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Influence of seasonality and gonad development on the nutritional quality and safety of the razor clam *Sinonovacula constricta* (Lamarck 1818)

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Abstract

Understanding the reproductive activity or seasonal variation in comprehensive evaluations of the nutritional quality and safety of the razor clam *Sinonovacula constricta* (Lamarck) is important for improving product quality and economic benefits. The contents of moisture (from 80.12±0.38 to 84.98±0.40%), protein (from 53.14±0.06 to 63.12±0.69% DW), lipid (from 4.55±0.19 to 5.69±0.35% DW), glycogen (from 5.40±1.11 to 16.13±1.55% DW), and ash (from 12.03±0.33 to 19.86±0.56% DW) varied significantly at different gonadal development cycles or seasons. Glycogen, lipid, and protein variations were closely associated with gonadal development, with the lowest levels of lipid and protein in the spawning period. Moreover, *S. constricta* consistently had a well-balanced essential amino acid profile throughout the year, indicating that it is a source of high-quality protein. The predominant free amino acids in *S. constricta* were taurine, glycine, lysine, and alanine, which are important contributors to the flavor. *S. constricta* was defined as lean fat throughout the gonad cycle with polyunsaturated fatty acid accounting for the majority in the active and maturing stages. Microbiological quality and total volatile basic nitrogen were associated with the alteration of seasons. The optimal harvesting time of *S. constricta* was the inactive stage of most gonads in spring (from March to April in Taizhou, Zhejiang Province, China).

Keywords: razor clam; seasonal variation; the gametogenic cycle; physicochemical variation; microbiological quality.

1. Introduction

The razor clam (*Sinonovacula constricta* Lamarck), belonging to Mollusca (Bivalvia: Veneroida: Solecurtidae), resides widely in the intertidal zones and estuarine waters along the coasts of the West Pacific Ocean. As one of the four most important and traditionally cultivated shellfish, *S. constricta* has been cultivated for more than 400 years using natural resources in southeastern China (Shen et al., 2013). *S. constricta* has a deep-burrowing lifestyle and utilizes a well-developed foot to move vertically in the upper substrate layer of coastal intertidal mudflat bottom sowing aquaculture, beach dam, and pond intensive culture systems in Zhejiang Province. It is one of the dominant species (including *Crassostrea gigas*, *Ruditapes philippinarum*, and *Tegillarca granosa*) of clam aquaculture in China (Xie, 2003). The global production of razor clams reached 869,251 tons in 2019, accounting for 37.6% of total marine shellfish yield (FAO, 2022). In addition, *S. constricta* has not only high nutritional value (e.g., essential amino acids, protein, polyunsaturated fatty acid [PUFA], and minerals), but also delicious meat and a high economic value (Ran et al., 2017b).

Seasonal variations of the gametogensis cycles in marine bivalves are influenced by environmental parameters, e.g., temperature, salinity, tidal changes, and food availability (Darriba et al., 2004; Dridi et al., 2007). Studies on the metabolism of bivalves have focused on the seasonal and reproduction cycle-related changes in glycogens, fats, proteins, amino acids, and fatty acids (Li et al., 2006; Liu et al., 2008; Park et al., 2001; Qin et al., 2021). Oysters (*Crassostrea rhizophorae*) are cultivated during the winter and have a higher nutritional value. They have a high protein level, functional nutrients (e.g., combination of EPA+DHA with PUFA), and a nutritional quality index for the fatty acids (Lira et al., 2013). Various studies have also shown that the reproductive cycles of marine bivalves are strongly associated with the alteration of seasons and energy storage and utilization cycles, which is beneficial for the large-scale artificial breeding and protection of natural populations (Isono et al., 2020; Liu et al., 2020; Santos et al., 2011). Decreases in the umami flavor and firmness of gonads can reduce the quality of the products, mainly due to the transformation that occurs during the reproductive cycle (Gao et al., 2021). However, there is still a lack of comprehensive evaluations on the nutritional/biochemical quality and healthiness/safety of *S. constricta* during the gametogenic cycles or alteration of seasons.

Market demand for the razor clam is high, and its aquaculture has considerable economic value in southern China (Chen

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et al., 2022). However, farmers often complain that the taste and meat quality of clams are poor in some periods of the year, which affects the prices and sales, and that the optimal harvesting time cannot be precisely predicted (Zhou et al., 2021). For *Mytilus galloprovincialis*, the best time for marketing mussels harvested in the North Adriatic Sea is before the gamete release (from April to October), when they exhibit the highest commercial and nutritional quality for direct consumption (Bongiorno et al., 2015). Therefore, understanding the reproductive activity or seasonal variation in nutritional quality, flavor, and microbiological quality of *S. constricta* is important for the adjustment of the harvest cycle, improvement of product quality, and economic benefits. Thus, this study aimed to (1) investigate the correlation between the seasons and the gonad development; (2) discuss the physicochemical and microbiological qualities of *S. constricta* during the gonadal development cycles or seasonal changes; and (3) identify the optimal harvesting time to achieve the best safety and nutritional quality. The effects of different physical and chemical compositions on the seafood quality of *S. constricta* were also discussed.

2. Materials and methods

2.1. Animals and environmental conditions

Wild Razor Clam *S. constricta* cultured in the beach dam of Sihai Village, Linhai, Taizhou, Zhejiang Province (28°44′14″, 121°18′54″) was collected monthly from May 2017 to May 2018 (with 80–100 samples being randomly collected each time). Samples were immediately transported to the laboratory of the Zhejiang Mariculture Research Institute under refrigeration (4±1°C). The temperature and salinity of the surface seawater were measured in situ during sampling with a mercury bulb thermometer and portable refractometer. The concentration of chlorophyll *a* at the sampling site (at 0–1 m depth) was measured in the laboratory (Parsons et al., 1984).

2.2. Histological analysis

Ten individuals from each group were used for histological examination. A transverse cut was made across the middle part of the soft body of *S. constricta*, and a 5-mm-thick section was fixed in Bouin's solution and processed by routine histological techniques. The tissues from the posterior region of the gonads were fixed in Bouin's fixation fluid for 24 h. Sections were dehydrated with an ascending alcohol series, embedded in paraffin, cut into 5-μm thick sections, stained with Harry's hematoxyl, and counterstained with eosin (Ibarra et al., 2017). The sections were examined to assess the sex and developmental stage of the gonad by using the microscope (Nikon Eclipse Ni, Japan) equipped with an image-analyzing system (Nikon PS-Riz, Japan). The diameter of 500 oocytes from five animals (50 oocytes per animal) was microscopically measured to determine the degree of maturity every month. The gametogenic stages were identified according to Ivell (1979).

2.3. Morphometric characteristics

The razor clams were externally cleaned by brushing and rinsing with distilled water in order to remove debris and epibionts. The external morphometrics, including shell height (SH), shell length (SL), and shell width (SW), were measured (mm) with a vernier caliper. The shells were opened with a knife, and the whole muscles were collected.

