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# Seasonal variations and comparative nutritional composition of hatchery-reared, hatchery-released, and wild black rockfish (*Sebastes schlegelii*)

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The black rockfish (*Sebastes schlegelii*) is a commercially important marine species in the Northwest Pacific Ocean. Its population has significantly declined due to overfishing and environmental changes. Stock enhancement has been launched in response to wild populations decline. However, limited evidence is available to confirm the ecological effects after release. Empirically, if the hatchery-released individuals are well-adapted to the wild environment, they should show good or similar nutritional conditions as well as their wild counterparts. Therefore, nutritional analysis was essential and conducted in the present study, by using 146 *S. schlegelii* individuals in order to compare the differences among hatchery-reared (before-release), hatchery-released, and wild conspecifics (post-release) groups in consideration of different environments during a whole stock enhancement practice. Our results exhibited that hatchery-reared *S. schlegelii* exhibited significantly lower levels of crude protein and amino acid content than that of hatchery-released and wild ones ( $P < 0.05$ ). Specifically, both the hatchery-released and wild *S. schlegelii* generally showed similar trends of nutrition profiling compared with their hatchery-reared counterparts, such as moisture, crude ash, crude lipid, crude protein, and amino acid profiles ( $P > 0.05$ ), indicating homogeneity in their nutritional contents. Our research suggested that *S. schlegelii* exhibited extensive ecological plasticity, and the variations in nutrition of a population was mainly influenced by environmental factors rather than the origin. However,

several differences in fatty acid composition between hatchery-released and wild *S. schlegelii* indicated that hatchery-released fish might not have fully adapted to the food supply in the wild. This study provided insights into promoting responsible stock enhancement of this species in the future.

#### KEYWORDS

*Sebastes schlegelii*, stock enhancement, before-release, post-release, nutritional composition

## 1 Introduction

The global capture fisheries production, except algae, was 92.3 million tonnes, with an evaluated value of United States dollars (USD) 159 billion in 2022 (FAO, 2024). Although world fisheries face many challenges, such as overfishing, habitat destruction, and pollution (Worm and Branch, 2012), the development of sustainable fishery has become a consensus to deal with the declining status of global fishery resources. As a sustainable fishery management approaches, stock enhancement refers to the practice of releasing hatchery-reared juveniles into existing populations to increase fishing and rebuild diminished resources in marine and freshwater (Blaxter, 2000). China is one of the significant aquaculture producers, accounting for 36% of the global aquaculture production now (FAO, 2024). The development of aquaculture contributes to the supply of abundant seedlings for stock enhancement in coastal waters to restore local population resources (Taylor et al., 2017). The stock enhancement is recognized as an effective short-term fisheries management strategy to increase fishery production or conservation in the wild (Richardson et al., 2023). This approach has been extensively implemented not only in China but also in other waters whenever salt water or freshwater throughout the world (Kitada, 2020). The negative impacts of large-scale stock enhancement programs are a global concern (Kitada, 2018). One primary concern is the ecological impacts, particularly the adaptation of hatchery-reared juveniles after release and their ecological interactions with wild conspecifics (Johnsson et al., 2014; Pinter et al., 2019). Stock enhancement activities generally lack investigation and monitoring, with insufficient informative data for evaluation. Regarding the estimation of economic benefits (Hunt et al., 2017) and genetic impacts (Liu et al., 2018), the ecological effects of stock enhancement are even unclear, primarily due to difficulties in tracking hatchery-released juveniles in natural habitats. In one case, hatchery-released juvenile Japanese Spanish mackerel (*Scomberomorus niphonius*) tended to be larger than wild fish and had higher growth performance than wild ones (Nakajima et al., 2013). Density-dependent growth caused by competition for food could decrease the growth rate of both of hatchery-released juveniles and wild ones, as well as other competitive species, or even replace the wild populations when stocking exceeded the carrying capacity of the environment in salmon (Kitada, 2018). In contrast,

other researches have revealed that hatchery-reared juveniles can adapt to the natural water in post-release and seem not to have negative impacts on wild populations (Wang et al., 2019). For example, a feeding ecology analysis from hatchery-released Japanese flounder (*Paralichthys olivaceus*) demonstrated that the growth status and the stomach contents of the hatchery-released and wild fish were almost identical. This inferred that post-release of *P. olivaceus* had well adaptability with their wild counterparts (Wang et al., 2019). Similar results were also reported in honmasu salmon (*Oncorhynchus rhodurus*) (Munakata et al., 2000), European grayling (*Thymallus thymallus*) (Turek et al., 2010), meagre (*Argyrosomus regius*) (Gil et al., 2014). Hence, research and monitoring are necessary to investigate the possible adaptation differences in hatchery-released fish.

Variations in fish nutrient composition, including proximate composition, amino acid contents, and fatty acid contents, are the result of adaptation to available food and environment (Grigorakis, 2007; Oh et al., 2023). It reflects the nutritional status of the fish itself and its interactions with the environment (Rosburg et al., 2019). Therefore, demonstration of the nutrition profiling of both hatchery-released fish and their wild conspecifics during the same period can indirectly respond to feeding selectivity for further exploring whether hatchery-released fish can exhibit similar feeding ecology with their wild counterparts and potential impacts of stock enhancement on wild fish after release (Tomiyama et al., 2011). Different life history or behavior may lead to differences in the specific nutritional components. Sprague et al. (2016) analyzed the fatty acid composition of over 3,000 Atlantic salmon (*Salmo salar*) farmed between 2006 and 2015 and found that terrestrial fatty acids of farmed populations have significantly increased alongside a decline in EPA and DHA levels, in comparison to wild populations (Sprague et al., 2016). Furthermore, Chakma et al. (2022) reported that the protein and carbohydrate content of farmed pangasius catfish (*Pangasius hypophthalmus*) was significantly higher than that of wild populations (Chakma et al., 2022). This indicated that the protein quality of farmed fish was relatively consistent, whereas wild fish exhibited significant fluctuations in feeding selectivity due to the diverse range of food sources available throughout their life cycle. In addition, research on nutritional compositions in one species among its different geographical populations, such as hilsa

(*Tenualosa ilisha*) (De et al., 2019) and southern stingray (*Hypanus americana*) (Hoopes et al., 2020) illustrated that the habitat environment conditions could affect fish nutritional quality. However, few have been reported regarding nutrition analyses to reveal the characteristics of hatchery-released fish so far.

The black rockfish (*Sebastes schlegelii*) is widely distributed along the bottom of coastal areas of northern China, Japan and Korea, with a wide temperature range of 5–28°C (Chin et al., 2013). It is also an economic fishery species due to its high value. However, its stocks are declining due to overfishing and environmental changes (McGreer and Frid, 2017). Large-scale stock enhancement of *S. schlegelii* in China has been initiated since 2016, with an average release of more than 30 million juveniles annually. From 2016 to 2019, more than 97% of the total released number in China was concentrated in the coastal waters of Shandong Province each year. There were no releases of *S. schlegelii* in Liaoning Province before 2019. Therefore, the whole process of *S. schlegelii* stock enhancement was first traced in Liaoning Province in 2019. In our previous study, the hatchery-released individuals from wild fish were distinguished by recapture investigation using DNA profiling (Liu et al., 2022). Based on nutritional components analysis, the present study aimed to evaluate (1) whether hatchery-released *S. schlegelii* exhibited similar nutritional quality with their wild counterparts, (2) whether hatchery-reared *S. schlegelii* could adapt to the native environment after release. This study offers crucial insights into understanding the ecological effects of stock enhancement and serves as a valuable reference for successful stock enhancement activities in the future.

