

分为水稻单作 D、稻鳖共作 B 和稻鳖鸭共作 Y 3 组, 每组稻田的面积约为 0.2 hm², 由一块约 0.6 hm² 的田分割而成, 田块呈长方形。共作田中鳖的放养规格约 150 g/只, 放养密度为 750~1 500 只/hm²。7 月下旬在 Y 田一边的岸上养殖雏鸭, 放养密度 150~300 只/hm²。养殖初期, 鸭子只在田埂活动, 养殖约一个月后, 鸭子自由进入田间。

1.2 杂草样品采集与处理

于 2020 年 7 月 18 日、8 月 24 日、11 月 6 日, 采 3 次样(分别对应水稻生长的分蘖期、齐穗期、再生稻期)。在每块稻田中随机选择 5 个样方, 每个样方面积为 1 m², 采集样方内所有杂草。再生稻期每个样方面积定为 2 m², 因为此时是冬季, 气温较低, 杂草较少。并记录下杂草盖度, 样品带回实验室称重并记录其种类。

1.3 数据分析

杂草的湿重、盖度的差异运用 SPSS 16.0 软件进行单因素方差(One Way ANOVA)分析, $P < 0.05$ 表示差异显著。

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2 结果与分析

2.1 水稻生长 3 个时期稻田杂草的种类

水稻生长 3 个时期各个稻田杂草种类见表 1。

(1)分蘖期。分蘖期共作田的优势种均为喜旱莲子草(*Alternanthera philoxeroides* Mart)和稗[*Echinochloa crusgalli* (L.) Beauv.],而水稻单作田的优势种为喜旱莲子草、稗和钻叶紫菀(*Aster subulatus* Michx.)。单作田并未发现马唐[*Digitaria sanguinalis* (L.) Scop.],可能是因为本次采样时间处于马唐抽穗初期,对外界条件反应敏感^[9],而单作田中狗牙根[*Cynodon dactylon* (L.) Pers.]竞争力极强,挤占马唐生存空间,因此水稻单作田马唐较少。

(2)齐穗期。齐穗期稻鳖共作田与稻鳖鸭共作田中,喜旱莲子草和双穗雀稗 [*Paspalum paspaloides* (Michx.)

Scribn.]为优势种。水稻单作田的杂草优势种为喜旱莲子草、三叶鬼针草(*Bidens pilosa* L.)和钻叶紫菀。

(3)再生稻期。再生稻期共作田杂草优势种均为通泉草[*Mazus japonicus* (Thunb.) O. Kuntze]和香附子(*Cyperus rotundus* L.),水稻单作田的杂草优势种为喜旱莲子草、狗牙草和三叶鬼针草 3 种。

由分蘖期和齐穗期结果可以看出,共作田的杂草种类均少于水稻单作稻田,并且稻田的杂草优势种也存在一定差异。而再生稻时期采样结果中两种稻田杂草种类均较少,并且差异不大。原因可能是采样时间为 11 月初,进入冬季,气温较低,大部分杂草因不能耐低温或耐低温能力较差而死亡,只留下耐低温能力较强的种类。总体来看,共作田杂草种类与水稻单作田杂草种类存在差异。

表 1 水稻生长 3 个时期稻田杂草种类^①

杂草种类	分蘖期			齐穗期			再生稻期		
	B	Y	D	B	Y	D	B	Y	D
白酒草[<i>Conyza japonica</i> (Thunb.) Less.]	✓	✓	✓	✓	✓	✓			
马唐[<i>Digitaria sanguinalis</i> (L.) Scop.]	✓	✓				✓			
雀稗[<i>Paspalum thunbergii</i> Kunth ex steud.)	✓	✓	✓	✓	✓				
牛鞭草[<i>Hemarthria altissima</i> (Poir.) Stapf et C. E. Hubb.]	✓	✓	✓						
稗[<i>Echinochloa crusgalli</i> (L.) Beauv.]	✓	✓	✓	✓	✓	✓			
钻叶紫菀(<i>Aster subulatus</i> Michx.)	✓	✓	✓	✓	✓	✓			
喜旱莲子草(<i>Alternanthera philoxeroides</i> Mart)	✓	✓	✓	✓	✓	✓			✓
双穗雀稗[<i>Paspalum paspaloides</i> (Michx.) Scribn.]	✓	✓	✓	✓	✓	✓			
扁穗牛鞭草[<i>Hemarthria compressa</i> (L. f.) R.Br.]	✓	✓	✓						
粉绿狐尾藻[<i>Myriophyllum aquaticum</i> (Vell.) Verdc.]	✓	✓	✓			✓			
狗牙根[<i>Cynodon dactylon</i> (L.) Pers.]			✓	✓	✓	✓			
水蜈蚣(<i>Kyllinga brevifolia</i> Rothb.)			✓						
千金子(<i>Euphorbia lathyris</i> L.)			✓	✓	✓	✓			
牛鞭草[<i>Murdannia loriformis</i> (Hassk.) Rolla Rao et Kammathy]			✓						
水蓼(<i>Polygonum hydropiper</i> L.)			✓						
蜈蚣草(<i>Pteris vittata</i> L.)			✓			✓			
紫菀(<i>Aster tataricus</i> L. f.)			✓						
龙葵(<i>Solanum nigrum</i> L.)			✓	✓	✓				
异型莎草(<i>Cyperus difformis</i> L.)			✓			✓			
鹅观草(<i>Roegneria kamoji</i> Ohwi)			✓						
香附子(<i>Cyperus rotundus</i> L.)				✓	✓	✓	✓	✓	
醴肠[<i>Eclipta prostrata</i> (L.) L., E. alba (L.) Hassk.]				✓	✓	✓			
华马唐[<i>Digitaria sanguinalis</i> (L.) Scop.]				✓	✓	✓			

稻—虾—蟹高值化混养技术

为探索水产养殖新模式，提高稻渔种养综合效益，促进稻渔综合种养产业高质量发展，笔者团队将池塘养殖虾蟹模式引入稻田，在重庆市开展了稻田—青虾—河蟹混养模式试验，取得较好效果，现将其技术总结成文，以期对相关模式应用推广提供技术参考。

文/孟文旭¹ 李敬雄¹ 闫胜华² 唐洪玉^{1*}

近年来，国家高度重视稻渔综合种养产业高质量发展，农业农村部等部门出台了一系列政策文件，政策文件强调稳粮促渔保供为前提，实现稻渔综合种养高值化发展，同时提出了具体的目标和措施，如丰富品种模式，加快适宜水稻品种的筛选、优化搭配模式、搭配比例等。

本团队就虾蟹搭配养殖模式做了大量的试验，结果表明水稻、青虾、河蟹混养通过时间差和空间差，充分利用阳光、气温、溶氧、饵料等资源，发挥立体种养的优势，提高资源转化率，有效提升了青虾、河蟹的养殖产量与品质，进一步满足了大众对于健康美味水产品的需求。青虾、河蟹同属于甲壳类动物，它们食性相近、生长特征相同，通过一次次的蜕壳来完成生长过程，且都富含优质蛋白，凭借其独特鲜美的风味，深受大众喜爱。

一、稻田选择与改造

（一）田块选择

试验农田应近水源，灌溉方便，水源水质符合国家渔业用水标准（GB 11607-1989），且农田交通便利，有硬化公路。

（二）田间工程改造

加固田埂，做好防漏、防逃相

关措施。开挖排水沟，面积不超过10%。

（三）选排水及防逃设施

在田块的对角设置进排水口，安装提水泵和进水管，每块田安装自动排水管，进水口高出田间最高水位50cm左右，安装过滤网防止野杂鱼进入。在田块四周铺设防渗膜、防垮塌网、防逃网、防护网，外围防逃网埋入土中10cm，确保地平面上高度达50cm，这样既可以防止稻田内养殖河蟹外逃，也可以防止蛇类、两栖类、鼠类、野猫等进入。

二、水稻选择与栽培

（一）水稻选择

选择生长期长、耐肥力强、茎秆坚挺、抗倒伏、抗病虫害、产量高的优良稻种，例如荃香优575，水稻栽培技术与常规水稻单作田一致，种植晚稻，于5月中下旬移栽育好的秧苗。

（二）插秧与收割

采取人工平田和栽插秧苗。人工插秧有利去除田间杂草，且秧苗存活率高。5月上旬，用生石灰（每亩75kg）全池泼洒消毒，杀灭敌害生物，改良池底。在8月下旬水稻收割前20天，逐渐排出田水，水稻成熟后采用人工收割方式。

三、虾蟹养殖与管理

（一）水体培养

在放养前，移栽水草，如虾藻（菹草）、轮叶黑藻或马来眼子菜等沉水性植物，移栽面积占环形沟的50%~60%，零星分布为宜。水草为虾蟹提供良好的栖息与蜕壳场所，同时也可作为部分饵料来源。

