Effects of starvation duration on growth, biochemical composition, digestive enzyme activity, and antioxidant indices in Hemifusus tuba (Postprint)

Authors: Wang Shuangjian, Ding Yuhuijian, Jiang Maowangjian, Jiang Xiaminjian, Han Qingxijian

Date: 2018-12-20T00:00:00+00:00

Abstract

360 replicates were established, each containing 20 individuals, with starvation durations of 0 (control), 5, 10, 20, 30, and 40 days at water temperatures of 29–31 °C. Results showed that starvation time had no significant effect on the survival rate, shell height, or shell width of *Hemifusus tuba* ($P>0.05$), with 100% survival in all groups; with prolonged starvation, the body weight of the snails gradually decreased, particularly during 20–40 days of starvation, with weight loss of 12.73%–21.14%. Except at 40 days of starvation, starvation time had no significant effect on moisture and crude protein content in the foot muscle ($P>0.05$); with prolonged starvation, crude fat content in the foot muscle gradually decreased, while crude ash content gradually increased. With prolonged starvation, glycogen content in the foot muscle decreased significantly ($P<0.05$), and hepatopancreatic glycogen content exhibited a decreasing trend; except for no significant differences between the 10- and 20-day starvation groups or between the 20- and 30-day starvation groups ($P>0.05$), significant differences were found among other groups ($P<0.05$). With prolonged starvation, the contents of saturated fatty acids and monounsaturated fatty acids in the foot muscle gradually decreased, while polyunsaturated fatty acid content increased relatively; the content of even-carbon fatty acids gradually decreased, whereas the content of odd-carbon fatty acids increased relatively. Hepatopancreatic lipase and trypsin activities were significantly higher than those in the control group after 5 days of starvation ($P<0.05$) and significantly lower than the control group after 40 days of starvation ($P<0.05$); hepatopancreatic amylase activity was significantly higher than the control and 5-day starvation groups after 10 days of starvation ($P<0.05$) and significantly lower than the control group after 40 days of starvation ($P<0.05$). Hepatopancreatic superoxide dismutase (SOD), catalase (CAT),

and glutathione peroxidase (GPx) activities increased with starvation duration during 5–30 days and were significantly higher than the control group ($P<0.05$), whereas after 40 days of starvation they were significantly lower than the control group ($P<0.05$); hepatopancreatic malondialdehyde (MDA) content showed no significant difference prior to 30 days of starvation ($P>0.05$) but increased significantly thereafter ($P<0.05$). These results suggest that *Hemifusus tuba* exhibits tolerance to starvation, but after 30 days of starvation at high temperature (29–31 °C), substantial alterations occurred in its biochemical parameters, indicating the onset of starvation stress.

Full Text

Effects of Starvation Time onGrowth, Biochemical Composition, Digestive Enzyme Activities, and Antioxidant Indices of Hemifusus tuba Gmelin

WANG Shuangjian, DING Yuhui, JIANG Maowang, JIANG Xiamin*, HAN Qingxi (School of Marine Sciences, Ningbo University, Ningbo 315211, China)

Abstract

This study investigated the effects of different starvation durations on the growth, biochemical composition, digestive enzyme activities, and antioxidant indices of Hemifusus tuba Gmelin. A total of 360 individuals with an average body weight of (28.33±1.27) g were randomly divided into six groups (three replicates per group, 20 snails per replicate) and subjected to starvation periods of 0 (control), 5, 10, 20, 30, and 40 days at water temperatures of 29–31 °C. The results showed that starvation time had no significant effect on survival rate, shell height, or shell width ($P>0.05$), with all groups achieving 100% survival. However, body weight gradually decreased with prolonged starvation, with losses of 12.73%–21.14% observed after 20–40 days of food deprivation. Except for the 40-day starvation group, moisture and crude protein contents in the pedal muscle were not significantly affected by starvation ($P>0.05$). Crude lipid content in the pedal muscle gradually decreased, while ash content progressively increased with extended starvation. Pedal muscle glycogen content declined significantly ($P<0.05$) as starvation progressed, and hepatopancreas glycogen content also showed a decreasing trend, with significant differences among most groups ($P<0.05$) except between the 10- and 20-day groups and between the 20- and 30-day groups. With prolonged starvation, saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) contents in the pedal muscle gradually decreased, whereas polyunsaturated fatty acid (PUFA) content relatively increased. Additionally, fatty acids with even carbon numbers decreased while those with odd carbon numbers relatively increased. Hepatopancreas lipase and protease activities were significantly higher than the control group after 5 days of starvation ($P<0.05$) but significantly lower after 40 days ($P<0.05$). Hepatopancreas amylase activity peaked at 10 days