## 2 Materials and methods

### 2.1 Ethical statement

Animal experiments were conducted at the Fisheries Resources Laboratory of Dalian Ocean University, in accordance with Chinese laws, regulations, and ethical principles.

### 2.2 Sample collection

From April to May 2019, around 800 inseminated female *S. schlegelii* were collected from the Northern Yellow Sea area of Liaoning Province (Dalian). The species typically engages in mating activities during November and December of 2018, utilizing a polyandrous mating pattern in the wild. Consequently, only female fish were selected as broodstock for subsequent stock enhancement. In conclusion, a total of 489 broodstock females spawned in May 2019, with approximately 30 broodstock females placed in each breeding pond (50 m<sup>3</sup>) and allowed to spawn in a shaded environment. The effective population size of the broodstock population was found to be sufficiently large ( $N_e = 4,593.5$ , using the linkage disequilibrium method,  $P_{crit} = 0.02$ ) to produce first-generation offspring with high genetic diversity, no significant genetic differentiation with local wild populations (Liu

et al., 2022). Subsequently, the offspring seeds were fed pellets in a running water system prior to release (Supplementary Table S1). Hatchery-reared *S. schlegelii* [ $n = 36$ , total length (TL):  $8.332 \pm 0.162$  cm, mean  $\pm$  standard error (S.E.)] were randomly selected as the before-release group for nutrition analysis. In addition, a total of 1.05 million *S. schlegelii* juveniles were released in the Northern Yellow Sea area of Liaoning Province in July 2019.

Fishery recapture investigations were then conducted monthly, and 710 *S. schlegelii* were captured by fishing trap from August to December post-release in 2019 (Figure 1). In our previous study, we used nine highly polymorphic microsatellite markers with Cervus 3.07 to assign parents. During recapture surveys, we distinguished 279 hatchery-released individuals and 431 wild ones based on likelihood calculations. The combined power of exclusion (CPE) of all the loci in this parentage assignment reached to 99.8% (Liu et al., 2022). From the 279 hatchery-released fish, 66 individuals (TL:  $7.856 \pm 0.356$  cm, mean  $\pm$  S.E.) were selected as the hatchery-released group based on their higher logarithm of the odds (LOD, ranging from 3 to 12) within the confidence interval for further analysis (Supplementary Table S2). In contrast, 44 of the 431 wild counterparts (TL:  $8.705 \pm 0.246$  cm, mean  $\pm$  S.E.) were also selected as the wild group due to their lower LOD scores (ranging from -5 to 2) outside the confidence interval (Supplementary Table S2). Therefore, 146 samples were divided into three groups (before-release, hatchery-released, and wild group) in order to analyze their nutritional profiles in the present study. In each group, due to different specimen sizes, 5 to 18 fish per month were used, respectively (Supplementary Table S3).

### 2.3 Proximate composition analysis

Prior to proximate composition analysis, in each month, the fish samples per group were randomly divided into three independent treatments as replicates for nutrient determinations. Each fish per group was prepared by removing the scales, skin and bones. The muscle tissues from each month were then chopped and mixed. Moisture was evaluated by using a hot air oven (DGG-9123A, Shanghai, China) for drying the sample at 95–105°C for 4 hours (Henken et al., 1986), and crude ash by high-temperature combustion at  $550 \pm 25^\circ\text{C}$  (AOAC, 2023). Crude lipid content was determined using the Soxhlet extraction method, with Methyl tert-Butyl Ether (MTBE, boiling point: 40–60°C, Sigma, USA) as a solvent (Katikou and Robb, 2001). Crude protein content was assessed using the Kjeldahl method, and crude protein was estimated by multiplying the nitrogen percentage of 6.25 (Anantkrishnan and Srinivasa Pai, 1952).

### 2.4 Amino acid analysis

Amino acids were determined using high-performance liquid chromatography (HPLC, Agilent 1260, Germany) (Alaiz et al., 1992). The tissue samples were stored in the laboratory at -25°C for 24 hours. Subsequently, they were lyophilized for 48 hours at -53°C under a vacuum condition of less than 20 Pa using a freeze dryer

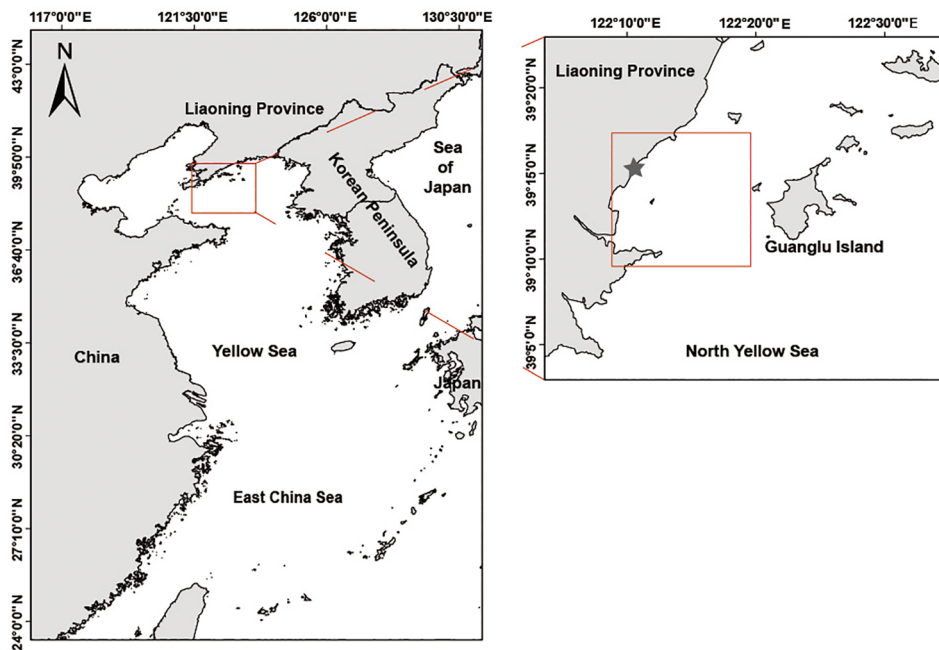


FIGURE 1

The Fishery recapture investigations of the *S. schlegelii* in North Yellow Sea. Hatchery-released and wild *S. schlegelii* were collected in the red dotted lines area after stock enhancement, grey star indicates the North Yellow Sea area of releasing *S. schlegelii*.

(SCIENTZ-18N/A, Ningbo, China). Weigh the appropriate amount of freeze-dried sample into a 50 mL hydrolysis tube, and then samples ( $\geq 0.05$  g) were hydrolyzed with 6 mol/L HCl at 110°C for 22 hours. Accurately, 100  $\mu$ L of the sample was taken into a 15 mL centrifuge tube, placed in a vacuum drying oven, and dried at 60°C for 2 hours (the solvent was dried). After drying, the sample was diluted to 0.5 mL with water, mixed well and passed through a 0.45  $\mu$ m organic membrane. After filtration and drying with nitrogen gas, the samples were neutralized and analyzed using a Hitachi amino acid analyzer (Hitachi L-8900, Tokyo, Japan) via EZChromElite software to detect amino acid contents. The tryptophan (Try) content was not measured in this study since Try was destroyed by acid hydrolysis.