（二）苗种选取

河蟹应选择规格整齐、体格健壮、无残缺、无伤病的优质品种，如中华绒螯蟹“诺亚1号”。蟹种在放养前需经过筛选和消毒，常用的消毒方法有3%~5%的食盐水浸浴5min~10min或20mg/L的高锰酸钾溶液浸浴10min~15min。青虾应选用“太湖2号”等高产抗病品种。

青虾规格为 (0.5 ± 0.05) g，河蟹平均规格为 (15.27 ± 0.30) g，虾的放养密度为7kg/亩~15kg/亩，河蟹的放养密度为500只/亩~1200只/亩。试验表明每亩10kg青虾+800只中河蟹组合时，对水肥平衡、虾蟹规格以及经济效益有更大帮助，所以推荐此放养密度。

为提升河蟹活动能力，于4月初，开始蟹种暂养，约50天。待6月中旬水稻分蘖后，选择晴天将蟹种分散投放至稻田中，以提高大规格蟹的比例。

表1 单位面积投入汇总表 (元/亩)

试验分组	田租	水稻苗种	蟹苗种	虾苗种	肥料、农药	饲料	水电、人工	合计
稻田—青虾—河蟹共作	530	200	1200	700	0	624	400	3654
水稻单作	530	200			60		60	850

表2 单位面积产出汇总表

试验分组	水稻产出			水产品产出				单位面积产出 (元/亩)
	单产 (kg/亩)	单价 (元/kg)	产值 (元/亩)	蟹产量 (kg/亩)	蟹产值 (元/亩)	虾产量 (kg/亩)	虾产值 (元/亩)	
稻田—青虾—河蟹共作	420	2.8	1176	21	2100	38	4180	7456
水稻单作	500	2.4	1200					1200

为保证青虾正常生长，于6月初一次性投放青虾虾苗，将其均匀投放至稻田，放养时间宜选择在阴雨天进行，晴天应在早晨或傍晚进行，以避免阳光直射、温度过高，影响放养虾苗的成活率。

（三）饲料投喂

科学投喂，坚持四看、四定原则（定时、定点、定质、定量），提供充足且营养均衡的食物。河蟹的饵料要求鲜活、适口、适量，以动物性饵料为主、植物性饵料为辅。日投喂量可参考：3月~4月为存塘虾蟹体重的1%左右，5月~6月为存塘虾蟹体重的3%~5%，7月~10月为存塘虾蟹体重的5%~10%。虾不单独投喂，稻田面积过大，则需要在稻田中间补投饲料，保证河蟹都能摄食，提高蜕壳期成活率，避免规格不齐。在蜕壳前，青虾、河蟹需要摄入足够的蛋白质和矿物质等营养物质，以支持新壳的生长和硬化。

（四）日常管理

1.水质调控

高温季节每天注水，平时根据水质变化情况适时换水，每次换水10cm左右，换掉底层老水，改善水质环境。高温期排水时间定为上半夜，注水时间定为午夜前后。合理控制水草数量，避免水草死亡造成水体污染。

加强日常巡塘，及时观察青虾、河蟹的生活状态，及时捞出病死虾蟹，避免水质损坏。

2.水位控制

稻渔综合种养期间，注意根据水稻的生长特点调节水位。青虾、河蟹蜕壳关键期，保持适宜的水温和水位。水稻收割前，将稻田水放去，留水在环沟，虾蟹全部归到环沟内，不影响水稻收割。水稻收割后，加深水位至20cm~50cm，虾蟹返稻田，保障水稻收割后虾蟹的继续增长，继续养殖至12月。及时捕捞达到上市规格成虾蟹。

（五）虾蟹捕捞

9月中旬开始，将地笼网放置沟中数小时后取捕一次，或在第一天晚上放置，第二天清晨取虾蟹。此方法简单方便，对虾蟹生长干扰小。将规格达到上市要求的虾蟹捕起，达不到上市要求的虾蟹留存过冬。收河蟹时，应检查其成熟度，选择体色青灰、腹部饱满、蟹爪有力的健康河蟹进行捕捞。

四、经济效益

由表1、表2单位面积投入、产出可知，稻田—青虾—河蟹共作净利润可达3802元，水稻单作利润为350元，利润是水稻单作的数倍。通过合理的养殖管理，不仅可以获得水稻的稳定产量，还能增加青虾、河蟹的产出，增加收益。

五、小结与展望

在本次试验过程中，青虾、河蟹混养并没有发生影响产量的残食现象，关键因素为人工投喂饲料充足，满足了日常摄食需求。稻田—青虾—河蟹混养模式是一种高效、生态的农业发展模式，经济效益高于稻虾、稻蟹以及水稻单作，具有广阔的市场前景和推广价值。同时，该模式在实际应用中还需进一步解决一些问题，如进一步优化养殖密度、提高饵料利用率、完善疾病防控体系等。未来，应加强科技研发和技术推广，促进该模式的规范化和标准化发展，为我国农业现代化和乡村振兴贡献力量。

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基于水稻直播下的稻鳖鱼鸭生态种养试验

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随着“大食物观”的不断深入落实, 稻渔综合种养产业越来越受到国家的重视, 从中央到地方都将发展稻渔综合种养, 作为保障粮食和水产品供给、促进农民增收和推进乡村振兴的重要产业。当前, 推进稻渔综合种养产业高质量发展, 加强科技支撑, 创新引领, 推出新技术和新模式, 为产业健康快速发展提供智力支持和技术保障尤为重要。

稻鳖鱼鸭综合生态种养模式, 是指在水稻种植过程中, 适时引入鳖、鱼、鸭共生、互利, 实现一水多用、一田多收的复合生态种养模式。稻田为鳖、鱼类、鸭等提供适宜的生长环境, 鳖、鱼和鸭能有效疏松土壤、清除杂草和害虫幼卵, 其粪便、代谢物为水稻生长提供营养, 从而实现稻田大幅减少甚至不用化肥和农药, 控制农业面源污染, 达到降低生产成本、改善水稻和水产品品质、保持水土等, 其经济效益、社会效益和生态效益显著。水稻直播因具有省工省时、节本高效等优势, 应用范围越来越广。现于2021年, 开展基于水稻直播下的稻鳖鱼鸭生态种养试验。

1 材料与方法

1.1 田块

选择水源充足、水质良好、排灌方便、地势平坦, 不受洪水威胁的田块, 面积 13 000 m²。

1.2 田间工程

1.2.1 开挖环沟及田埂

为施工和日常管理方便, 开挖“一”字形宽沟或“L”形沟, 沟宽 3~5 m, 深 1.0~1.5 m, 沟坑比为 10%, 并在主干道田头留足收割机下田通道。在养殖动物不伤害水稻时, 加深田水, 养殖动物可自由进入稻田活动, 疏松土壤, 摄食杂草、虫害等天然饵料。

1.2.2 设置食台及晒台

稻田养殖鳖(甲鱼)可不设置固定晒台和食台, 尤其是放养密度较低时, 鳖可在田埂或稻田浅水区域晒背休息。放养密度高时, 设置固定的食台和晒台, 供甲鱼摄食和晒背。冬眠时, 水位下降, 鳖会在田中间打洞直接冬眠, 也有一部分鳖会在沟里冬眠。

1.2.3 进排水口及防逃设施

进排水口设置在稻田对角, 保证池塘水能充分交换、流动。进、排水口用铁丝网或栅栏围住, 以防养殖水产动物逃逸, 同时向内设置瓦片或网片, 或在田埂上设置围栏, 防止养殖动物逃逸。

1.2.4 灭虫设备

利用太阳能灭虫灯进行物理灭虫, 2 000~2 700 m²稻田设置 1 台太阳能灭虫灯, 即可达到较好的灭蚊效果, 同时, 鳖、鱼、鸭子等也可捕食蚊虫。

资助项目: 重庆市生态渔产业技术体系项目(4322000112201903); 重庆市水产科技创新重点攻关项目——山地特色生态稻渔综合种养产业化关键技术体系构建与应用(4322300285)

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重庆中海拔地区稻田黄颡鱼养殖技术

本文重点介绍了重庆中海拔地区稻田养殖黄颡鱼模式中水稻种植技术、黄颡鱼投放养殖驯化技术等。经过团队实践证明稻田养殖黄颡鱼经济效益显著，供相关从业者参考。

◎ 文/李敬雄¹ 罗雪峰² 孟文旭¹ 郑永华¹ 唐洪玉^{1*}

近年来，稻渔综合种养实施范围不断扩大，种养技术不断向广度和深度发展，养殖模式不断创新，水产养殖品种更加多样化，稻田综合效益明显提高。2022年，我国有稻渔综合种养面积统计的省（区、市）27个，种养面积4295.56万亩，水产品产量387.22万吨，在各水域养殖水产品总产量中位居第二，仅次于池塘养殖产量。

《稻渔综合种养通用技术要求》（GB/T 43508-2023）发布和执行，对稻渔综合种养模式要求更明确、更规范。如何因地制宜、分类制定稻渔生态高效种养技术，对充分发挥水稻、水产品互利共生作用，提高稻田综合种养经济效益，促进稻渔综合种养健康可持续发展非常重要。根据重庆中海拔地区稻田水温、水质特点，广温性水产品适宜生长期一般只有3个~5个月，生长期相对较短。

