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Original Article

Flow-Regime Conditioning for Wild-Adaptation in Juvenile *Sebastes Schlegelii*

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Abstract:
Sebastes schlegelii is an economically important fish distributed in northern China, but its populations are declining. To support stock enhancement and assess the effects of swim-conditioning on juveniles' wild-adaptation, we conducted swim-training on juvenile *Sebastes schlegelii* (total length 3.05 ± 0.10 cm, body weight 0.58 g) to improve their preparedness for the natural environment. Constant-flow swim training was applied at three speeds (2BL s^{-1}, 4BL s^{-1}, and 6BL s^{-1}) for 30 days. After the training, the following parameters were measured: survival rate, terminal body mass, weight gain efficiency, visceral somatic index, condition factor, specific growth rate; as well as the activity of alkaline phosphatase, catalase, glutamate oxaloacetate transaminase, lysozyme, and total amino acids. The results showed that after swim training, the 2BL s^{-1} flow rate affected juveniles' growth performance and biochemical parameters by significantly increasing terminal body mass, weight gain efficiency, specific growth rate and condition factor. The 4BL s^{-1} flow rate produced no significant differences compared with the control. The 6BL s^{-1} flow rate caused significant negative effects relative to the control. The research indicates that moderate swimming exercise training can enhance the growth performance and biochemical indicators and improve the muscle quality, increase the antioxidant capacity, and regulate the functions in juvenile *Sebastes schlegelii*, thereby promoting the healthy growth and helping them adapt to the complex conditions of the wild.

Keywords: Wild-adaptation training, *Sebastes schlegelii*, Swim training, Conservation stocking

1. Introduction

Sebastes schlegelii is primarily distributed along Northeast Asian coastal shelves, with confirmed populations in Bohai Sea, Yellow Sea, and East China Sea. This species is a cold-water, demersal, carnivorous fish and is one of the most economically important fish species in northern China, characterized by its strong adaptability, rapid growth, and rich nutritional value (Liu et al., 2018). However, increased production efficiency and various human activities - such as overfishing, water conservancy project construction, and industrial and agricultural production - have led to a decline in the species (Zhang et al., 2023). As a result, fisheries increasingly rely on aquaculture, in order to

maintain sustainable fishing populations (Brown & Laland, 2001).

Stock enhancement through artificial release is a crucial method for maintaining natural biological resources, restoring aquatic ecosystems, and protecting endangered species. This process involves artificially cultivating juvenile fish and releasing them into target marine areas to increase population size (Johnston et al., 2018). The juveniles feed on natural prey and grow to specified sizes within a short period, yielding economic benefits upon capture. Some of these released juveniles can also breed with wild populations, thereby contributing positively to the ecology of natural populations (Shi et al., 2012). Juveniles are individuals that have developed from larvae and possess an appearance similar to adults. The shapes of the fins are well defined, and there are no significant differences in appearance from adult fish. Compared with larvae, juveniles show marked improvements in activity and condition. They exhibit a strong appetite and considerable food intake, consuming the same feed as adults. Body growth and skeletal development reach a peak during this stage. Generally, after a 4 - 5 month rearing period, these juveniles can develop a body shape similar to that of adult fish. Mass mortality during stock enhancement occurs due to incomplete development of critical survival behaviors (reef-oriented behavior, predator avoidance, swimming competency) in hatchery-reared juveniles, leading to impaired environmental adaptation post-release. Concurrently, transport-induced thermal fluctuations and hydrodynamic shocks exacerbate mortality rates. Artificial selection-induced morphological divergence in cultured conspecifics significantly reduces post-release survival compared to wild populations (Maynard et al., 1995).

Wild-adaptation training is defined as a husbandry-based conditioning protocol for hatchery-reared target organisms, integrating behavioral conditioning methodologies with environmental enrichment strategies. This approach aims at enhancing survival competency through simulated natural conditions of wild conspecifics, while promoting ontogenetic behavioral development and improving post-release viability (Gao et al., 2017). Pre-release wild-adaptation training constitutes a validated methodology for enhancing stock enhancement success rates, with technical development emphasizing hydrodynamic variability conditioning to optimize swimming performance. This protocol rectifies critical behavioral deficits in hatchery-reared organisms through simulated ecological challenges, including migratory orientation precision, predator avoidance response latency, and prey capture efficiency. Cultured fish released into natural environments typically exhibit experiential deficiencies in navigating complex habitat structures, executing successful predation on variable prey morphologies, and implementing anti-predation strategies. Post-training physiological convergence demonstrates measurable improvements in swimming capacity, with select cultured populations achieving 85% critical swimming speed parity with wild conspecifics. These adaptive behavioral refinements validate wild-adaptation training as an effective intervention to recalibrate laboratory-reared fish behaviors toward ecological competence, ultimately enhancing post-release survival through improved environmental adaptability (Brown, Jones, & Braithwaite, 2007).

Different species of fish have their own unique swimming modes, which mainly include burst swimming, sustained swimming, and prolonged swimming. These correspond to short bursts of fast swimming, long periods of slow swimming, and swimming for extended durations, respectively (Blake, 2004). Critical swimming speed refers to the maximum sustained swimming speed of fish and is often used to assess their swimming ability (Kristan III, 2003).

Hydrodynamic wild-adaptation training has been systematically implemented in riverine ecosystem rehabilitation programs, utilizing flow regime replication to stimulate species-specific behavioral ontogeny and locomotor performance optimization (Tudorache et al., 2007).Hydrodynamic wild-adaptation training enhances locomotor capacity in cultured fish populations while increasing field survival probability. This methodology not only improves overall survival rates and reproductive capabilities of artificially reared fish but also facilitates rational exploitation and conservation of aquatic biological resources, providing a regulatory framework for sustainable fisheries development (Radchuk et al., 2019). Therefore, hydrodynamic wild-adaptation training plays a pivotal role in fisheries ecological conservation and resource augmentation, merits further research validation, and warrants widespread application in aquatic management practices.

2 Materials and Methods

2.1 Fish Used in the Experiment

The experimental fish were juvenile *Sebastes schlegelii* artificially bred by a company. The fish were raised in indoor aquaculture tanks for three months before the experiment. During the rearing period, they were fed with pellet feed twice per day at 8:00 AM and 6:00 PM respectively. Residual feed was removed using a siphoning method 30 minutes after feeding. The water quality was maintained as recirculating seawater with a salinity of 29-32 ‰, a controlled temperature of (17±1) ℃, dissolved oxygen concentration above 6.0 mg/L, pH levels of 7.9-8.2, and light intensity of 500-1000 lx. Test specimens exhibited body length of 3.05±0.1 cm compliant with minimum stocking size requirements for release fry.