of starvation, significantly exceeding both the control and 5-day groups ($P<0.05$), but dropped significantly below control levels by 40 days ($P<0.05$). Hepatopancreas superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities increased with starvation duration during 5–30 days, significantly surpassing control values ($P<0.05$), but declined sharply at 40 days, falling significantly below control levels ($P<0.05$). Hepatopancreas malondialdehyde (MDA) content remained stable until 30 days ($P>0.05$) but increased significantly thereafter ($P<0.05$). These results indicate that H. tuba Gmelin exhibits strong starvation tolerance, but dramatic changes in biochemical markers after 30 days of starvation at high temperature (29–31 °C) suggest the onset of severe starvation stress.

Keywords: Hemifusus tuba Gmelin; starvation stress; survival rate; growth; nutritional components; enzyme activity

Introduction

In natural aquatic environments, food resources are spatially heterogeneous and temporally variable due to seasonal changes, making starvation a common phenomenon throughout the life history of aquatic animals. Starvation significantly affects physiological metabolic activity and the consumption of endogenous energy reserves [1]. Hemifusus tuba Gmelin, belonging to Mollusca, Gastropoda, Mesogastropoda, Galeodidae, and genus Hemifusus, is a relatively large economically important marine gastropod. As a carnivorous species, it offers delicious meat and high nutritional value, making it a prized seafood delicacy that has attracted considerable attention from researchers worldwide. Domestic studies on H. tuba Gmelin have primarily focused on artificial breeding [2–3], reproductive biology [4–5], ecological habits [6–7], nutritional composition analysis [8–9], and antitumor activity [10], while international research has concentrated on shell structure [11], mechanical properties [12], and molecular biology [13]. Due to resource scarcity and soaring prices, particularly during fishing moratorium periods when supply cannot meet demand, fishermen in Zhoushan and Xiangshan coastal areas of Zhejiang Province frequently collect or purchase adult H. tuba Gmelin in large quantities during March–April. These snails are concentrated in net cages suspended at 3–8 m depth for temporary culture without feeding, to be sold during the June–July fishing moratorium. However, this practice of prolonged starvation and high-density suspended culture yields inconsistent economic returns. Therefore, this study employed a single-factor starvation experiment to investigate the effects of different starvation durations on growth, nutritional composition, and enzyme activities in H. tuba Gmelin, providing a theoretical basis for large-scale offshore temporary culture of this species.

1.1 Experimental Materials

Experimental H. tuba Gmelin were collected from the Xiangshan sea area (122.13°E, 28.54°N) in August 2016 through a single batch of bottom trawling, totaling 280 kg. The snails were temporarily cultured for one week in cement tanks (6.0 m × 4.0 m × 1.4 m, water depth 65 cm) at Xiangshan Laifa Aquaculture Seedling Farm. Culture conditions were maintained at 29–31 °C water temperature, 19‰–25‰ salinity, and pH 7.6–8.4. The snails were fed once daily with fresh clams (*Sinonovacula constricta*) ad libitum; feeding ceased when visual observation indicated the snails stopped consuming the bait. Water was completely exchanged daily with removal of residual bait, and continuous aeration was provided throughout the one-week acclimation period.