## 2.5 Fatty acid analysis

The determination of fatty acids in *S. schlegelii* was performed using a gas chromatography-mass spectrometry (GC-MS) system (Trace 1310 ISQ, Waltham, USA). Briefly, a precisely measured homogenized sample was dispensed into a 100 mL tube and mixed with 2 mL of 95% ethanol, 4 mL of water, and 10 mL of 8.3 mol/L HCl. The mixture was hydrolyzed in an 80°C water bath for 40 minutes with periodic shaking, then cooled to room temperature. The hydrolyzed sample was mixed with 10 mL of 95% ethanol and extracted three times with 100 mL of an ether-petroleum ether mixture for fat extraction. The extract was evaporated to obtain the fat, which was then saponified and methylated by adding 4 mL of 2% sodium hydroxide in methanol and heating at 45°C for 30 minutes. Next, 4 mL of 14% boron trifluoride methanol solution (CNW) was added, and the mixture was heated at 45°C for another

30 minutes. After cooling, 3 mL of hexane was added, and the mixture was shaken for 2 minutes to extract fatty acid methyl esters (FAME). The upper layer of the supernatant was removed and filtered through a 0.45  $\mu$ m membrane filter before analysis.

The GC-MS column used was a 50 m  $\times$  0.25 mm diameter column with a 0.25  $\mu$ m film thickness. The gas chromatography conditions were as follows: initial temperature of 80°C held for 1 minute, increased to 160°C at a rate of 20°C/min and held for 1.5 minutes, then increased to 230°C at a rate of 5°C/min and held for 6 minutes. The injector temperature was set to 260°C, and the split ratio was 100:1. Fatty acid distribution was determined using FAME standards (Sigma, USA).

## 2.6 Data analysis

Statistical analysis was conducted using IBM SPSS 25.0. Prior to further analysis, all data were subjected to a test for normality based on the Shapiro-Wilk test and a test for homoscedasticity via the Levene test. The differences in moisture, crude ash, crude lipid, crude protein, amino acids, and fatty acids among the three groups were compared by one-way analysis of variance (ANOVA) with *post hoc* comparisons based on Tukey's test or Nonparametric tests (Kruskal-Wallis Test). All analyses were performed at the significance level of  $P < 0.05$  and  $P < 0.01$ , respectively. The results are presented as the mean  $\pm$  S.E. of the mean unless otherwise stated. A paired sample *t*-test, otherwise, Wilcoxon signed-rank test was used to determine the significant differences in the nutritional value between hatchery-released fish and wild fish during the same month. The significance values have been adjusted by the Bonferroni correction for multiple tests.

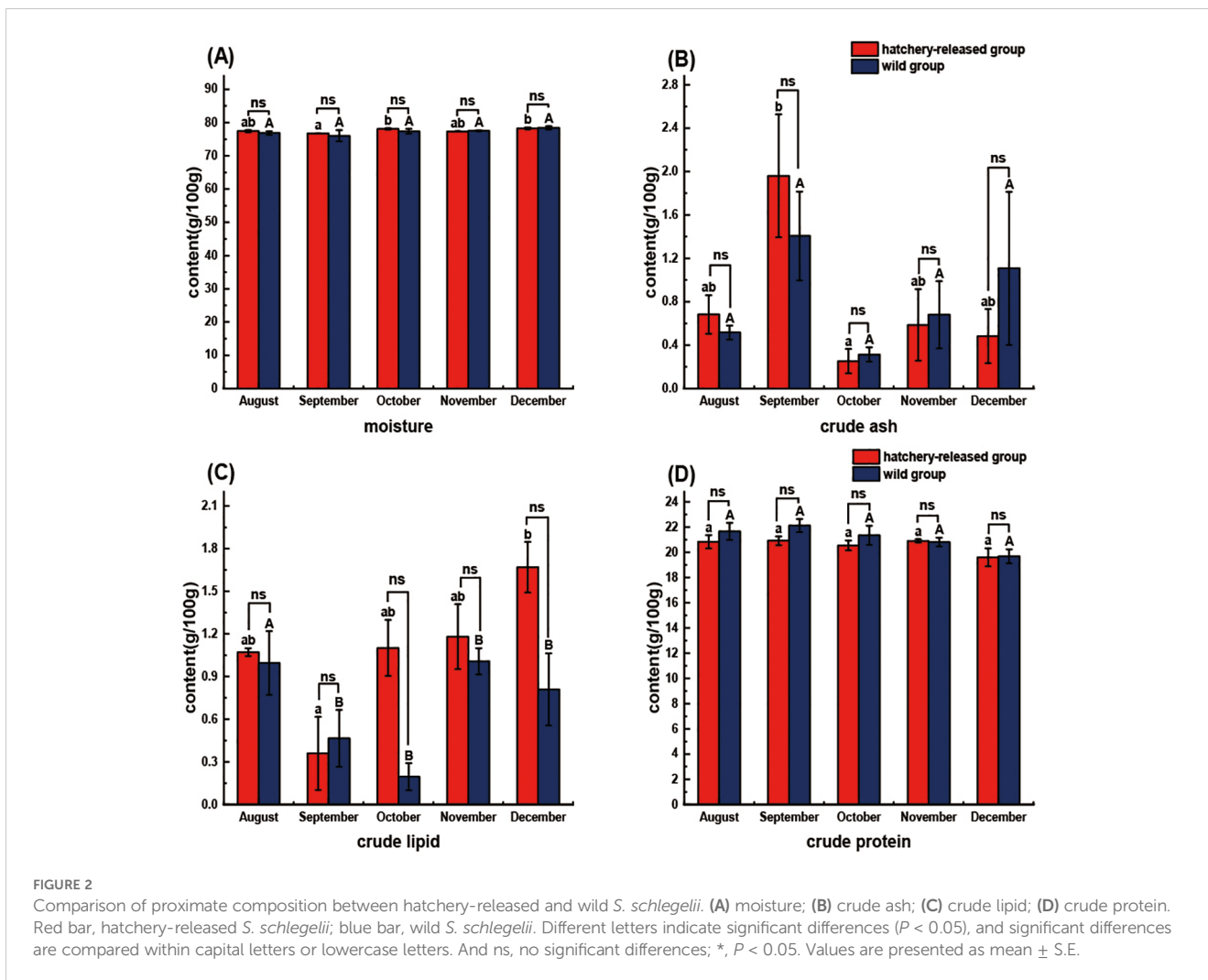


FIGURE 2 Comparison of proximate composition between hatchery-released and wild *S. schlegelii*. (A) moisture; (B) crude ash; (C) crude lipid; (D) crude protein. Red bar, hatchery-released *S. schlegelii*; blue bar, wild *S. schlegelii*. Different letters indicate significant differences ( $P < 0.05$ ), and significant differences are compared within capital letters or lowercase letters. And ns, no significant differences; \*,  $P < 0.05$ . Values are presented as mean  $\pm$  S.E.

### 3 Result

#### 3.1 Proximate composition analysis

The proximate composition of the hatchery-reared, hatchery-released and wild *S. schlegelii* was presented in Table 1 and Figure 2. All groups were characterized by a high moisture content, consistently exceeding  $77.225 \pm 0.398$  g/100 g wet weight (ww). Furthermore, the crude protein content was consistently higher than  $16.936 \pm 0.194$  g/100 g (ww), while the lipid content was

consistently higher than  $0.848 \pm 0.089$  g/100 g (ww). In contrast, the crude ash content was always very low, not surpassing  $1.482 \pm 0.191$  g/100 g (ww). Overall, in the comparison of all samples, there was no significant difference in crude ash content among hatchery-reared, hatchery-released and wild *S. schlegelii*, while the hatchery-reared *S. schlegelii* had relatively higher values ( $P > 0.05$ ). The hatchery-reared *S. schlegelii* exhibited significantly higher moisture and crude lipid contents, but significantly lower crude protein content, compared to wild *S. schlegelii* ( $P < 0.05$ ). The results showed a similar trend of mean value for proximate composition

TABLE 1 Proximate composition of hatchery-reared, hatchery-released and wild *S. schlegelii* (g/100 g, wet weight).