Prior to weighing, each animal was placed on the surface of an absorbent tissue for 10 min to remove the extraneous water. Afterward, the total wet weight (Twe) of each animal was measured (precision: 0.01 g) using an electronic scale. The meat yield (MY) was estimated for each animal as it represents the percentage of edibility for the species. MY was calculated with Equation 1 (Celik et al., 2012):

$$MY=[\text{wet meat weight(g)}/\text{total weight(g)}]\times 100 \quad (1)$$

2.4. Chemical analysis

Each biochemical compound was determined in triplicate on pooled tissues from 30 individuals. The composition of the body tissue was determined by measuring the content of moisture, crude protein, and lipids according to the methods of AOAC (2005). Briefly, moisture content was measured by drying the samples to a constant weight at 105°C. Total protein content was determined by the semi-automatic Kjeldahl System (BUCHI, Kjielflex K-360, Switzerland) after acid digestion. The ammoniac nitrogen was absorbed in boric acid by adding sodium hydroxide solution and then titrated with hydrochloric acid. Finally, protein content was estimated by multiplying nitrogen content by a factor of 6.25. Crude lipid was extracted from 1 g of each sample using 20 mL of an extraction solution consisting of chloroform and methanol (2:1, v/v). Ash content was determined by using a muffle furnace (Thermo Scientific Lindber Blue M, USA) at 640°C for 4 h. According to the rule from GB/T 9695.31-2008, the glycogen content was determined by the anthrone-sulfuric acid method with minor modifications (Horikoshi, 1958).

2.5. Identification and quantification of amino acids, free amino acids, and fatty acids

The content of amino acids was measured as described by Wu et al. (2021). Briefly, approximately 20 mg of freeze-dried muscles were hydrolyzed by 5 mL of 6M HCl in sealed glass tubes filled with nitrogen for 22 h at 110°C. After hydrolysis, the hydrolysate was dried in a vacuum oven, dissolved in HCl solution (0.02 N), centrifuged at 11,000×g for 20 min at 4°C, and then filtered with a 0.25 μm millipore nylon membrane. Amino acid profiles of muscle samples were analyzed by using an L-8900 High-speed Amino Acid Analyzer (Hitachi, Tokyo, Japan). All samples were measured in triplicate, and the results were presented as mg g^{-1} matter. The composition of free amino acids was analyzed as described by Yin et al. (2022). Each sample (dry weight: 100 mg) was homogenized with an Ultraturrax T10 (IKA-Werke, Staufen, Germany) in 3 mL of deionized water added with hydrochloric acid (0.1 mol L^{-1}) and maintained in an ultrasound bath for 30–60 min (40°C) before being centrifuged at 6,000 rpm for 10 min (4°C). The supernatant was added with trifluoroacetic acid or sulfosalicylic acid (10% W/V) and

carefully shaken for 1 min. After being maintained at 4°C for 1 h, the mixtures were centrifuged at 12,000 rpm for 30 min (4°C). The supernatant was then added to a NaOH solution (8 mol L^{-1}) to adjust the pH to 1.7–2.2. The homogenates were centrifuged at 12,000 rpm for 15 min at 4°C and filtered with a 0.25 μm Millipore nylon membrane. Free amino acid profiles were detected by using an L-8900 High-speed Amino Acid Analyzer (Hitachi, Tokyo, Japan). All samples were measured in triplicate, and the results were presented as mg g^{-1} matter. The preparation of fatty acids was carried out according to the methods described by Ran et al. (2017a).

2.6. Microbiological analysis

Samples of 25 g were aseptically dissected from clams, placed in a sterile masticator bag with 225 mL of 0.1% sterile peptone water, and homogenized in a masticator machine (Wiggens homogenius, HG400W, Wiggens). After homogenization of the meat (any liquor present in the shell was discarded), the microbial count was determined in 10^{-1} to 10^{-6} dilutions of the homogenates. Aerobic plate counts were determined by the pour plate method using Plate Count Agar (3M Petrifilm, USA) incubated at 30±1°C for 72±3 h (Chung et al., 2021). Microbiological data were transformed into logarithms of the number of colony-forming units (CFU/g). The analysis was performed in triplicate for each sampling point.

2.7. Determination of the total volatile basic nitrogen

The TVB-N of clam was estimated using a modified semi-micro steam distillation method as described by Zhang et al. (2011). A 5 g of sample was homogenized with 45 mL of deionized water and mixed for 30 min. The mixture was then filtered, and the filtrates were collected and transferred to a digestion tube. MgO (10 g L^{-1}) was added immediately into the tube, and samples were distilled using a semi-automatic Kjeldahl System (BUCHI, Kjielflex K-360, Switzerland). The distilled ammonia was then entrapped in 10 mL of boric acid solution containing methylene blue and methyl red as indicators. Distillation was determined as completed when the distillate reached a final volume of 150 mL and was titrated with a 0.01 mol L^{-1} hydrochloric acid (HCl) solution. The TVB-N content was expressed as mg/100 g of sample.

2.8. Determination of pH

The pH values were determined using the method described previously (Woyewoda et al., 1986). Briefly, 10 g of blended clams were homogenized with 100 mL of ultrapure water using a rotary suspension mixer (Vortex Genie 2, MOBIO, USA) and centrifuged (5,000×g, 5 min, 4°C) to collect supernatant. The pH of the supernatant was measured using a digital Aqua-pH meter (Orion STAR A211, Thermo Fisher Scientific, USA).

2.9. Evaluation of taste activity value

TAV was calculated as the ratio between the concentrations of component molecules and threshold values measured in water or in a sample matrix (Liu et al., 2018; Yue et al., 2016). Generally, compounds with a TAV>1 are considered active compounds in food taste analysis, while compounds with a TAV<1 are thought to have less effect on taste (Zhang et al., 2019) (Equation 2).

$$TAV = \frac{Concentration\ of\ free\ amino\ acid}{taste\ threshold\ corresponding\ to\ free\ amino\ acid} \quad (2)$$

2.10. Statistical analysis

Results were presented as mean±standard deviation (SD), and statistical analyses were performed using IBM SPSS Statistics 18.0. All analyses were carried out in triplicate. Data were submitted to one-way analysis of variance (ANOVA) tests, and means were compared by Tukey's test. A p-value of <0.05 was considered significant. The degree of association between the different parameters was estimated by the Spearman rank-order correlation coefficients (rs).

3. Results

3.1. Environmental parameters

A seasonal cycle in seawater temperature was observed with the highest temperature of 33°C in August and the lowest temperature of 0°C in January. Salinity remained relatively stable throughout the year, ranging from 22.0 to 24.0. The concentration of chlorophyll *a* was variable throughout the year, ranging from 6.60 to 28.92 μg L-1. The partial coefficients showed that the salinity was not significantly correlated with temperature ($p>0.05$), but the temperature and chlorophyll *a* were positively correlated (Spearman correlative test, $rs=0.651$, $p<0.05$). There was a clear seasonal pattern in the temperature and chlorophyll *a*, but the salinity did not show any seasonal pattern.

3.2. The gonadal development cycle

Figure 1 shows the microscopic features of the gonads during the gametogenic cycle of *S. constricta* over a period of one year. In stage I (the initiation stage), a few small isolated follicles were present in females, and acini were present in males, with groups of protogonia and gonia in mitosis. The sexes could be differentiated according to the differences between spermatogonia and oogonia. In stage II (the growing stage), the number of follicles rapidly increased, and the original reproductive cells continued to differentiate. A few reproductive cells appeared, and a rapidly increasing number of gonocytes was observed. In stage III (the maturing stage), follicles and acini were predominantly composed of mature gametes. Macroscopically, the gonad completely covered the digestive gland and extended into the foot area, and gametes were liberated freely. Mature oocytes free in the follicles had a polygonal-shaped profile due to the packing in the females. Spermatozoa were manifested as streaks. In stage IV (the spawning stage), the male gonads were characterized by the loss of the radial arrangement of the spermatozoa. In females, empty spaces were observed in the follicles, and mature oocytes at a lower density were observed. In stage V (the resting stage), the sexes were unidentifiable in most of the specimens, and all of the space of the gonad was occupied by the connective tissues without any gametes in it.