1.2 Experimental Design

Based on acclimation results, five experimental groups were established with starvation durations of 0 (control), 5, 10, 20, 30, and 40 days. Each group comprised three replicates, with each replicate containing 20 snails cultured in white foam boxes (44 cm × 34 cm × 25 cm). The experimental snails had an average body weight of (28.33±1.27) g, shell width of (36.28±1.27) mm, and shell height of (67.27±1.88) mm. During the experiment, water temperature was maintained at 29–31 °C, salinity at 19‰–25‰, pH at 7.6–8.4, with continuous aeration ensuring dissolved oxygen concentration above 4.0 mg/L. Half of the water volume was exchanged daily using pre-stored seawater that had undergone sedimentation, sand filtration, and cotton filtration. Body weight, shell width, and shell height were measured for each snail before and after starvation treatment. At the end of each starvation period, three snails were randomly selected from each replicate (nine snails per group) for dissection, and the pedal muscle and hepatopancreas were separated, snap-frozen in liquid nitrogen, and stored at –80 °C.

1.3 Analysis of Proximate Composition, Glycogen Content, and Fatty Acid Profile in Pedal Muscle

To eliminate individual variation, pedal muscle samples from three snails in each of the three replicates were pooled after mincing. Moisture content was determined by oven drying at 105 °C, crude protein by the Dumas combustion method, crude lipid by Soxhlet extraction with anhydrous ether, and ash content by muffle furnace incineration at 550 °C. Glycogen content was measured using an assay kit (anthrone reagent colorimetric method) purchased from Nanjing Jiancheng Bioengineering Institute. Fatty acid composition was analyzed according to GB/T 9695.2-2008 using a Shimadzu GCMS-QP2010 gas chromatography-mass spectrometer (Shimadzu, Japan).

1.4 Determination of Digestive Enzyme Activities, Antioxidant Indices, and Glycogen Content in Hepatopancreas

To eliminate individual variation, hepatopancreas samples from three snails in each replicate were pooled after mincing. The hepatopancreas was homogenized, and the supernatant was aliquoted and stored at -80 °C until analysis. All indices were determined using assay kits from Nanjing Jiancheng Bioengineering Institute following the manufacturer's protocols. Protease activity was measured by the Folin-phenol method, lipase activity by titration of fatty acid reaction products with dilute NaOH to determine acid value, amylase activity by the 3,5-dinitrosalicylic acid colorimetric method, superoxide dismutase (SOD) activity by the WST-1 method, catalase (CAT) activity by ammonium molybdate colorimetry, glutathione peroxidase (GPx) activity by the dithionitrobenzoic acid (DTNB) method, malondialdehyde (MDA) content by the thiobarbituric acid (TBA) method, and glycogen content by the anthrone reagent colorimetric method.

1.5 Statistical Analysis

All data are expressed as mean ± standard deviation (mean±SD). One-way ANOVA was performed using SPSS 13.0 statistical software. When significant differences were detected ($P<0.05$), Tukey' s-b(k) multiple comparison test was used to analyze differences among groups.

2.1 Effects of Starvation Time on Growth of H. tuba Gmelin

As shown in Table 1 , starvation time had no significant effect on survival rate ($P>0.05$), with no mortality observed in any group throughout the experiment. Similarly, shell height and shell width were not significantly affected by starvation ($P>0.05$). Body weight did not differ significantly among the 0-, 5-, and 10-day groups ($P>0.05$), but all differed significantly from the 20-, 30-, and 40-day groups ($P<0.05$). After 40 days of starvation, body weight decreased by 21.14%.

Table 1 Survival rate and changes in body weight, shell width, and shell height of Hemifusus tuba Gmelin during starvation

Starvation time (d)	Survival rate (%)	Weight after starvation (g)	Shell width before starvation (mm)	Shell width after starvation (mm)	Shell height before starvation (mm)	Shell height after starvation (mm)
0	100	28.07±1.10a	36.67±1.46	36.65±1.76	67.97±1.53	67.89±1.71
5	100	27.69±1.12a	37.52±1.50	37.91±1.45	67.98±1.49	68.40±1.44
10	100	26.38±1.17a	36.54±2.02	36.50±1.73	66.21±2.01	66.22±1.69
20	100	24.41±1.34b	36.60±1.28	36.60±2.46	68.32±1.27	68.32±2.35
30	100	23.60±1.35b	35.54±2.42	35.54±1.93	65.24±2.39	65.25±1.91

Starvation time (d)	Survival rate (%)	Weight after starvation (g)	Shell width before starvation (mm)	Shell width after starvation (mm)	Shell height before starvation (mm)	Shell height after starvation (mm)
40	100	22.68±1.44a	37.13±1.62	37.18±2.17	68.17±1.64	68.19±2.17

Note: In the same column, values with different letter superscripts indicate significant differences (P<0.05), while values with the same or no letter superscripts indicate no significant difference (P>0.05). The same applies to Table 2 .

