

EFFECTS OF LONG-TERM AND HIGH-DENSITY NET-PEN CAPTIVITY ON MEAT QUALITY, NUTRITIONAL COMPOSITION OF WILD-CAUGHT BIGHEAD CARP (*Hypophthalmichthys nobilis*)

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ABSTRACT

This study investigates the impact of temporary net-pen captivity on the fresh meat quality and nutritional composition of wild bighead carp (*Hypophthalmichthys nobilis*) caught from Qiandao Lake over an 18-week experimental period. The research aimed to assess changes in body size, muscle texture, and the nutritional profile of the fish during captivity, which are critical factors for the freshwater fishery industry. The research methodology encompassed biometric measurements, texture profile analysis, and assessments of the muscle's nutritional profile, including protein and fatty acid content. Results indicate significant alterations in the body size and nutritional composition of bighead carp, with notable effects on the muscle texture, potentially affecting the meat's taste and chewiness. Variations in protein and fatty acid content were observed, likely influenced by physical activity restrictions, feed limitations, and water quality changes during captivity. The study concludes that temporary net-pen captivity significantly impacts the meat quality and nutritional value of bighead carp, with implications for sustainable fisheries management. It is recommended that the captivity period for wild bighead carp be curtailed to within 3 weeks, extending to a maximum of 12 weeks to ensure product quality. These findings are essential for guiding the freshwater fishery industry towards sustainable practices that balance the health of the fish and the quality of the final product.

Keywords: bighead carp *Hypophthalmichthys nobilis*; wild-caught; temporary net-pen captivity; fresh meat quality; nutritional composition.

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INTRODUCTION

Bighead carp (*Hypophthalmichthys nobilis*) is one of the four major Chinese traditional domesticated fishes alongside Black Carp (*Mylopharyngodon piceus*), Grass Carp (*Ctenopharyngodon idella*), and Silver Carp (*Hypophthalmichthys molitrix*) with an annual production over 3.27 million tonnes (Yuan *et al.*, 2022; Peng *et al.*, 2023b). Bighead carp and other fish occupy an important place in the human diet due to their excellent nutritional value (Kakoolaki *et al.*, 2013; Talas *et al.*, 2014). As a famous big-scale freshwater fish, bighead carp is widely distributed throughout China and other Asian regions. Aquatic products and animals are essential nutrients in the human diet and are also present in the global aquatic product industry for consumers. Therefore, it is imperative that we take measures to protect our aquatic environment against pollution and various environmental and ecological effects. The aquatic ecosystems and living organisms, including bighead carp, suffer from environmental impacts such as emissions of volatile

organic substances and water pollution by oil chemicals and many hazardous agents (Ates *et al.*, 2008; Orun *et al.*, 2008; Talas *et al.*, 2008, 2014). In the last few decades, bighead carp is used as an effective tool for restoring eutrophic lakes and rivers due to its filter-feeding behavior (Li *et al.*, 2018; Yu *et al.*, 2019; Shen *et al.*, 2020; Newton *et al.*, 2021). As a result, it has been successfully introduced to more than 70 countries and regions worldwide, including Europe and North America (Luo *et al.*, 2022; Yuan *et al.*, 2022). However, the management and control of bighead carp escapement have become pressing issues due to their invasive status in numerous North American lakes and rivers (Camacho *et al.*, 2019; Ivan *et al.*, 2020; Broaddus and Lamer, 2022). In contrast, bighead carp is a popular ingredient in gourmet cuisine due to its tender, snow-white flesh and delicious flavor in China, particularly the head portion of the fish (Alahmad *et al.*, 2022; Shi *et al.*, 2022). The bighead carp captured from Qiandao Lake is highly regarded for its unique growing environment and excellent quality, and is not only popular locally, but also

throughout China, where it is the first officially certified organic fish.

As it is customary in China to purchase live fish for cooking and consumption, bighead carp is primarily sold fresh (Peng *et al.*, 2023b). Fishing operations were restricted by seasonal and time constraints in open water fishery production during the early stages. This could result in a concentration of catches on the market, resulting in backlogs of fish, difficulties in preserving freshness, transportation issues, lower prices, and other related problems. Thus, temporary net-pen captivity has become a popular technical means of aquaculture, particularly in open water fishery production. It effectively regulates the listing cycle, ensures fish freshness, reduces transportation losses, and improves economic benefits. In the capture fishery production of Qiandao Lake, wild bighead carp need to be temporarily reared in net-pens after being caught to reduce their wildness. This practice helps to reduce transportation mortality and improve the fish quality. The duration of net-pen captivity might range from a few days to several months. However, it is important to note that high-density and long-term net-pen captivity may result in decreased locomotion and lack of food for the wild bighead carp. The exact impact of long-term net-pen captivity on wild bighead carp is currently not well understood and requires further investigation.

Similar technical methods are frequently used in intensive aquaculture, particularly in closed-containment systems, which involves transferring farmed fish to clean water or micro-flowing water system for a period of time (Palmeri *et al.*, 2008a, 2009; Du *et al.*, 2021; Podduturi *et al.*, 2021; Lindholm-Lehto *et al.*, 2023). The process is commonly referred to as depuration, and is primarily intended to eliminate any possible off-flavors from the fish and improve the quality of farmed fish (Palmeri *et al.*, 2008a). Depuration and starvation improve flesh quality of fish (Lv *et al.*, 2018). A minimum 12-day purging process improves the sensory quality and nutritional composition of intensively farmed Murray cod, particularly by increasing polyunsaturated fatty acids and enhancing flavor (Palmeri *et al.*, 2008b; 2009). Depuration process without feed for 10-15 days significantly reduced off-flavors in Atlantic salmon cultured in recirculating aquaculture systems (RAS), weight loss and decreases in lipid content were observed while moisture content increased (Burr *et al.*, 2012; Davidson *et al.*, 2014). Similar findings have been reported in other intensively farmed species, including common carp (Zajic *et al.*, 2013), grass carp (Lv *et al.*, 2018; Du *et al.*, 2020), crucian carp (Du *et al.*, 2021), European whitefish (Lindholm-Lehto *et al.*, 2019), and pikeperch (Podduturi *et al.*, 2021). However, it is also necessary to investigate whether prolonged periods of excessive starvation may have negative impact on fish welfare, meat quality, and nutritional composition.

The objective of this study is to investigate the survival conditions and characteristics changes of wild bighead carp in the high-density, long-term net-pen captivity environment. The study aims to reveal changes in muscle texture, nutritional composition, and other meat qualities of bighead carp under net-pen captivity conditions. Thus, the challenges and problems that bighead carp may face in long-term and high-density net-pen captivity could be well understood. This could provide theoretical support and practical guidance for scientific fishing, net-pen captivity management, and quality improvement of bighead carp. The results will aid in enhancing the temporary net-pen captivity technology of bighead carp, optimizing its meat quality, improving net-pen captivity efficiency, protecting wild bighead carp resources, maintaining aquatic ecological balance, and satisfying consumer demand for high quality and delicious bighead carp products. Fish is an essential nutrient in the human diet and is also present in the global aquatic product industry for consumers (Ates *et al.*, 2008; Kakoolaki *et al.*, 2013). Therefore, we need to improve our aquatic products and ensure their health and well-being, aligning with the growing consumer demand for organic and sustainably sourced aquatic food.

MATERIALS AND METHODS

Experimental fish and treatment: The wild bighead carp used in the experiment were naturally grown in Qiandao Lake for over five years after artificial stock enhancement and releasing, with a fishing specification of over four kilograms. On August 2, 2023, they were transported to the Danzhu temporary net-pen captivity base of the Hangzhou Qiandao Lake Development Group Co., Ltd. by live-water transport vessels after being harvested (Fig. 1). The net-pen was specified to be 12m × 8m × 10m (L × W × H) with a mesh size of 12cm. Each net-pen was stocked with 1500 bighead carp. Five bighead carps were randomly selected from the net-pen as the experimental fish on August 3. The study was conducted over a period of 18 weeks, from August 3 to December 2. Six sampling times were scheduled during this period, on August 3, August 9, August 24, September 15, October 22, and December 2, representing 1, 3, 6, 12 and 18 weeks of temporary net-pen captivity, respectively.