本团队通过调研，选择生长速度快、市场接受度高、价格较稳定的单性化全雄黄颡鱼作为养殖品种，在重庆市秀山县、城口县等地开展中海拔（600m左右）地区稻田养殖单性化黄颡鱼技术研究。结果表明：该模式综合效益显著，是中高海拔地区稻渔综合养的创新高效模式，现总结如下，以供广大种养户参考。



一、稻田选择与改造

（一）田块选择

选择水源充足、进排水方便，平坦、易管理的田块，稻田面积在20亩左右。

（二）田间工程

充分利用池塘精养理论技术，以方便管理和捕捞上市及运输等为原则，在稻田开挖“一”字鱼沟或“L”形鱼沟，沟上沿宽3m~5m、底部宽2m~4m、深1.5m以上，沟中挖出的土用来加固田埂和内埂，田埂高出稻田50cm~60cm，留出收割机进入田间的便道。有条件的可用空心砖、防渗膜等护坡，沟坑面积不超过稻田10%。

表1稻田黄颡鱼养殖与单作水稻经济效益对比

种植模式	投入 (元/亩)							产出 (元/亩)			
	种植成本			养殖成本				稻谷产值	黄颡鱼产值	蔬菜利润	总利润
	土地	稻种	化肥农药	鱼种	饲料	鱼药	工程、人工、电费等				
稻渔养殖	500	30	120	809	1062	50	1159	1456	4172	100	1998
单作水稻	500	100	140	0	0	0	400	1540	0	0	400

上,投喂量为鱼体重的3%~5%,采用“四定”原则,即定点、定时、定质、定量投喂,每天投喂两次,水温高时宜在9:00、17:00左右投喂。

该模式必须做好鱼类摄食驯化,投喂时可敲盆(或其他能发出声响的器具)驯化一周,驯化成功才在稻田打开缺口,让鱼自由进出。驯化好的鱼类,每次敲盆发出声响后很快集中到沟壑摄食,摄食后又慢慢进入稻田活动、觅食,充分发挥稻渔共生互利。但如果没驯化好,稻田内鱼类长势不均匀、规格不整齐,且不容易集中,不方便捕捞和出售,增加劳动力成本。

(二) 病害防治

每天巡塘,观察田间情况,发现田间问题及时解决。根据天气变化、鱼的吃食量和水质变化等情况及时调整饲料投喂量,防止剩余饲料污染水体。6月中旬和7月上旬可在饲料中添加中草药预防肠炎和寄生虫,如三黄散(大黄、黄连、黄芩)和板黄散(板蓝根、大黄)等。

(三) 成鱼捕捞

7月下旬~8月初,黄颡鱼尾平均重量达到80g,开始捕捞上市。一般8月底可全部上市。没达到上市的黄颡鱼,收割前晒田时,可集中在鱼沟里继续饲养,等水稻收割后加深田水,在大田中养殖,年底前上市。

五、效益分析

黄颡鱼是我国重要的淡水经济鱼类,其肉质细嫩、营养丰富、味道鲜美,深受广大消费者喜爱。将黄颡鱼

引入稻田养殖,尤其是安装增氧机后,可明显提高黄颡鱼产量,是融合池塘精养技术的稻渔综合种养创新模式,不仅改善稻田环境,还提高了水产品品质和生长速度,经济效益显著。黄颡鱼是小型肉食性鱼类,食性比较杂,进入稻田后可摄食稻田中的昆虫和幼虫,减少了稻田病虫害,同时其排泄物也可以为稻田和鱼沟种植的水生植物提供养分,养殖水体能够自然净化,生态效益明显。

以重庆市秀山县某农业专业合作社试验稻田为例,该合作社核心区100亩,经测产并计算经济效益,稻渔综合种养模式中水稻产量520kg/亩,售价2.8元/kg,黄颡鱼产量149kg/亩,稻田黄颡鱼价格市场平均价格28元/kg,利润平均1998元/亩。单作水稻产量在550kg/亩,售价2.8元/kg,利润仅为400元/亩,详见表1。由于稻渔综合种养模式降低了化肥农药使用量,一定程度提高了水稻和水产品质量,更受消费者欢迎,如果能做好宣传、打造品牌,经济效益更高。同时,在中高海拔地区,黄颡鱼鱼价稳定且相对偏高,能较好确保种养户利润。

六、小结讨论

因地制宜发展中海拔地区稻田养殖单性化黄颡鱼,不仅水产品能及时上市、资金周转快、经济效益好,还可减少稻田病害,提高土壤肥力,促进稻田良性循环,实现稻渔综合种养模式可持续发展。



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重庆地区冬季上市小龙虾稻田养殖关键技术

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冬季因春节等节日因素，是消费高峰季节，在渝西温度适宜地区发展反季节小龙虾养殖，不仅可以打破小龙虾供应时间的限制，平衡消费市场供需，满足人们日益增长的生活需求，还可以带动小龙虾产业相关技术的发展及运用。基于此，作者团队在渝西温度适宜地区开展了冬虾养殖研究。本文从田块选择、苗种来源与放养、饲养管理、捕捞与收获等方面，对冬季上市小龙虾稻田养殖技术进行总结，以供相关从业者参考。

近年来，小龙虾因其营养价值高，肉质鲜美而受到广大消费者的热爱，市场需求居高不下。随着越来越多的人投身小龙虾养殖行列，稻虾综合种养模式的应用也越来越广泛。这种养殖模式可以充分利用水体资源，挖掘生产潜力，达到稻虾双收、渔农双赢的目的。但由于生物学特性限制和养殖规模扩大，目前小龙虾上市时间主要集中在春夏季，且局部出现了一些滞销现象，而其冬季上市较少，供不应求。过于集中的上市时间在一定程度上阻碍了小龙虾产业持续发展，在新冠肺炎疫情防控常态化下矛盾更为突出。在温度适宜地区开展冬季上市小龙虾（以下简称“冬虾”）养殖，具有反季节、错峰销售的特点，能较好平衡消费市场供需，大幅提高养殖效益，值得探索与实践。

一、田块选择

冬虾稻田养殖在田块选择上与常规的稻虾养殖模式基本一致，但需水源充足，保水性能好，能够维持一定水位，有利于保持适宜的水温。小龙虾在冬季活

动能力不强，但仍要做好防逃措施，在池埂四周水位线上1m处固定由尼龙网片制作的围栏，形成水上防逃网以阻止小龙虾“潜逃”，这种防逃网还可提高小龙虾起捕率，保护池堤。

二、准备工作

（一）放养前准备

1. 稻田清整

虾苗放养前首先清除田边杂草，避免遗留草种和虫卵。其次，排干田水，晾晒20d~30d，使田底呈龟裂状，增加稻田透气性。

养管理的关键点,加深水位可以调节水温,12月~次年1月低气温期间水位应保持在1.2m~1.5m。此外,还可采用有机肥发酵的方式,将鸡粪、猪粪、羊粪等有机肥用编织袋封装好后,散放在池中用木桩固定,其发酵过程散热可提高水体的温度。

2. 水体循环

冬季养殖期间,早期中午温度较高,注意对养殖池塘进行水体循环,循环标准为水体总量的1/3。其次,应适时、适量进行换水,在小龙虾生长旺季,半个月进行一次换水,每次换水量占总水量的10%左右,提高水体溶氧量,避免小龙虾窒息死亡。

(二) 饲料投喂

放养后的第一周,小龙虾主要摄食粉料、豆浆等,投喂饲料中植物性蛋白和动物性蛋白的配比为6:4,第二周加大投喂量,配比变为5:5,增加小鱼虾、蚯蚓等动物性饲料,早、晚各投1次,晚上投喂日饵量的70%。投喂早期,每万尾稚虾日投饲量为0.3kg~0.5kg,中后期按池内虾体重的10%左右投饲,具体投喂量根据长势而定。小龙虾食性广,摄食杂,喜食动物性饵料,有争食、贪食习性,在不同生长发育阶段食性有所差异,应根据其生长发育阶段、生活习性、摄食特点、环境变化进行灵活调整,科学合理投喂。

(三) 巡塘

在反季节小龙虾养殖的整个过程中,要严格做好巡塘工作,在巡塘期间检查小龙虾的生长情况,记录好池塘水温、pH、溶解氧等等,控制水体pH在7~8.5,透明度在30cm左右,保持水体溶氧量在4mg/L以上。其次,观察小龙虾的摄食情况及生长状况,查看有无死亡的现象,观察死亡数量和死亡症状是否异常,如有异常及时采取合理的解决措施。此外,应注意观察小龙虾体形情况,若出现大头,

可能由于饵料投喂效果不佳所致;若虾壳出现发黑,应及时进行补钙,促进脱壳,否则会影响小龙虾品质与产量。与普通小龙虾养殖不同的是,若冬季池塘水体不结冰封冻,初春气温回升容易导致病菌及小球藻、硅藻、青苔等藻类过量生长,应注意在2月份左右使用生石灰进行全池泼洒,泼洒量为20kg/667m²。