2.2 Experimental Apparatus and Experimental Design

2.2.1 Experimental Apparatus

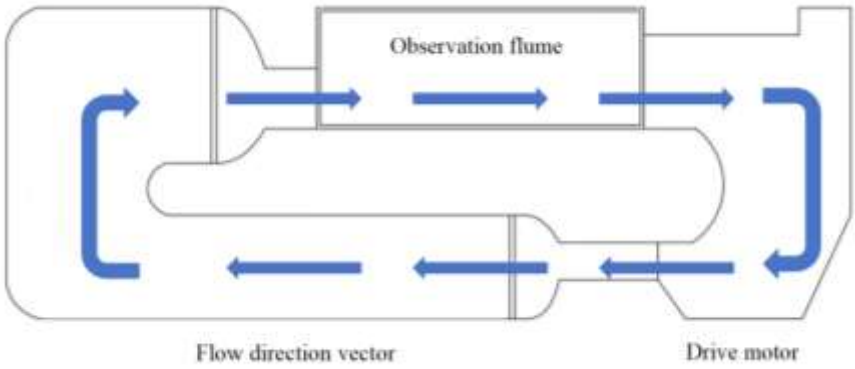

Figure 1 Experimental Apparatus

A vertical circulating water channel is used in the experiment. The testing tank is 255 cm in length, 110 cm in height, and 32 cm in width. The observation section measures 100 cm in length, 25 cm in width, and 25 cm in height. A flow velocity meter (VR-201H, KENEK, Japan) is used to measure the flow velocity.Measurement range 0.00-2.00 m/s, resolution 0.01 m/s. Before the experiment and after each run, the flow velocity meter was calibrated at two points using a standard towing method, with recorded drift less than ±0.01 m/s; a zero check was performed every 2 hours during the experiment. The measurement point was located at the longitudinal center of the observation section (50 cm from the inlet), with the lateral and vertical positions at the section center (12.5 cm from the sidewall and bottom, respectively).

2.2.2 Experimental Design

In preliminary experiments, the maximum endurance threshold was determined by recording individual time-to-exhaustion under uniform flow conditions, defined as cessation of continuous swimming with failure to resume after gentle caudal stimulation. Each fish was tested only once to avoid training effects; groups were randomized, and water temperature, dissolved oxygen, and photoperiod were kept consistent. At the optimal flow velocity identified in pilot trials (2BL s^{-1}), individuals sustained longer training durations with minimal mortality.The experimental design included four parameters: flow velocity, single-session duration, daily training frequency, and total training duration. Six test groups and one control group were established (n = 20 per group). Fish in each experimental group were placed daily in a micro-channel within the same water body (0.05BL s^{-1}). Continuous aeration was provided by air pumps during training.

After training, fish were fasted for 24 h to minimize short-term feeding effects on metabolic and biochemical indices. Subsequently, 10 individuals per group were randomly selected for biometric measurements (body length/weight), followed by euthanasia and dissection under an approved anesthesia protocol, with collection of viscera and dorsal muscle. All samples were handled under pre-chilled conditions; tissues were weighed, snap-frozen in liquid nitrogen, and stored at -80℃ to prevent degradation. For each biochemical assay, 10 technical replicates were performed to meet sample size requirements. Training capacity calculated as flow velocity × single-session duration × daily training frequency × total training duration (Rimmer et al., 1985).

Table 1 Formula for calculating total capacity

Flow velocity (BL s^{-1})	Duration of each training session(min)	Number of training sessions per day (times)	Total number of training days (d)	Total training capacity
2	15	4	15	1800
2	15	2	30	1800
4	15	2	15	1800
4	15	1	30	1800
6	20	1	15	1800
6	10	1	30	1800

Growth physiological measurement indicators:

Survival rate = Nt/N0 × 100%

Organ to body ratio = Wh/Wb × 100%

Condition factor = Wb/L^3 × 100%

Specific growth rate = (ln Wt – ln W0) / t × 100%

Note: N0 is the initial number of fish tails; Nt is the final number of fish tails; W0 (g) is the initial average body weight; Wt (g) is the final average body weight; t (d) is the training duration in days; Wb (g) is the final weight of each fish; L (cm) is the final length of each fish; Wh (g) is the final weight of the internal organs of each fish.

Visceral Biochemical Measurement Indicators:

The experimental detection indicators are measured using reagent kits from Nanjing Jianchen Bioengineering Institute. The detection instruments include a spectrophotometer, constant temperature water bath, centrifuge, vortex mixer, micropipette and etc.

Alkaline phosphatase (AKP): Enzyme activity of AKP in the tissue = $\frac{A\_measure}{A\_standard} \times m_S \div (Cpr \times V\_sample)$

A_measure: The true measured value of enzyme activity in the sample

A_standard: The standard used for calibration to establish the standard curve

m_S: The amount of phenol in the standard tube, 0.005 mg

V_sample: The volume of the sample tube, 0.03 mL

Cpr: Protein concentration in the tissue homogenate, g_prot/mL ("prot" refers to protein)

Catalase (CAT): Enzyme activity of CAT in the tissue = ΔA × 271 ÷ V_sample ÷ T ÷ Cpr

ΔA: Change in absorbance of the sample

271: The reciprocal of the slope, a constant used directly

V_sample: Sample volume, 0.05 mL

T: Reaction time, 60 seconds

Cpr: Protein concentration in the homogenate, mg_prot/mL ("prot" refers to protein)

Glutamate oxaloacetate transaminase (GOT): Enzyme activity of GOT in the tissue = Enzyme activity of homogenate obtained from the standard curve ÷ Protein concentration of the homogenate to be measured.

Lysozyme (LMZ): Lysozyme content (μg/mL) = $\frac{\Delta T\_measure}{\Delta T\_standard} \times C\_standard \times N$

ΔT_measure: The change in absorbance in the test tube is calculated by subtracting the absorbance at 5 seconds from that at 2 minutes and 5 seconds after adding the bacterial solution.

ΔT_standard: The change in absorbance in the standard tube is calculated by subtracting the absorbance at 5 seconds from that at 2 minutes and 5 seconds after adding the bacterial solution.

C_standard: Concentration of the standard solution, 2.5 μg/mL, which is equivalent to 200 U/mL (1 μg = 80 U).

N: Dilution factor before testing the sample.

Total amino acids determination: The total amino acid content in the tissue (μmol/mg protein) = $\frac{A\_measure - A\_blank}{A\_standard - A\_blank} \times C\_standard \div Cpr$

A_measure: Absorbance value of the sample, which reflects the concentration of amino acids.

A_blank: Absorbance value of the blank sample used for calibration to ensure the accuracy of the experiment.

A_standard: Absorbance value of the standard solution with known amino acid concentration, used to establish the standard curve for calculating the total amount of amino acids in the sample.

C_standard: Concentration of the standard solution, 50 μmol/mL.

Cpr: Protein concentration of the tissue sample, mg/mL.