Biometric parameters: The experimental bighead carp were measured and weighed in situ, and the measurements included body length, body weight, visceral weight, liver weight, visceral fat weight and carcass weight. After anesthetizing the experimental fish with MS-222, a stainless-steel tape measure was used to measure the body length of the experimental fish, and an electronic weighing scale (Meilen MT201) was used to measure the weight of the fish, whereas the fish were

dissected with a dissecting knife to decompose the visceral mass, liver, and visceral fats successively. The visceral masses, liver, and visceral fats were measured sequentially using an electronic balance (Sartorius BSA822-CW) and an electronic weighing scale (Meilen MT201) were used to measure the carcass weight.

Texture profile analysis: After dissection, take the whole muscle of the dorsal of the experimental fish, and take 3 samples from the front, middle and back of the experimental fish's dorsal muscle respectively. Cut the samples into small pieces of 2cm × 2cm × 1cm, and balance them in a 4°C refrigerator for 1h before testing. In texture testing, 5 experimental fish were taken for each treatment, and 15 data were obtained for each texture parameter, and their mean and standard deviation were calculated. The Brookfield CT3 texture analyzer (Brookfield Engineering Laboratories, INC., USA) was used for texture detection. The measurement mode was selected as texture profile analysis (TPA). The probe type was TA44, the test speed was 5.00 mm/sec, the target mode: strain (compression ratio 75.00%, time 5.00 sec), trigger mode: Button. The texture parameters measured include: hardness, springiness, adhesiveness, cohesiveness, gumminess and chewiness.

Nutritional composition, amino acid and fatty acid analysis: The content of moisture, crude protein, crude fat and ash in dorsal muscle of the experimental fish were determined by 105°C constant temperature drying method, Kjeldahl nitrogen method, ether extraction method and muffle furnace 550°C ashing method respectively, according to the AOAC recommendations; amino acids and fatty acids were determined by amino acid analyzer (Sykam S433D) and gas chromatography-mass spectrometry (Agilent 7890B) respectively.

Data calculation and statistical analysis: The calculation methods of related indicators in the experiment are as follows:

$$\text{Visceral fat index (VFI, \%)} = 100 \times W_{VF} / W_B$$

$$\text{Condition factor (K, g/cm}^3\text{)} = 100 \times W_B / L_B^3$$

$$\text{Hepatosomatic index (HSI, \%)} = 100 \times W_H / W_B$$

$$\text{Viscerosomatic index (VSI, \%)} = 100 \times W_V / W_B$$

$$\text{AAS} = [\text{amino acid content in sample protein (mg/g)}] / [\text{corresponding essential amino acid content in the ideal pattern (mg/g)}]$$

$$\text{CS} = [\text{amino acid content in sample protein (mg/g)}] / [\text{corresponding essential amino acid content in eggs (mg/g)}]$$

$$\text{EAAI} = [(100A/AE) \times (100B/BE) \times \dots \times (100H/HE)]^{1/n}$$

Where: W_B is body weight, L_B is body length, W_H is liver weight, W_V is visceral mass weight; W_{VF} is visceral fat weight; A, B, C,, H are essential amino acid contents (% dry weight); AE, BE, CE,, HE are

essential amino acid contents of whole egg protein (% dry weight); n is the number of amino acids compared.

Data were sorted using Excel 2019 (Microsoft Office) and analyzed statistically with SPSS 19.0 (IBM SPSS Statistics), with results presented as (Mean ± S.D.). A one-way ANOVA was utilized to assess the effects of temporary net-pen captivity duration on bighead carp, employing Duncan's test for multiple comparisons, where significance was set at $P < 0.05$. Graphical representations were generated using Origin 2022 (Origin Lab) software.

RESULTS

Biometric parameters Variation: The biometric data of experimental bighead carp, captured at different time intervals in temporary net-pens, are presented in Table 1. Throughout the duration of their captivity, the body weight, carcass weight, visceral weight, liver weight, and visceral fat weight of the experimental bighead carp decreased. However, the fish's body length remained stable. Significant differences were observed in the body weight at the 12th week, carcass weight at the 18th week, and visceral weight at the 6th week ($P < 0.05$). Moreover, both the liver weight and visceral fat weight of the experimental fish showed significant differences compared to the 1st week during their temporary net-pen captivity.

Figure 2 shows the visceral fat index (VFI), condition factor (K), hepatosomatic index (HSI), and viscerosomatic index (VSI). These indexes, along with their reduction rates, exhibited a significant decreasing trend throughout the duration of the temporary net-pen captivity. The VFI decreased by 69.96% during the experiment, with a significant difference found in the 1st week ($P < 0.05$). The reduction rate of VFI increased with the duration of the temporary net-pen captivity, averaging 0.56% per day. The condition factor, HSI, and VSI decreased by 20.83%, 64.09%, and 18.54%, respectively, over the 18-week period of temporary net-pen captivity. The condition factor demonstrated a significant variance from the 3rd week ($P < 0.05$), exhibiting a reduction rate of 0.17% per day. Furthermore, the HSI and VSI displayed notable differences when compared to the 1st and 12th weeks respectively ($P < 0.05$). The average reduction rates for HSI and VSI were 0.51% and 0.15%, respectively.

Muscle Composition Variation: Table 2 presents the contents of moisture, crude protein, crude fat, and ash in dorsal muscle of the experimental bighead carp during the temporary net-pen captivity. The results showed that the moisture content varied between 79.00% and 79.75%, the crude protein content ranged from 16.09% to 16.71%, the crude fat content fluctuated between 0.71% and 3.44%, and the ash content spanned from 1.16% to

1.39%. The moisture, crude protein, and ash contents remained relatively stable throughout the entire temporary net-pen captivity cycle. However, there was a significant decreasing trend in crude fat content over this period, with a significant difference ($P < 0.05$) observed after the 12-week of captivity. After the 18-week of temporary net-pen captivity, the crude fat content in the dorsal muscle of bighead carp decreased from 3.44% to 0.71%, representing a decrease of 79.36%. On average, the crude fat content decreased by 0.63% per day during the experimental cycle. The reduction rate decreased as the duration of temporary net-pen captivity increased, with rates of 2.88%, 2.01%, 1.34%, and 0.78% per day observed in the 1st, 3rd, 6th, and 12th weeks, respectively.

TPA Variation: The results of texture profile analysis (TPA) are displayed in Figure 3. Significant differences in hardness, cohesiveness, springiness, gumminess, and chewiness were observed during the period of temporary net-pen captivity ($P < 0.05$). All results decreased throughout the 18-week net-pen captivity period. The highest reduction rates of hardness and adhesiveness were recorded in the 1st week, at 3.71% per day and 7.60% per day, respectively, and continued to decrease with an averaging 0.30% per day and 0.42% per day as the duration of temporary net-pen captivity increased. The cohesiveness, springiness, gumminess, and chewiness of the experimental bighead carp showed a tendency to increase and then decrease during the period of temporary net-pen captivity. The mean reduction rates for these parameters were 0.32% per day for cohesiveness, 0.15% per day for springiness, 0.40% per day for gumminess, and 0.51% per day for chewiness.

Amino acid Variation: The variation of amino acids in the dorsal muscle of experimental bighead carp during different duration of temporary net-pen captivity are shown in Figure 4. In totally, 17 amino acids were detected in all samples, which were categorized into essential, non-essential, and semi-essential amino acids. The contents of Glu, Gly, Thr, Met, His, Arg, Cys, Tyr, and Pro showed significant changes during the duration of temporary net-pen captivity ($P < 0.05$). The Cys content decreased the most throughout the experiment, by 71.45%, followed by Met, Arg and Glu at 31.80%, 22.97%, and 11.93%, respectively. The contents of Lys, His, and Pro showed an increasing tendency, with Pro content increased by 20.90% during the duration of temporary net-pen captivity.