(四) 防病

冬季气温低,病害少。遵循以防为主、防治结合的原则,提高小龙虾免疫力、改善和优化生长环境、切断病原体传播途径及加强检疫制度。做好养水,调水,种植水草,培育有益菌,为小龙虾生长繁殖提供一个良好、稳定的生态环境。

四、捕捞与收获

为了获得更高的养殖效益,冬季上市的小龙虾要适时捕捞。时刻关注市场需求,早捕、多捕,1月~3月中旬开始起捕规格达到25g左右的大虾,4月份可全部上市。冬季小龙虾销售市场广阔,及时捕捞上市规格的虾,能合理降低养殖密度,为小规格虾提供

充足的生长资源,有利于提高小规格虾生长速度、供应量、供应频率,进而提高经济效益。

五、总结

冬季上市的小龙虾稻田养殖技术基于稻虾养殖,不仅技术操作简单,还具备投资成本低,病虫害少的特点,其核心技术在于提前获取优质种虾或虾苗,以及控温控水。

冬季上市小龙虾稻田养殖一是改变了养殖周期,改善了传统情况下过于集中上市的养殖模式,打破了小龙虾销售时间限制,稳定了小龙虾市场销售链,带来较高的经济效益;二是满足了消费者不断提高的物质需求,改变了市场上春、冬季吃鲜活小龙虾难的局面;三是可以进一步推动相关养殖技术研究,对我国小龙虾产业健康高质量发展具有积极推动作用。

此外由于经济效益较高,冬季上市小龙虾稻田养殖可为助力乡村振兴、产业兴旺增砖加瓦,并进一步促进乡村旅游、餐饮等一二三产业融合发展,值得相关从业者借鉴参考。



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山地特色平板田稻虾高值化生态养殖关键技术

平板田稻虾高值化生态养殖技术是在原有稻虾养殖模式基础上,通过去除环沟,实现稻田资源更好利用的一种新模式。作者团队在重庆地区进行了该技术的研究与实践,表明该技术模式可以提升水产品产量且经济效益明显。本文重点介绍了山地条件下平板田生态养殖技术中水稻栽培技术、小龙虾投放养殖技术、水草种植管理和捕捞技术等,供相关从业者参考。

◎ 文/李敬雄¹ 李昆鹏² 孟文旭¹ 郑永华¹ 唐洪玉^{1*}

近年来,在政府推动、技术进步和消费需求旺盛等多重因素带动下,我国稻渔综合种养产业持续稳定发展,生产规模和产量逐年扩大,养殖综合效益和竞争力稳步提升。2022年,我国稻渔综合种养面积4295.56万亩,水产品产量387.22万吨,其中稻虾种养(特指稻田养殖小龙虾)面积2350万亩,占全国稻渔综合种养面积的54.71%。

重庆市地处长江上游、四川盆地东南部,2022年全市稻渔综合种养面积39.89万亩,水产品产量1.9178万吨,其中,小龙虾产量达到1.5万吨。“十四五”期间,重庆市将大力推进稻渔综合种养产业发展,力争2025年面积达到120万亩,稻田综合种养潜力巨大。但重庆市以丘陵、山地为主,规模化的稻田工程建设较为困难,尤其在沟坑占比不超过10%、稳定水稻单产、保护稻田耕作层等的严格要求下,三合土、砖等硬物护坡受到很大限制,常规方式稻田开挖的沟渠容易垮塌,需要每年维护,养殖成本增加。

如何在重庆山地特色条件下,实现稻田不挖沟渠、稻虾双丰收,本团队经过几年的实践,总结出重庆市山地特色的高值化生态平板田稻虾养殖模式,供同行参考。

一、稻田选择及改造

(一) 田块选择

选择水源充足、排灌方便、无污染、连片易管理的田块,面积在20亩~50亩为宜。

(二) 改造田埂

加固加高加宽田埂,以便小龙虾打洞和捕捞及饲料投喂等操作。一般田埂高出田面80cm~100cm,加宽为90cm,留出收割机械进入田间的便道。田埂呈梯形状,坡度为1:1.5,缓坡利于小龙虾打洞和较好防垮塌。

利用田埂种植蔬菜、水果等经济农作物,田埂土壤肥沃不会缺水,不仅蔬菜水果生长效益好,而且可以防病害,同时起到固土作用。可根据当地蔬菜种植品种、气候环境及市场需求选择合适的果蔬品种,宜种植品质

优良、抗病性较强、有市场的品种,如辣椒、南瓜、玉米、沃柑等,根据果蔬品种选择适宜时间种植,通常在6月水稻种植后,种植果蔬需注意保留操作通道,一般以田埂一侧种植为宜。

(三) 进排水设施与防逃设施

根据稻田面积与形状设置进排水口,一般设置在稻田对角,进水口应当高出田间最高水位40cm~60cm,进排水口安装铁丝网防逃且保证水流正常通过。

在整块稻田四周装上护栏网,隔离虾塘与外界环境,起防逃和防止老鼠、蛇等敌害生物进入等作用。一般可用60目聚乙烯网制作护栏网,高60cm。具体与常规稻虾模式相同。

二、水稻栽培技术

(一) 播种

稻虾综合种养模式水稻栽培技术与常规水稻单作田基本一致。但要求选择产量高、耐肥力强,抗倒伏的品种,如荃香优575、宜香优2115等,于4月中旬进行育苗,待苗种达到8cm即

10kg~20kg。也可直接在水稻收割、消毒清整后,放养繁育分离培育的300尾/kg~400尾/kg规格幼虾,10000尾/亩左右。具体放养密度根据养殖技术和稻田条件而定。

(四) 饲料投喂

小龙虾游泳能力弱,需要全田投喂,即四周及田中均需要投喂饲料,现有专用投饲机可满足投饲需求,有条件的还可用无人机遍撒。饲料以专用颗粒饲料配合黄豆,效果较好,饲料蛋白质含量在35%以上,一般一天投喂1次,阴雨天不投喂,投喂量一般占虾总重的3%左右,具体根据天气、温度、吃食情况增减。

(五) 日常管理

1. 水位控制管理

由于本模式采用不开沟的平板田模式,水位管理极其重要,尤其是水稻、小龙虾共生期。收割水稻后到插秧前水位管理主要是根据小龙虾生长及控制水温的需求而定。水稻收割稻田清整后逐渐灌水,根据小龙虾出洞情况,逐步加深田水,水位可保持在30cm左右。12月~2月,由于水温低,水位控制在40cm~50cm,甚至更深,便于水温稳定。3月~5月水位控制在30cm左右,有利水温提升。5月初为插秧准备期,稻田开始逐渐排水至3cm~4cm,促进小龙虾钻入土壤底层或打洞,以便人工平田和插秧时不伤害小龙虾苗种。待秧苗返青,逐渐灌水,灌水速度以小龙虾不伤稻、符合水稻生长需求即可。6月~7月水位控制在40cm~50cm,有利降温、维持水质良好。7月中下旬逐渐排水,小龙虾开始在田埂周围打洞,可在田埂周围铺玉米秆保持湿润,促进小龙虾在秸秆下集中打洞,方便管理。至水稻田块完全晒干,有裂口出现,方便机械进行收割。

2. 种苗补放

初次养殖时苗种投放一般在

表1 平板田稻虾养殖与单作水稻经济效益对比

种植模式	投入 (元/亩)					产出 (元/亩)			
	土地	稻种	虾苗	饲料	工程、人工等	稻谷产值	小龙虾产值	蔬菜利润	总利润
稻虾养殖	500	100	2600	800	1300	1000	7650	150	3500
单作水稻	500	100	0	0	300	1250	0	0	350

6月~7月,此时秧苗已经分蘖完成,水稻茂盛,为小龙虾提供了栖息场所,小龙虾对水稻无伤害,且避免了小龙虾疾病高发季节。多年养殖的稻田小龙虾存量不够需要补充苗种或交换原良种也可在该阶段,但此时温度较高,操作应仔细,放养密度也不宜过大。

3. 水质管理

加强水质管理,注重水体调节,适时观察小龙虾吃食情况,防止饲料投喂过多导致底质恶化;合理控制水草数量,避免水草死亡造成水体污染;加强日常巡塘,及时观察小龙虾生活状态,及时捞出病死虾。

(六) 小龙虾捕捞

多年养殖的稻田及繁育分离有合适规格虾苗供应的稻田,成虾可在10月开始捕捞售卖,主要集中在11月~4月底。第二批主要在7月~8月捕捞。采用地笼捕捞方式,地笼网眼规格在2.5cm~3cm,能够保证成虾被捕捞,幼虾顺利通过网眼,确保小龙虾上市规格和苗种的充足。

四、经济效益分析

以重庆长寿区某种养殖专业合作社试验地为例,该试验地亩产水稻400kg~500kg,400kg/亩算,水稻出售价格为2.5元/kg,水稻产值为1000元。小龙虾170kg/亩,小龙虾价格浮