2.3 Data Processing

All data were presented as Mean ± S.D. following verification of normal distribution and homogeneity of variances. Statistical analysis was conducted using One-Way ANOVA and Paired sample T-test to compare growth performance metrics and enzyme activity variations among flow velocity treatments ($P<0.05$ significance level), with data processed through SPSS 22.0, Excel, and Origin software packages.

3 Results

3.1 Growth Performance Indicators

Survival Rate:

Result: After 15 days of training, the survival rate in all groups was 100%. After 30 days of training, with a water flow rate of 2BL s^{-1}, the survival rate was also 100%, while at flow rates of 4BL s^{-1} and 6BL s^{-1}, the survival rates were 90%. The survival rate in the control group was also 100%.

Terminal Body Mass:

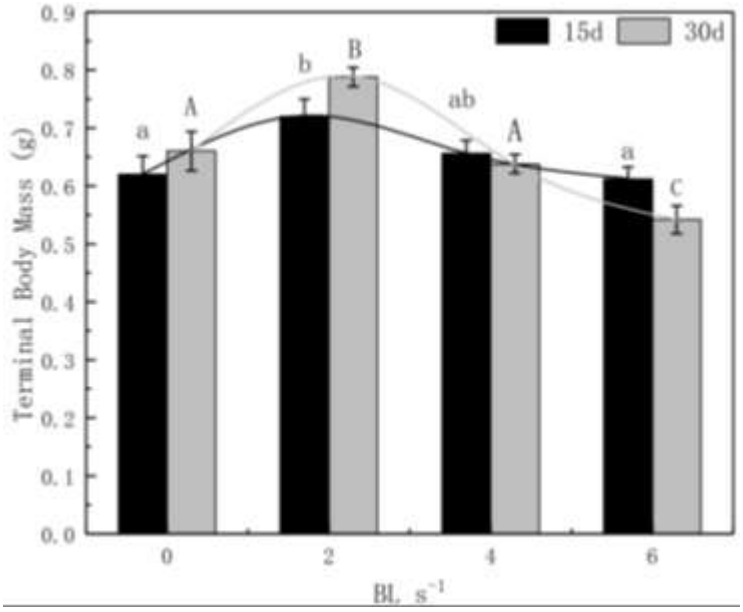

Figure 2 The effect of exercise training on the terminal body mass of juvenile fish of the S.schlegelii

Note. Different lowercase letters in figures indicate significant differences (P<0.05) among 15-day training groups, while uppercase letters denote significant variations (P<0.05) between 30-day training groups. Asterisks (*) represent significant inter-group differences (P<0.05) across different training durations under identical flow velocities. The definition of the above symbols are applicable to subsequent figures.

Result: After 15 and 30 day training periods, the 2BL s^{-1} group attained the maximum mean terminal body mass, contrasted by the minimum value in the 6BL s^{-1} group (P<0.05). At 15 days, significant differences (P<0.05) were observed between the control group and 2BL s^{-1} group, and between the 2BL s^{-1} group and 6BL s^{-1} group, with no significant differences among the other groups. After 30 days, all groups differed significantly from the control group except for the 4BL s^{-1} group. Comparatively, no significant differences in body weights were found in the same flow-rate groups at 15 vs. 30 days.

Weight Gain Efficiency

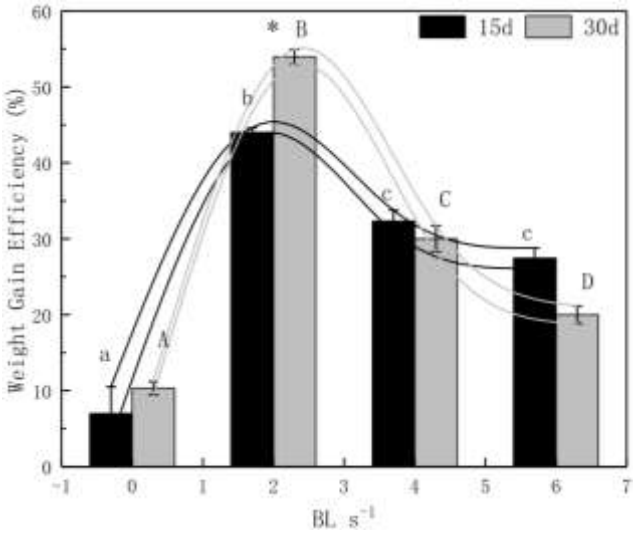

Figure 3 The effect of exercise training on the weight gain efficiency of juvenile S.schlegelii

Result: The 2BL s^{-1} group achieved the maximum weight gain efficiency after 15 and 30 day training periods. At the 15 day mark, pairwise comparisons reached statistical significance ($P<0.05$) except for the 4BL s^{-1} vs. 6BL s^{-1} contrast. After 30 days, all inter-group differences became significant. Comparison between 15 and 30 day periods showed significant differences only in the 2BL s^{-1} flow group, while no significant differences were detected in remaining groups.

Visceral Somatic Index:

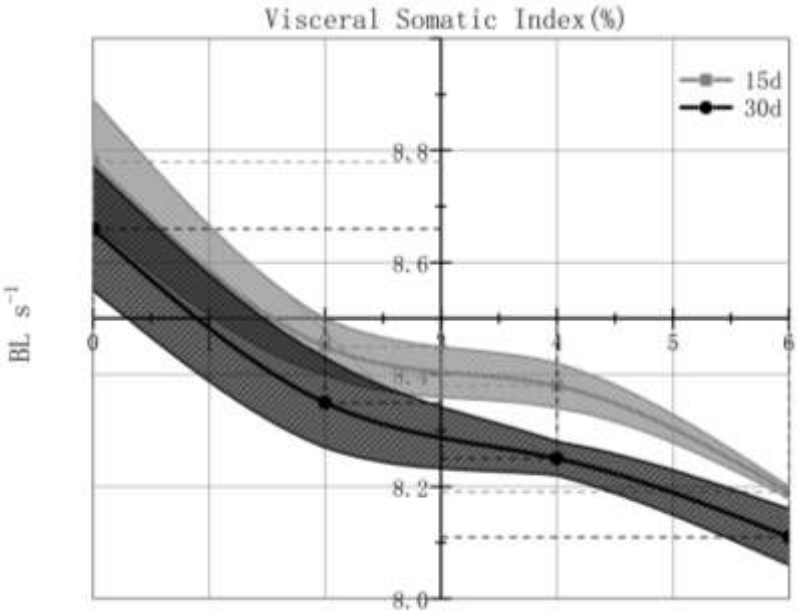

Figure 4 Effect of exercise training on the visceral somatic index of juvenile S.schlegelii
Note. Different colored regions represent the standard deviation for each group

Result: After 15 day training period, significant differences were observed except for non-significant differences between 2BL s^{-1} and 4BL s^{-1} flow groups, and between 4BL s^{-1} and 6BL s^{-1} flow groups. After 30-day training, significant differences were found between control group vs.