Proximate Composition	Groups			P-value
	hatchery-reared	hatchery-released	wild	
moisture*	$80.118^b \pm 0.176$	$77.573^a \pm 0.168$	$77.225^a \pm 0.398$	0.000
crude ash	$1.482 \pm 0.191$	$0.793 \pm 0.203$	$0.805 \pm 0.183$	0.116
crude lipid*	$1.464^b \pm 0.105$	$1.076^{ab} \pm 0.134$	$0.848^a \pm 0.089$	0.016
crude protein*	$16.936^a \pm 0.194$	$20.558^b \pm 0.220$	$21.121^b \pm 0.397$	0.000

Values are presented as mean  $\pm$  S.E. In the same row with different letters indicate significant differences ( $*P < 0.05$ ). No letter in the same row indicates no significant difference ( $P > 0.05$ ). The significance values have been adjusted by the Bonferroni correction for multiple tests.

content in the hatchery-released *S. schlegelii* compared to wild *S. schlegelii* (Table 1).

Significant differences were observed in the study of seasonal variations in proximate composition among hatchery-released *S. schlegelii* across different months. Specifically, *S. schlegelii* exhibited significantly higher levels of moisture in October and December compared to those in September ( $P < 0.05$ ), whereas the crude ash content was notably lower in October relative to September ( $P < 0.05$ ). Additionally, the crude lipid content in December was significantly higher than that in September ( $P < 0.05$ ). The proximate composition of wild *S. schlegelii* was relatively steady from August to December. No significant differences were observed in moisture, crude ash, crude lipid, and crude protein content ( $P > 0.05$ ). In contrast to the wild fish, the hatchery-released *S. schlegelii* exhibited an opposing trend in moisture and crude ash content variation (Figure 2). Comparison of hatchery-released and wild *S. schlegelii* in the same months revealed no significant difference in all the proximate composition content ( $P > 0.05$ ) (Figure 2). It is worth noting that hatchery-released and wild *S. schlegelii* almost share the

similar proximate profile trends during the post-release period in the wild, which can be inferred that hatchery-reared *S. schlegelii* can adjust quickly from “hatchery” to their wild counterparts in order to adapt to the natural environment.

### 3.2 Amino acids analysis

In this study, 17 amino acids were detected in *S. schlegelii*, including eight non-essential amino acids (NEAA: Asp, Glu, Cys, Ser, Ala, Pro, Tyr, His) and nine essential amino acids (EAA: His, Arg, Thr, Val, Met, Ile, Leu, Phe, Lys). The amino acid composition of hatchery-reared, hatchery-released, and wild *S. schlegelii* is displayed in Table 2. There was a significant difference in the NEAA content among these three groups, whereas intermediate value was also observed in hatchery-released *S. schlegelii* ( $P < 0.05$ ),  $7.985 \pm 0.203$  g/100 g (ww) (hatchery-reared) vs  $9.327 \pm 0.105$  g/100 g (ww) (hatchery-released) vs  $9.762 \pm 0.083$  g/100 g (ww) (wild), which is in agreement with the results of proximate

TABLE 2 Amino acid composition of hatchery-reared, hatchery-released and wild *S. schlegelii* (g/100 g, wet weight).

Amino acid	Groups			P-value
	hatchery-reared	hatchery-released	wild	
Aspartic acid**	1.841 <sup>a</sup> ± 0.054	2.167 <sup>b</sup> ± 0.022	2.190 <sup>b</sup> ± 0.019	0.000
Glutamic acid**	2.581 <sup>a</sup> ± 0.081	3.075 <sup>b</sup> ± 0.040	3.084 <sup>b</sup> ± 0.027	0.000
Cysteine**	0.031 <sup>a</sup> ± 0.008	0.067 <sup>b</sup> ± 0.007	0.064 <sup>b</sup> ± 0.005	0.005
Serine**	0.716 <sup>a</sup> ± 0.018	0.863 <sup>b</sup> ± 0.011	0.884 <sup>b</sup> ± 0.007	0.007
Alanine**	1.037 <sup>a</sup> ± 0.027	1.231 <sup>b</sup> ± 0.011	1.262 <sup>b</sup> ± 0.011	0.000
Proline**	0.262 <sup>a</sup> ± 0.044	0.231 <sup>a</sup> ± 0.021	0.551 <sup>b</sup> ± 0.054	0.014
Tyrosine **	0.544 <sup>a</sup> ± 0.032	0.650 <sup>b</sup> ± 0.011	0.662 <sup>b</sup> ± 0.009	0.004
Glycine**	0.972 <sup>a</sup> ± 0.011	1.043 <sup>b</sup> ± 0.012	1.065 <sup>b</sup> ± 0.011	0.000
NEAA*	7.985 <sup>a</sup> ± 0.203	9.327 <sup>b</sup> ± 0.105	9.762 <sup>c</sup> ± 0.083	0.000
Histidine*	0.360 <sup>a</sup> ± 0.005	0.392 <sup>ab</sup> ± 0.008	0.396 <sup>b</sup> ± 0.008	0.044
Arginine**	1.145 <sup>a</sup> ± 0.032	1.316 <sup>b</sup> ± 0.015	1.308 <sup>b</sup> ± 0.019	0.000
Threonine*	0.782 <sup>a</sup> ± 0.035	0.911 <sup>b</sup> ± 0.013	0.924 <sup>b</sup> ± 0.011	0.013
Valine	0.871 ± 0.030	0.928 ± 0.024	0.923 ± 0.034	0.542
Methionine**	0.455 <sup>a</sup> ± 0.019	0.582 <sup>b</sup> ± 0.015	0.587 <sup>b</sup> ± 0.010	0.000
Isoleucine	1.008 ± 0.018	1.061 ± 0.033	1.072 ± 0.045	0.274
Leucine**	1.418 <sup>a</sup> ± 0.050	1.691 <sup>b</sup> ± 0.022	1.689 <sup>b</sup> ± 0.021	0.003
Phenylalanine*	0.918 <sup>a</sup> ± 0.006	1.021 <sup>b</sup> ± 0.016	1.051 <sup>b</sup> ± 0.029	0.007
Lysine**	1.507 <sup>a</sup> ± 0.060	1.720 <sup>b</sup> ± 0.024	1.777 <sup>b</sup> ± 0.032	0.000
EAA**	8.463 <sup>a</sup> ± 0.220	9.621 <sup>b</sup> ± 0.141	9.726 <sup>b</sup> ± 0.174	0.000
TAA**	16.449 <sup>a</sup> ± 0.420	18.949 <sup>b</sup> ± 0.229	19.489 <sup>b</sup> ± 0.237	0.000

Values are presented as mean ± S.E. In the same row with different letters indicate significant differences (\*\* $P < 0.01$ ; \* $P < 0.05$ ); the same letter or no letter indicates no significant difference ( $P > 0.05$ ). NEAA, sum of non-essential amino acid content; EAA, sum of essential amino acid content; TAA, sum of amino acid content.

composition. Except for the Pro, there was no significant difference in the levels of the other 16 types of amino acids between hatchery-released and wild *S. schlegelii* ( $P > 0.05$ ). These 16 types of amino acid contents were significantly higher in both hatchery-released and wild *S. schlegelii* than in hatchery-reared *S. schlegelii* ( $P < 0.05$ ). In fact, the hatchery-released and wild *S. schlegelii* were significantly richer in NEAA, EAA and total amino acid (TAA) than that of the hatchery-reared ones ( $P < 0.05$ ) (Table 2). The results showed that the living environment obviously affected the amino acid contents of *S. schlegelii*.