动大,但本模式采用错峰上市,小龙虾捕捞时间主要在11月~4月,成虾捕捞量占75%,7月~8月成虾捕捞量仅占25%,总体售价较高,其平均价格可达45元/kg,水产品产值约7650元。从多年田埂种植蔬菜水果的实际收入看,田埂纯收入至少150元/亩。因此该模式利润达到3500元/亩,经济效益明显,具体见表1。如果打造品牌,加强宣传,提升稻米和蔬菜等价格,经济效益还有较大提升空间。在养殖过程中不使用农药和化肥,减少了农业环境污染,其生态效益显著。

五、结语

稻虾高值化平板田生态养殖技术以不开挖田间沟,仅加高加固加宽田埂为基础,既能充分保证耕地面积,又方便田间管理和饲料投喂等,还可充分利用田埂种植经济作物,综合效益显著提高,与稳粮兴渔增收的方针政策吻合,较好地诠释了大食物观。

随着稻田综合种养的发展与农业科技的进步,越来越多智能化设备投入到稻田综合种养中,高值生态化的稻虾平板田养殖模式为智能化机器提供了良好的应用条件,无人机饲料投喂和机械化水稻收割可更方便使用,极大提高了农业生产效率,管理更加精准、快捷,有效促进了稻田综合种养高质量发展。

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2015年长江上游油溪江段宜昌鳅鲃早期资源量及空间分布

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摘要: 为保护长江上游渔业资源, 2015年5–7月, 在重庆江津油溪段对宜昌鳅鲃(*Gobiobotia filifers*)的卵苗进行了采集和分析, 结果显示: 该断面的宜昌鳅鲃鱼卵总径流量为 4.33×10^8 ind., 平均径流量为 0.79×10^7 ind./d, 最大径流量为 1.94×10^7 ind./d, 监测期间均能采集到鱼卵, 且无显著峰值; 宜昌鳅鲃的鱼卵水平分布左岸和右岸的差异不显著, 左岸与中间、右岸与中间差异极显著, 整体水平分布两岸高于中间; 整体垂直分布上差异不显著。对比历史数据发现, 近年来宜昌鳅鲃早期资源量呈现波动性增加, 宜昌鳅鲃鱼卵所占比例升高, 这一结果可能与鳅鲃生物学特性和对环境的适应能力有关系。

关键词: 长江上游; 宜昌鳅鲃(*Gobiobotia filifers*); 空间分布

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The early resource quantity and spatial distribution of *Gobiobotia filifers* in Youxi section of the upper Yangtze River in 2015

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Abstract: The eggs of *Gobiobotia filifers* were collected from Youxi river of Jiangjin District in Chongqing province from May to July in 2015 to investigate and protect fishery resources in the upper of the Yangtze River. The total runoff of eggs was 4.33×10^8 ind., the mean total runoff was 0.79×10^7 ind. with the max runoff density of 1.94×10^7 ind. The eggs were collected and existed no significant peak during the monitoring period. The difference between the left bank and the right bank was not significant. The difference between the left bank and the middle was significant. Meanwhile, the left bank showed significant difference with the right bank. The overall horizontal distribution was higher in both sides than that in the middle; The difference in the overall vertical distribution was not significant. Analogous historical data in recent years, the proportion of *G. filifers* eggs increased and the early resource quantity of *G. filifers* showed increasing in fluctuation, which might be related to its biological characteristics and the adaptation ability to the environment.

Key words: the upper Yangtze River; *Gobiobotia filifer*; spatial distribution

长江上游是长江流域中一个特殊的水域生态系统, 具有特殊的地质、地貌、气候和自然生态环境, 鱼类资源尤为丰富, 达286种^[1], 其中上游特有鱼类124种^[2]。长江上游也是水资源开发利用的重点区域, 这些区域生态环境敏感而又脆弱, 水资源特别是水能开发可能对生态环境产生较大影

响^[3], 所以对长江上游的鱼类早期资源变化调查在对鱼类保护和环境监测中尤为重要。重庆油溪江段水资源丰富, 水情状况稳定, 河滩缓和开阔, 便于作为鱼类资源调查的采样断面。

宜昌鳅鲃(*Gobiobotia filifer*)隶属于鲤形目鲤科鳅鲃亚科鳅鲃属, 又称沙婆子、沙胡子, 广布于

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长江干支流,喜栖息于江河的沙石底下^[4]。近年来,长江中上游流域由于一些水利工程的建设和频繁的人类活动影响,主要经济鱼类资源量呈下降趋势,而宜昌鳅鲇在渔获物中的比例却逐渐升高^[5],其早期资源量现状尚未有深入研究。鱼类早期生活史阶段的资源量是造成鱼类种群规模变动和年龄结构变化的主要原因,能很好反映鱼类的繁殖规模及种群规模的变动趋势,也是鱼类生态学与保护生态学研究的重要内容之一^[6]。本研究对2015年长江上游油溪江段的宜昌鳅鲇的早期资源量、卵苗发育情况和时间空间分布作了调查。结合近几年相关研究数据作为参比,以期对长江上游梯级开发对宜昌鳅鲇等产漂流性卵鱼类的影响研究提供基础数据支撑,为宜昌鳅鲇的进一步研究与利用提供基础数据。

1 材料与方法

1.1 断面设置

2015年5月20日-7月16日在重庆市江津区油溪断面进行调查,监测断面位于江津区油溪镇油溪码头上游约1 km处,约距江津区朱杨溪65 km,下距重庆小南海40 km,期间共监测55 d,106次。该断面江面宽度560 m左右,设置左岸(N29°12.0598', E106°09.0559')、中间(N29°12.1175', E106°09.2667')、右岸(N29°12.1420', E106°09.2930')三个采样点(图1,图2),左岸、右岸两个采样点距岸边20~30 m,水深约为3~5 m,江中为左右两岸之间偏右。每个采集点采集表(0.3 m)、中(0.5 m)和底层(1.0 m)3个不同水层,每天分上、下午两次采集。定性采集设置在左右两岸水流平缓处,每次采集2 h左右。

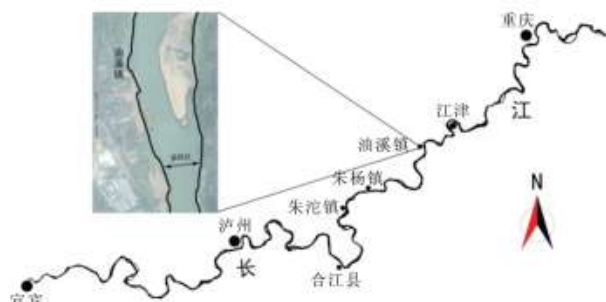


图1 长江江津油溪江段断面采样点断面设置
Fig. 1 Collection sample sections in Youxi of the upstream of Yangtze River

1.2 样品采集与鉴定

监测方法按照《内陆水域渔业自然资源调查

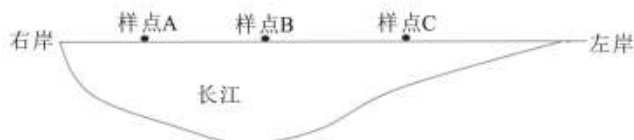


图2 长江江津油溪江段断面采样点断面剖面图

Fig. 2 Cross section drawn of collection sample sections in Youxi River of the upstream of Yangtze River

手册》^[7]和《河流水生生物调查指南》^[8]进行。样品的采集用网网目为0.50 mm的尼龙筛绢制成的圆锥网,网口开口面积为0.21 m²,网衣长2.0 m;采样时网口流速的测量用装在圆锥网上HR-2型流速测算仪;每天测定水温、pH值和透明度等^[9];水文数据(水位、径流量)在重庆水文水资源信息网(<http://www.cqwater.gov.cn/>)获取朱沱水文站的流量信息。

对采集的鱼卵通过肉眼和解剖镜观察其发育期、卵膜径和主要性状,并记录;在解剖镜或显微镜下观察采集到的鱼苗,主要基于肌节数、身体色素、膘的形状位置、鳍条位置等性状特征来进行鉴定;部分鱼苗保存于5%的福尔马林溶液中,以便实验室内进一步鉴定和验证。

1.3 数据处理

试验的数据采用SPSS22.0软件处理,水平和垂直分布的差异采用W符号秩次检验^[10]($P > 0.05$,不显著; $0.01 < P < 0.05$ 显著, $P < 0.01$,极显著),其他相关数据的计算方法以易伯鲁等^[11]的调查方法作参照,具体方法如下:

1.3.1 宜昌鳅鲇产卵场位置推算

产卵场距离公式:

$$L = VT$$

式中: L -鱼卵(或仔、稚鱼苗)的漂流距离,单位为(m); V -采集江段的平均流速,单位为(m/s); T -胚胎发育所经历的时间,单位为(s)。

1.3.2 卵、苗径流量估算

整个调查期间,调查江段鱼卵、鱼苗断面径流量包括每次采集时的径流量和非采集期间的径流量。

1.3.2.1 每次采集时卵苗径流量计算

依据所采集到的鱼卵和鱼苗数量、采集时间、网口流速,结合水文数据按下列公式估算。

$$M = \frac{Q}{sv} \times m \times C$$

$$s = a \cos \left(\frac{\theta}{180} \times 3.14 \right)$$

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四大家鱼幼鱼运动能力对捕食胁迫的响应

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摘要 捕食胁迫(胁迫时长和胁迫强度)影响鱼类的运动能力,遭遇捕食者时猎物鱼的逃逸策略不尽相同。为考察鱼类运动能力对捕食胁迫的响应,本实验选取乌鳢(*Channa argus*)和南方大口鲶(*Silurus meridionalis*)为捕食者,青鱼(*Mylopharyngodon piceus*)、草鱼(*Ctenopharyngodon idellus*)、鲢(*Hypophthalmichthys molitrix*)、鳙(*Aristichthys nobilis*)四大家鱼幼鱼为猎物鱼。猎物鱼分别在无捕食(对照)、低捕食(隔网胁迫)和高捕食(直接胁迫)压力下接受捕食胁迫,胁迫时长分0、7和14 d三个水平,随后对比四大家鱼种间运动能力的差异,并考察四大家鱼幼鱼的稳定和非稳定游泳能力在不同捕食胁迫水平(胁迫时长和胁迫强度)下的适应性改变。结果表明:四大家鱼的稳定游泳和非稳定游泳能力之间存在权衡,应对捕食胁迫时以增强快速启动游泳能力为主,临界游泳速度有下降趋势;四大家鱼均表现为“C”型快速启动游泳模式,在快速启动过程中身体旋转主要在第1阶段完成,速度性能在第2阶段达到最大;在捕食压力下,四大家鱼的逃逸策略存在种间差异;青鱼和草鱼以缩短反应时滞为主要逃逸策略,随着捕食强度增加,反应时滞下降幅度增大;鲢在低捕食胁迫水平下即表现出显著的缩短反应时滞和增大逃逸角度,高捕食胁迫水平下增加表现为增大逃逸速度;鳙在受到捕食胁迫后以提高逃逸速度为主要应对策略,反应时滞有降低趋势,但响应不显著。

关键词 捕食胁迫; 临界游泳; 快速启动; 四大家鱼

Responses of locomotor ability of juveniles of the four major Chinese carps to predation stress. LONG Zhen-man^{1,2}, ZHU Feng-yue², DUAN Xin-bin², GUO Jie^{2,3}, YU Li-xiong², ZHENG Yong-hua¹, TANG Hong-yu^{1*} (¹College of Fisheries, Southwest University, Chongqing 400715, China; ²Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Wuhan 430223, China; ³Wuxi Fisheries College, Nanjing Agricultural University, Wuxi 214081, Jiangsu, China).

Abstract: Predation stress (both duration and intensity of the stress) affects locomotor ability of fish. The escape strategies of prey fish are different when encountering predators. We investigated the responses of locomotor ability of the juvenile black carp (*Mylopharyngodon piceus*), grass carp (*Ctenopharyngodon idellus*), silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) (four major Chinese carps) to predation stress from snakehead fish (*Channa Argus*) and Southern catfish (*Silurus meridionalis*). The prey fish were subjected to predation stress under no-predation (control), low-predation (net-separated stress, i.e. indirect stress) and high-predation (direct stress), with three duration levels of 0, 7, and 14 d. The differences of locomotor ability among the four Chinese carps were compared, and the adaptability of steady and unsteady swimming ability under different levels of predation stress was investigated. The results showed that there was a trade-off between steady swimming and unsteady swimming for the four Chinese carps. In response to the predation stress, the fast-start swimming performance was mainly enhanced, whereas the critical swimming performance tended to decrease. The four Chinese carps all showed the C-type fast-start swimming pattern, with body rotation was mainly completed in stage 1 and the speed performance was maximized in stage 2 during the fast-start process. Under the predation pressure, there were interspecific differences in the escape strategies of the four Chinese carps. The main escape strategy of black carp and grass carp was to shorten the response latency, which showed stronger reduction with increasing predation

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intensity. Silver carp showed a significant reduction in response latency and an increase in escape angle under low predation stress, and an increase in escape velocity under high predation stress. Bighead carp adopted increasing escape velocity as the main response strategy after predation stress, and the response latency tended to decrease, but the response was not significant.

Key words: predation stress; critical swimming; fast-start; four major Chinese carps.

在自然界中,几乎所有的物种都面临着被捕食的风险并采取相应的防御策略,以增强其适应性(Caro *et al.*, 2005; Tien *et al.*, 2012)。猎物反捕食的策略体现在形态、行为、生理或生态等方面(Buskirk *et al.*, 2000; Eklöv *et al.*, 2006; Stankowich *et al.*, 2014; Kortet *et al.*, 2019),就鱼类而言,其反捕食的行为策略主要分为3类:躲避捕食者,遭遇捕食者之后逃跑或主动防御。有研究发现,当被捕食风险较高时,猎物鱼会通过减少活动量(Belgrad *et al.*, 2016),调整活动时间(Bach *et al.*, 2021),增加或改变栖息地的利用(Lvarez *et al.*, 2010)等行为来降低与捕食者相遇的概率;遭遇捕食者时,猎物鱼会提高快速启动游泳能力来保证其顺利逃脱(Ramasamy *et al.*, 2015)。此外,当猎物鱼被捕食者发现或袭击时,有些鱼类会改变身体形态(Carlos *et al.*, 2020)或利用身体进化出的棘和刺进行防御(Price *et al.*, 2015)。上述行为的改变通常能成功降低猎物鱼被捕食者直接捕杀的概率,但就成功逃避捕食的猎物鱼个体而言,对捕食者的恐惧及为此作出的反捕食响应是有代价的,可能对其觅食(Stoks *et al.*, 2005; Eaton *et al.*, 2016)、生长(Rahman *et al.*, 2021)和繁殖(Mukherjee *et al.*, 2014)产生负面影响。因此,猎物鱼会根据当前应对的捕食压力的强度和持续时间来调整自己的反捕食策略,最大化反捕食效益。例如,在遭遇捕食者后,澳氏雀鲷(*Pomacentrus wardi*)会降低冒险行为(Lönnstedt *et al.*, 2012),高捕食压力环境中的埃氏短棒鲈(*Brachyrhaphis episcopi*)种群对胁迫表现出更高的活跃性和探索性(Archard *et al.*, 2012)。此外,运动能力的调整对于猎物鱼遭遇捕食者后逃跑至关重要,高捕食压力环境中的黑鲫(*Carassius carassius*)、平头米诺鱼(*Pimephales promelas*)比低捕食压力环境种群表现出更快的逃逸速度(Ferrari *et al.*, 2006; Domenici *et al.*, 2008)。猎物鱼维持较高的运动状态,对捕食者保持警惕能提高生存能力,但捕食胁迫水平与运动能力响应程度之间的关系尚未具有统一论。

青鱼(*Mylopharyngodon piceus*)、草鱼(*Ctenopharyngodon idellus*)、鲢(*Hypophthalmichthys molitrix*)和

鳙(*Aristichthys nobilis*)合称为“四大家鱼”,是中国长江流域常见的鲤科鱼类。在其幼鱼阶段,捕食胁迫普遍存在,且多年来由于水力建设、环境污染、过度捕捞等诸多原因导致其野外种群数量急剧下降(Duan *et al.*, 2009; 刘飞等, 2019),增殖放流在恢复和维持其自然种群数量方面起到了十分重要的作用(石小涛等, 2012; 张照鹏等, 2021)。为了深入了解捕食胁迫对放流四大家鱼幼鱼的影响,同时探索天然捕食环境中猎物鱼如何调整其运动能力以应对捕食压力,本研究选择青鱼、草鱼、鲢和鳙作为猎物鱼,以其水域常见的天敌乌鳢(*Channa argus*)和南方大口鲶(*Silurus meridionalis*)作为捕食者,研究了不同捕食胁迫水平下“四大家鱼”幼鱼的运动能力对捕食胁迫的响应方式和强度,考察四大家鱼在应对捕食压力时其运动能力的优化模式,比较其种间差异,为优化鱼类增殖放流方案、增加鱼类放流存活率提供科学依据,为探索捕食者与被捕食者间的生态互作效应提供科学参考。

1 材料与方法

1.1 实验鱼来源与驯养

本实验于2021年5—7月在长江四大家鱼监利老江河原种场开展,猎物鱼四大家鱼幼鱼(青鱼、草鱼、鲢、鳙)及捕食者(乌鳢和南方大口鲶)均取自老江河原种场,其中四大家鱼幼鱼为原种场人工繁殖用于增殖放流的幼鱼。实验鱼分别放入规格为4 m×2 m×1.2 m的长方形水泥养殖池中驯养2周。驯养期间,“四大家鱼”幼鱼每天09:00和17:00以商业饲料投喂两次,投喂量为池内实验鱼体重总和的3%;乌鳢、南方大口鲶与不用于实验的“四大家鱼”幼鱼混养,不额外投喂饲料,用于混养投喂捕食者的“四大家鱼”幼鱼与用于测定的实验鱼取自同一批次幼鱼,比例、规格均相近。养殖用水取自老江河,驯化温度为25~29℃,溶氧为7~8 mg·L⁻¹。

