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SEASONAL VARIATION ON GENERAL COMPOSITION, FREE AMINO ACIDS AND FATTY ACIDS IN THE GONAD OF TAIWAN'S SEA URCHIN *TRIPNEUSTES GRATILLA*

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Key words: *Tripneustes gratilla*, seasonal variation, free amino acids, fatty acids.

ABSTRACT

The seasonal variation of chemical composition in sea urchin *Tripneustes gratilla* gonad from Taiwan is investigated. The level of both gonad index (GI) and body weight in the sea urchins showed seasonal variations and were higher during spring and summer. The main saturated fatty acids (SFA) of sea urchin gonad were C14:0 and C16:0, and both varied with season. Arachidonic acid and eicosapentaenoic acid were the dominant poly-unsaturated fatty acids (PUFA), while C16:1, C18:1 and C22:1 were the predominant monoenoic fatty acids (MFA). The levels of total fatty acids were higher in summer, and decreased to the lowest in December (13.2 ± 2.3 mg/g). The SFA level was higher in summer, while the PUFA level was higher in winter. The most dominant free amino acid (FAA) of sea urchin gonad was glycine, where alanine, proline, arginine and glutamic acid were the predominant FAAs. Arginine and valine were in increasing from September to February. Meanwhile glycine, alanine and glutamic acid also presented seasonal change. Overall, the gonad of Taiwan's sea urchin *T. gratilla* offered higher gonad content, better color and higher level of lipid in summer. It suggested the better period for consuming *T. gratilla* in Taiwan is from May to July.

I. INTRODUCTION

Sea urchin is one of the most highly prized fishery product in the world because of its unique flavor. The largest market of sea urchin is primarily in Japan, but there is also demand in the markets of Europe and other countries for both live and processed sea urchin [25, 28]. The sea urchin *Tripneustes gratilla*, which is widely distributed in Taiwan, is one of the most valuable fishery products, and it is a fast-growing species in subtropical ocean areas. The sea urchin's gonad, which is of highest quality, has excellent market acceptance [12]. The edible part of the sea urchin is its gonad, called "uni" in Japan and the price depends on gonad color, quality, appearance and nutritional value [36, 37]. Many studies have suggested that these factors are affected by season, temperature, photoperiod, food intake and other variables [22, 27, 34, 40, 43]. The quality of wild-harvested sea urchin is directly affected by season, and gonad yield depends on the sea urchin's developmental stage. In recent decades, there have been several studies on sea urchins that have examined the effects of controlling the diet of sea urchin to enhance the gonad quality [12, 21, 41].

Fatty acids and free amino acids (FAA) are among the most important indicators of gonad quality, these factors mainly affect nutrient value and flavor. Cook *et al*. [8] suggested that the fatty acid composition of *Psammechinus miliari* gonad is related to dairy diet. Fatty acids are abundant in sea urchin *Strongylocentrotus droebachiensis*, for example, rich in triacylglycerols, sterol, phosphatidylcholine and phosphotidylethanolamine. Although fatty acid compositions are variable with species [5], the main saturated fatty acids (SFA) are myristic acid (C14:0) and palmitic acid (C16:0) [23], while arachidonic acid (C20:4n6) and eicosapentaenoic acid (C20:5n3, EPA) are the dominant poly-unsaturated fatty acid [32]. On the other hand, FAA also varied with diet and developing stage [29]. Free amino acids affect the flavor of sea urchin gonad [14]. By consecutive omission tests, it proved that FAA affect sweetness, umami and aftertaste of sea urchin gonad. These fatty acids and FAA play important roles in physiological aspects such as osmotic regulations and juvenile growth, they also provide the sea urchin with energy during development. This study aimed to determine the seasonal variation of the chemical composition of the sea urchin *Tripneustes gratilla* gonad, and offer a consult for consumers the better period for eating sea urchin in Taiwan.