When comparing amino acid composition between months within the hatchery-released or wild group, separately, we also only found limited significant contrasts, such as Phe and Lys in wild fish and Ser in hatchery-released fish, respectively (Table 2 and Figure 3). Concerning the whole investigation period from August to December, we actually found relatively slight differences in the amino acid profile between hatchery-released and wild *S. schlegelii* ( $P < 0.05$ ), only for Ser in October,  $0.819 \pm 0.009$  g/100 g (ww) (hatchery-released) vs  $0.909 \pm 0.023$  g/100 g (ww) (wild); Pro in October,  $0.206 \pm 0.055$  g/100 g (ww) (hatchery-released) vs  $0.401 \pm 0.059$  g/100 g (ww) (wild), November  $0.263 \pm 0.043$  g/100 g (ww) (hatchery-released) vs  $0.655 \pm 0.025$  g/100 g (ww) (wild), and December  $0.257 \pm 0.087$  g/100 g (ww) (hatchery-released) vs  $0.624 \pm 0.055$  g/100 g (ww) (wild); Val in August  $0.903 \pm 0.044$  g/100 g (ww) (hatchery-released) vs  $1.020 \pm 0.019$  g/100 g (ww) (wild), as well as Lys in August  $1.681 \pm 0.038$  g/100 g (ww) (hatchery-released) vs  $1.932 \pm 0.016$  g/100 g (ww) (wild).

The amino acid profile indicated that hatchery-released *S. schlegelii* had obviously similar amino acid compositions with the wild *S. schlegelii*, but sharply in contrast to the hatchery-reared ones, and this finding is in accord with the results of proximate composition.

### 3.3 Fatty acids analysis

A total of 35 common fatty acids were detected in this study, of which 15 fatty acids were successfully identified. These include four saturated fatty acids (SFA: C14:0, C16:0, C18:0, C24:0), five monounsaturated fatty acids (MUFA: C16:1, C20:1, C24:1, C18:1n9c, C22:1n9) and six polyunsaturated fatty acids (PUFA: C18:2n6c, C18:3n3, C20:3n3, C20:4n6 (ARA), C20:5n3 (EPA), C22:6n3 (DHA)). The results are displayed in Table 3. The fatty acids profile demonstrated obvious differences among groups, being the general distribution between SFA, MUFA, and PUFA variables. Our analysis suggested that the hatchery-reared *S. schlegelii* had significantly higher SFA and MUFA content than that of hatchery-released and wild ones,  $0.188 \pm 0.016$  g/100 g (ww) (hatchery-reared) vs  $0.132 \pm 0.012$  g/100 g (ww) (hatchery-released) vs  $0.116 \pm 0.008$  g/100 g (ww) (wild) for SFA;  $0.23 \pm 0.021$  g/100 g (ww) (hatchery-reared) vs  $0.125 \pm 0.013$  g/100 g (ww) (hatchery-released) vs  $0.096 \pm 0.006$  g/100 g (ww) (wild) for MUFA, respectively ( $P < 0.05$ ). In addition, hatchery-released and wild *S. schlegelii* also had less PUFA and total fatty acid contents than the hatchery-reared ones. In the first case (SFA), there was also a significant difference

that hatchery-released and wild *S. schlegelii* was lack of C16:0 than hatchery-reared fish,  $0.125 \pm 0.011$  g/100 g (ww) (hatchery-reared) vs  $0.085 \pm 0.008$  g/100 g (ww) (hatchery-released) vs  $0.075 \pm 0.005$  g/100 g (ww) (wild) ( $P < 0.05$ ). In MUFA, whereas the hatchery-released and wild *S. schlegelii* exhibited significantly less contents of C16:1 and C18:1n9c, in contrast of hatchery-reared ones,  $0.038 \pm 0.005$  g/100 g (ww) (hatchery-reared) vs  $0.014 \pm 0.002$  g/100 g (ww) (hatchery-released) vs  $0.011 \pm 0.001$  g/100 g (ww) (wild) in C16:1;  $0.145 \pm 0.014$  g/100 g (ww) (hatchery-reared) vs  $0.060 \pm 0.007$  g/100 g (ww) (hatchery-released) vs  $0.052 \pm 0.004$  g/100 g (ww) (wild) in C18:1n9c, respectively ( $P < 0.01$ ). For PUFA, hatchery-reared fish also showed the highest values, whether in C18:2n6c, EPA, DHA, n-3 PUFA, and n-6 PUFA contents ( $P < 0.05$ ). On the contrary, hatchery-reared fish has less ARA than that of hatchery-released and wild ones,  $0.012 \pm 0.001$  g/100 g (ww) (hatchery-reared) vs  $0.026 \pm 0.002$  g/100 g (ww) (hatchery-released) vs  $0.019 \pm 0.003$  g/100 g (ww) (wild) in PUFA ( $P < 0.05$ ).

In the study of hatchery-released *S. schlegelii* during the whole investigation period, the fish is significantly richer C24:0, DHA, n-3 PUFA, PUFA, and total fatty acid contents in September than that of December ( $P < 0.05$ ), while C24:1 content in August was significantly higher than that of November and December, respectively ( $P < 0.05$ ). Furthermore, the hatchery-released *S. schlegelii* in August were also significantly richer C22:1n9 than in October ( $P < 0.05$ ) (Figure 4). Additionally, the *S. schlegelii* was significantly richer n-6 PUFA content in September than in August, October, and December ( $P < 0.05$ ). Concerning the wild *S. schlegelii*, there was no significant difference in fatty acid profile during the whole investigation period, except for C18:0 in SFA (Figure 4).

In contrast to the fatty acid profile between hatchery-released and wild *S. schlegelii* from August to December, it was possible to suggest that hatchery-released and wild fish almost own the same SFA and MUFA, except for C22:1n9. Nevertheless, in fact, hatchery-released *S. schlegelii* was significantly richer in PUFA than the wild ones,  $0.214 \pm 0.021$  g/100 g (ww) (hatchery-released) vs  $0.112 \pm 0.019$  g/100 g (ww) (wild). This led to the finally that hatchery-released *S. schlegelii* had significantly higher total fatty acid content than wild *S. schlegelii*. Furthermore, the PUFA of hatchery-released *S. schlegelii* exhibited a certain degree of similarity, particularly in EPA and DHA, to that of hatchery-reared fish. In conclusion, the present findings indicate that hatchery-released *S. schlegelii* has not yet attained the same fatty acid profile as the wild *S. schlegelii*. Therefore, considering of the proximate compositions and amino acids, the observed changes in fatty acid composition appear to be a delay in response to environmental and dietary alterations.