As Table 3 indicates, the UAA contents of experimental bighead carp decreased from 5.45% to 4.96%. The EAA contents, NEAA contents, and TAA contents decreased from 5.69% to 5.26%, 8.94% to

8.21%, 14.63% to 13.47%, respectively. Significant differences in UAA/TAA were observed during the duration of temporary net-pen captivity ($P < 0.05$), with significantly lower levels found in the 12th week compared to other weeks. The ratio of EAA/TAA ranged from 38.83% to 39.35%, EAA/NEAA ranged from 63.48% to 64.89%, and the differences in variation during the duration of temporary net-pen captivity were not significant.

Protein Quality: The amino acid score (AAS), chemical score (CS), and essential amino acid index (EAAI) for the dorsal muscles of the experimental bighead carp during different duration of temporary net-pen captivity are presented in Table 4. Throughout the 18-week net-pen captivity, all the indexes exhibited decrease, although significant differences were only observed in the levels of methionine (Met) + cysteine (Cys) ($P < 0.05$). Regardless of whether the AAS or CS method was used, the first limiting amino acids were Met + Cys, and the second limiting amino acids were valine (Val).

Fatty acid Variation: The fatty acid analysis presented in Table 5 reveals the presence 20 different types of fatty acids in the dorsal muscle of experimental bighead carp under varying duration of temporary net-pen captivity. The fatty acid content demonstrated variation across varying durations of temporary net-pen captivity, with notable differences noted for C14:0, C18:0, C14:1, C15:1, C16:1, C17:1n7, C18:1n9t, C18:3n6, C20:4n6, C20:5n3, and C22:6n3 ($P < 0.05$).

As the length of temporary net-pen captivity extended, there was a relative stability in the SFAs content within the experimental bighead carp. The MUFAs content of the experimental bighead carp decrease, while the PUFAs content increase, and the variations in MUFAs and PUFAs were statistically significant ($P < 0.05$). The MUFAs content decrease 19.33% and the PUFAs increase 25.91% after an 18-week period of temporary net-pen captivity.

Correlation and similarity: Figure 5 (a) presents the results of a principal component analysis (PCA) applied to all data, as well as an analysis of similarities (ANOSIM). Significant differences were shown between the experimental bighead carp sampling in the 0 weeks and the 18th week, the 1st week and the 18th week, and the 3rd week and the 18th week (ANOSIM, $P < 0.05$). The figure 5 (b) depicts the outcomes of Pearson's correlation analysis performed on the entire dataset. The analysis revealed negative correlations between the biometric parameters of the experimental bighead carp and both moisture content and several specific fatty acids, namely C16:0, Σ SFAs, C18:2n6c, and C20:5n3 (EPA).

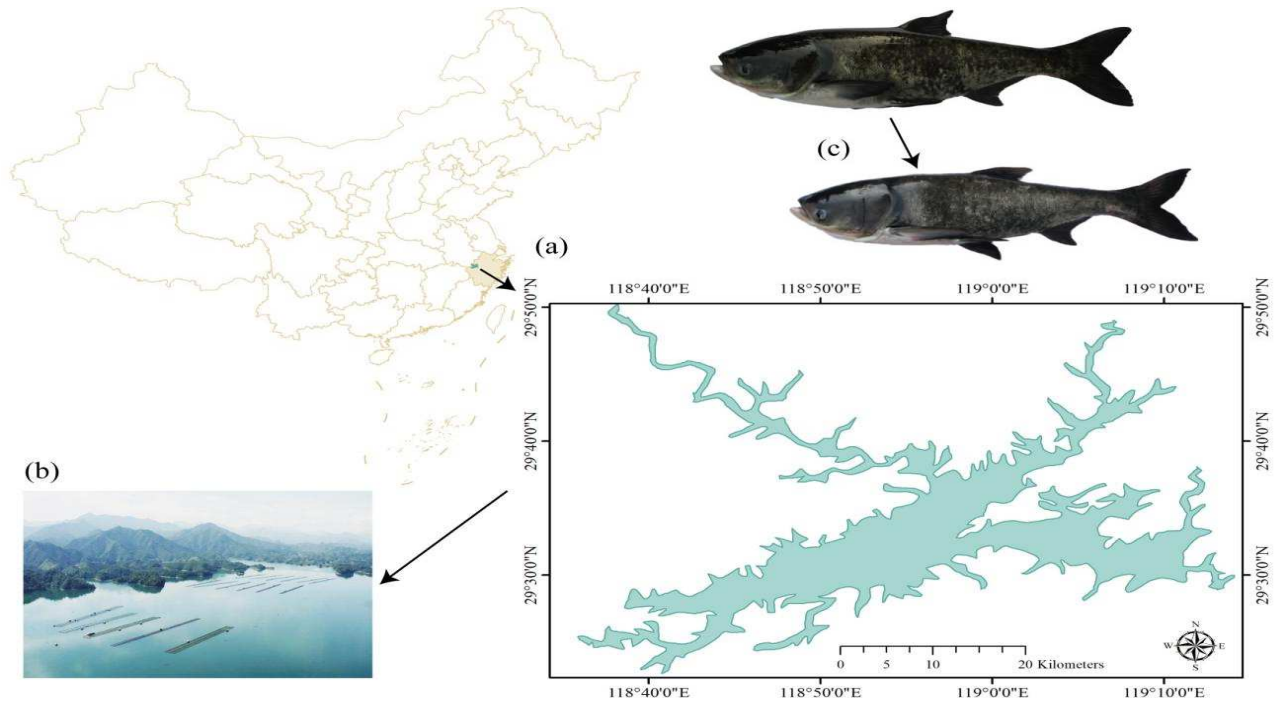


Fig. 1 The Danzhu temporary net-pen captivity base. (a) The location of temporary net-pen captivity base; (a) The net-pen used in present research; (c) The change in the experimental bighead carp.