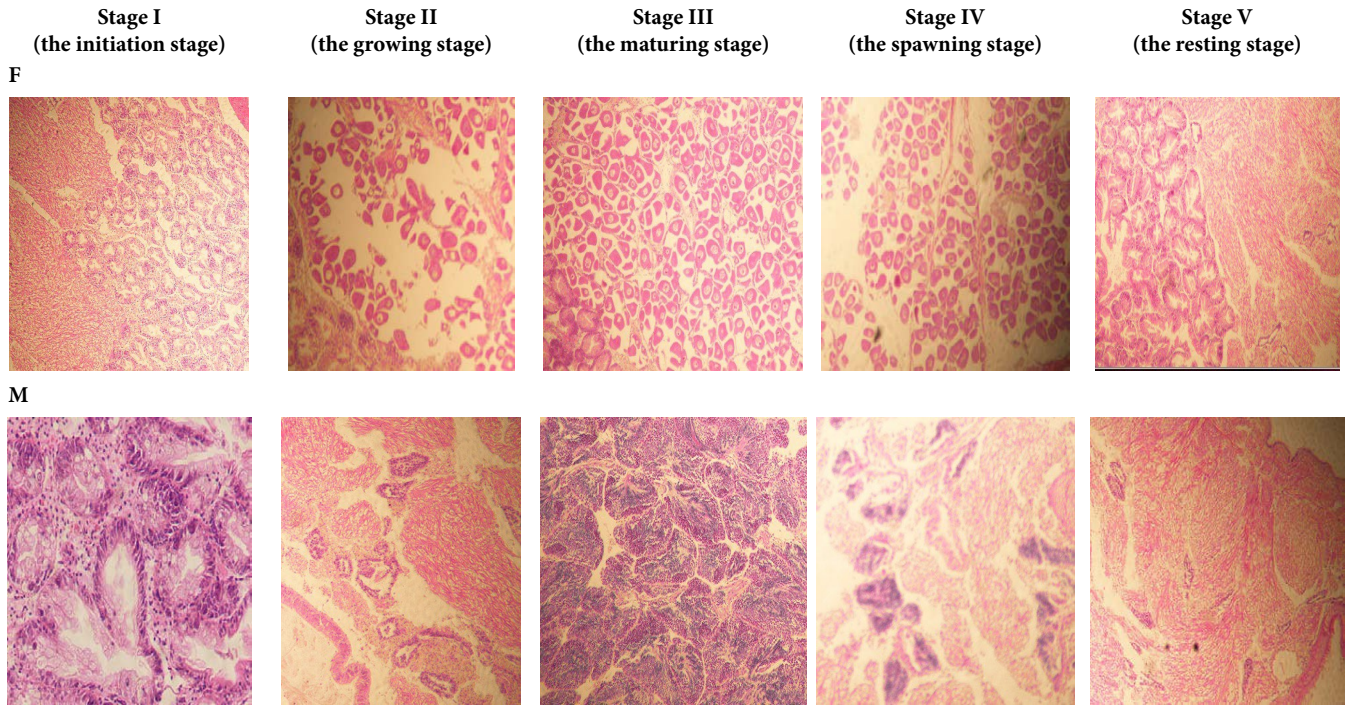
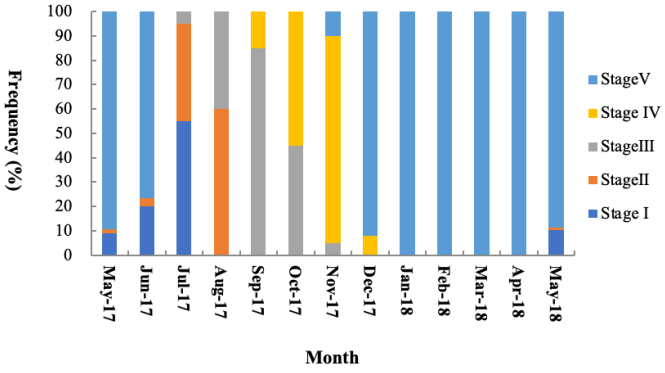


Figure 1. Photomicrographs of the gonadal stages of *S. constricta*. M: male; F: female.

The percentage of different gonadal development stages over the study period is shown in Figure 2. In May and June, female gonads were mainly in the proliferating stage. In May 2017, 59.43% of *S. constricta* were in the inactive stage, 8.90% were in the initiation stage, and 1.67% were in the growing stage. Female gonads were mainly in the growing stage in July and August, and mature individuals began to appear. In September, gonads were mainly in the growing and maturing stages, and some individuals began spawning. By September, 78.15% of the individuals had matured. The spawning activity was observed mainly between September and November. By December, 92% of the individuals had returned to the inactive stage after the release of gametes. Between January and April, all individuals were in the inactive stage.



Stage I: initiating stage; II: growing stage; III: maturing stage; IV: spawning stage; V: resting stage.

Figure 2. Seasonal distribution of the five stages of gonad development in *S. constricta*.

3.3. Morphometric characteristics

The monthly changes in the morphometric characteristics of *S. constricta* are shown in Figure 3. Shell length, shell width, body weight, and wet weight varied between 28.50±1.90 mm (May 2017) and 55.38±1.13 mm (May 2018), 5.31±0.48 mm (May 2017) and 13.30±0.55 mm (May 2018), 1.24±0.01 g (May 2017) and 7.44±1.11 g (May 2018), and 0.33±0.05 g (May 2017) and 4.03±0.13 g (May 2018), respectively. Physical parameters (SL, SW, BW, WW, and MY) showed very similar patterns through the whole year, with the highest and lowest values occurring at the end of the growth cycle and at the beginning of the growth cycle, respectively. MY of *S. constricta* was significantly affected by the alteration of seasons and reproductive activity ($p<0.05$). The highest rate of MY was 43.41±4.82% in September, followed by 37.92±3.71% in August, and the lowest rate was 29.56±2.56% in November (the spawning stage). From the growing stage to the maturing stage, MY was significantly increased ($p<0.05$), and from the maturing stage to the spawning stage, it was significantly decreased ($p<0.05$).

3.4. Variation of biochemical parameters

The approximate chemical composition of *S. constricta* was determined over a period of one year, and the results are shown in Figure 4. The moisture content of the body tissue ranged between 80.12±0.38 and 84.98±0.40% with an average monthly content of 82.96±0.14%. The moisture content declined from the inactive stage to the maturing stage and then increased from the maturing stage to the spawning stage. Protein content

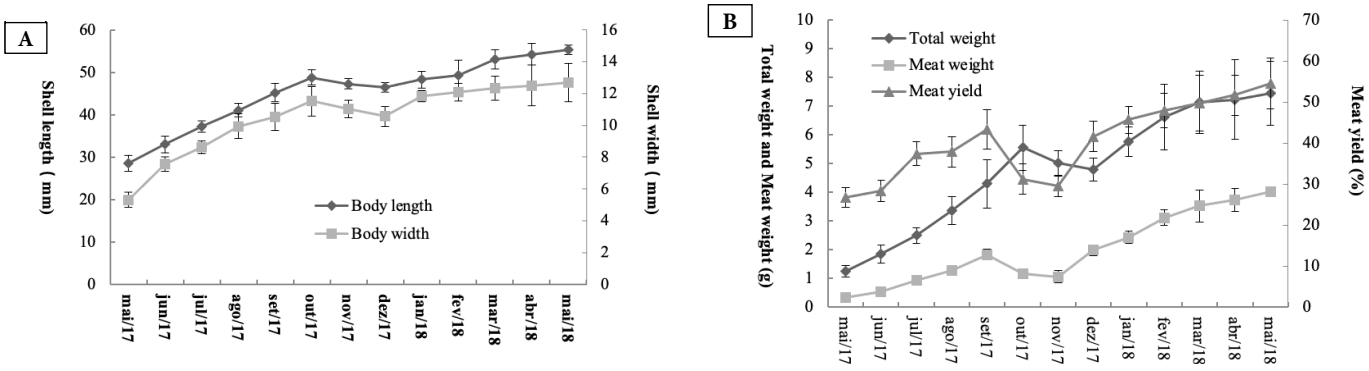


Figure 3. Variation of the (A) mean shell length and shell width; and (B) total body weight, wet weight, and meat yield in each month for *S. constricta*.