1.2 实验设计

2周驯养结束后,每种实验鱼各随机选择体长相近的样本(青鱼:8.81±0.22 cm, 11.73±0.62 g, n=

400; 草鱼: 8.33 ± 0.19 cm, 10.23 ± 0.72 g, $n = 400$; 鲢: 8.43 ± 0.15 cm, 9.10 ± 0.36 g, $n = 400$; 鳙: 8.50 ± 0.11 cm, 11.10 ± 0.36 g, $n = 400$) 分类别按捕食胁迫程度将每种鱼随机分为3组: 无捕食组(空白对照), 低捕食组(隔网胁迫)和高捕食组(直接捕食), 共12组。如图1所示, D、E、H、I 各放入100尾同种实验鱼, D、E 为对照组, H 为低捕食组, I 为高捕食组, 为和捕食组保持驯养密度一致, D、E 各放入100尾实验鱼。对照组中拦网两侧不放入捕食者, 捕食组中将乌鳢(400~500 g)和南方大口鲈(400~500 g)各一尾作为共同捕食者一起放入。低捕食组实验鱼与捕食者被拦网隔开, 无直接接触, 高捕食组实验鱼与捕食者处于拦网同侧, 捕食者可直接捕食高捕食组实验鱼(图1)。捕食胁迫期间, “四大家鱼”幼鱼的投喂同驯养期间一致, 捕食者不额外投喂, 其食物来源为混养的用于测定的实验鱼。捕食胁迫处理0、7、14 d 后随机选取身体健康、大小接近的实验鱼, 分别测定其体长、体重、临界游泳速度($n = 6$)和快速启动游泳能力($n = 12$)。

1.3 临界游泳速度的测定

“四大家鱼”幼鱼在测定前禁食24 h 后转移至鱼类游泳速度测定设备, 仪器的原理结构详见已发表论文(王晓等, 2021)。临界游泳速度采用流速递增法进行测定(Brett, 1964)。正式试验之前首先预估实验鱼的绝对临界游泳速度(U_e), 再根据预估值确定试验的速度增量 ΔU ($15\% U_e$)。选取一尾健康且无损的实验鱼转移至水槽, 测定前在低流速($5 \text{ cm} \cdot \text{s}^{-1}$) 环境下进行1 h 的适应, 以消除转移过程对鱼体的胁迫。适应结束后, 以 $5 \text{ cm} \cdot \text{s}^{-1}$ 作为起始流速, 每2 min 增加 $0.4 \text{ BL} \cdot \text{s}^{-1}$ ($\text{BL} = \text{Body Length}$),

直至实验鱼疲劳, 此时的流速值即为 U_e 。

正式试验时, 将单尾实验鱼放入试验装置, 使其在低流速($5 \text{ cm} \cdot \text{s}^{-1}$) 环境下适应1 h。适应结束后, 以 $5 \text{ cm} \cdot \text{s}^{-1}$ 作为起始流速, 每隔5 min 水流速度增加 $0.5 \text{ BL} \cdot \text{s}^{-1}$ 至 $60\% U_e$, 后每隔15 min 增加流速 $15\% U_e$, 直至实验鱼疲劳力竭, 此时结束试验, 记录流速值和游泳时间。实验鱼疲劳的判断标准为实验鱼被水流冲至水槽尾部拦网上无法游动且持续时间超出20 s。临界游泳速度计算公式如下:

$$U_{\text{crit}}^a = U_{\text{max}} + (t/T) \Delta U$$

$$U_{\text{crit}}^r = U_{\text{crit}}^a / \text{BL}$$

式中, U_{crit}^a 是绝对临界游泳速度($\text{cm} \cdot \text{s}^{-1}$), U_{max} 是实验鱼在力竭时达到的最大游泳速度($\text{cm} \cdot \text{s}^{-1}$), t 是实验鱼力竭时在设定时间内的实际游泳时间(min), T 是设定时间(15 min), ΔU 是设定时间内的流速增量($15\% U_e$), U_{crit}^r 是相对临界游泳速度($\text{BL} \cdot \text{s}^{-1}$), BL 是实验鱼的体长(cm)。本研究实验鱼的最大横截面积不超过游泳水槽横截面积的10%, 可忽略水流阻挡效应, 故无需校正(Jain, 2003)。

1.4 快速启动游泳能力的测定

本研究中快速启动游泳能力的测定装置参考雀鲷类幼鱼(*Acanthochromis polyacanthus*) 快速启动测定装置(Ramasamy et al., 2017)。将实验鱼放入测定水槽中适应30 min, 待实验鱼开始规则的绕壁游动至水槽中央时释放重物刺激, 同时以480 帧/秒(索尼, PXW-FS5/FS5K) 的速度记录实验鱼的快速启动过程, 拍摄时间持续3秒。以重物接触水面后实验鱼头部方向改变作为第1阶段(Stage 1, S1) 的开始, 通过逐帧观察, 将鱼体弯曲角度最大所对应的

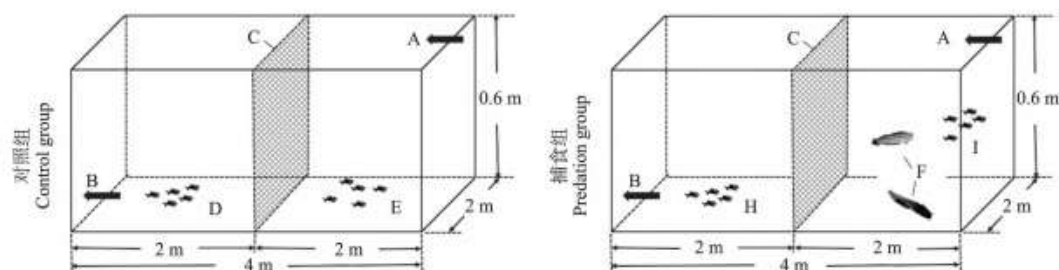


图1 试验装置示意图

Fig.1 Schematic diagram of the test device

注: A: 进水口; B: 出水口; C: 尼龙拦网(孔径为 $0.5 \text{ cm} \times 0.5 \text{ cm}$); D、E: 对照组, 各放100尾实验鱼, 不放入捕食者; F: 捕食者(乌鳢和南方大口鲈各一尾); H: 低捕食组, 实验鱼($n = 100$)与捕食者分隔网可见; I: 高捕食组, 实验鱼($n = 100$)中放入双捕食者: 乌鳢和南方大口鲈各一尾。

Note: A: Water inlet; B: Water outlet; C: Nylon block ($0.5 \text{ cm} \times 0.5 \text{ cm}$); D, E: control group, each put 100 experimental fish, and no predators were added; F: Predator (one snakehead and one southern catfish); H: low-predation group, experimental fish ($n = 100$) were separated from the predator and visible in separate nets; I: high-predation group, experimental fish ($n = 100$) were placed in a double predator: one snakehead and one southern catfish each.

乌杨调节坝运行初期汉丰湖浮游植物群落结构 及其与环境因子的相关性分析

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摘要: 为了解乌杨调节坝正式运行初期汉丰湖水体情况, 2016年10月—2017年10月对汉丰湖浮游植物群落及水质状况进行了调查。结果显示, 调查期间共检出浮游植物7门58属114种, 其中绿藻门和硅藻门最多, 分别占39.47%和38.60%。尖针杆藻、梅尼小环藻和嗜蚀隐藻等为优势种。浮游植物平均密度为 8.64×10^5 ind./L; 平均生物量为2.03 mg/L。其中, 平均密度和生物量在2016年12月最小, 2017年2—8月不断增大, 10月份有所下降; 四个采样点的平均密度和生物量差异均不显著。各采样点浮游植物 Margalef 物种丰富度指数(d_{Ma})为1.69~5.23; Shannon-Wiener 指数(H')为1.12~2.75; Pielou 均匀性指数(J_e)为0.45~0.96。水温、高锰酸盐指数、总磷、总氮、氨氮的变化范围分别为12.7~31.5℃、1.54~4.92 mg/L、0.01~0.18 mg/L、0.20~1.11 mg/L、0.10~0.81 mg/L。冗余分析(RDA)结果表明, 汉丰湖浮游植物与高锰酸盐指数、总磷、亚硝态氮和氨氮呈正相关, 与透明度呈负相关。

关键词: 汉丰湖; 浮游植物; 时空变化; 冗余分析

中图分类号: S932.4

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Phytoplankton community structure and its redundancy correlation with environmental factors in Hanfeng Lake during the early stage of the operation of Wuyang Regulating Dam

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Abstract: The phytoplankton community and water quality of Hanfeng Lake was investigated from October 2016 to October 2017 in order to understand the situation of Hanfeng Lake in the early stage of the Wuyang regulating dam officially put into operation. A total of 114 species from 7 phyla of phytoplankton were detected. The dominant algae were Chlorophyta and Bacillariophyta with a ratio of 39.47% and 38.60%, respectively. *Synedra acus*, *Cyclotella meneghiniana* and *Cryptomonas erosa* were dominant species. The average density of phytoplankton was 8.64×10^5 ind./L. Meanwhile, the average biomass was 2.03 mg/L. The Margalef index (d_{Ma}) of phytoplankton was between 1.69~5.23, the Shannon-Wiener index (H') of phytoplankton was between 1.12~2.75 and the Pielou's uniformity index (J_e) was between 0.45~0.96. Scope of the change for water temperature, permanganate index, total phosphorus, total nitrogen and ammonia nitrogen were 12.7~31.5℃, 1.54~4.92 mg/L, 0.01~0.18 mg/L, 0.20~1.11 mg/L, 0.10~0.81 mg/L, respectively. Redundancy analysis (RDA) results showed that phytoplankton in Hanfeng Lake was positively correlated with permanganate index, total phosphorus, nitrate nitrogen and ammonia nitrogen, and negatively correlated with transparency.