2BL s^{-1} flow group, control group vs. 4BL s^{-1} flow group, and control group vs. 6BL s^{-1} flow group, while the remaining flow groups showed no significant differences. No significant differences were observed between identical flow groups when comparing 15 and 30 day periods.

Specific Growth Rate and Condition Factor:

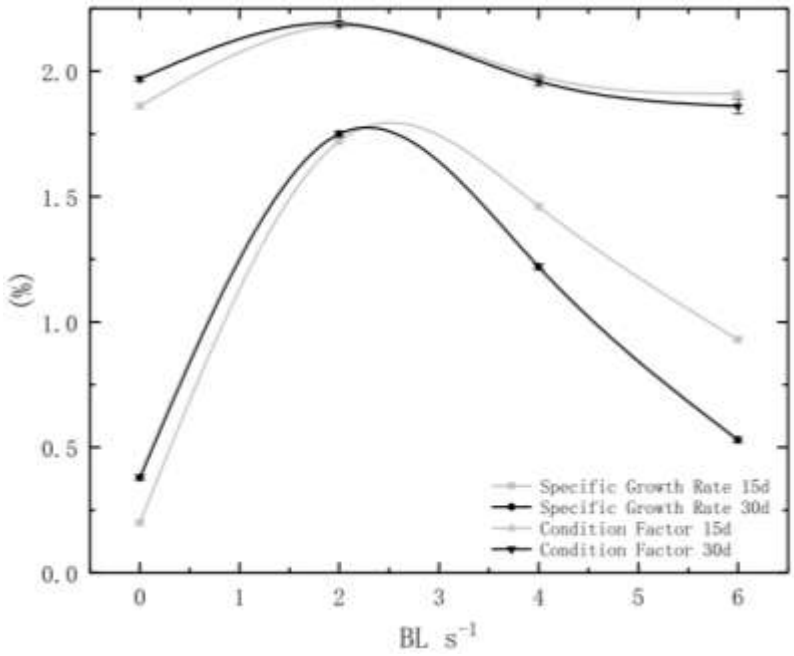

Figure 5 The effect of exercise training on specific growth rate and condition factor of S.schlegelii

Result: After 15 and 30 day training periods, the 2BL s^{-1} flow group exhibited the highest specific growth rate and condition factor. During 15 day training, all flow groups showed significant differences from each other. After 30 day training, all flow groups demonstrated significant differences in specific growth rate. Regarding condition factor, no significant differences were observed between the control group and 4BL s^{-1} flow group, while significant differences were found among the remaining groups. When comparing 15 and 30 day periods, significant differences were found in the control group, the 4BL s^{-1} flow group for specific growth rate, and the 6BL s^{-1} flow group, whereas no significant differences were observed in the 2BL s^{-1} flow group. Comparisons among different condition factors within identical flow groups showed no significant differences.

3.2 Viscera Biochemical Indicators

Alkaline Phosphatase (AKP):

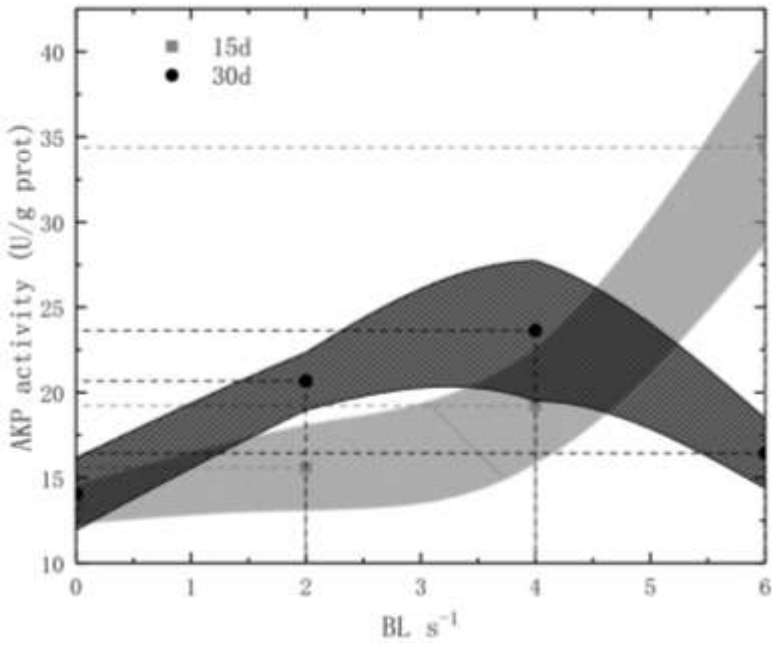

Figure 6 The effect of exercise training on alkaline phosphatase activity in juvenile fish of S.schlegelii

Result: After 15 days of training, the activity of alkaline phosphatase (AKP) increased with the increasing intensity of training. The 2BL s^{-1} flow rate group had the lowest activity at 15.59 (U/g prot), but there was no significant difference compared to the 4BL s^{-1} flow rate group. Significant differences were observed between the 2BL s^{-1} and 6BL s^{-1} flow rate groups, as well as between the 4BL s^{-1} and 6BL s^{-1} flow rate groups. After 30 days of training, there were no significant differences among all groups. When comparing 15 days to 30 days of training, only the 6BL s^{-1} flow rate group showed a statistically significant decrease in enzyme activity, while the other two groups did not show significant differences.

Catalase (CAT):

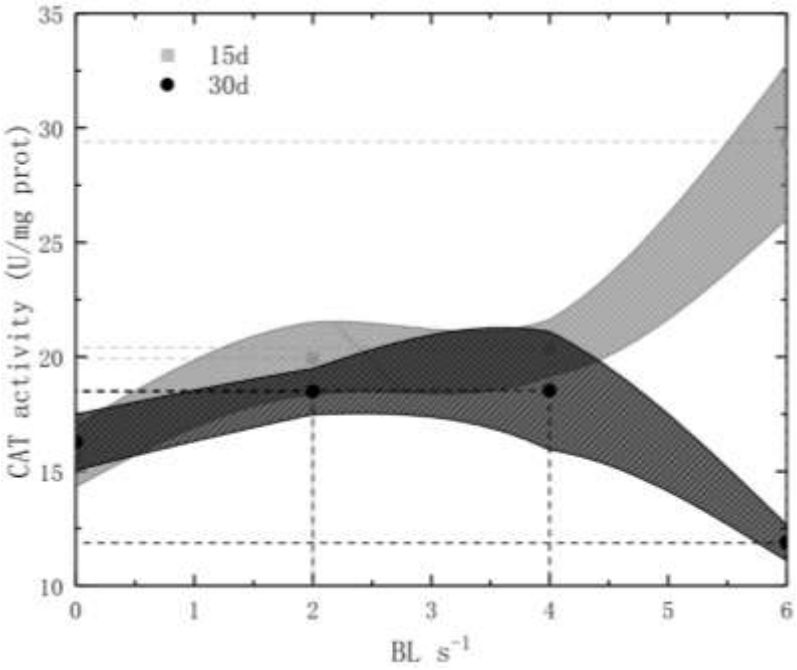

Figure 7 Effect of exercise training on catalase activity in juvenile fish of S.schlegelii