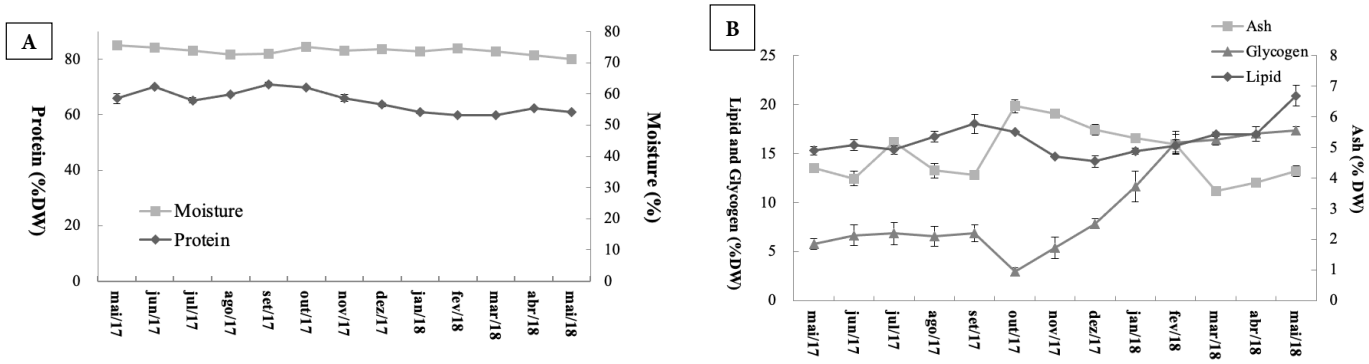


Figure 4. Bimonthly variations of (A) moisture and protein; and (B) glycogen, lipid, and ash in *S. constricta*.

ranged from 53.14±0.06% DW to 63.12±0.69% DW with an average monthly content of 57.64±0.49% DW. The protein content consistently exceeded 53% DW, indicating that it is the major biochemical component of *S. constricta* throughout the year. Moreover, the protein content increased gradually from 57.19±0.83% DW in July to a maximum of 63.12±0.69% DW in September and then decreased after October. The lipid content in *S. constricta* varied from 4.55±0.19% DW to 5.69±0.35% DW with an average monthly content of 5.25±0.15% DW. The minimum content of total lipid was observed in November (the spawning period). The lowest glycogen content occurred in October (the partial spawning stage), with a content of 2.96±0.40% DW, then continued to increase in the inactive stage. From December 2017 to May 2018, the glycogen content increased significantly from 5.40±1.11% DW to 16.13±1.55% DW during non-reproductive stages. Ash content ranged from 12.03±0.33% DW to 19.86±0.56% DW. The ash content formed a unimodal distribution with a peak content of 19.86±0.66% DW during the spawning period.

3.5. Variation in amino acid content and free amino acid composition

The amino acid levels in *S. constricta* throughout the year are shown in Table 1. The total amino acid content of the razor clam tissue ranged from 40.40±5.23% DW to 65.20±2.60% DW, and the essential amino acid content ranged from 14.60±1.83% DW to 25.31±1.00% DW. Similar to the pattern for protein content, the total amino acid and essential amino acid content of the body tissue decreased from January 2018 to May 2018 (the inactive stage).

Glutamic acid, glycine, aspartic acid, and alanine were the major amino acids, accounting for 38.20–43.59% of total amino acids during the sampling period and contributed to the good flavors of bivalves. The highest levels of favorable amino acids reached 43.59% in August 2017 (the maturing stage), indicating that variations of the amino acids contributing to good taste are closely associated with the gonad development.

Non-protein nitrogenous compounds, e.g., the total FAA content, in *S. constricta* from May 2017 to May 2018 are shown in Figure 5. The total FAA content in the summer (June, July, and August) was significantly higher than that in the other seasons. The total FAA content increased with gonad formation and maturation and peaked in August. Meanwhile, taurine was the most prominent and accounted for 7.79–23.09% of the total FAA in *S. constricta*. Figure 6 shows the percentage of flavored amino acids in total free amino acids over a period of one year. In this study, 27 FAAs were estimated by an amino acid analyzer. Further analysis showed that umami-taste free amino acids (FAAs) accounted for 5.98–12.20% of the total FAAs, sweet free amino acids (SFAA) accounted for 32.03–70.70% of the total FAAs, and bitter FAAs accounted for 11.24–20.69% of the total FAAs. The content of TAV in the body tissue of *S. constricta* throughout the year is shown in Table 2. There were 11 kinds of

Table 1. Monthly variation of amino acid (AA) content (% DW) of *S. constricta*.