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Key words: Hanfeng Lake; phytoplankton; spatial and temporal changes; redundancy analysis

浮游植物是水体中初级生产者之一^[1], 同时也是浮游动物和鱼类的食物, 其在水域生态系统中起着重要作用。研究表明浮游植物受水体环境因子影响较大, 且浮游植物种类组成和分布一定程度上能反映水体环境^[2]。因此, 研究水体浮游植物群落结构及其与环境因子的关系, 对水域生态环境的保护和治理具有一定意义。

汉丰湖地处重庆市开州区, 因三峡工程蓄水后倒灌形成, 是三峡库区最大的库中人工湖^[3]。汉丰湖是当地旅游开发的主要景点, 关系着开州区的经济发展。然而由于汉丰湖水位在三峡水库“蓄清排浑”运行方式的影响下, 库区内每年都会形成30 m消落区^[4], 影响湖内的水域生态环境。为缓解消落区带来的负面影响, 开州区政府在丰乐镇乌杨村修建了调节坝来控制湖内水位变化, 保证汉丰湖常年性稳定蓄水。调节坝于2016年8月31日正式运行, 调节坝运行后汉丰湖内的水域生态环境可能发生改变, 目前有关汉丰湖浮游植物的研究均

集中在调节坝正式运行前^[5,6], 有关调节坝运行初期汉丰湖的浮游植物现状及其与水质的关系还未见报道。因此, 通过对其运行初期的浮游植物群落结构和水质关系进行调查分析, 了解调节坝运行后汉丰湖内水域生态环境情况尤为重要。

1 材料与方法

1.1 采样时间和采样点

2016年10月—2017年10月, 每两个月对汉丰湖进行采样调查。依据《内陆水域浮游植物监测技术规程》, 结合汉丰湖实际情况, 本次调查共设置4个采样点(见图1), 分别为南河汇入口C1(镇安, 108°20′34.01″E, 31°9′8.63″N), 汉丰湖中心C2(故津广场, 108°23′18.22″E, 31°11′2.62″N), 东河汇入口C3(丰乐街道, 108°25′18.12″E, 31°12′6.30″N)和出水口C4(调节坝, 108°27′36.12″E, 31°10′59.83″N)。

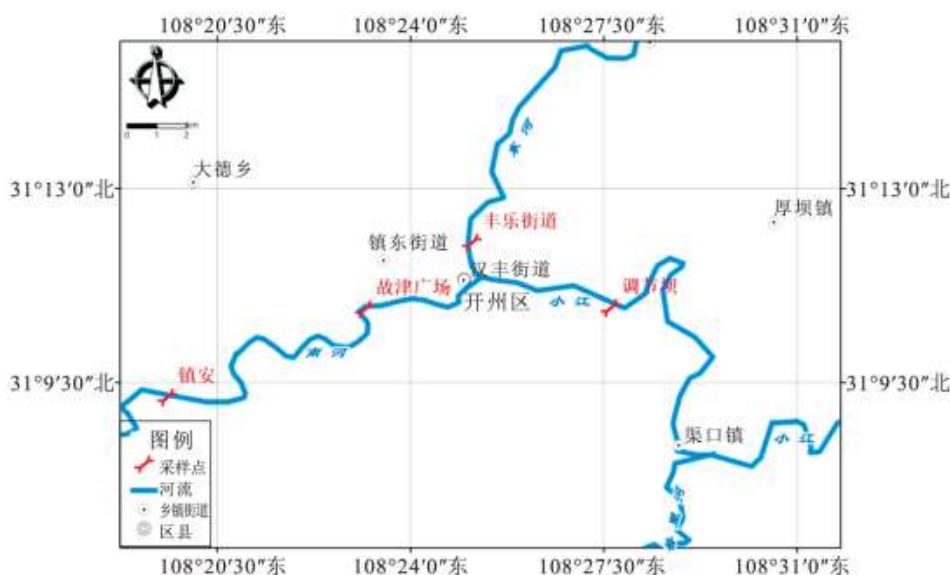


图1 汉丰湖采样点分布图

Fig. 1 The stations of the survey in Hanfeng Lake

1.2 样品采集与分析

水质因子按照《水和废水监测分析方法》^[7]和《地表水环境质量标准》^[8], 现场测定水温(WT)和pH等指标, 溶解氧(DO)、总磷(TP)、总氮(TN)、氨氮(NH₃-N)、高锰酸钾指数(COD_{Mn})等指标利用采集回去的水样, 在实验室及时进行检测。

浮游植物样品采集遵循《高级水生生物学》^[9]要求进行操作, 定性样品采集用25#浮游生物网在水面下作“∞”形拖动约3~5 min, 将网底的浓缩水样倒入标本瓶中, 加1%鲁哥氏液; 定量样品采集直接采1 L水于水样瓶中, 并加入15 mL鲁哥氏液充分摇匀固定带回。浮游植物鉴定和计算参考

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黑藻 (*Hydrilla verticillata*) 在铅、锌胁迫下的代谢组学研究

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摘要: 黑藻(*Hydrilla verticillata*)作为我国淡水水域常见的沉水植物,对水环境重金属污染修复有着重要作用。通过超高效液相色谱串联质谱(UPLC-MS/MS)技术检测了黑藻中 342 个初级代谢产物,并采用单变量、主成分分析(PCA)和正交偏最小二乘法判别分析(OPLS-DA)多元统计分析方法分析代谢物,以探究在不同浓度铅、锌胁迫下黑藻初级代谢产物的变化特性,弄清铅、锌对生物体代谢活动的影响。结果表明,铅单一胁迫主要促进黑藻机体酚酸类和糖类物质的生成,抑制脂类的生成,N-苯乙酰基-L-谷氨酰胺和异水杨酸-O-葡萄糖显著上调,相比单一铅胁迫,铅锌复合胁迫主要促进黑藻氨基酸、糖类、有机酸和脂质等代谢物的合成,抑制酚酸类、维生素和脂质等代谢物的合成;锌单一胁迫主要促进氨基酸、核苷酸、糖类和有机酸等代谢物的生成,抑制部分酚酸类和脂质代谢物的生成,相比单一锌胁迫,高浓度铅锌复合胁迫(Pb0.10+Zn2.00)可主要促进酚酸类、核苷酸、有机酸和脂质等代谢物的合成,抑制以谷胱甘肽为代表的氨基酸等代谢物的合成;超高浓度铅锌复合胁迫(Pb0.20+Zn4.00)可主要促进对苯二甲酸(C₈H₆O₄)和去鼠李糖异洋丁香酚苷 B(C₂₃H₂₆O₁₁) 2 种酚酸类代谢物的合成,抑制氨基酸、核苷酸、维生素和有机酸等代谢物的合成。另外,N-苯乙酰基-L-谷氨酰胺(C₁₃H₁₆N₂O₄)、酪氨酸(C₉H₉NO)、对香豆酰咖啡酰酒石酸(C₂₂H₁₈O₁₁)、没食子鞣质(C₁₃H₁₆O₁₀)和异水杨酸-O-葡萄糖(C₁₃H₁₆O₈)可作为铅胁迫的代谢标记物,5-氨基戊酸(C₅H₁₁NO₂)、L-(+)-精氨酸(C₆H₁₄N₄O₂)和 L-焦谷氨酸(C₅H₇NO₃)等可作为锌胁迫的代谢标记物。

关键词: 铅; 锌; 黑藻; 植物毒性; 代谢组学

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Metabolomics Study of *Hydrilla verticillata* under Heavy Metal Stress of Lead and Zinc

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Abstract: *Hydrilla verticillata*, a common submerged plant in freshwater of China, has a certain adsorption effect on heavy metals, which made it play an important role in water environment protection. Excessive lead accumulation could affect the growth and physiological metabolism in plants, meanwhile, even cause plant death. As the associated metal of lead, zinc is one of the necessary elements of organisms, but when its content exceeds the micro-demand required, it will cause a certain degree of damage to the body. Three hundred and forty-two primary me-

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