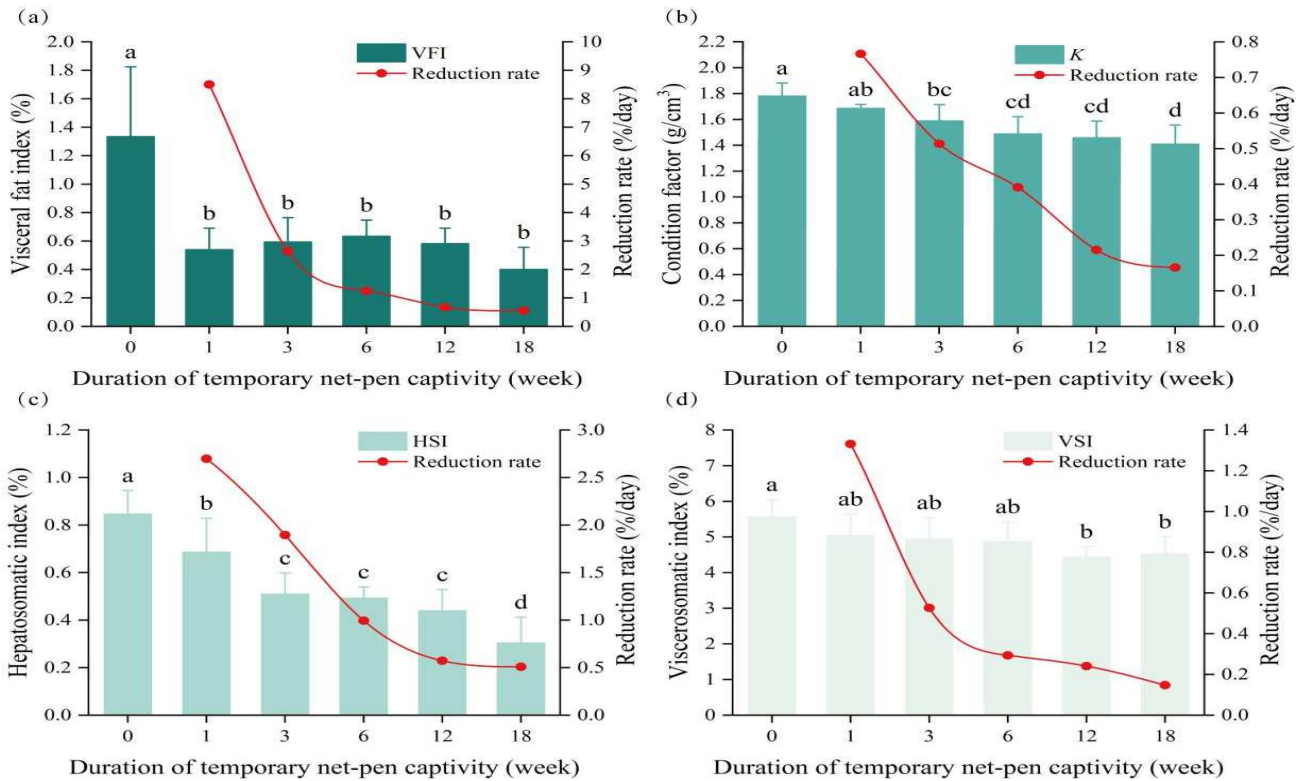


Fig. 2 The variation of visceral fat index (VFI), condition factor (K), hepatosomatic index (HSI), viscerosomatic index (VSI), and their reduction rate during the duration of temporary net-pen captivity. (a) The variation of VFI and reduction rate; (b) The variation of condition factor and reduction rate; (c) The variation of HSI and reduction rate; (d) The variation of VSI and reduction rate. Distinct letters denote statistically significant differences ($P < 0.05$).

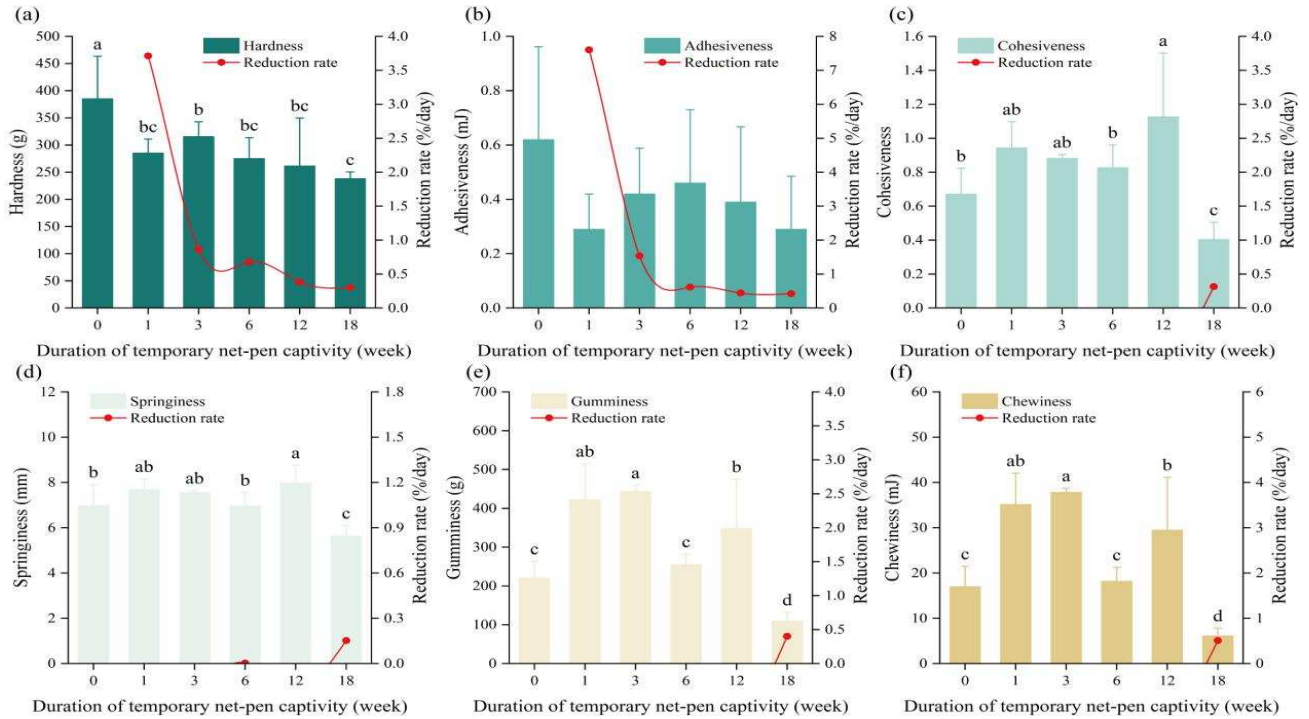


Fig. 3 The variation of TPA in the dorsal muscle of experimental bighead carp during the duration of temporary net-pen captivity. (a) The variation of hardness and reduction rate; (b) The variation of adhesiveness and reduction rate; (c) The variation of cohesiveness and reduction rate; (d) The variation of springiness and reduction rate; (e) The variation of gumminess and reduction rate; (f) The variation of chewiness and reduction rate. Distinct letters denote statistically significant differences ($P < 0.05$).

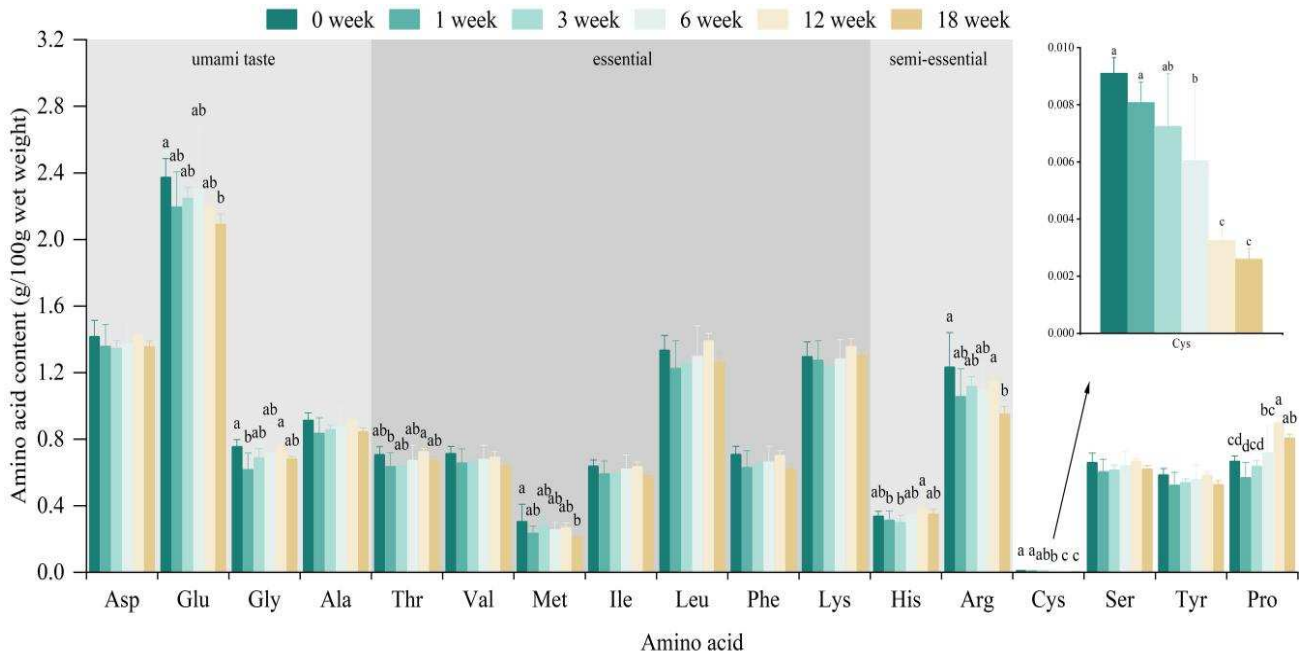


Fig. 4 The variation of amino acids in the dorsal muscle of experimental bighead carp during the duration of temporary net-pen captivity. Asp: Aspartic acid. Glu: Glutamic acid. Gly: Glycine. Ala: Alanine. Thr: Threonine. Val: Valine. Met: Methionine. Ile: Isoleucine. Leu: Leucine. Phe: Phenylalanine. Lys: Lysine. His: Histidine. Arg: Arginine. Cys: Cysteine. Ser: Serine. Tyr: Tyrosine. Pro: Proline. Distinct letters denote statistically significant differences ($P < 0.05$).