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II. MATERIALS AND METHODS

1. Sample Collection

At least 10 specimens of the sea urchin *Tripneustes gratilla* were collected monthly from the Bi-sha seafood market in Keelung from July, 2008 to June, 2009. To mimic the consumption of consumers, the sex of collected sea urchin was not determined in this experiment. After collecting the sea urchins in different months, all samples were weighed and dissected, and the gonad of each specimen was homogenized and then stored at -20°C for use.

2. Gonad Index (GI) Determination

A gonad index (GI) was calculated from the wet weight of individuals as follows:

$$GI = (\text{gonads (g wet)}/\text{whole urchin (g wet)}) \times 100\%$$

3. Determination of Proximate Composition

Proximate compositions were determined according to the standard procedure of AOAC [1], with objects of analysis including moisture, ash, crude protein and crude fat. The carbohydrate level was calculated by the following formula: Carbohydrate (%) = 100-(moisture + ash + crude protein + crude fat).

The total energy was counted by the following formula:

$$\text{Energy (kcal/100 g)} = (\text{Crude protein} \times 5.65) + (\text{Crude lipid} \times 9.5) + (\text{Carbohydrate} \times 3.9).$$

4. Gonad Color Assessment

Gonad color was tested by colour method using colorimeter (TC-1800 MK-II, Tokyo Denshoku). Each individual was tested under standard light conditions supplied by a light cabinet (D65) and assessed using a quantitative colour method of CIE (L*a*b*) [38]. To measure gonad color, ten replicate measurements were taken and averaged to determine CIE L* (intensity or lightness), a* (hue or redness) and b* (chroma or yellowness).

5. Fatty Acid Composition Determination

1) Lipid Extraction

The lipid extraction procedure was referenced and modified from the method of Folch *et al*. [13]. Two grams of homogenized gonad sample were mixed with 10 ml chloroform/methanol (v:v, 2:1). These samples were well mixed and filtered, and the above-mentioned steps were repeated three times. Filtrates were then collected and vacuum- dried. The extracted lipids were stored at -20°C with 0.01% butylated hydroxytoluene (BHT) for use.

2) Fatty Acid Esterification

The method of fatty acid esterification was referenced from A.O.A.C [1]. Briefly, the extracted lipids were mixed with 0.1 ml internal standard C13:0 (10 mg/ml, Sigma, U.S.A.) and 5 ml 0.5N NaOH/methanol and boiled for 10 min. Then, boron trifluoride and methanol (v:v, 14:86%) were added and boiled for 2 min for esterification. After cooling, methyl esters were extracted with 5 ml hexane. The hexane layer, which contained the fatty acid methyl esters, was evaporated to dryness and preserved at -20°C for fatty acid analysis.

3) Fatty Acid Analysis

The methyl ester samples were analyzed using gas chromatography (Shimadzu GC-14A, Kyoto, Japan) equipped with an injector, a flame ionization detector and a Rtx-2330 column (10% cyanopropylphenyl and 90% biscyanopropyl polysiloxane, RESTK, Bellefonte, PA, U.S.A.). The inlet and detector temperature were both set at 250°C. The column temperature was kept at 140°C for 5 min and then programmed from 140°C to 240°C with an increment of 4°C/min and kept at 240°C for 30 min. Nitrogen gas with high purity was used as the carried gas. To identify the species of fatty acid, the standard F.A.M.E. Mix C4-C24 (Supelco, Bellefonte, PA, U.S.A.) was used to compare the retention time with the samples, and the level was calculated for each fatty acid.

6. Free Amino Acid Composition Determination

1) Extraction of Free Amino Acids

The extraction of FAA was carried out according to the method described by Konosu *et al*. [26]. A 10 g sea urchin gonad was homogenized with 30 ml of 7% cold trichloroacetic acid (TCA) using Polytron PT-MR 3100 homogenizer for 3 min. The homogenate was centrifuged at 4,000 *g* for 20 min at 4°C. The supernatant was filtered through Adventec Toyo No. 2 filter paper. The precipitate was then extracted twice with TCA by the same procedure. The supernatants were combined and made up to 100 ml with 7% TCA. The TCA extract was mixed with an equal amount of diethyl ether to remove TCA and fat. This procedure was repeated five times. The aqueous layer was evaporated to dryness. The dried matter was diluted with redistilled water and made up to 25 ml for FAA analysis.