2.2 Effects of Starvation Time on Proximate Composition of Pedal Muscle in H. tuba Gmelin

As shown in Table 2, moisture and crude protein contents in pedal muscle did not differ significantly among 0–30 day starvation groups (P>0.05). However, after 40 days of starvation, moisture content was significantly higher than in 0–5 day groups (P<0.05), and crude protein content was significantly lower than in 0–30 day groups (P<0.05). Crude lipid content gradually decreased with prolonged starvation, with significant differences among the 0-, 5-, and 10-day groups (P<0.05), though no significant differences were observed between the 10- and 20-day groups, 20- and 30-day groups, or 30- and 40-day groups (P>0.05). Ash content gradually increased with starvation duration, though no significant differences were detected during 5–20 days (P>0.05).

Table 2 Changes in proximate nutritional components in pedal muscle of Hemifusus tuba Gmelin during starvation (%)

Starvation time (d)	Moisture	Crude protein (DM basis)	Ether extract (DM basis)	Ash (DM basis)
0	73.53±1.05a	67.40±0.18a	3.08±0.06a	9.15±0.51d
5	73.95±2.16a	67.37±0.12a	2.56±0.09b	11.32±0.62c
10	74.43±1.04a	67.43±0.33a	2.03±0.08c	11.23±0.39c
20	75.51±0.66a	67.55±0.20a	1.86±0.11cd	12.47±0.21c
30	76.42±1.44a	67.56±0.18a	1.71±0.08de	14.04±0.76b
40	77.41±1.02a	66.56±0.49b	1.32±0.06e	17.11±0.71a

2.3 Effects of Starvation Time on Glycogen Content in Pedal Muscle of H. tuba Gmelin

Pedal muscle glycogen content decreased significantly with prolonged starvation (P<0.05). After 40 days of starvation, glycogen content was only (1.83±0.28)

mg/g, representing 26.52% of the control group value of (6.90±0.15) mg/g (Figure 1 [Figure 1: see original paper]).

Note: Data columns with different letter superscripts indicate significant differences (P<0.05), while columns with the same or no letter superscripts indicate no significant difference (P>0.05). The same applies to Figure 2 [Figure 2: see original paper].

Figure 1 Effects of starvation time on glycogen content in pedal muscle of Hemifusus tuba Gmelin

2.4 Effects of Starvation Time on Glycogen Content in Hepatopancreas of H. tuba Gmelin

Hepatopancreas glycogen content showed a declining trend with prolonged starvation, with significant differences among most groups (P<0.05) except between the 10- and 20-day groups and between the 20- and 30-day groups (P>0.05). After 40 days of starvation, hepatopancreas glycogen content decreased to (7.26±0.77) mg/g, equivalent to 38.82% of the control group value of (18.70±0.90) mg/g (Figure 2 [Figure 2: see original paper]).

Figure 2 Effects of starvation time on glycogen content in hepatopancreas of Hemifusus tuba Gmelin

2.5 Effects of Starvation Time on Fatty Acid Composition in Pedal Muscle of H. tuba Gmelin

As shown in Table 3 , saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) contents gradually decreased with prolonged starvation, while polyunsaturated fatty acid (PUFA) content relatively increased. Within SFAs, fatty acids with even carbon numbers decreased, whereas those with odd carbon numbers relatively increased. After 40 days of starvation, C15:0 and C17:0 contents increased from 0.89% and 1.87% to 3.92% and 4.52%, respectively.

Table 3 Effects of starvation time on fatty acid composition in pedal muscle of Hemifusus tuba Gmelin (%)

Fatty acids	Starvation time (d)
	0
C14:0	
C15:0	
C16:0	
C17:0	
C18:0	
C20:0	
SFA	36.26a
C16:n-3	

Fatty acids	Starvation time (d)
C18:n-6	
C20:n-3	
MUFA	17.90a
C18:2n-3	
C20:2n-6	
C20:4n-3	
C20:5n-3 (EPA)	
C22:2n-3	
C22:6n-3 (DHA)	
PUFA	45.84f

Note: In the same row, values with different letter superscripts indicate significant differences (P<0.05), while values with the same or no letter superscripts indicate no significant difference (P>0.05). The same applies below.