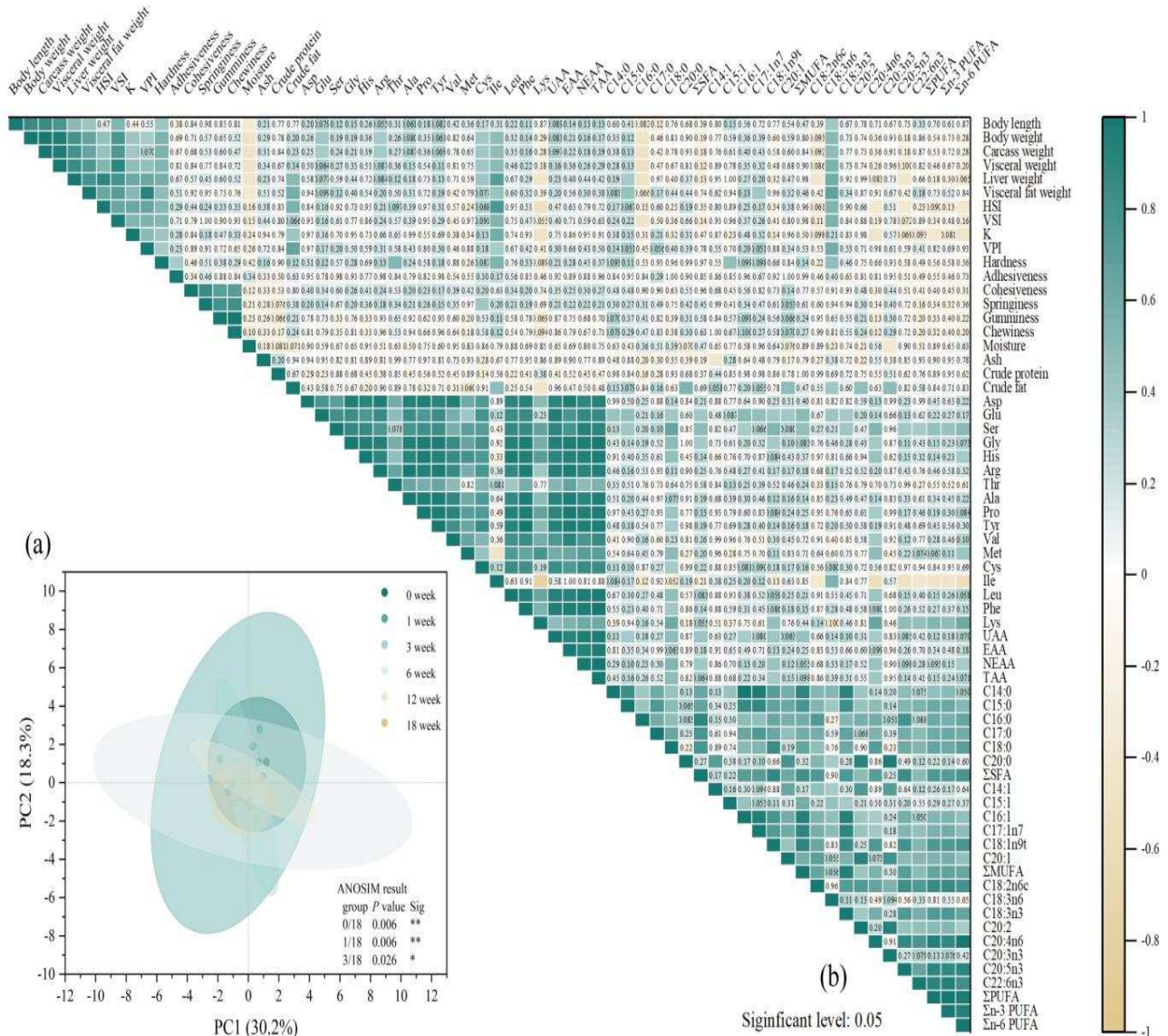


Fig. 5 The correlation and similarity of all detected data. (a) Principal Component analysis of all data and analysis of similarities (ANOSIM); (b) Pearson's correlation analysis of all data.

Table 1. Biometric parameters of the experimental bighead carp in different duration of temporary net-pen captivity (% wet weight).

Index	Duration of temporary net-pen captivity					
	0-week	1-week	3-week	6-week	12-week	18-week
Body length (cm)	66.14±5.74	65.10±4.39	64.50±4.26	64.30±5.39	64.10±4.93	64.00±3.69
Body weight (g)	5243.00±1375.86 ^a	4712.00±1046.29 ^{ab}	4310.00±961.38 ^{ab}	3968.00±713.53 ^{ab}	3852.00±663.91 ^b	3676.00±302.70 ^b
Carcass weight (g)	4679.40±1242.95 ^a	4277.20±938.87 ^{ab}	3890.20±832.37 ^{ab}	3581.20±631.86 ^{ab}	3498.80±639.06 ^{ab}	3353.20±301.72 ^b
Visceral weight (g)	295.57±103.51 ^a	241.66±79.08 ^{ab}	217.14±77.09 ^{ab}	195.09±50.41 ^b	172.29±40.63 ^b	165.99±17.56 ^b
Liver weight (g)	43.81±10.36 ^a	32.58±10.24 ^b	21.56±3.82 ^c	19.74±4.76 ^{cd}	17.17±5.89 ^{cd}	11.13±4.08 ^d
Visceral fat weight (g)	69.52±29.91 ^a	24.56±4.52 ^b	26.82±13.86 ^b	25.13±6.64 ^b	22.67±7.01 ^b	14.74±5.61 ^b

Note: Distinct letters denote statistically significant differences ($P < 0.05$).

Table 2. Essential nutrient components of dorsal muscle of the experimental bighead carp in different duration of temporary net-pen captivity (% wet weight).

Index	Duration of temporary net-pen captivity					
	0-week	1-week	3-week	6-week	12-week	18-week
Moisture	79.20±0.98	79.00±0.88	79.26±0.54	79.67±0.93	79.33±0.61	79.75±0.73
Crude protein	16.53±1.61	16.09±0.68	16.23±0.73	16.69±1.05	16.71±0.41	16.53±0.70
Crude fat	3.44±1.71 ^a	2.74±1.99 ^{ab}	1.99±1.66 ^{abc}	1.50±1.14 ^{abc}	1.18±0.65 ^{bc}	0.71±0.37 ^c
Ash	1.37±0.18	1.33±0.17	1.16±0.06	1.30±0.14	1.25±0.29	1.39±0.11

Note: Distinct letters denote statistically significant differences ($P < 0.05$).

Table 3. The amino acid content in dorsal muscle of the experimental bighead carp in different duration of temporary net-pen captivity (% wet weight).

Index	Duration of temporary net-pen captivity					
	0-week	1-week	3-week	6-week	12-week	18-week
UAA	5.45±0.24	5.00±0.53	5.13±0.17	5.26±0.79	5.28±0.13	4.96±0.13
EAA	5.69±0.42	5.24±0.68	5.30±0.18	5.46±0.71	5.76±0.22	5.26±0.21
NEAA	8.94±0.57	8.06±0.98	8.33±0.32	8.62±1.29	8.96±0.25	8.21±0.25
TAA	14.63±0.90	13.30±1.66	13.63±0.41	14.08±1.99	14.72±0.43	13.47±0.45
UAA/TAA (%)	37.30±0.82 ^a	37.64±0.67 ^a	37.62±0.62 ^a	37.34±0.63 ^a	35.90±0.44 ^b	36.86±0.42 ^a
EAA/TAA (%)	38.91±1.41	39.35±0.41	38.88±1.07	38.83±0.59	39.11±0.68	39.03±0.42
EAA/NEAA (%)	63.77±3.87	64.89±1.10	63.66±2.91	63.48±1.57	64.26±1.82	64.03±1.12

Note: UAA: umami taste amino acids. EAA: essential amino acids. NEAA: non-essential amino acids. TAA: total amino acids. Distinct letters denote statistically significant differences ($P < 0.05$).