Amino acid	May-17	Jun-17	Jul-17	Aug-17	Sep-17	Oct-17	Nov-17	Dec-17	Jan-18	Feb-18	Mar-18	Apr-18	May-18
Isoleucine	2.93±0.07	3.00±0.12	2.49±0.19	2.18±0.2	2.03±0.06	2.15±0.02	2.1±0.03	2.13±0.01	1.92±0.07	1.86±0.15	2.02±0.02	1.79±0.04	1.73±0.22
Leucine	4.79±0.14	5.15±0.21	4.19±0.32	3.67±0.35	3.36±0.12	3.49±0.02	3.44±0.06	3.44±0.02	3.22±0.10	3.08±0.27	3.3±0.03	2.93±0.07	2.85±0.36
Lysine	5.11±0.17	5.35±0.18	4.49±0.37	4.00±0.32	3.93±0.19	4.02±0.06	3.87±0.06	3.8±0.05	3.68±0.11	3.42±0.27	3.72±0.03	3.15±0.07	3.10±0.40
Methionine	1.61±0.06	1.6±0.08	1.42±0.13	1.17±0.02	1.11±0.04	1.23±0.02	1.19±0.1	1.2±0.01	1.18±0.05	1.07±0.11	1.19±0.02	1.08±0.03	0.94±0.13
Phenylalanine	2.87±0.04	2.81±0.11	2.53±0.19	2.14±0.23	1.97±0.06	2.16±0.01	2.12±0.01	2.15±0.03	2.04±0.24	1.94±0.15	2.03±0.04	1.81±0.04	1.66±0.21
Threonine	3.19±0.08	3.41±0.14	2.96±0.23	2.6±0.24	2.39±0.08	2.52±0.01	2.51±0.03	2.47±0.02	2.34±0.09	2.25±0.2	2.35±0.03	2.02±0.04	1.94±0.25
Valine	3.25±0.07	3.3±0.12	2.85±0.22	2.5±0.22	2.33±0.08	2.54±0.02	2.5±0.02	2.55±0.01	2.44±0.14	2.26±0.17	2.38±0.02	2.05±0.03	1.93±0.25
Tryptophan	0.59±0.02	0.52±0.04	0.50±0.02	0.50±0.02	0.49±0.01	0.51±0.01	0.47±0.05	0.47±0.04	0.45±0.03	0.42±0.01	0.49±0.02	0.47±0.02	0.44±0.02
Aspartic acid	6.62±0.21	6.89±0.28	5.83±0.49	5.12±0.5	4.7±0.16	5.03±0.01	5.02±0.08	4.96±0.01	4.60±0.22	4.41±0.38	4.7±0.07	4.2±0.10	4.03±0.52
Serine	3.22±0.14	3.21±0.14	3.13±0.23	2.81±0.25	2.74±0.08	2.52±0.02	2.55±0.04	2.53±0.03	2.43±0.15	2.31±0.21	2.43±0.03	2.05±0.05	1.91±0.24
Glutamic acid	9.52±0.45	10.25±0.43	8.66±0.7	7.69±0.48	7.16±0.23	7.38±0.03	7.4±0.13	7.37±0.03	7.02±0.55	6.65±0.59	7.1±0.08	6.17±0.14	6.01±0.77
Glycine	3.68±0.07	3.62±0.16	5.16±0.45	4.38±0.41	3.75±0.16	3.64±0.02	3.63±0.03	3.14±0.01	2.95±0.16	2.61±0.18	2.63±0.07	2.58±0.06	2.56±0.32
Alanine	4.45±0.22	4.15±0.15	7.02±0.64	6.52±0.37	5.2±0.21	3.68±0.02	4.47±0.14	4.71±0.01	4.22±0.22	3.95±0.36	4.08±0.07	4.25±0.08	4.09±0.55
Cystine	0.62±0.02	0.69±0.04	0.63±0.05	0.55±0.03	0.49±0.03	0.62±0.02	0.58±0.04	0.55±0.01	0.52±0.02	0.49±0.05	0.43±0.02	0.41±0.05	0.41±0.09
Tyrosine	2.16±0.07	2.36±0.11	1.91±0.14	1.61±0.2	1.44±0.05	1.6±0.03	1.55±0.01	1.47±0.02	1.39±0.08	1.36±0.13	1.4±0.01	1.35±0.03	1.33±0.15
Histidine	1.38±0.03	1.44±0.04	1.26±0.1	1.12±0.1	1.07±0.03	1.2±0.03	1.1±0.02	1.09±0.04	1.05±0.05	0.98±0.04	1.07±0.01	0.86±0.02	0.81±0.11
Arginine	5.09±0.24	4.95±0.17	4.52±0.34	4.02±0.33	3.91±0.15	3.01±1.59	3.62±0.1	3.75±0.04	3.43±0.22	3.35±0.29	3.72±0.01	3.27±0.09	3.13±0.41
Proline	2.48±0.08	2.5±0.08	2.19±0.14	1.81±0.11	1.75±0.10	2.6±1.12	1.91±0.04	1.8±0.03	1.72±0.08	1.59±0.1	1.67±0.04	1.61±0.06	1.51±0.24
TAA, %	63.54±2.18	65.2±2.60	61.75±4.95	54.39±4.39	49.81±1.83	49.88±3.08	50.04±0.95	49.58±0.29	46.60±2.56	44±3.66	46.72±0.63	42.04±1.02	40.4±5.23
EAA, %	24.32±0.65	25.14±1.00	21.45±1.66	18.76±1.59	17.61±0.64	18.61±0.17	18.21±0.35	18.22±0.13	17.27±0.55	16.3±1.32	17.48±0.22	15.29±0.34	14.6±1.83
NEAA, %	39.22±1.53	40.06±1.60	40.31±3.29	35.63±2.80	32.21±1.19	31.27±2.91	31.83±0.59	31.36±0.16	29.33±0.69	27.71±2.34	29.24±0.42	26.75±0.68	25.80±3.39
EAA/TAA	38.27	38.56	34.73	34.49	35.35	37.31	36.4	36.74	37.06	37.04	37.42	36.38	36.14
EAA/NEAA, %	62.01	62.75	53.21	52.65	54.67	59.53	57.22	58.08	58.89	58.82	59.8	57.18	56.59
Total taste-active AA, %	38.2	38.21	43.18	43.59	41.76	39.55	41.01	40.69	40.32	40.05	39.61	40.92	41.29

flavor-active substances, and the order of their contribution to the overall flavor was Ala, Glu, Arg, Gly, Ser, His, Val, Lys, Met, Asp, Thr, Ile, Leu, Pro, and Phe. In addition, TAV values were greater than 1, followed by alanine, glutamic acid, and arginine.

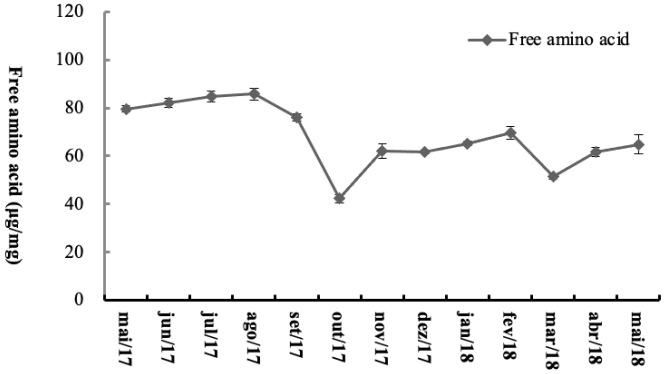


Figure 5. Bimonthly variations of total free amino acid in *S. constricta*.

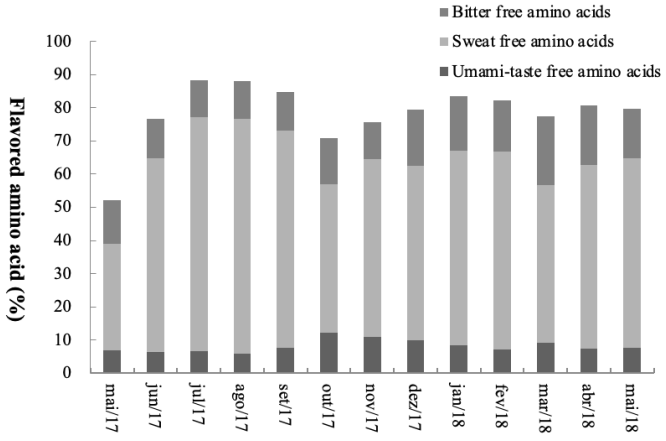


Figure 6. Monthly variation of umami-taste free amino acids, sweat free amino acids, and bitter free amino acids (%) in *S. constricta*.

3.6. Fatty acids composition

During the annual change of the fatty acid constituent, the body tissue of the constituent contained 26 fatty acids of the same type, including 8 saturated fatty acids (SAFA) and 18 unsaturated fatty acids (UFA), of which there were 8 kinds of monounsaturated fatty acids (MUFA) and 10 kinds of polyunsaturated fatty acids (PUFA). The fatty acid composition of *S. constricta* throughout the year is shown in Table 3 and Figure 7. SFA ranged from 29.16% (May 2017) to 55.31% (March 2018). The most abundant SFA in the body was C14:0, C16:0, and C18:0, and their content was not significantly changed throughout the seasons. MUFA ranged from 25.61% (March 2018) to 34.69% (November 2018). The fatty acids C16:1n-7, C18:1n-9c, and C20:1 were the most abundant forms in body tissue. The C16:1n-9 content was significantly lower in the months between June and August than in other months. The content of the fatty acids C18:1n-7 and C20:1n-7 decreased during the maturing period. PUFA ranged from 19.08% (March 2018) to 44.02% (May 2017) in body tissues. The most abundant and interesting fatty acids were C22:6n3 (DHA) and C20:5n3 (EPA).

3.7. Microbiological quality, total volatile basic nitrogen, and pH

As shown in Figure 8, the total bacterial count (TBC) in *S. constricta* ranged between 3.57±0.04 and 6.16±0.04 log (CFU/g) through the whole cycle. TBC in *S. constricta* was significantly correlated with the seawater temperature (Spearman correlative test, rs=0.773, $p<0.05$). TBC in *S. constricta* was at a high level in the summer (6.16±0.04 log CFU/g).