2) Analysis of Free Amino Acids

Free amino acids were separated by ion exchange chromatography and analyzed by a Hitachi L-8500 high speed amino acid analyzer with a Hitachi 2622 SC packed column (4.6 × 60 mm) (Hitachi Ltd, Tokyo, Japan). The standard analytical method applied for physiological fluid assay was performed according to the instruction manual provided by the manufacturer. The levels of FAA were estimated on the basis of peak area of the known concentration of a standard (Wako Ltd, Osaka, Japan) by a Hitachi D-2850 Chromato data processor.

7. Statistical Analysis

The data were presented as mean ± SD. The differences

Table 1. Proximate composition of sea urchin gonad.

	2008 Jul	Aug	Sep	Oct	Nov	Dec	2009 Jan	Feb	Mar	Apr	May	Jun	Average
Moisture (%)	$80.7 \pm 3.0^{a,b*}$	79.6 ± 1.5^{a}	$81.5 \pm 0.1^{a\text{-}c}$	$82.1 \pm 1.3^{b,c}$	$82.4 \pm 1.1^{b,c}$	$84.8 \pm 0.2^{c,d}$	$84.6 \pm 0.3^{c,d}$	$83.5 \pm 0.2^{c,d}$	$80.9 \pm 0.8^{a,b}$	$82.2 \pm 0.3^{b,c}$	$81.7 \pm 1.0^{a\text{-}c}$	$81.4 \pm 0.8^{a\text{-}c}$	82.1 ± 1.6
Ash (%)	2.8 ± 0.5^{b}	3.1 ± 0.2^{b}	3.1 ± 0.3^{b}	2.8 ± 0.2^{b}	2.7 ± 0.1^{b}	3.1 ± 0.6^{b}	3.1 ± 0.3^{b}	2.8 ± 0.1^{b}	$2.6 \pm 0.2^{a,b}$	2.7 ± 0.5^{b}	2.1 ± 0.2^{a}	2.1 ± 0.1^{a}	2.8 ± 0.3
Crude protein (%)	11.7 ± 0.3^{b}	$8.3 \pm 3.0^{a,b}$	7.3 ± 0.5^{a}	$8.5 \pm 2.8^{a,b}$	11.5 ± 0.5^{b}	$9.4 \pm 0.5^{a,b}$	6.6 ± 1.6^{a}	8.0 ± 2.6^{a}	11.7 ± 1.6^{b}	$9.7 \pm 2.6^{a,b}$	7.1 ± 1.7^{a}	7.9 ± 0.1^{a}	9.0 ± 1.8
Crude lipid (%)	$2.8 \pm 0.3^{b\text{-}e}$	$2.9 \pm 0.2^{b\text{-}e}$	$3.3 \pm 0.4^{e,f}$	$2.5 \pm 0.3^{b,c}$	$2.6 \pm 0.1^{b\text{-}d}$	1.8 ± 0.1^{a}	$2.3 \pm 0.1^{a,b}$	3.6 ± 0.4^{f}	3.8 ± 0.2^{f}	$3.5 \pm 0.4^{e,f}$	$3.2 \pm 0.4^{d\text{-}f}$	$3.2 \pm 0.8^{c\text{-}f}$	3.0 ± 0.6
Carbohydrate (%)	2.0 ± 0.2^{a}	6.1 ± 1.3^{e}	$4.8 \pm 0.8^{c,d}$	$4.0 \pm 1.6^{b,c}$	0.8 ± 0.2^{a}	0.9 ± 0.5^{a}	3.5 ± 0.3^{b}	2.0 ± 0.1^{a}	1.0 ± 0.4^{a}	1.9 ± 0.2^{a}	$5.9 \pm 0.3^{d,e}$	$5.5 \pm 0.2^{d,e}$	3.2 ± 2.0
Energy (Kcal/100 g)	100.8 ± 0.2^{f}	$98.7 \pm 1.2^{e,f}$	$91.2 \pm 2.3^{b,c}$	87.7 ± 3.1^{b}	$93.1 \pm 0.8^{c,d}$	73.7 ± 2.6^{a}	72.7 ± 1.3^{a}	87.7 ± 3.2^{b}	106.1 ± 0.6^{g}	$95.1 \pm 2.4^{c\text{-}e}$	$93.9 \pm 1.7^{c,d}$	$96.0 \pm 3.3^{d,e}$	91.4 ± 10.0