2.6 Effects of Starvation Time on Digestive Enzyme Activities in Hepatopancreas of H. tuba Gmelin

As shown in Table 4 , hepatopancreas lipase and protease activities were significantly higher than the control group after 5 days of starvation (P<0.05), then gradually decreased, becoming significantly lower than the control group at 40 days [at (31.9±0.9) U/g prot and (408.8±64.1) U/g prot, respectively] (P<0.05), dropping to (18.2±0.1) U/g prot and (1,143.5±86.4) U/g prot. Hepatopancreas amylase activity was significantly higher than both the control and 5-day groups at 10 days (P<0.05), but significantly lower than the control group at 40 days [(148.5±6.9) U/g prot] (P<0.05), decreasing to (117.5±7.9) U/g prot.

Table 4 Effects of starvation time on digestive enzyme activities in hepatopancreas of Hemifusus tuba Gmelin (U/g prot)

Starvation time (d)	Lipase	Protease	Amylase
0	31.9±0.9b	408.8±64.1b	148.5±6.9b
5	39.1±1.2a	686.3±94.8a	150.3±7.9b
10	26.3±0.7c	1,343.3±19.2b	215.0±12.6a
20	27.8±1.0c	1,329.9±28.6b	198.3±1.2a
30	27.7±2.2c	1,376.5±41.3b	154.9±3.3b
40	18.2±0.1d	1,143.5±86.4c	117.5±7.9c

2.7 Effects of Starvation Time on Antioxidant Indices in Hepatopancreas of H. tuba Gmelin

As shown in Table 5 , hepatopancreas SOD, CAT, and GPx activities increased during 5–30 days of starvation, significantly exceeding control values

(P<0.05), but declined sharply at 40 days, falling significantly below control levels (P<0.05). Hepatopancreas MDA content remained stable until 30 days (P>0.05) but increased significantly thereafter (P<0.05), reaching (28.99±1.12) nmol/mg at 40 days.

Table 5 Effects of starvation time on antioxidant indices in hepatopancreas of Hemifusus tuba Gmelin

Items	Starvation time (d)
	0
SOD (U/mg prot)	21.13±1.02d
CAT (U/mg prot)	1.51±0.08d
GPx (U/mg prot)	34.64±0.19d
MDA (nmol/mg prot)	25.09±0.82c

3.1 Effects of Starvation Time on Growth of H. tuba Gmelin

During starvation, most animals mobilize stored substances, leading to body weight reduction. Zhang et al. [14] reported that red sea bream (*Pagrosomus major*) lost 7.05% of body weight after 15 days of starvation without mortality. Du et al. [15] found that *Penaeus vannamei* lost 10.55% of wet weight after 6 days of starvation. In this study, H. tuba Gmelin lost 21.14% of body weight after 40 days of starvation without mortality, indicating strong starvation tolerance. Mollusk shells are primarily composed of calcium carbonate and small amounts of shell matrix secreted by mantle epithelial cells [16]; the formation mechanism remains unclear. Few studies have examined starvation effects on mollusk shell dimensions, though Guo et al. [17] reported partial compensatory growth in shell height of *Pomacea canaliculata* under short-term alternating wet-dry conditions, achieved primarily through increased post-starvation feeding. In contrast, this study found no significant differences in shell height or width before and after starvation, suggesting that starvation does not significantly affect these morphological parameters in H. tuba Gmelin.