Result: After 15 days of training, the activity of catalase (CAT) increased with the increasing intensity of training. The CAT activity in the 2BL s^{-1} flow rate group was 19.92 (U/mg prot), which showed no significant difference compared to the 4BL s^{-1} flow rate group. However, there were significant differences between the 2BL s^{-1} and 6BL s^{-1} flow rate groups, as well as between the 4BL s^{-1} and 6BL s^{-1} flow rate groups. For the 30 days of training, the CAT activity decreased with the increase in training intensity. The CAT activity in the 2BL s^{-1} flow rate group was 18.48 (U/mg prot), which again showed no significant difference compared to the 4BL s^{-1} flow rate group. Significant differences were found between the 2BL s^{-1} and 6BL s^{-1} flow rate groups, as well as between the 4BL s^{-1} and 6BL s^{-1} flow rate groups. When comparing the results between 15 days and 30 days of training, only the 6BL s^{-1} flow rate group showed significant decrease in enzyme activity from 29.39 (U/mg prot) after 15 days to 11.87 (U/mg prot) after 30 days. The other two groups did not show significant differences.

Glutamate Oxaloacetate Transaminase (GOT):

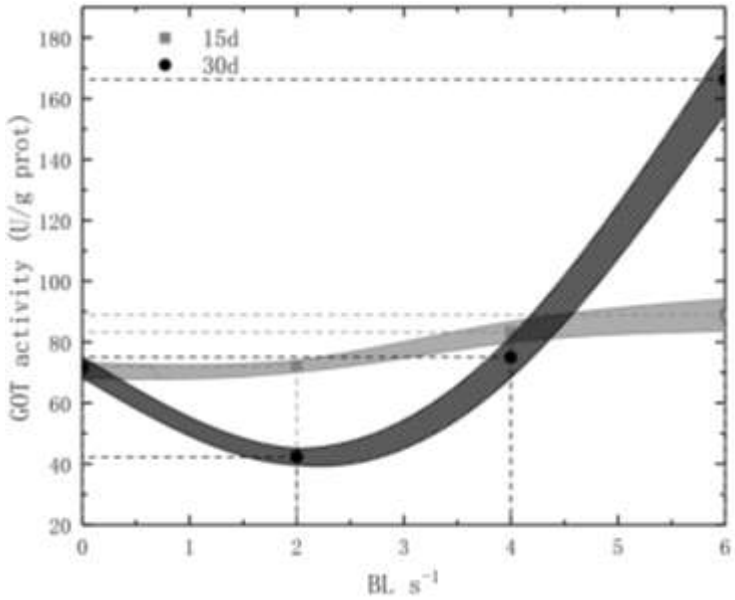

Figure 8 Effect of exercise training on the activity of glutamate oxaloacetate transaminase in juvenile fish of S.schlegelii

Result: After 15 days of training, the activity of GOT (Glutamate Oxaloacetate Transaminase) increased with the increasing intensity of training, with no significant difference between the 2BL s^{-1} flow rate group and the 4BL s^{-1} flow rate group. However, significant differences were observed between the 2BL s^{-1} and 6BL s^{-1} flow rate groups, as well as between the 4BL s^{-1} and 6BL s^{-1} flow rate groups. After 30 days of training, all groups showed significant differences. When comparing the training periods of 15 days and 30 days, the GOT activity in the 2BL s^{-1} flow rate group was at its lowest at 42.34 (U/g prot) at 30 days, and the GOT activities observed after 30 days in both the 2BL s^{-1} and 4BL s^{-1} flow rate groups were lower than those observed after 15 days. Conversely, the GOT activity in the 6BL s^{-1}S flow rate group was greater than that observed at 15 days and was the highest among all groups. There was a significant difference between the 2BL s^{-1}S and 6BL s^{-1} flow rate groups.

Lysozyme (LMZ):

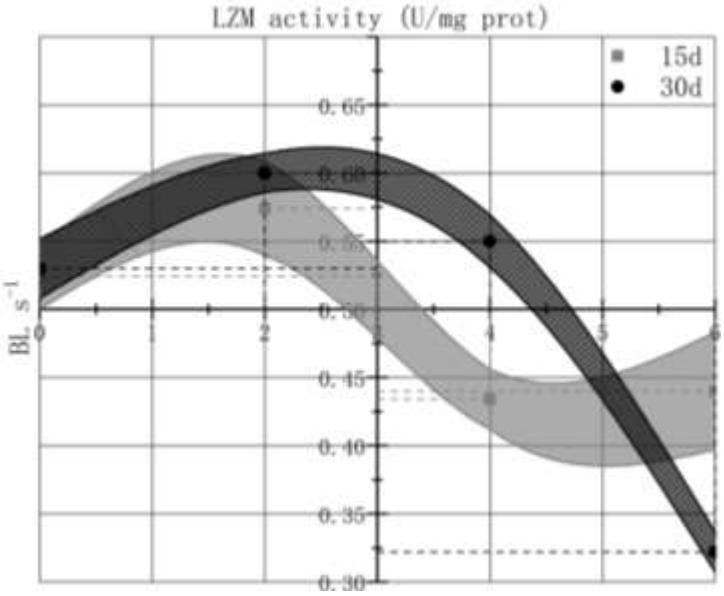

Figure 9 Effect of exercise training on lysozyme activity in juvenile fish of S.schlegelii

Result: After 15 days of training, the activity of lysozyme (LZM) decreased with increasing training intensity, and there was no significant difference between the 4BL s^{-1} flow rate group and the 6BL s^{-1} flow rate group. However, there were significant differences between the 2BL s^{-1} flow rate group and the 4BL s^{-1} flow rate group, as well as between the 2BL s^{-1} flow rate group and the 6BL s^{-1} flow rate group. After 30 days of training, the LZM activity continued to decrease with increasing training intensity, and there were significant differences among all groups. The 2BL s^{-1} flow rate group exhibited the highest enzyme activity at 0.6 (U/mg prot), while the 6BL s^{-1} flow rate group had the lowest enzyme activity at 0.32 (U/mg prot). When comparing the results between 15 days and 30 days of training, only the 2BL s^{-1} flow rate group showed no significant difference, while the other two groups demonstrated significant differences.