Table 4. The AAS and CS of the experimental bighead carp in different duration of temporary net-pen captivity.

Score model	EAA	Duration of temporary net-pen captivity					
		0-week	1-week	3-week	6-week	12-week	18-week
AAS	Thr	1.08±0.19	0.99±0.15	0.99±0.06	1.01±0.16	1.08±0.05	1.01±0.07
	Val	0.88±0.13 ^{▲▲}	0.82±0.12 ^{▲▲}	0.81±0.05 ^{▲▲}	0.82±0.13 ^{▲▲}	0.83±0.06 ^{▲▲}	0.78±0.06 ^{▲▲}
	Ile	0.97±0.14	0.92±0.14	0.91±0.06	0.93±0.15	0.95±0.07	0.87±0.07
	Leu	1.16±0.18	1.08±0.17	1.09±0.06	1.11±0.18	1.18±0.06	1.08±0.08
	Lys	1.45±0.22	1.46±0.17	1.40±0.07	1.42±0.16	1.49±0.09	1.44±0.09
	Met + Cys	0.54±0.17 ^{a▲}	0.43±0.09 ^{ab▲}	0.51±0.10 ^{a▲}	0.44±0.08 ^{ab▲}	0.46±0.05 ^{ab▲}	0.36±0.03 ^{b▲}
	Phe + Try	1.30±0.21	1.18±0.20	1.20±0.07	1.21±0.21	1.26±0.07	1.14±0.08
	Thr	0.93±0.16	0.84±0.13	0.85±0.05	0.86±0.14	0.93±0.04	0.87±0.06
CS	Val	0.66±0.10 ^{▲▲}	0.62±0.09 ^{▲▲}	0.61±0.04 ^{▲▲}	0.62±0.10 ^{▲▲}	0.63±0.04 ^{▲▲}	0.59±0.04 ^{▲▲}
	Ile	0.74±0.11	0.69±0.11	0.69±0.05	0.70±0.12	0.72±0.05	0.66±0.05
	Leu	0.95±0.15	0.89±0.14	0.90±0.05	0.91±0.15	0.97±0.05	0.89±0.06
	Lys	1.12±0.17	1.12±0.13	1.08±0.05	1.09±0.12	1.15±0.07	1.11±0.07
	Met + Cys	0.31±0.10 ^{a▲}	0.24±0.05 ^{ab▲}	0.29±0.06 ^{a▲}	0.25±0.05 ^{ab▲}	0.26±0.03 ^{ab▲}	0.21±0.02 ^{b▲}
	Phe + Try	0.87±0.14	0.79±0.14	0.81±0.05	0.81±0.14	0.85±0.05	0.76±0.06
EAAI		74.46±11.96	68.44±10.63	69.78±4.16	69.33±11.04	72.35±4.45	65.64±4.57

Note: ▲ represents the first limiting amino acid, ▲▲ denotes the second limiting amino acid. Distinct letters denote statistically significant differences ($P < 0.05$).

Table 5. Fatty acid composition and content of dorsal muscle of the experimental bighead carp in different duration of temporary net-pen captivity (mg/kg).

Index	Duration of temporary net-pen captivity					
	0-week	1-week	3-week	6-week	12-week	18-week
C14:0	9.63±4.06 ^{abc}	5.80±3.07 ^{bcd}	12.78±6.64 ^a	11.42±4.52 ^{ab}	4.36±4.09 ^{cd}	3.06±1.61 ^d
C15:0	1.48±0.78	0.80±0.49	1.13±0.86	1.05±0.70	0.71±0.69	0.59±0.38
C16:0	45.61±25.08	49.43±22.51	58.96±12.93	61.57±19.81	56.10±12.62	56.48±6.89

C17:0	2.81±1.21	1.65±1.12	2.31±1.26	2.27±1.11	2.06±0.32	1.96±0.33
C18:0	56.64±7.14 ^{ab}	42.65±9.86 ^b	53.87±7.35 ^{ab}	59.43±15.52 ^a	61.19±12.59 ^a	54.02±2.74 ^{ab}
C20:0	4.53±0.37	5.06±2.37	4.35±0.54	4.49±0.29	3.89±0.08	3.98±0.09
∑SFAs	120.71±25.94	105.39±30.41	133.41±28.04	140.22±41.47	128.31±27.98	120.09±11.14
C14:1	4.44±1.10 ^{ab}	5.25±3.58 ^{ab}	3.90±0.69 ^b	6.73±0.84 ^a	4.18±1.74 ^b	3.58±0.88 ^b
C15:1	16.79±6.84 ^{ab}	15.63±4.66 ^{ab}	20.23±1.88 ^a	23.11±8.62 ^a	11.32±7.42 ^b	15.70±2.38 ^{ab}
C16:1	22.46±6.89 ^{ab}	15.00±4.84 ^b	29.02±13.81 ^a	24.07±12.24 ^{ab}	12.99±8.59 ^b	11.61±2.63 ^b
C17:1n7	2.60±1.11 ^a	1.17±1.03 ^{ab}	2.70±1.65 ^a	2.68±1.19 ^a	0.98±1.06 ^b	0.81±0.49 ^b
C18:1n9t	28.97±7.35 ^{ab}	18.94±7.98 ^b	37.18±13.04 ^a	40.42±19.31 ^a	35.72±15.69 ^{ab}	29.25±4.67 ^{ab}
C20:1	1.88±0.56	2.09±1.76	2.07±0.53	2.13±1.05	1.87±0.74	1.30±0.11
∑MUFAs	77.15±12.01 ^{abc}	58.08±18.69 ^c	95.09±29.16 ^{ab}	99.14±38.59 ^a	71.05±25.77 ^{abc}	62.24±7.47 ^{bc}
C18:2n6c	13.15±4.17	15.54±13.30	19.59±9.87	21.04±8.72	17.13±5.73	22.48±3.85
C18:3n6	4.14±0.97 ^a	3.06±0.60 ^{bcd}	3.27±0.74 ^{abc}	3.63±0.38 ^{ab}	2.34±0.61 ^d	2.49±0.27 ^{cd}
C18:3n3	12.58±6.15	7.78±3.35	17.01±9.64	16.19±8.39	8.93±6.28	11.19±1.45
C20:2	1.02±0.26	2.24±3.83	1.31±1.07	1.78±1.52	0.84±0.71	0.85±0.43
C20:4n6	100.26±21.32 ^{abc}	68.42±16.91 ^c	87.44±22.47 ^{bc}	105.79±41.55 ^{ab}	110.08±29.06 ^{ab}	128.44±14.54 ^a
C20:3n3	1.09±0.66	2.11±4.08	1.10±0.75	1.42±0.93	0.63±0.32	0.66±0.32
C20:5n3	65.47±19.25 ^{ab}	56.86±19.44 ^b	77.03±22.38 ^{ab}	80.06±40.46 ^{ab}	72.75±18.24 ^{ab}	91.67±11.30 ^a
C22:6n3	138.36±58.52 ^{ab}	106.17±47.02 ^b	113.92±16.71 ^{ab}	140.25±41.15 ^{ab}	133.54±35.56 ^{ab}	165.36±20.19 ^a
∑PUFAs	336.08±83.62 ^{ab}	262.17±104.78 ^b	320.67±78.49 ^{ab}	370.16±140.74 ^{ab}	346.24±92.53 ^{ab}	423.15±48.17 ^a
∑n-3 PUFA	217.50±65.69 ^{ab}	172.92±72.13 ^b	209.06±47.28 ^{ab}	237.93±89.54 ^{ab}	215.85±58.25 ^{ab}	268.88±30.78 ^a
∑n-6 PUFA	117.56±24.24 ^{ab}	87.01±29.58 ^b	110.30±31.02 ^{ab}	130.46±50.22 ^{ab}	129.55±33.98 ^{ab}	153.41±17.74 ^a

Note: SFAs: saturated fatty acids. MUFAs: monounsaturated fatty acids. PUFAs: poly-unsaturated fatty acids. DHA: docosahexaenoic acid, C22:6n3. EPA: eicosapentaenoic acid, C20:5n3. Distinct letters denote statistically significant differences ($P < 0.05$).