## 4 Discussion

The *S. schlegelii* is a critical species for aquaculture and stock enhancement in the Northwest Pacific Ocean region. The nutritional value of fish is influenced by several factors, including the environment, the intrinsic characteristics of the fish, and the bait

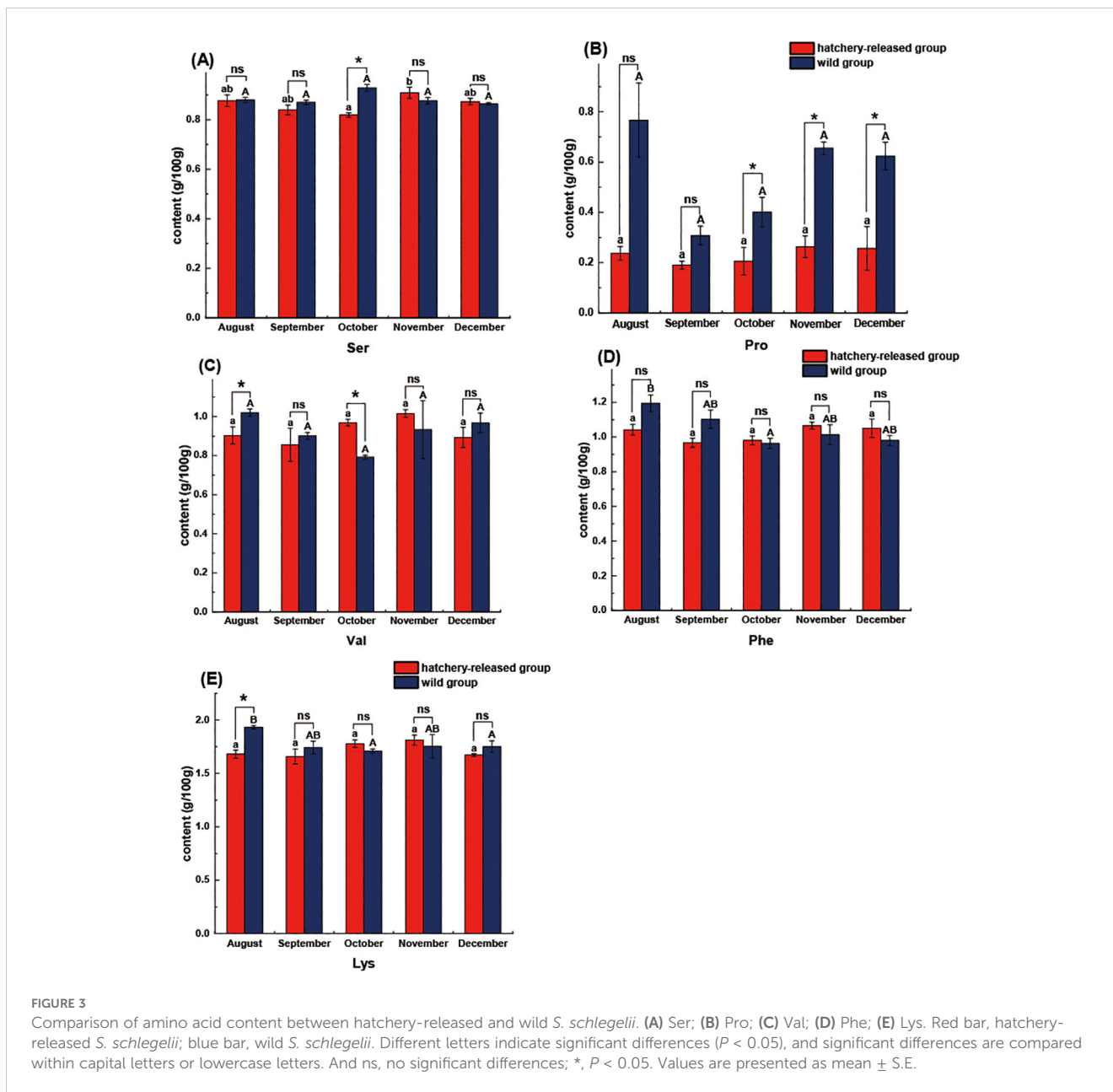


FIGURE 3

Comparison of amino acid content between hatchery-released and wild *S. schlegelii*. (A) Ser; (B) Pro; (C) Val; (D) Phe; (E) Lys. Red bar, hatchery-released *S. schlegelii*; blue bar, wild *S. schlegelii*. Different letters indicate significant differences ( $P < 0.05$ ), and significant differences are compared within capital letters or lowercase letters. And ns, no significant differences; \*,  $P < 0.05$ . Values are presented as mean  $\pm$  S.E.

organisms (Alberdi et al., 2019). Fishes generally exhibited diverse plastic throughout their life history and gene-environment interactions, such as epigenetics could influence their phenotypes. Hence, fish physiology and biological phenotypes will develop differently depending on their living environment (Johnsson et al., 2014). These fish phenotypes could be regarded as ecologically adaptation indicators for hatchery-released fish (Johnsson et al., 2014). The success of a stock enhancement program requires the maintenance of economic profit while minimizing negative genetic and ecological effects (Taylor et al., 2017). On the other hand, a successful stock enhancement depends on the capacity of fish phenotypes that domesticated population readapts to the natural environment (Gil et al., 2014). Changes in phenotypes, such as nutritional compositions post-release, could be monitored to

determine of whether hatchery-released fish adapt well to the wild environment or fail to adapt to wild conditions (Grigorakis, 2007). Considering limited study cases of ecological effects during stock enhancement at present, therefore, exploring the differences in nutritional profiles before-release (hatchery-reared) and post-release (hatchery-released vs wild) will contribute to clarifying the ecological effects and ultimately be beneficial for stock enhancement.

#### 4.1 Proximate composition analysis

The present results showed that the crude protein content of hatchery-reared *S. schlegelii* was significantly lower than that of wild *S. schlegelii* ( $P < 0.05$ ) (Table 1). This finding between hatchery-reared



TABLE 3 Fatty acid composition of hatchery-reared, hatchery-released and wild *S. schlegelii* (g/100g, wet weight).

Fatty acid	Groups			P-value
	hatchery-reared	hatchery-released	wild	
C14:0	0.016 ± 0.002	0.004 ± 0.001	0.002 ± 0.001	–
C16:0*	0.125 <sup>b</sup> ± 0.011	0.085 <sup>a</sup> ± 0.008	0.075 <sup>a</sup> ± 0.005	0.007
C18:0	0.038 ± 0.002	0.035 ± 0.002	0.036 ± 0.002	–
C24:0	0.009 ± 0.001	0.009 ± 0.001	0.003 ± 0.001	–
SFA*	0.188 <sup>b</sup> ± 0.016	0.132 <sup>a</sup> ± 0.012	0.116 <sup>a</sup> ± 0.008	0.002
C16:1**	0.038 <sup>b</sup> ± 0.005	0.014 <sup>a</sup> ± 0.002	0.011 <sup>a</sup> ± 0.001	0.006
C20:1	0.017 ± 0.002	0.006 ± 0.002	0.002 ± 0.001	–
C24:1	0.008 ± 0.001	0.009 ± 0.001	0.008 ± 0.000	0.382
C18:1n9c**	0.145 <sup>b</sup> ± 0.014	0.060 <sup>a</sup> ± 0.007	0.052 <sup>a</sup> ± 0.004	0.004
C22:1n9**	0.022 <sup>ab</sup> ± 0.002	0.036 <sup>b</sup> ± 0.004	0.022 <sup>a</sup> ± 0.002	0.009
MUFA*	0.230 <sup>b</sup> ± 0.021	0.125 <sup>a</sup> ± 0.013	0.096 <sup>a</sup> ± 0.006	0.000
C18:2n6c*	0.062 <sup>c</sup> ± 0.006	0.009 <sup>b</sup> ± 0.001	0.004 <sup>a</sup> ± 0.001	0.000
C20:4n6(ARA)*	0.012 <sup>a</sup> ± 0.001	0.026 <sup>b</sup> ± 0.002	0.019 <sup>ab</sup> ± 0.003	0.019
n-6 PUFA*	0.075 <sup>b</sup> ± 0.007	0.035 <sup>a</sup> ± 0.003	0.022 <sup>a</sup> ± 0.004	0.000
C18:3n3	0.007 ± 0.001	0.002 ± 0.001	ND	–
C20:3n3	ND	ND	0.001 ± 0.001	–
C20:5n3(EPA)*	0.034 <sup>b</sup> ± 0.003	0.018 <sup>b</sup> ± 0.002	0.009 <sup>a</sup> ± 0.002	0.000
C22:6n3(DHA)*	0.213 <sup>b</sup> ± 0.011	0.159 <sup>b</sup> ± 0.016	0.079 <sup>a</sup> ± 0.015	0.000
n-3 PUFA*	0.255 <sup>b</sup> ± 0.015	0.179 <sup>b</sup> ± 0.019	0.090 <sup>a</sup> ± 0.016	0.010
PUFA*	0.329 <sup>b</sup> ± 0.022	0.214 <sup>b</sup> ± 0.021	0.112 <sup>a</sup> ± 0.019	0.000
Total fatty acid*	0.743 <sup>b</sup> ± 0.061	0.470 <sup>b</sup> ± 0.040	0.323 <sup>a</sup> ± 0.028	0.012