Total Amino Acids:

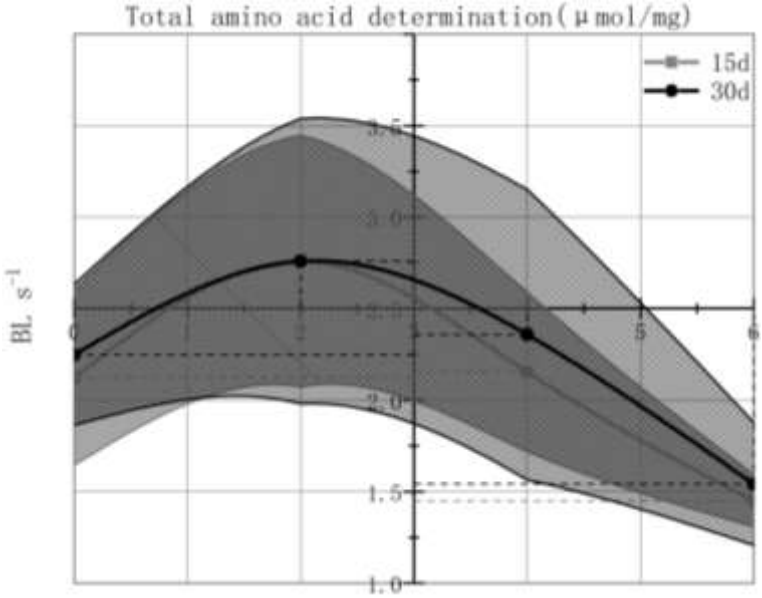

Figure 10 Effect of exercise training on total amino acids in juvenile fish of S.schlegelii

Result: After 15 days of training, there was no significant difference between the 2BL s^{-1} flow rate group and the 4BL s^{-1} flow rate group. However, significant differences were observed between the 2BL s^{-1} flow rate group and the 6BL s^{-1} flow rate group, as well as between the 4BL s^{-1} flow rate group and the 6BL s^{-1} flow rate group. After 30 days of training, there was again no significant difference between the 2BL s^{-1} flow rate group and the 4BL s^{-1} flow rate group, while significant differences were found between the 2BL s^{-1} flow rate group and the 6BL s^{-1} flow rate group, and between the 4BL s^{-1} flow rate group and the 6BL s^{-1} flow rate group. When comparing the results between 15 days and 30 days of training, there were no significant differences among all groups.

4 Discussion

4.1 Effects of wild-adaptation on growth performance in juvenile fish

Environmental quality has a crucial impact on the health of fish, as lower vertebrates living in water (Lorenzen & Camp, 2018). Exercise is considered one of the means to enhance fish's nutrient absorption, disease resistance, predator avoidance, and skills for field training, and thus has received considerable attention (Brown & Laland, 2001).In 2003, China's Jiangxi Province implemented an artificial propagation and release program to enhance and restore wild *Squaliobarbus curriculus* populations. Before release, fish-containing cages were installed in microflow ponds with external water flow velocity approximately 0.3 m s^{-1}, exposed to continuous water flow for 24-30 hours. Following flow-based domestication training, experimental fish demonstrated significantly enhanced hypoxia tolerance surpassing untrained individuals, thereby improving the quality, environmental adaptability, and survival rates in released fish(Kong et al., 2008).In 2018, the wild resources of the *Schizothorax lissolabiatus Tsao*, distributed in the Pearl River system, were extremely scarce. After successful artificial breeding, appropriate training was conducted. The flow intensity was increased by 15 days, 10 days, and 5 days before release, respectively. After the acclimatization training, the growth rate and post-release survival rate were improved (Gao & Xiao, 2019).Due to the impacts by hydropower development and overfishing, the resources of wild *Percocypris pingi* are suffering a severe decline. In response to this situation, the Institute of Hydrobiology of the Chinese Academy of Sciences selected the excellent strain of hybrid silver carp 'Zhongaoke No. 3' (*Carassius auratus gibelio* var. CAS III) as the research subject and conducted targeted swimming ability training. After 8 weeks of swimming training and cultivation, the study found that moderate swimming exercise could significantly improve the muscle texture and quality of hybrid silver carp (Wang, 2019). In this study, exercise training at 2BL s^{-1} flow velocity significantly ($p<0.05$) enhanced terminal body mass, specific growth rate, and weight gain efficiency in experimental juvenile fish after both 15 day and 30 day training periods, with survival rate reaching 100%. Under constant total training volume conditions, prolonged training duration significantly enhanced weight gain efficiency. This indicates that extended training time under 2BL s^{-1} flow velocity - a relatively low flow velocity level - induced more pronounced growth effects. The findings demonstrate that exercise training improved the fish' physical fitness, As swimming capacity improves, the metabolic profile of juvenile fish may also undergo changes (Liu et al., 2009). Juveniles may gradually adapt to higher-intensity activities.Enhancing energy conversion and utilization efficiency. This thereby promotes juvenile growth and subsequently increases specific growth rate (Brown et al., 2011), thus enhancing their environmental adaptability and promoting post-release survival.

The Martínez team conducted a 42-day speed training study on the muscle fibers (red and white) of *Anoplopoma fimbria* and observed various effects on muscle growth. The results indicated that reasonable training can significantly promote the growth and development of fish muscle. The increase in slow muscle fibers and the proliferation of myofibrillar cells are crucial for improving muscle quality (Martínez et al., 2003). In 2019, Yunnan Province in China began implementing a program for the flow-based wild training of *Wallago attu* in the Lancang River. The training scheme included increasing the flow intensity for three days before release, further increasing the flow intensity for two days before release, and designating a control group that did not undergo increased flow intensity. The results of the study indicated that this training method is unfavorable for the survival rate in weaker individuals. For individuals that are physically weaker, it is important to carefully select the training intensity to avoid excessive strain that could lead to a decrease in survival rate. By adjusting the flow intensity of the training, it is possible to better enhance the adaptability of the experimental fish and improve their survival rate after release (Fu et al., 2019). This indicates that high-flow wild training can deplete energy, which is detrimental to the survival of weaker individuals; prolonged wild training with flowing water excessively consumes the energy of juvenile fish, leading to weakened physical condition. Conversely, flow increase can diminish the fish's ability to adapt to natural flowing water as well as is also unfavorable for the survival of juvenile fish. Therefore, selecting an appropriate flow rate for wild training is crucial to avoid impairing the fish's physiological functions (Team, 2022). In this experiment, the 4BL s^{-1} flow velocity group showed increases in terminal body mass, specific growth rate, and weight gain efficiency, but these effects were less pronounced compared to the 2BL s^{-1} group, with survival rate decreasing to 90%. Concurrently, the specific growth rate of fish trained for 30 days significantly decreased compared to the 15 day training group. The 6BL s^{-1} flow velocity exercise training exhibited inhibitory effects on growth, with specific growth rate and weight gain efficiency significantly lower than both 2BL s^{-1} and 4BL s^{-1} groups, while survival rate remained at 90% (lower than the 2BL s^{-1} group). Compared to the 15 day training group, the specific growth rate of fish trained for 30 days showed significant decline. This indicates that under constant total training volume conditions, prolonged training duration exerts significant detrimental effects on specific growth rate in both 4BL s^{-1} and 6BL s^{-1} flow velocity groups. It is hypothesized that the swimming capacity and training regimen are closely related to the growth and development in fish. Excessive training may cause fish to excessively consume energy for swimming activities, thereby reducing energy allocation for growth and resulting in inferior growth performance compared to other groups, while insufficient training intensity in experimental design may also lead to suboptimal growth outcomes compared to other groups, failing to achieve desired training effects (Zhou et al., 2019).Research results indicate that excessive training intensity renders swimming unsustainable and excessively stressful, resulting in excessive energy depletion within the fish's body, causing physical damage, and may ultimately lead to fish mortality (Tunçelli & Memiş, 2024). Experimental observations revealed that increased flow velocity correlated with reduced condition factor in experimental fish, while no significant differences were observed between 15 day and 30 day training groups under identical flow velocities. This phenomenon may be related to the influence of training flow velocity on fish exercise intensity. When flow velocity exceeds a certain threshold, enhanced exercise intensity increases energy expenditure from lipid reserves, resulting in reduced indices such as condition factor (Ashraf, Van Wassenbergh, & Verma,