DISCUSSION

Biometric parameters Variation: This study investigated the effects of temporary net-pen captivity on bighead carp. The body weight, carcass weight, visceral weight, liver weight, and visceral fat weight of the experimental bighead carp decreased during the captivity period. Graphical analysis was shown a significant declining trend in the visceral fat index (VFI), condition factor (K), hepatosomatic index (HSI), and viscerosomatic index (VSI). These findings indicate that temporary net-pen captivity significantly affected the physiological indicators of bighead carp.

The decrease in body weight and carcass weight may be attributed to reduced food intake due to environmental changes and limited feeding conditions. The decrease in visceral weight, liver weight, and visceral fat weight may be related to changes in fish metabolism and energy allocation. The declining trend of VFI, K , HSI, and VSI suggests physiological adjustments in response to the net-pen captivity. Insufficient energy intake may lead to the consumption of fat reserves, resulting in decreased visceral fat. Changes in fish body shape and the impact of rearing environment may contribute to the decline in the condition factor. The reduction of HSI and VSI may reflect metabolic status and organ function adjustments in fish.

In addition, other research results also support these findings. Murray cod was observed for the body weight, condition factor (K), hepatosomatic index (HSI) loss after purging process (Palmeri *et al.*, 2008b; 2009).

Atlantic salmon had lost 7.3% mass and the condition factor had decreased from 1.23 to 1.06 at the end of an 8-week fasting period (Hvas *et al.*, 2022). A study in Nile tilapia showed that fasting resulted in weight loss, muscle and liver histological changes, the severity of which increased with the duration of the fast (Elbialy *et al.*, 2022).

Further research is needed to investigate specific reasons and mechanisms behind these changes, such as monitoring food intake, metabolic rate, and environmental factors. Improving rearing environments, feed management, and management practices during net-pen captivity should be studied to ensure the health and meat quality of bighead carp. Ensuring the health and meat quality of bighead carp as human food is not only crucial for the fish itself but also directly impacts human health and nutrition (Mesut, 2021).

Overall, this study provides valuable information for understanding the effects of temporary net-pen captivity on bighead carp. These findings have implications for catch-and-market management, aquaculture practices, and further research on fish ecology and physiology. Fish is an essential nutrient in the human diet and is also present in the global aquatic product industry for consumers. Therefore, we need to improve our aquatic products and their health well (Zeliha, 2018).

Muscle Composition Variation: The moisture content in dorsal muscle of the experimental bighead carp ranged from 79.00% to 79.75%, crude protein content ranged

from 16.09% to 16.71 %, crude fat content ranged from 0.71% to 3.44 %, and ash content ranged from 1.16% to 1.39%. These results are consistent with previous studies, where the moisture content in bighead carp fresh muscle samples was found to be 77.31%, crude protein content was 15.78%, crude fat content was 1.68%, and ash content was 1.34% (Alahmad *et al.*, 2022). Additionally, another study reported that the moisture content in the dorsal muscle of bighead carp was 79.51%, ash content was 1.26%, crude protein content was 17.51%, and crude fat content was 1.17% (Peng *et al.*, 2023b). These findings align with existing research.

The moisture, crude protein, and ash contents remained relatively stable throughout the entire temporary net-pen captivity cycle. This indicates that these nutritional components are not significantly affected by the captivity duration. However, there was a notable decrease in the crude fat content over the course of temporary net-pen captivity, and the decrease was statistically significant after the 12-week captivity. This may have implications for the nutritional value of the fish and its suitability for certain culinary applications. Similar results were found in the purging process of common carp, where the lipid content decreased continuously during the purging process (Zajic *et al.*, 2013). Further studies could focus on understanding the underlying mechanisms behind this decrease in fat content and explore potential strategies to mitigate or manage this reduction. Firstly, an examination of the potential influence of environmental conditions during temporary net-pen captivity on the observed decline in crude fat content is warranted. Moreover, an investigation into the correlation between the activity levels of captive fish and the reduction in lipid content could provide additional insights. Constraints on fish movement within net pens may affect their lipid metabolism. Lastly, the implementation of management strategies to mitigate the decrease in fat content is worth considering.

TPA Variation: Based on the results of the texture profile analysis (TPA) of the dorsal muscles of the experimental bighead carp during the period of temporary net-pen captivity, significant differences were observed in hardness, cohesiveness, springiness, gumminess, and chewiness. These findings indicate that the texture properties of the samples were affected by the duration of net-pen captivity. Throughout the 18-week net-pen captivity period, all the measured texture parameters showed a decreasing trend. This suggests that the texture of the fish samples became progressively softer and less cohesive as the duration of captivity increased. The variation of reduction rates indicated that the initial changes in texture were more pronounced, but became less significant over time. These findings highlight the dynamic changes in texture properties that occur during the captivity of fish in net-pens. The decrease in hardness

and adhesiveness could be attributed to the natural degradation of muscle tissue over time. The initial surge in factors such as cohesiveness, springiness, gumminess, and chewiness could potentially be attributed to alterations in protein structure or water-holding capacity. These changes may be induced by stress or confinement within the net-pen captive environment. Nonetheless, comprehensive research is required to elucidate the precise mechanisms driving these observed alterations.

Previous studies have demonstrated that swimming exercise has a substantial impact on muscle texture, which is closely associated with muscle fiber and collagen content (Harimana *et al.*, 2019). Additionally, environmental factors within the living environment, such as water salinity, can also influence fish muscle texture (Du *et al.*, 2022). Specifically, an increase in muscle fiber density and a decrease in fiber diameter result in higher levels of hydroxyproline and collagen, ultimately leading to firmer fish filets (Rasmussen *et al.*, 2011). Another study reported that exercised fish exhibited lower lipid deposition in their body muscles, which may contribute to enhanced muscle texture (Periago *et al.*, 2005; Cai *et al.*, 2023). These previous studies support our current findings regarding the TPA results during temporary net-pen captivity. The observed decreases in hardness, cohesiveness, springiness, gumminess, and chewiness can be attributed to the lower muscle fiber density, larger fiber diameter, and reduced collagen and hydroxyproline content resulting from the captive environment. Taken together, these findings highlight the importance of swimming exercises in maintaining and enhancing muscle texture in fish. But a long-term and high-density net-pen captivity have seriously affected the swimming movement of fish. Incorporating swimming exercises into aquaculture practices can therefore be a valuable strategy for optimizing both fish welfare and product quality.

Overall, this study provides valuable insights into the temporal changes in texture properties of fish during temporary net-pen captivity. The findings contribute to our understanding of the quality changes that occur in fish muscle under different aquaculture conditions and can inform strategies for optimizing fish welfare and product quality in aquaculture operations.

Amino acid Variation: Among the amino acids analyzed, Glu, Gly, Thr, Met, His, Arg, Cys, Tyr, and Pro exhibited notable variations during the duration of temporary net-pen captivity. The most substantial decrease was observed in Cys content, which decreased by 71.45% throughout the experiment. This was followed by Met, Arg, and Glu, which decreased by 31.80%, 22.97%, and 11.93%, respectively. Conversely, the contents of Lys, His, and Pro showed an increasing trend, with Pro content increasing by 20.90% during the duration of temporary net-pen captivity. Umami taste

amino acids (UAA) content decreased from 5.45% to 4.96%, similarly, the contents of essential amino acids (EAA), non-essential amino acids (NEAA), and total amino acids (TAA) also decreased during the experimental period. This suggests that temporary net-pen captivity may have a negative impact on the overall amino acid profile of bighead carp muscle tissue. Notably, the UAA/TAA ratio significantly decreased during the duration of temporary net-pen captivity.