* Values in the same column with different superscripts are significantly different at $p < 0.05$ (n = 10).

Table 2. Lightness, red, and yellow (*L*,*a*,*b*) for *Tripneustes gratilla* gonad during seasonal change.

	2008 Jul	Aug	Sep	Oct	Nov	Dec	2009 Jan	Feb	Mar	Apr	May	Jun	Average
L	$57.21 \pm 10.75^{d*}$	$54.88 \pm 6.60^{b\text{-}d}$	$55.69 \pm 3.55^{c,d}$	$50.28 \pm 5.48^{a\text{-}d}$	40.56 ± 2.62^{a}	$44.16 \pm 5.69^{a,b}$	$52.77 \pm 1.74^{b\text{-}d}$	$44.88 \pm 9.75^{a\text{-}c}$	$54.45 \pm 2.52^{b\text{-}d}$	$55.81 \pm 4.78^{c,d}$	59.70 ± 4.30^{d}	59.39 ± 4.56^{d}	52.48 ± 6.23
a	6.97 ± 1.90^{b}	4.05 ± 1.93^{a}	4.04 ± 1.78^{a}	3.46 ± 1.82^{a}	3.30 ± 0.89^{a}	$4.77 \pm 1.28^{a,b}$	3.87 ± 1.05^{a}	$5.49 \pm 1.59^{a,b}$	$6.02 \pm 0.33^{a,b}$	$4.48 \pm 0.90^{a,b}$	6.87 ± 1.48^{b}	$6.54 \pm 1.08^{a,b}$	4.99 ± 1.34
b	$34.09 \pm 5.77^{d,e}$	$31.91 \pm 3.37^{b\text{-}e}$	$31.25 \pm 2.52^{b\text{-}e}$	$29.39 \pm 3.27^{a\text{-}d}$	23.93 ± 1.67^{a}	$26.29 \pm 2.93^{a,b}$	$31.28 \pm 1.48^{b\text{-}e}$	$27.49 \pm 6.04^{a\text{-}c}$	$31.91 \pm 0.99^{b\text{-}e}$	$32.99 \pm 2.30^{c\text{-}e}$	36.87 ± 2.60^{e}	36.19 ± 2.96^{e}	31.13 ± 3.86

* Values in the same column with different superscripts are significantly different at $p < 0.05$ (n = 10).

between mean values were analyzed by a one-way analysis of variance (ANOVA) followed by the Duncan test.