Values are presented as mean ± S.E. In the same row with different letters indicate significant differences (\*\* $P < 0.01$ ; \* $P < 0.05$ ); the same letter or no letter indicates no significant difference ( $P > 0.05$ ). ND, not detected. –, not statistically significant. SFA, sum of saturated fatty acids; MUFA, sum of monounsaturated fatty acids; n-6 PUFA, sum of n-6 polyunsaturated fatty acids; n-3 PUFA, sum of n-3 polyunsaturated fatty acids; PUFA, sum of polyunsaturated fatty acids.

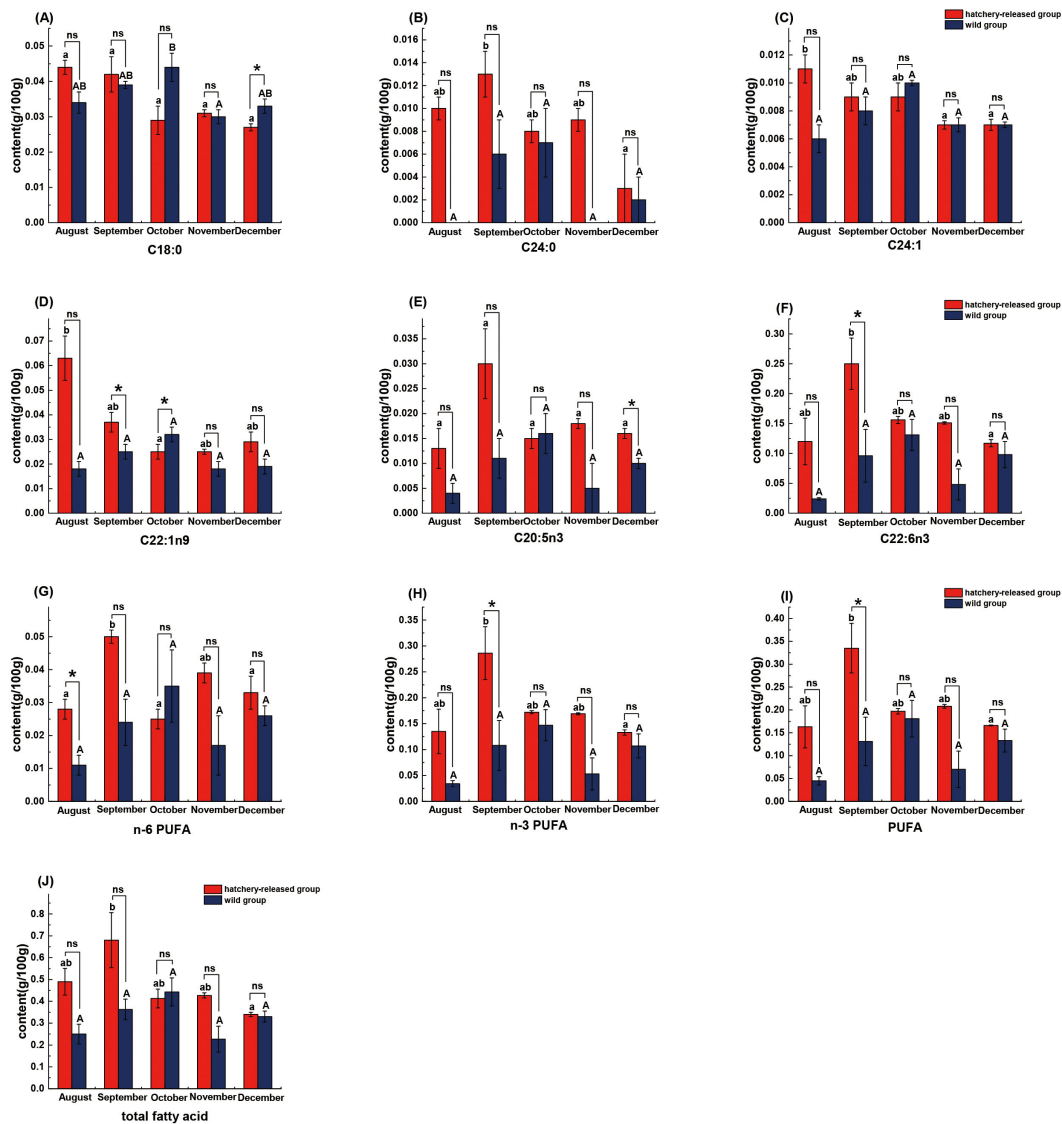
and wild *S. schlegelii* was in accordance with previous results of other species, such as silver pomfret (*Pampus argenteus*) (Zhao et al., 2010), large yellow croaker (*Larimichthys crocea*) (Guo et al., 2019) and *S. schlegelii* (Gao et al., 2023). Additionally, hatchery-reared *S. schlegelii* had significantly higher moisture and crude lipid content compared to wild ones ( $P < 0.05$ ). Previous research showed that environmental factors such as temperature, salinity, and food availability could significantly contribute to the biochemical composition of an organism (Kim et al., 2015), which led to an environment-dependent biochemical composition within the species.

Interestingly, all of the moisture, crude lipid, and crude protein contents of the hatchery-released *S. schlegelii* exhibited intermediate values between hatchery-reared and wild ones in this research. It is possible to indicate that hatchery-released *S. schlegelii* seems to be a swift transition from “hatchery” to the native environment. Moreover, there was no significant difference in proximate profile from August to December between hatchery-released and wild fish, which also confirmed that hatchery-released *S. schlegelii* could adapt to the natural environment post-release.

## 4.2 Amino acid analysis

Amino acids are the fundamental components of proteins and play crucial roles in cell construction, antibody protein production, and tissue repair (Hou et al., 2024). There was no significant difference in most of the amino acid contents between hatchery-released and wild groups ( $P > 0.05$ ), except that the hatchery-released fish exhibited a significantly lower Pro and then resulted in decreased NEAA content than that of wild counterparts ( $P < 0.05$ ) (Table 2). Pro involves collagen biosynthesis and cellular metabolism (De et al., 2019). The cultural practice before release, including almost feeding a single pellet source, may ultimately cause differences in Pro levels between the hatchery-released and wild *S. schlegelii* in post-release (Oztekin et al., 2020).