The total number of colonies increased with the increase in temperature. TVB-N content in *S. constricta* fluctuated between 5.09±0.86 mg/100 g and 8.55±1.06 mg/100 g throughout the year, without significant seasonal changes (rs=0.313, $p>0.05$) (Figure 9). As shown in Figure 10, pH in *S. constricta* varied between 6.85±0.09 and 7.23±0.01 throughout the year. The partial

Table 2. The TAV of free amino acids (FAA) in *S. constricta* in different months.

FAA	Taste attribute	Taste threshold (mg/100 g)	TAV												
			May-17	Jun-17	Jul-17	Aug-17	Sep-17	Oct-17	Nov-17	Dec-17	Jan-18	Feb-18	Mar-18	Apr-18	May-18
Aspartic acid	Sweet/fresh(+)	100	0.08	0.02	0.11	0.10	0.10	0.09	0.14	0.16	0.12	0.09	0.09	0.08	0.09
Glutamic acid	Fresh(+)	30	1.57	0.69	1.51	1.37	1.57	1.41	1.76	1.50	1.45	1.40	1.26	1.27	1.35
Threonine	Sweet(+)	260	0.03	0.02	0.05	0.06	0.04	0.02	0.04	0.05	0.07	0.10	0.05	0.04	0.03
Proline	Sweet/bitter(+)	300	0.01	0.01	0.03	0.03	0.03	0.01	0.03	0.02	0.01	0.01	0.01	0.01	0.01
Serine	Sweet(+)	150	0.19	0.24	0.33	0.38	0.42	0.13	0.19	0.27	0.33	0.41	0.27	0.24	0.19
Glycine	Sweet(+)	130	0.59	0.78	1.45	1.45	1.12	0.46	0.84	0.45	0.65	0.50	0.52	0.42	0.59
Alanine	Sweet(+)	60	3.06	4.22	5.62	5.61	4.48	1.69	2.94	3.45	4.03	4.32	2.64	3.97	4.22
Valine	Sweet/bitter(-)	40	0.07	0.09	0.16	0.19	0.11	0.08	0.22	0.29	0.23	0.16	0.18	0.13	0.08
Methionine	Bitter/sweet(-)	30	0.02	0.03	0.08	0.07	0.03	0.02	0.06	0.24	0.21	0.22	0.27	0.26	0.01
Isoleucine	Bitter(-)	90	0.02	0.03	0.04	0.05	0.03	0.03	0.07	0.08	0.05	0.04	0.04	0.04	0.03
Leucine	Bitter(-)	190	0.01	0.02	0.02	0.02	0.02	0.01	0.04	0.04	0.03	0.02	0.02	0.01	0.01
Phenylalanine	Bitter(-)	90	0.01	0.02	0.02	0.01	0.01	0.01	0.04	0.02	0.01	0.02	0.01	0.01	0.01
Lysine	Sweet/bitter(-)	50	0.11	0.14	0.15	0.14	0.14	0.09	0.25	0.15	0.11	0.07	0.08	0.08	0.13
Histidine	Bitter(-)	20	0.13	0.08	0.11	0.10	0.16	0.18	0.20	0.17	0.18	0.25	0.21	0.26	0.16
Arginine	Sweet/bitter(-)	50	1.80	1.54	1.34	1.38	1.35	0.82	0.51	1.17	1.22	1.55	1.50	1.61	1.55

The taste threshold (mg/100 g) represents the lowest concentration of free amino acid that can be tasted in aqueous solution (+ pleasant, - unpleasant).

Table 3. Monthly variation of fatty acid composition (μg mg-1) of *S. constricta*.

Fatty acid	May-17	Jun-17	Jul-17	Aug-17	Sep-17	Oct-17	Nov-17	Dec-17	Jan-18	Feb-18	Mar-18	Apr-18	May-18
C14:0	0.24	0.25	0.37	0.53	0.66	0.64	0.42	0.40	0.53	0.82	0.41	0.44	0.52
C15:0	0.12	0.15	0.12	0.19	0.19	0.17	0.10	0.09	0.12	0.15	0.10	0.11	0.14
C16:0	1.08	1.25	1.76	2.76	2.66	1.89	1.42	1.44	1.90	2.70	2.01	2.06	2.23
C17:0	0.12	0.18	0.16	0.21	0.20	0.18	0.14	0.12	0.13	0.15	0.12	0.12	0.14
C18:0	0.88	0.98	0.70	0.91	0.96	1.32	1.11	0.91	0.98	1.06	0.90	0.88	0.92
C20:0	0.02	0.02	0.04	0.06	0.05	0.04	0.04	0.03	0.03	0.02	0.03	0.03	0.04
C22:0	0.02	0.01	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.03	0.02	0.03	0.03
C24:0	0.03	0.05	0.02	0.03	0.03	0.02	0.02	0.01	0.02	0.03	0.03	0.04	0.03
∑SFA	2.50	2.90	3.20	4.71	4.78	4.29	3.28	3.02	3.73	4.96	3.61	3.71	4.05
cis-9-C16:1	0.31	0.31	0.71	1.03	1.05	1.09	0.56	0.42	0.53	0.65	0.34	0.48	0.55
cis-11-C16:1	0.02	0.02	0.02	0.04	0.05	0.06	0.03	0.04	0.04	0.09	0.02	0.02	0.03
trans-4-C18:1	0.02	0.01	0.04	0.08	0.07	0.04	0.02	0.02	0.05	0.08	0.05	0.06	0.08
trans-9-C18:1	0.40	0.53	0.52	0.66	0.62	0.88	0.49	0.36	0.44	0.50	0.31	0.36	0.45
trans-11-C18:1	0.28	0.33	0.51	0.62	0.62	0.59	0.54	0.44	0.40	0.43	0.35	0.36	0.39
cis-11-C20:1	0.60	0.77	0.83	0.87	0.81	0.96	0.80	0.33	0.65	0.24	0.21	0.27	0.38
cis-13-C20:1	0.37	0.38	0.26	0.30	0.29	0.31	0.35	0.29	0.28	0.31	0.24	0.26	0.27
cis-9-C20:1	0.25	0.33	0.36	0.36	0.34	0.39	0.40	0.25	0.27	0.29	0.16	0.15	0.19
∑MUFA	2.25	2.68	3.25	3.96	3.85	4.32	3.20	2.15	2.66	2.59	1.67	1.97	2.35
cis-9,12-C18:2n6	0.07	0.09	0.10	0.13	0.13	0.13	0.08	0.06	0.07	0.08	0.05	0.06	0.07
cis-11,14-C20:2n6	0.24	0.25	0.21	0.18	0.16	0.17	0.16	0.13	0.11	0.06	0.10	0.13	0.13
cis-5,8,11,14-C20:4n6	0.44	0.57	0.50	0.50	0.49	0.56	0.27	0.15	0.13	0.08	0.10	0.12	0.18
trans-5,8,11,14,17-C20:5n3	0.61	0.57	0.56	0.62	0.70	0.76	0.43	0.28	0.38	0.32	0.16	0.20	0.24
cis-5,13-C22:2n6	0.51	0.45	0.30	0.29	0.27	0.46	0.41	0.32	0.28	0.25	0.22	0.24	0.24
cis-13,16-C22:2n6	0.26	0.26	0.28	0.27	0.25	0.38	0.48	0.27	0.25	0.21	0.15	0.14	0.13
cis-7,10,13,16-C22:4n6	0.18	0.23	0.24	0.25	0.24	0.35	0.15	0.07	0.06	0.03	0.05	0.06	0.06
cis-4,7,10,13,16-C22:5n6	0.22	0.25	0.21	0.19	0.19	0.27	0.16	0.07	0.08	0.04	0.05	0.07	0.07
cis-7,10,13,16,19-C22:5n3, EPA	0.22	0.24	0.16	0.17	0.19	0.24	0.16	0.08	0.10	0.07	0.05	0.06	0.06
cis-4,7,10,13,16,19-C22:6n3, DHA	1.08	1.05	0.41	0.45	0.52	0.63	0.46	0.44	0.42	0.37	0.33	0.37	0.34
∑PUFA	3.82	3.95	2.97	3.07	3.14	3.97	2.75	1.86	1.88	1.51	1.25	1.45	1.52
DHA/EPA	4.90	4.44	2.52	2.58	2.73	2.59	2.95	5.74	4.20	5.31	6.96	5.88	5.63
EPA+DHA	1.30	1.28	0.58	0.63	0.71	0.88	0.61	0.51	0.52	0.44	0.37	0.43	0.40
n-3 PUFA	1.91	1.85	1.14	1.24	1.41	1.64	1.04	0.79	0.90	0.76	0.53	0.63	0.64
n-6 PUFA	1.92	2.10	1.84	1.83	1.73	2.33	1.71	1.07	0.98	0.75	0.72	0.82	0.88
n-6:n-3	1.00	1.14	1.61	1.47	1.22	1.42	1.64	1.34	1.09	0.99	1.36	1.30	1.38