3.2 Effects of Starvation Time on Nutritional Composition of H. tuba Gmelin

During starvation, animals consume stored substances to maintain physiological metabolism, primarily utilizing glycogen and lipid reserves, with some species consuming protein. As stored substances are depleted, moisture content increases [18–21]. This study found that pedal muscle moisture content in H. tuba Gmelin increased from 73.53% to 77.41% after 10 days of starvation. Crude protein content remained stable during the first 30 days but decreased significantly in the 40-day group. Crude lipid content began declining after 5 days, from 3.08% to 1.32% at 40 days. Ash content progressively increased from 9.15% to 17.11% at 40 days. These results indicate that H. tuba Gmelin first utilizes lipid and glycogen reserves, consuming small amounts of lipid but primarily glycogen

during 10–30 days of starvation, while relatively preserving protein as a structural component—consistent with findings by Xue et al. [1]. Regarding fatty acid utilization, this study revealed decreased SFA and MUFA contents but relatively increased PUFA content in pedal muscle, suggesting preferential utilization of SFAs and MUFAs during starvation stress while PUFAs are relatively preserved. This may be because abundant PUFAs are essential components of phospholipid bilayers necessary for maintaining cell membrane structure and function [22]. Furthermore, accumulating evidence indicates that fatty acids, particularly PUFAs, regulate gene expression to maintain lipid metabolism balance [23], consistent with Liu et al.' s [24] findings in *Miichthys miiuy*. Within SFAs, even-carbon fatty acids decreased while odd-carbon fatty acids relatively increased, indicating preferential utilization of even-carbon fatty acids during starvation stress. From an energy utilization perspective, -oxidation of even-carbon fatty acids is more economical, whereas odd-carbon fatty acids yield propionyl-CoA that requires additional energy for conversion to succinyl-CoA for further oxidation [25]. Additionally, odd-carbon fatty acids may be less involved in energy storage and more important for biomembrane structure [24].

3.3 Effects of Starvation Time on Digestive Enzyme Activities and Antioxidant Indices of H. tuba Gmelin

Most aquatic animals regulate digestive enzyme activities to mobilize stored substances during starvation stress [1,26–27]. This study found that all three digestive enzymes (protease, lipase, and amylase) in H. tuba Gmelin hepatopancreas generally decreased during starvation, consistent with Xue et al.' s [1] findings in *Babylonia areolata*. However, protease and lipase activities increased significantly at 5 days of starvation, possibly reflecting enhanced enzyme secretion in early starvation to improve conversion of residual food, which may be related to the complex digestive system structure of H. tuba Gmelin [28]. After 10 days, both lipase and protease activities decreased, likely because residual food was exhausted and lack of external food stimuli reduced enzyme secretion. As a carnivore with low carbohydrate utilization capacity, H. tuba Gmelin has low amylase activity, yet this study found higher amylase than lipase activity, possibly due to residual herbivorous digestive system characteristics from early benthic diatom feeding [29]. The increase in amylase activity after 10 days may reflect enhanced utilization of hepatic and muscle glycogen for metabolism, while the decrease below control levels after 40 days likely results from glycogen depletion.

Oxygen free radicals are produced during metabolism and stress responses. Under normal conditions, a dynamic equilibrium is maintained as radicals are continuously generated and scavenged by antioxidant systems comprising enzymes like SOD and low-molecular-weight antioxidants such as vitamins C and E [30–31]. Environmental stress disrupts this balance, and excessive free radicals cause disease. This study demonstrated that hepatopancreas SOD, CAT, and GPx activities in H. tuba Gmelin showed good consistency and upward trends

before 30 days of starvation, significantly exceeding control values ($P<0.05$). This consistency has been observed in other studies; Morales et al. [32] reported that common dentex (*Dentex dentex*) showed significantly increased liver SOD, CAT, and GPx activities after 5 weeks of starvation, which returned to control levels after 3 weeks of refeeding. The decline in these enzyme activities below control levels after 40 days of starvation indicates that prolonged starvation caused oxygen radical accumulation to exceed the capacity of the antioxidant system, disrupting metabolism, damaging biomembranes and enzyme systems, and inhibiting related gene expression. MDA, a lipid peroxidation product of free radical action, is cytotoxic, and its level indirectly reflects the severity of cellular damage [33]. In this study, hepatopancreas MDA content remained stable at approximately 25 nmol/mg prot until 20 days, reflecting adaptation of the antioxidant system and other defense mechanisms. However, significant increases after 30 days, particularly at 40 days, indicated that the antioxidant system could no longer handle excess free radicals, resulting in oxidative stress.