Assessing the nutritional quality of muscle proteins involves considering the composition, quantity, and proportion of amino acids. The tenderness of muscle tissue is predominantly affected by umami amino acids, namely Asp, Glu, Gly, and Ala, among which the Glu content holds particular significance in contributing to the perception of umami taste (Peng *et al.*, 2023b). In our study, significant changes were observed in the levels of Glu and UAA/TAA ratio during the duration of temporary net-pen captivity. Specifically, we noted a continuous decrease in Glu content, which may have implications for the flavor profile of bighead carp. According to the guidelines established by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), an ideal protein source is characterized by a maintained EAA/TAA of approximately 40%, and an EAA/NEAA exceeding 60%, indicating a higher quality protein composition (Energy and protein requirements: report of a joint FAO-WHO ad hoc expert committee. Rome, 22 March - 2 April 1971., 1973). In our study, the protein of experimental bighead carp exhibited a high nutritional value, as evidenced by the EAA/TAA ratio ranging between 38.83% and 39.35%, and the EAA/NEAA ratio ranging between 63.48% and 64.89%.

The changes in amino acid composition suggest that temporary net-pen captivity may influence the nutritional quality of the fish. As such, efforts should be made to optimize management practices to mitigate these effects and ensure the production of high-quality bighead carp. Further research could focus on investigating the underlying mechanisms behind these changes and exploring strategies to minimize the negative impact of temporary net-pen captivity on amino acid composition. Additionally, sensory evaluations could be conducted to assess the impact of these variations on the texture and taste of bighead carp muscle tissue, providing insights for enhancing consumer acceptance and marketability.

Protein Quality: The observed decreases in the AAS, CS, and EAAI of the dorsal muscles of bighead carp during net-pen captivity suggest a decline in nutritional quality. These results were mainly attributed to limited space, high stocking densities, and suboptimal bait biomass densities during temporary net-pen captivity. The significant decrease in Met + Cys levels is of particular concern, as these amino acids are essential for

protein synthesis and play important roles in various metabolic processes. The observed decline in Met + Cys content may result from inadequate dietary intake and/or increased catabolism due to stress or other physiological factors associated with captivity. Moreover, the identification of Met + Cys and valine (Val) as the first and second limiting amino acids, respectively, which aligns with the findings of previous studies examining amino acids in bighead carp muscle (Peng *et al.*, 2023b).

Therefore, effective management practices are necessary to optimize the nutrition and health of fish temporary captivity in net-pens. Future research should focus on determining the ideal management practices to maximize protein quality while minimizing nutritional deficiencies in temporary captive fish. Given the significance of fish as a vital nutrient in human diets and its prominence in the international aquatic produce market, it is essential that we continuously refine our aquatic products and prioritize their health (Zeliha, 2018; Mesut, 2021).

Fatty acid Variation: The fatty acid analysis conducted in this study offers significant insights into the alterations in fatty acid composition observed in bighead carp subjected to temporary net-pen captivity. Previous research has demonstrated the presence of a diverse range of fatty acids in the muscles of bighead carp (Hong *et al.*, 2015; Peng *et al.*, 2023a). In our study, a total of 20 fatty acids were identified in the dorsal muscle of the experimental bighead carp. Notably, the concentrations of C14:0, C18:0, C14:1, C15:1, C16:1, C17:1n7, C18:1n9t, C18:3n6, C20:4n6, C20:5n3, and C22:6n3 demonstrated significant variations throughout the temporary net-pen captivity period. The result might indicate that the duration of temporary net-pen captivity influenced the fatty acid composition of the fish. These findings suggest that environmental factors associated with net-pen captivity, such as changes in diet or stress levels, can impact the synthesis or metabolism of specific fatty acids in the bighead carp.

Furthermore, the changes observed in MUFAs and PUFAs are particularly noteworthy. Previous studies have found that common carp mainly metabolized MUFAs, initially, but with prolonged starvation, fish started to metabolize more PUFAs during the purging process (Zajic *et al.*, 2013). Other studies have demonstrated that freshwater fish, such as zander, have been observed to demonstrate a preference for the accumulation of PUFA over SFA and MUFA during periods of ample food availability (Gokalp *et al.*, 2007). In our study, on the other hand, the content of MUFAs showed a decreasing trend, while the content of PUFAs exhibited an increasing trend with prolonged duration of temporary net-pen captivity. In Murray cod, a similar trend was noted wherein the PUFAs content escalated in correlation with the duration of purification (Palmeri *et*

al., 2008a). Dietary inclusions of PUFAs have several health benefits for humans and other animals. Many animal studies have shown that PUFAs have cardiovascular health, antineoplastic and anti-inflammatory properties. Therefore, the increase in PUFA content, especially omega-3 fatty acids (e.g. C20:5n3 and C22:6n3), in the experimental bighead carp is of great significance. The observed decrease in MUFAs could potentially be attributed to alterations in dietary composition or heightened lipid oxidation during the temporary net-pen captivity process. Alternatively, the increase in PUFAs may be due to dietary modifications that promote accumulation of these fatty acids or enhanced endogenous synthesis.

The fatty acid composition could potentially influence the nutritional quality of bighead carp. The surge in PUFAs, particularly omega-3 fatty acids, holds significant promise from a human health standpoint as it augments the nutritional value of the fish. Nonetheless, further research is imperative to ascertain the optimal duration of net-pen captivity and to devise management strategies that can optimize the beneficial fatty acid composition in bighead carp without adversely affecting their overall health.

Correlation and similarity: The results of the principal component analysis (PCA) and the analysis of similarities (ANOSIM) provide important insights into the temporal changes of the experimental bighead carp under temporary net-pen captivity. The significant differences observed between the various time points indicate that the duration of captivity has a significant effect on the bighead carp. For wild-caught bighead carp, a temporary net-pen captivity period of less than three weeks is considered optimal, with a maximum duration of 12 weeks.

The negative correlations observed between the biometric parameters of the bighead carp and moisture content suggest that as the duration of net-pen captivity increases, there is a decrease in both the biometric parameters and the moisture content of the fish. This finding implies that prolonged net-pen captivity may lead to physiological changes in the fish, resulting in decreased overall body size and water content.

Moreover, the significant negative correlations between the biometric parameters of the bighead carp and specific fatty acids, including C16:0, Σ SFAs, C18:2n6c, and C20:5n3 (EPA), are noteworthy. These results suggest that the fatty acid composition in fish is affected by net-pen captivity duration. This implies that extended periods of captivity may lead to metabolic or availability alterations in these fatty acids, potentially impacting the nutritional quality of the fish. The observed shifts in fatty acid composition and biometric parameters can be ascribed to various factors linked with net-pen captivity,

such as dietary changes, stress levels, and physical activity.

The next phase entails evaluating the duration of captive farming for farmed fish and its subsequent impact on their nutritional quality and physiological state. A thorough examination of the physiological responses exhibited by these fish during varying durations of temporary net-pen captivity, encompassing stress hormone measurements and gene expression pattern analyses, can furnish a more nuanced comprehension of the health ramifications associated with such confinement. Such insights are pivotal in formulating sustainable aquaculture practices that optimally balance the welfare of the fish with the quality of the resultant product.

Conclusion: Temporary net-pen captivity significantly affects the wild bighead carp. During temporary net-pen captivity, the physiological indicators of bighead carp changed, including biomass, visceral fat index, muscle composition, muscle texture, amino acid composition, and fatty acid composition. It suggests that long-term temporary net-pen captivity has adverse effects on the physiological status of bighead carp, which may have negative impacts on its health and product quality. For wild-caught bighead carp, a temporary net-pen captivity period of less than three weeks is considered optimal, with a maximum duration of 12 weeks.

These research results remind us to pay more attention to the effects of temporary net-pen captivity on the physiological and nutritional characteristics of fish and to develop scientific and reasonable aquaculture management practices to ensure the health and product quality of bighead carp. Future research should further explore the mechanisms of temporary net-pen captivity on fish and investigate the best management strategies to minimize negative impacts.

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Authors' contribution: Y.X. D and Y.X. W conceived and designed the study. N. X, Y.L. D, J.P. W, H.J. M J.Y. P and J.Q. S executed the experiment and analyzed the samples. Y.X. D and W. G analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

Conflicts of Interest: The authors declare no conflict of interest.

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