coefficients analysis showed that the seawater temperature was negatively correlated with the pH (r_s=-0.617, $p<0.05$).

4. Discussion

4.1. Seasonal variations of gonad development stages

Although the time and period of spawning and the duration of gametogenesis of shellfish species varied between locations due to different latitude, temperature, salinity, and food availability, the overall trend of the gametogenic cycle is consistent (Ayache et al., 2016; Marshall et al., 2012; Zhao et al., 2022). It is likely that this consistency is due to the relatively narrow range of distribution of *S. constricta*, which is confined to coastal areas of southern China. In this study, gametogensis begins in May when the temperature is increasing, and spawning starts in September when the temperature and chlorophyll *a* levels are high. The gonad development of *S. constricta* in southern China could be divided into two major phases: the gametogenesis phase from May to November (including maturing and spawning) and the reproductive inactivation phase from December to April, during which males and females were indistinguishable. Xue et al. (2021) found that more than half of the *S. constricta* individuals initiate gonad development when the temperature reaches 20–21°C. Yan et al. (2010) demonstrated that gametogensis of *S. constricta* begins in June and July, when the water temperature is 22–27°C in the Yellow River delta in China.

4.2. Association of biochemical parameters with gametogenesis or seasonal change

The moisture content of the body generally reduces during the period of growth. Moisture content typically decreases during the matured gametes (summer) and then increases during the spawning period (autumn). Lipid and protein levels are closely associated with gamete development, with the

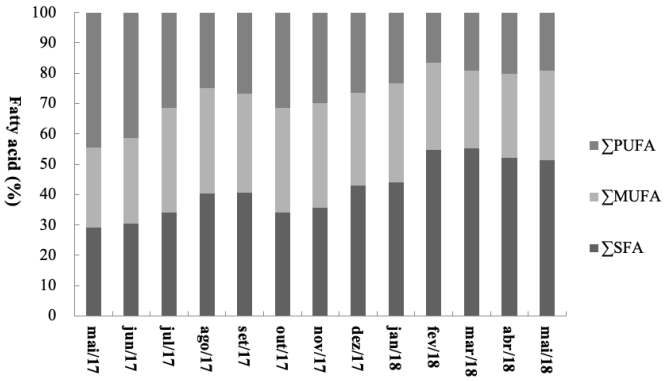


Figure 7. Monthly variations of fatty acid ratios in *S. constricta*.

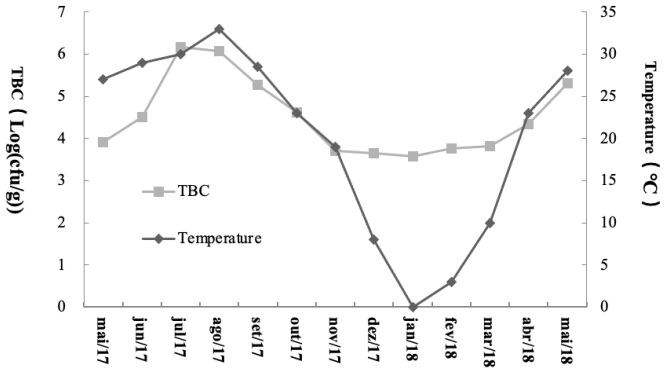


Figure 8. Bimonthly variations of total bacteria count (TBC) and temperature in *S. constricta*.

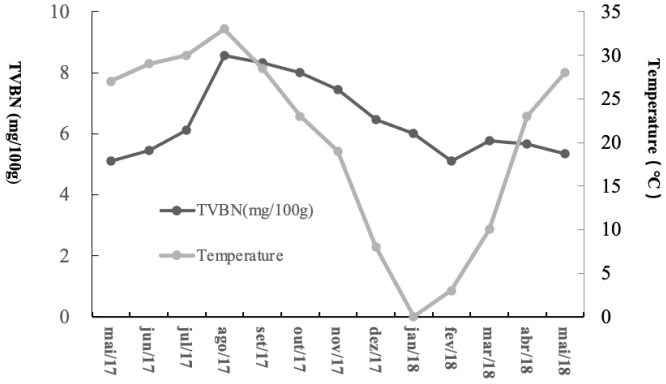


Figure 9. Bimonthly variations of total volatile basic nitrogen (TVBN) and temperature in *S. constricta*.

lowest levels of lipid or protein in the spawning period. Similar results were observed in the Pacific oyster *Crassostrea gigas*, in which lipid and protein contents decrease after spawning and increase again as the gametes mature (Bethelin et al., 2000; Ren et al., 2003). The protein content slightly increases during sexual maturation and then declines after spawning, which is consistent with the proliferation and maturation of gametes and indicates that proteins are accumulated in the gametes (Celik et al., 2014; Cotter et al., 2010). In this study, *S. constricta* usually contains a lean level of fat throughout the cycle (average 0.89% wet weight).

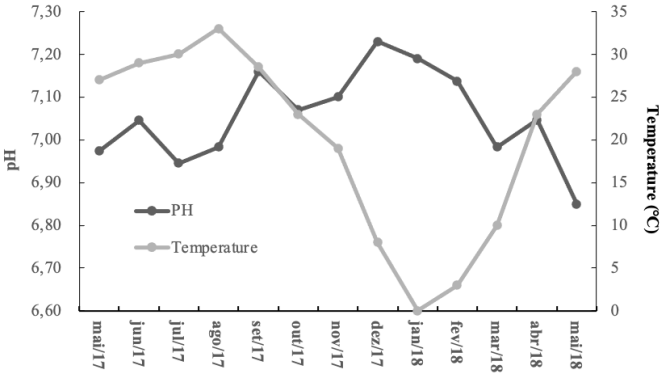


Figure 10. Bimonthly variations of pH and temperature in *S. constricta*.

Based on fat content, bivalves can be categorized into lean fat (<2%), medium fat (4–8%), and high fat (>8%) (Tan et al., 2020). Glycogen level decreases during the period of gamete formation and proliferation from June to September when high energy is demanded, and reaches the lowest level when most gonads are mature, indicating that glycogen reserves are utilized during the reproductive phase of *S. constricta*. This is consistent with previous studies showing that variations in the glycogen levels of bivalves are closely related to the reproductive activity and the glycogen concentrations peak during non-reproductive periods (Mondol et al., 2016; Normand et al., 2008). On the contrary, glycogen content is an important factor affecting the taste (sweetness) (Fernandez et al., 2015), indicating that *S. constricta* tastes better (sweeter) during January to April when most razor clams are reproductively inactive.