Conclusions

1. H. tuba Gmelin exhibits strong starvation tolerance, showing no mortality during 40 days of starvation at 29–31 °C.
2. Body weight began to decrease significantly after 20 days of starvation, with a 21.14% reduction after 40 days. For economic viability, temporary culture starvation should not exceed 20 days to avoid economic losses.
3. During 10–30 days of starvation, H. tuba Gmelin consumes small amounts of lipid but primarily glycogen, while relatively preserving protein. Fatty acid utilization preferentially targets saturated and monounsaturated fatty acids, while polyunsaturated fatty acids are relatively preserved, with even-carbon fatty acids utilized before odd-carbon fatty acids. Nutritional components begin to decline after 10 days of starvation, indicating that feeding should commence after this period during temporary culture.
4. During starvation, hepatopancreas digestive enzyme activities generally decrease, though protease and lipase activities increase significantly at 5 days. After 40 days of starvation, SOD, CAT, and GPx activities decrease significantly while MDA content increases significantly, indicating oxidative stress development in H. tuba Gmelin.

References

[1] Xue M, Ke CH, Wei YJ. Effects of starvation on biochemical composition and digestive enzyme activities in juvenile *Babylonia areolata* [J]. Journal of Tropical Oceanography, 2010, 29(3): 120–125.

[2] Pan Y, Wang QZ, Pang YP, et al. Complete artificial breeding experiment of *Hemifusus tuba* Gmelin [J]. Fisheries Science & Technology Information, 2007, 34(2): 84–85.

[3] Zhang ZD. Artificial breeding of *Hemifusus tuba* Gmelin [J]. Scientific Fish Farming, 2001(4): 25.

[4] Pan Y, Pang YP, Luo FG, et al. Reproductive biology of *Hemifusus tuba* Gmelin [J]. Journal of Fisheries of China, 2008, 32(2): 217–222.

[5] Lin ZH, Wang TX, Xia CG. Observation on ecology and reproductive habits of *Hemifusus tuba* Gmelin [J]. Marine Sciences, 1998, 13(5): 11–12.

[6] Luo J, Liu CW, Huang XH. Effects of salinity on embryonic development of *Hemifusus tuba* Gmelin [J]. Journal of Guangdong Ocean University, 2007, 27(3): 24–28.

[7] Du T, Luo J, Liu CW, et al. Effects of temperature on embryonic development of *Hemifusus tuba* (Gmelin) [J]. Marine Sciences, 2010, 34(6): 44–49.

[8] Zhu AY, Zhao XJ, Yang YQ. Analysis of nutritional components in the soft body part of *Hemifusus tuba* Gmelin from Dongji sea area [J]. South China Fisheries Science, 2008, 4(2): 63–68.

[9] Tao P, Xu QL, Tan SR. Analysis of nutritional components of several gastropods and bivalves from Dalian coastal waters [J]. Journal of Liaoning Normal University (Natural Science Edition), 2000, 23(2): 182–186.

[10] Fu YQ, Gu QQ, Liu R, et al. Study on chemical composition and antitumor activity of neutral glycoprotein from *Hemifusus tuba* muscle [J]. Chinese Journal of Marine Drugs, 2002, 21(6): 20–24.

[11] Liang Y, Zhao J, Wu CW. The micro/nanostructure characteristics and the mechanical properties of *Hemifusus* conch shell [J]. Journal of Bionic Engineering, 2010, 7(4): 307–313.

[12] Zhao J, Chen C, Liang Y, et al. Mechanical properties and structure of *Haliotis discus hannai* Ino and *Hemifusus tuba* conch shells: a comparative study [J]. Acta Mechanica Sinica, 2010, 26(1): 21–25.

[13] Wu L, Li RH, Wang CL, et al. Isolation and characterization of 42 microsatellite loci from the *Hemifusus tuba* Gmelin [J]. Conservation Genetics Resources, 2014, 6(3): 707–710.

[14] Zhang B, Sun Y, Tang QS. Effects of starvation on growth and biochemical composition of red sea bream (*Pagrosomus major*) [J]. Journal of Fisheries of China, 2000, 24(3): 206–210.

[15] Du XT, Zhou XZ, Yu HN, et al. Effects of starvation on biochemical composition and compensatory growth of *Penaeus vannamei* [J]. Journal of Fisheries of China, 2004, 28(1): 47–53.