In addition, the habitat and feeding behaviors were also markedly different between the natural environment and hatchery, which can quickly influence the nutrition profile of fish (Cardoso et al., 2023). The previous research found that the above factors had a profound effect on the amino acid profile in fish (De



**FIGURE 4** Comparison of fatty acid content between hatchery-released and wild *S. schlegelii*. (A) C18:0; (B) C24:0; (C) C24:1; (D) C22:1n9; (E) C20:5n3; (F) C22:6n3; (G) n-6 PUFA; (H) n-3 PUFA; (I) PUFA; (J) total fatty acid. Red bar, hatchery-released *S. schlegelii*; blue bar, wild *S. schlegelii*. Different letters indicate significant differences ( $P < 0.05$ ), and significant differences are compared within capital letters or lowercase letters. And ns, no significant differences; \*,  $P < 0.05$ . Values are presented as mean  $\pm$  S.E.

et al., 2019). The present results indicated that the hatchery-reared *S. schlegelii* generally exhibited significantly lower levels of amino acids than that of wild counterparts. This similar phenomenon was also observed in other fish species, such as *P. hypophthalmus* (Chakma et al., 2022), Axillary seabream (*Pagellus acarne*) (Oztekin et al., 2020), and Ussuri catfish (*Pseudobagrus ussuriensis*) (Wang et al., 2013). Although no significant difference was found in Val and Iso ( $P > 0.05$ ) between before-release and post-release groups (Table 2), the most surprising finding is that hatchery-released *S. schlegelii* after release showed almost similar amino acid profiles with their wild counterparts ( $P > 0.05$ ), but significantly different with hatchery-reared ones ( $P < 0.05$ ). Previous research reported changes in amino acid levels within species could contribute to apparent seasonal variations in wild sea bass (*Dicentrarchus labrax*) (Özyurt and Polat, 2006).

Nevertheless, no significant differences in NEAA, EAA and TAA in present research were observed regardless of hatchery-released or wild *S. schlegelii* from August to December. The observed discrepancies are likely to be attributable to differences in species.

On the other hand, amino acid comparative analyses of the hatchery-released and wild *S. schlegelii* in the same months revealed only a few specific amino acids (4 out of 17 amino acids), including Ser, Pro, Val, Lys exhibited significant differences ( $P < 0.05$ ) (Figure 3), no significant differences were found in most of amino acid composition as well as the levels of NEAA, EAA, or TAA across months.

Adaptation to natural habitats and food sources is critical for the survival of hatchery-released fish (Tomiyama et al., 2011). Post-release predation in hatchery-released fish can be expected to occur in the wild in order to survive and adapt to the environment and

ultimately exhibit similar nutrition profiles to wild fish. The similarity amino acid composition profile in this research between hatchery-released and wild *S. schlegelii* was consistent with the above proximate composition finding, which indicated that the hatchery-released *S. schlegelii*, after release, could transfer from “hatchery” to the wild in order to adapt to their natural environment.

### 4.3 Fatty acid analysis

The fatty acid results of the present study were in accordance with previous research about *S. schlegelii* (Han et al., 2019). The primary fatty acids for SFA, MUFA, and PUFA in *S. schlegelii* are C16:0, C18:1n9c, and DHA, respectively (Han et al., 2019). In our study, the levels of SFA and MUFA were significantly higher in the hatchery-reared group compared to the hatchery-released and wild groups ( $P < 0.05$ ). This similar tendency has also been shown in other fish species, such as rainbow trout (*Oncorhynchus mykiss*) (Fallah et al., 2011) and yellow tail (*Seriola lalandi*) (O'Neill et al., 2015). The observed variations in the results can likely be attributed to a complex interplay of several key environmental variables, including fluctuations in temperature, variations in salinity levels, and the availability and diversity of food sources (Silva et al., 2021). In our results, the n-6 PUFA content of the hatchery-released *S. schlegelii* was close to the wild *S. schlegelii*, but significantly lower than the hatchery-reared fish ( $P < 0.05$ ). In *O. mykiss* (Fallah et al., 2011), *S. lalandi* (O'Neill et al., 2015), and *P. hypophthalmus* (Chakma et al., 2022) the hatchery-reared group has obviously more n-6 PUFA than that of the wild group. In contrast, we found both the hatchery-released and hatchery-reared *S. schlegelii* displayed similar n-3 PUFA (for instance, EPA and DHA), as well as the PUFA content, but significantly higher than that of the wild fish ( $P < 0.05$ ). It may be attributed to the fact that the food resource of hatchery-reared *S. schlegelii* was rich in PUFA, regardless of n-6 PUFA or n-3 PUFA before release. However, the PUFA availability in the wild seemed to be limited, resulting in a decline in the PUFA content of the hatchery-released fish subsequently (Cardoso et al., 2023). In general, the DHA and EPA are essential for biological membranes, nerves, and vision, and are not easily facilitated for organism energy supply (Zavorka et al., 2023). It is worth noting that hatchery-released *S. schlegelii* consumed a relatively greater proportion of n-6 PUFA and a lower proportion of n-3 PUFA in the wild. Consequently, the n-6 PUFA content of the hatchery-released *S. schlegelii* is close to the wild counterparts, while the n-3 PUFA is close to the hatchery-reared counterparts. Furthermore, the total fatty acid content in the hatchery-released group was also similar to that of the wild group. These findings indicate that the hatchery-released *S. schlegelii* is adapting to fatty acid changes from “hatchery” to the “wild” in consideration of the district food supply and living environment after release (Lee et al., 2021).

A five-month investigation in the present study revealed that fatty acid profiles within hatchery-released and wild *S. schlegelii* exhibited seasonal variation. However, the trends of fatty acid contents between them were distinctly different. There were 4 out of 15 types of fatty acid (C24:0, C24:1, C22:1n9, and DHA) showed

significant differences in the hatchery-released *S. schlegelii*. In contrast, the fatty acid level of wild *S. schlegelii* was relatively steady from August to December, except C18:0 in October and November, respectively ( $P < 0.05$ ). Since the availability of food in the natural environment is subject to variations in temperature, salt content, influx of freshwater, and exposure to solar energy (Xu et al., 2022), changes in the above environmental conditions in different seasons may have an influence on the abundance of prey (Nakajima et al., 2013). And wild *S. schlegelii* seemed to have developed an effective adaptive mechanism in response to such seasonal fluctuations in fatty acid levels, but hatchery-released *S. schlegelii* were not yet. Several fatty acid compositions, such as C18:0, C22:1n9, and DHA, showed significant differences between hatchery-released and wild counterparts, which may also be due to the above reasons.

## 5 Conclusion

This study has provided nutritional profile information on the before-release and post-release of *S. schlegelii*, which can provide us with a deep insight into the ecological effects derived from stock enhancement. The hatchery-reared *S. schlegelii* exhibited contrasting nutritional characteristics to their wild counterparts, with significantly higher moisture, crude lipid, and fatty acids, but significantly lower protein and amino acids. However, above distinct variations in nutritional composition were not observed between hatchery-reared and hatchery-released fish. Hatchery-released fish exhibited intermediate nutritional composition values among these three groups. The nutritional trends observed in both hatchery-released and wild *S. schlegelii* were found to be almost similar at different times post-release. Our results suggest that hatchery-reared *S. schlegelii* can adjust from “hatchery” to their wild counterparts and become adaptable to the natural environment after release. However, several significant differences in fatty acid composition indicate that hatchery-released fish did not seem to adapt to the food supply post-release completely. Future studies should pay more attention to understanding the ecological effects of stock enhancement.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

## Ethics statement

The animal study was approved by Animal experiments were conducted at the Fisheries Resources Laboratory of Dalian Ocean University, in accordance with Chinese laws, regulations, and ethical principles. The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

LW: Data curation, Investigation, Methodology, Writing – original draft. YH: Investigation, Methodology, Writing – review & editing. XW: Investigation, Methodology, Writing – review & editing. XL: Methodology, Writing – review & editing. RZ: Methodology, Writing – review & editing. QL: Conceptualization, Investigation, Methodology, Writing – review & editing. CW: Investigation, Writing – review & editing. JC: Investigation, Writing – review & editing. ML: Methodology, Writing – review & editing. ZM: Resources, Writing – review & editing. SQ: Resources, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2024.1536508/full#supplementary-material>

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