4.3. Variations in amino acid and free amino acid content during the gametogenic cycle or seasons

The amount and quality of proteins in food are very important for human nutrition. Protein quality is determined by the level of essential amino acid (Murata et al., 2020). Both total essential amino acids and total non-essential amino acids peak in September, which is consistent with the maximum protein level in this period. The total amino acid content increased with the formation and maturation of gonad. Particularly, the essential amino acid levels increase significantly. The amino acid content decreases after gametes are released by *S. constricta*, similar to the results of previous studies in *C. gigas* (Murata et al., 2020) and *C. hongkongensis* (Qin et al., 2021). The annual variation of EAA/TAA in the body tissue ranges from 34.73 to 38.56%, and EAA/NEAA range from 52.65 to 62.75%. The United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO) judge high-quality protein by ∑EAA/∑AA of about 0.4 and ∑EAA/∑NEAA of 0.6 or higher. With reference to this standard, *S. constricta* is in line with the food with high-quality proteins. Overall, in this study, we show that *S. constrict* not only contains a high level of proteins, but also has a relatively well-balanced essential amino acid profile throughout the year, indicating that it is a high-quality protein source.

FAAs are one of the most important biologically active molecules. FAAs are not only nutrients and basic materials for

cells and tissues during the growth and development of organisms, but also indicators that are widely used for evaluation of the flavor of fish and shellfish (Bermudez et al., 2014; Liu et al., 2021). In this study, we show that the level of total FAA in September to November (in the spawning phase) is lower than that in other phases. In particular, the level of total flavor-active components is very low in the spawning phase. Chen et al. (2021) showed that the content of FAAs and 5′-nucleotides in blue mussels (*Mytilus edulis* L.) was significantly lower when females were in the reproductive period. In addition, the largest proportion of SFAA in the body is also one of the reasons for the sweet flavor of the razor clam. The content of flavor amino acids in the body fluctuates greatly during a period of 1 year, particularly when it is more sensitive to the process of gonad maturation. The proportion of SFAA is increasing during the maturing period, and the proportion of SFAA in the spawning period is decreasing.

When determining the flavor intensity of food and exploring the contribution of a single component to its overall flavor, TAV is the most classic and objective method, and it is widely used in the evaluation of food flavor (Liu et al., 2020; Liu et al., 2022). The alanine content in *S. constricta* is the highest, followed by glutamic acid and arginine, which are all fresh and sweet, and their TAV values are greater than 1, indicating that alanine, glutamic acid, and arginine are important contributors to the umami taste of *S. constricta*. Among them, the TAV values of the sweet amino acids Gly and Ala are increasing during gonadal maturation. Glutamic acid, aspartic acid, alanine, and glycine are the main free amino acids with the highest content and stronger flavor, which may contribute to the delicious flavor of Manila clams (*Ruditapes Philippinarum*) (Zhou et al., 2021). Arg are slightly sweet, bitter amino acids whose bitterness can be masked by sodium chloride, sodium glutamate, or AMP. Arg has a freshness-boosting effect and provides seafood with a suitable overall flavor (Yang et al., 2001). Meanwhile, the TAV values of the body tissue of *S. constricta* are less than 1, indicating that it does not contribute much to the flavor.

4.4. Association of fatty acid composition with gametogensis or season

The fatty acid profile of bivalves is influenced by the reproductive cycle (Bongiorno et al., 2015). During the growth and development of *S. constricta*, the content of SFA as the main energy supply gradually increases with the increase of the individual, while the content of PUFA gradually decreases. From the perspective of major fatty acids, the proportion of PUFA to the total fatty acids in the active and maturing stages was higher than that in the spawning and resting stages. Increasing levels of n-3 PUFA during the maturing stage, mainly due to 22:6n-3 (DHA), are also observed. The peak in DHA concentration corresponds with gonad maturation, and the lowest concentration occurs when gonads are inactive. DHA plays an important role in the membrane structure and function of cells involved in oogenesis and embryogenesis, and it influences larval survival (Liu et al., 2020).

When the nutritional quality indicators of foods, including lipid content, n-3:n-6 ratio, and PUFA/SFA ratio, are taken into account, most marine bivalves appear to meet the recommended nutritional quality range (Tan et al., 2019). In this study, we show that the n-3:n-6 ratio ranges between 0.61 and 0.82 in the whole cycle. n-3 PUFAs increase with gonad development in *S. constricta*, resulting in a gradual increase in the n-3:n-6 ratio, with the highest ratio observed in September (most gonads have matured). It is recommended that the n-3:n-6 PUFA ratio should be at least 0.1–0.2 or more, and a higher proportion (>0.2) is considered to be nutritionally balanced and very beneficial to human health (FAO & WHO, 2017).

PUFA/SAFA values for *S. constricta* vary from 0.30 to 1.53 (monthly average value: 0.72), indicating that *S. constricta* is suitable for consumers who are interested in low-fat food selection. Foods with a PUFA/SAFA ratio below 0.45 are considered to be detrimental to human diets because they may cause the elevation of blood cholesterol levels (Prato et al., 2019).

4.5. Variation of TBC, TVB-N, and pH during the seasons

The microflora of shellfish is closely associated with the environmental conditions, microbiological quality of the water, water temperature, salt content, natural concurrence of bacteria, ingestion of food, methods of catch, and chilling conditions (Burkhardt et al., 1992; Gulusan & Duygu, 2009). In this study, *S. constricta* collected in the summer had higher microbial concentrations than those collected in the winter, probably because the seawater temperature in the summer is warmer than that in the winter. Therefore, temperature management is also crucial for the control of microbiological quality. An increase in the temperature may lead to an increase not only in the population of hygienic indicators but also in the population of potentially hazardous microorganisms and lactic acid bacteria (Kim et al., 2018).

TVB-N and pH are widely used to determine the freshness of aquatic products, and their sensitivity is higher for shellfish with high protein content; the pH values of fresh high-quality bivalves are fluctuated between 6 and 7, as described by previous literature (Khan et al., 2005). Seafoods with lower TVB-N content would indicate higher freshness (Kachele et al., 2017). TVB-N content in *S. constricta* increases with temperature, reaching its highest level (8.55±1.06 mg/100 g) in August 2017, followed by a decrease in tidal flat temperature. In a previous study, it was shown that clams with a TVB-N value lower than 30 mg/100 g are considered acceptable for human consumption (Lopez-Caballero et al., 2000). The pH was significantly associated with seasonal changes, indicating that seafood with a higher pH is considered good quality with less organic acids. The pH of white-scar oyster (*C. belcheri*) ranged from 6.2 to 6.4 during frozen storage by freezing method or antioxidant treatment (Songsaeng et al., 2010). The pH values of Pacific oysters (*Crassostrea gigas*) were significantly correlated with temperature fluctuations (Bi et al., 2023).

5. Conclusion

The present study provides an in-depth understanding of the effect of seasonal variations and gonad development on the nutrition, flavor, and safety of *S. constricta*. The reproductive

cycle of *S. constricta* can affect the lipid and protein composition and the degree of lipid unsaturation. *S. constricta* is most flavorful in its inactive stage when it contains more umami-tasting FAAs and glycogen. TVBN and TBC can be affected by the change of seasons. In summary, our study shows that the optimal harvesting time of *S. constricta* is spring (from March to April at Taizhou, Zhejiang Province, China), when most of the razor clams have inactive gonads.

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