[16] Liu LY, Zheng GM. General Zoology [M]. 3rd ed. Beijing: Higher Education Press, 2006: 198–199.

[17] Guo Q, Zhang JE, Luo H, et al. Effects of alternating wet-dry conditions on feeding and growth of *Pomacea canaliculata* [J]. Ecology and Environmental

Sciences, 2013, 22(5): 774-779.

[18] Jiang ZQ, Jia ZM, Han YB. Compensatory growth and its mechanism in red drum (*Sciaenops ocellatus*) after starvation [J]. Journal of Fisheries of China, 2002, 26(1): 67-72.

[19] Barclay MC, Dall W, Smith DM. Changes in lipid and protein during starvation and the moulting cycle in the tiger prawn, *Penaeus esculentus* Haswell [J]. Fisheries Research Board Canada, 1983, 68(3): 229-244.

[20] Kim MK, Lovell RT. Effect of restricted feeding regimens on compensatory weight gain and tissue changes in channel catfish *Ictalurus punctatus* [J]. Aquaculture, 1995, 135(4): 285-293.

[21] Maddock DM, Burton MP. Some effects of starvation on the lipid and skeletal muscle layers of the winter flounder, *Pleuronectes americanus* [J]. Canadian Journal of Zoology, 1994, 72(9): 1672-1679.

[22] Gylfason GA, Kntsóttir E, Ásgeirsson B. Isolation and biochemical characterization of lipid rafts from Atlantic cod (*Gadus morhua*) intestinal enterocytes [J]. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 2010, 155(2): 86-95.

[23] Jump DB. Dietary polyunsaturated fatty acids and regulation of gene transcription [J]. Current Opinion in Lipidology, 2002, 13(4): 155-164.

[24] Liu MH, Luo HZ, Fu RB, et al. Effects of short-term starvation stress on biochemical composition, fatty acid, and amino acid composition of *Miichthys miiuy* [J]. Acta Hydrobiologica Sinica, 2009, 33(2): 230-235.

[25] Mai KS, Li P, Zhao JM, et al. Nutritional Requirements of Fish and Crustaceans [M]. Beijing: Science Press, 2015: 115-116.

[26] Qiao QS. Effects of cyclic starvation and refeeding on growth performance, body composition, digestive enzymes, and antioxidant enzymes in blunt snout bream and Jian carp [D]. Master' s thesis. Nanjing: Nanjing Agricultural University, 2011: 34-35.

[27] Li ZH, Xie S, Wang JX, et al. Effects of intermittent starvation on growth and several digestive enzymes in *Macrobrachium nipponense* [J]. Journal of Fisheries of China, 2007, 31(4): 456-462.

[28] Zhang LL, Wan JJ, Mu CK, et al. Histological study of the digestive system of *Hemifusus tuba* Gmelin [J]. Journal of Coastal Research, 2011, 30(4): 540-545.

[29] Jin LB, Zhang LL, Li RH, et al. Effects of different diets on growth, digestive enzyme activities in liver, and nutritional composition of soft body part in *Hemifusus tuba* Gmelin [J]. Marine Sciences, 2013, 37(6): 66-72.

[30] Fang YZ, Yang S, Wu GY. Free radical homeostasis [J]. Progress in Physiological Sciences, 2004, 35(3): 199-204.

[31] Pascual P, Pedrajas JR, Toribio F, et al. Effect of food deprivation on oxidative stress biomarkers in fish (*Sparus aurata*) [J]. Chemico-Biological Interactions, 2003, 145(2): 191–199.

[32] Morales AE, Pérez-Jiménez A, Hidalgo MC, et al. Oxidative stress and antioxidant defenses after prolonged starvation in *Dentex dentex* liver [J]. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 2004, 139(1/2/3): 153–161.

[33] Grundy JE, Storey KB. Antioxidant defenses and lipid peroxidation damage in estivating toads, *Scaphiopus couchii* [J]. Comparative Biochemistry and Physiology B: Biochemical and Molecular Biology, 1998, 168(2): 132–142.

Note: Figure translations are in progress. See original paper for figures.