2020).The increase in condition factor indicates altered lipid reserves. Enhanced condition factor is typically associated with efficient nutrient utilization and effective energy storage capacity, implying larger body volume under equivalent weight conditions. Additionally result indicates improved food acquisition and digestive absorption capabilities, enabling juvenile fish to allocate energy more efficiently toward growth (Brown et al., 2011). These findings highlight the importance of exercise intensity and flow velocity in fish growth and lipid metabolism, advance understanding of the relationship between fish training and growth patterns, and hold potential implications for developing optimized aquaculture management strategies.

4.2 Effects of Wild-Adaptation on Viscera Biochemical in Juvenile Fish

Fish exhibit considerable plasticity in response to acute or chronic exercise training-induced stress, particularly in their ability to regulate endogenous antioxidant enzyme levels (Esbaugh et al., 2014). The activities of key glycolytic enzymes and mitochondrial metabolic enzymes play a crucial role in the body's metabolic capacity. These key enzymes can accelerate the metabolism and conversion of substrates, thereby improving the efficiency of energy generation. Therefore, by modulating the activity of these key enzymes, it is possible to effectively enhance the metabolic capacity of the body, providing adequate energy support for swimming training, while also contributing to improved exercise performance and the prevention of fatigue. Understanding and optimizing the activity of these key enzymes is vital for the effectiveness of swimming training. During exercise, both enzyme and non-enzyme antioxidants play an important role in protecting tissues from excessive oxidative damage (Boisclair & Tang, 1993). Numerous studies have confirmed that exercise training helps to enhance the antioxidant capacity of skeletal muscle in fish. These findings emphasize the importance of exercise in improving fish health, immunity, and survival capacity, providing valuable insights for future fish farming management and health protection (Ji, 1995).Through swimming training, the non-specific immunity of fish can be enhanced, and moderate exercise training is crucial for the health and survival of fish (Yousaf et al., 2023). The immune capacity of fish is closely related to their swimming ability. During swimming training, fish consume a large amount of energy, and the production of this energy is influenced by metabolic substrates and the organism's metabolic capacity. In animals, muscle glycogen and liver glycogen are important forms of energy storage, which can be broken down into glucose when needed to provide energy for the muscles (Vincent et al., 2000).

Alkaline phosphatase is an enzyme present in the liver that is excreted through bile. Its main function is to catalyze the removal of the 5` phosphate group from nucleic acid molecules, converting the 5'-P end of DNA or RNA segments into a 5'-OH end(Congleton & Wagner, 2006). This enzyme is directly involved in the metabolism of phosphorus and is closely related to the metabolism of RNA, DNA, lipids, and proteins, playing a crucial role in immune responses (Guardiola et al., 2014). In addition, alkaline phosphatase plays an essential role in immune reactions.In this experiment, Alkaline phosphatase activity in the 15 day 6BL s^{-1} group was higher than in the 2BL s^{-1} and 4BL s^{-1} groups, which could be explained by acute metabolic responses induced by higher flow velocity. Short-term stronger exercise stimulation increased hepatic metabolic load, leading to greater Alkaline phosphatase release. With extended training to 30 days, Alkaline phosphatase activity in the 6BL s^{-1} group significantly decreased, possibly reflecting hepatocellular functional impairment, oxidative stress accumulation, or reduced synthetic capacity, thereby suppressing enzyme synthesis or

secretion. Compared to the 15 day period, enzyme activity in the 2BL s^{-1} and 4BL s^{-1} flow groups showed increases but remained non-significant, suggesting that moderate-intensity sustained training primarily induces adaptive regulation, maintaining or gradually enhancing hepatic metabolism and immune function rather than functional exhaustion. This indicates that appropriate water flow training can improve immune function through enhanced growth and non-specific immune enhancement. However, when training intensity or duration exceeds physiological tolerance thresholds, it may shift toward chronic stress responses and metabolic suppression.

Catalase is a highly efficient enzyme that decomposes hydrogen peroxide, primarily converting hydrogen peroxide into water and oxygen, thereby reducing the concentration of hydroxyl free radicals in the body and protecting cells from hydrogen peroxide damage (Abdel-Tawwab et al., 2017). This enzyme plays a crucial role in the biological defense system, maintaining internal stability and health (Del Maestro, 1980). Hackbarth found that after 72 days of training at 1.0 BL s^{-1}, the serum enzyme activities, such as catalase, in the blood of *Brycon cephalus* changed significantly (Hackbarth & Moraes, 2006). In this experiment, catalase activity in the 15 day 6BL s^{-1} group was significantly higher than in the 2BL s^{-1} and 4BL s^{-1} groups, which could be explained by stronger exercise stimulation and metabolic oxygen consumption induced by higher flow velocity. Short-term elevated mitochondrial respiration and metabolic rates triggered upregulation of catalase to rapidly eliminate hydrogen peroxide, resulting in increased enzyme activity. By day 30, catalase activity in the 6BL s^{-1} group significantly decreased and became lower than low-flow groups, possibly reflecting antioxidant system exhaustion, oxidative damage-induced enzymatic inactivation, or reduced hepatic synthesis capacity for catalase production. In contrast, the 2BL s^{-1} and 4BL s^{-1} groups showed marginal decreases in catalase activity at day 30 that remained non-significant, suggesting that moderate-intensity sustained training primarily induces adaptive regulation with relatively stable antioxidant defenses or establishment of new steady states.

Although glutamate oxaloacetate transaminase is primarily distributed in the myocardium, this enzyme is also present in hepatic, renal, and skeletal muscle tissues. Hepatic damage can lead to significant enzyme release into the bloodstream, with elevated activity typically indicating hepatic injury severity. Studies have demonstrated that moderate aerobic training effectively reduces serum glutamate oxaloacetate transaminase levels, restoring them to normal ranges (Vincent et al., 2000).In this experiment, glutamate oxaloacetate transaminase levels in the 15 day 6BL s^{-1} group were significantly higher than in the 2BL s^{-1} and 4BL s^{-1} groups, possibly reflecting acute stress induced by short-term high flow velocity, increased metabolic and exercise loads in fish, elevated metabolic activity and membrane permeability in hepatic and skeletal muscle tissues, leading to glutamate oxaloacetate transaminase release and subsequent enzyme activity elevation. By day 30, glutamate oxaloacetate transaminase levels in moderate flow groups showed declines compared to day 15, with significant reduction in 2BL s^{-1} group, potentially indicating adaptive changes induced by chronic low-intensity training, gradual normalization of membrane stability and metabolic functions, enhanced hepatic detoxification and repair capabilities, and establishment of new physiological homeostasis where enzyme synthesis and consumption reached equilibrium (Wu et al., 2017). However, significant elevation in 6BL s^{-1} group at day 30 suggested cumulative damage exceeding physiological compensation capacity from sustained high-intensity loads, potentially resulting from persistent oxidative

stress, inflammatory responses, mitochondrial dysfunction, or cellular necrosis that caused excessive enzyme release, reflecting substantive hepatic or organ damage with inflammation, ultimately compromising individual health and physiological performance.

Lysozyme serves as the first line of defense in the humoral immune system, secreted by macrophages, neutrophils, and mucosal epithelial cells, and widely present in fish blood and various lymphocytes (Marsh & Rice, 2009). This enzyme hydrolyzes bacterial cell wall peptidoglycans, rapidly exerting antibacterial, anti-inflammatory, and antiviral effects as a crucial component of nonspecific immunity, playing a key role in fish immune systems (Di Yu et al 2015). Research indicates that lysozyme activity in crucian carp *Barbodesschwanengeldi* increased with flow velocity under 1.0BL s^{-1} and 3.0BL s^{-1} flow conditions (Song et al., 2008). Additionally, studies observed enhanced immune indices in *Atlantic salmon* at 0.8BL s^{-1} flow velocity, while sustained exercise training improved their survival rates (Castro et al., 2011).In this experiment, lysozyme levels in the 2BL s^{-1} group showed significant elevation compared to other groups. Low-intensity, sustained exercise typically induces mild physiological stress, acting as adaptive stimulation that enhances macrophage and other innate immune cell activation, promoting lysozyme synthesis and secretion. Moderate metabolic load allows sufficient energy allocation for immune substance production, thereby strengthening nonspecific immune function. Levels at day 30 demonstrated a slight but non-significant increase compared to day 15, indicating stable saturation. The 4BL s^{-1} and 6BL s^{-1}flow groups showed comparable enzyme activity at day 15, but significant elevation occurred in the 4BL s^{-1} group by day 30, suggesting medium-intensity training requires longer adaptation periods with delayed immune activation. Progressive lysozyme elevation observed over training duration demonstrated improved enzyme levels at day 30, though remaining lower than optimal low-intensity groups. However, significant decline in 6BL s^{-1} group at day 30 indicated chronic physiological stress from sustained high-intensity training, triggering endocrine responses that ultimately suppress immune cell function, reducing macrophage and neutrophil lysozyme synthesis and secretion.

Amino acids serve as the most fundamental structural units of living organisms, comprising various types of amino acids. Among the 20 essential amino acids, each possesses distinct biological functions and plays crucial roles in protein synthesis (Feng et al., 2016). When appropriately supplemented, they directly participate in diverse protein synthesis processes, playing key roles in enhancing immunity and strengthening physical health. Furthermore, amino acids contribute to maintaining nitrogen balance through conversion into lipids or carbohydrates, or by participating in enzyme formation (Gómez-Requeni et al., 2003).At 15 day training duration, no significant differences were observed between 2BL s^{-1} and 4BL s^{-1} flow groups, while significant differences were found between 2BL s^{-1} and 6BL s^{-1} groups as well as between 4BL s^{-1} and 6BL s^{-1} groups. At 30 day training duration with no significant differences between 2BL s^{-1} and 4BL s^{-1} groups, but significant differences persisting between 2BL s^{-1} and 6BL s^{-1} groups and between 4BL s^{-1} and 6BL s^{-1} groups. Comparison between 15 day and 30 day training periods showed no significant differences across all groups. These findings indicate that fish achieve metabolic homeostasis of total amino acids within 15 days under equivalent training intensities. Low to moderate flow velocities maintain relative metabolic equilibrium, while moderate-intensity swimming exercise promotes muscle protein synthesis and tissue repair. However, excessive training

intensity induces reaction that reduce amino acid content, ultimately compromising fish health with increasing flow velocity.

5 Conclusion

An overall analysis of experimental results demonstrated different effects of swimming training at various flow velocities on growth development in *Sebastes schlegelii* juveniles. The 2BL s^{-1} group showed positive impacts on both growth performance and biochemical indicators, significantly increasing terminal body mass, specific growth rate, weight gain efficiency, visceral somatic index, and condition factor compared to controls, while enhancing alkaline phosphatase, catalase, glutamate oxaloacetate transaminase, lysozyme, and total amino acid activities, thereby promoting healthy growth of *Sebastes schlegelii* juveniles. The 4BL s^{-1} group exhibited mostly non-significant differences in growth and biochemical parameters relative to controls, demonstrating suboptimal overall effects. The 6BL s^{-1} group caused substantial health impairment. Notably, 30 day training regimen produced better outcomes than 15 day training for both 2BL s^{-1} and 4BL s^{-1} groups, with 2BL s^{-1} juveniles showing an overall improvement across all measured parameters.

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Author Contributions

Shihong Chen and Tao Tian designed, conducted, and analyzed the experiments, contributed to the study design, and prepared the manuscript.Qi Jiang, Jiamin YAN, Zhenyu Xu and Jingyuan Yang were involved in the study design and result discussions. Yong Han conceived the study, coordinated the experiments, and assisted in manuscript writing. All authors contributed to and approved the final version of the article

Data Availability

All data generated and analyzed during this study are included in this published article and its additional files.

Ethics Approval and Consent to Participate

The experimental protocols of this study were approved by the Animal Ethics Committee of Ocean University of China (OUC-AE-2023047).

Consent for Publication

Informed consent for publication was obtained from all participants.

Conflict of Interests

The authors declare that they have no conflict of interests.

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