III. RESULTS

The proximate compositions of sea urchin gonad collected from July 2008 to June 2009 are shown in Table 1. Overall, the average levels of moisture, ash, crude protein, crude lipid, carbohydrate and energy were 82.1 ± 1.6%, 2.8 ± 0.3%, 9.0 ± 1.8%, 3.0 ± 0.6%, 3.2 ± 2.0% and 91.4 ± 10.0 kcal/100 g, respectively. The highest value of moisture was in December (84.8 ± 0.2%), and the lowest was in August (79.6 ± 1.5%). Ash was the highest in August, September, December and January, and it was the lowest in May and July. Crude protein was the highest in March and July (11.7 ± 1.6% and 11.7 ± 0.3%, respectively) and the lowest in January (6.6 ± 1.6%). Crude lipid was the highest in March (3.8 ± 0.2%) and the lowest in December (1.8 ± 0.1%). Carbohydrate was the highest in August (6.1 ± 1.3%) and the lowest in November (0.8 ± 0.2%). Energy was the highest in March (106 ± 0.6 kcal/100 g) and the lowest in January (72.7 ± 1.3 kcal/100 g). The average gonad index (GI) and body weight were 7.7 ± 3.5% and 143.1 ± 32.0 g, respectively. The seasonal variations of both GI and body weight in the sea urchins were higher during spring and summer (Fig. 1). The GI value was the highest in July (16.5 ± 3.7%) and the lowest in February (3.3 ± 1.3%). The body weight of the sea urchins was the highest in April (193.6 ± 39.5 g) and the lowest in December (93.6 ± 17.5g).

The variation of sea urchin gonad color were determined by colorimeter with CIE (L*a*b*) system. Overall, the averages of the percentages of L*, a* and b* were 52.48 ± 6.23, 4.99 ± 1.34 and 31.13 ± 3.86, respectively (Table 2). L*, a* and b* value were higher from May to July, and the lowest value detected from this research was in November (L* = 40.56 ± 2.62, a* = 3.30 ± 0.89, b* = 23.39 ± 3.27).

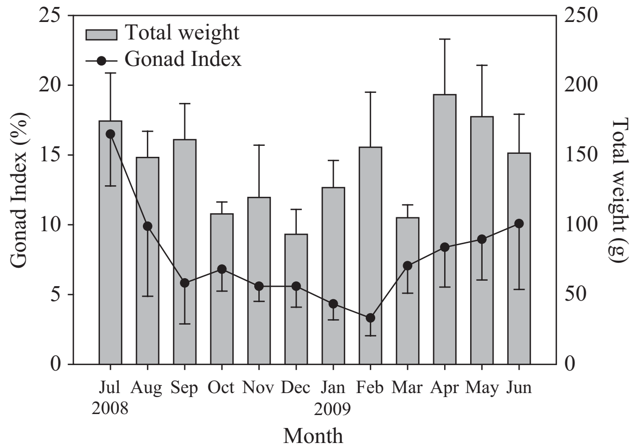

Fig. 1. Seasonal change of gonad index and total body weight of *Tripneustes gratilla* (n = 10). The gonad index (GI) was calculated as: GI = (gonads (g wet)/whole urchin (g wet)) × 100.

The total content of fatty acid compositions in the sea urchin *T. gratilla* gonad are shown in Table 3. The total fatty acid level was the highest in July (47.3 ± 14.5 mg/g) and the lowest in December (13.2 ± 2.3 mg/g) (Fig. 2). For better understanding the variation trend of the fatty acid content in sea urchin gonad, the fatty acid content were then convert into percentage. The percentage of fatty acid compositions in the sea urchin *T. gratilla* gonad are shown in Table 4. Overall, the averages of the percentages of SFA, MFA and PUFA are 47.2 ± 6.1%, 24.9 ± 2.7% and 27.9 ± 5.5% respectively. This analysis revealed that myristic acid (C14:0) and palmitic acid (C16:0) are the dominant saturated fatty acid (SFA); palmitoleic acid (C16:1), oleic acid (C18:1) and erucic acid (C22:1) are abundant in monoenoic fatty acid (MFA); and arachidonic acid (C20:4n6) and eicosapentaenoic acid

Table 3. The seasonal change of fatty acid composition (mg/g) of *Tripneustes gratilla* gonad.

	2008 Jul	Aug	Sep	Oct	Nov	Dec	2009 Jan	Feb	Mar	Apr	May	Jun	Average
SFA	$23.4 \pm 7.6^{d*}$	23.9 ± 5.8^{d}	22.5 ± 8.9^{d}	$13.3 \pm 3.6^{a-c}$	$11.6 \pm 1.9^{a,b}$	5.8 ± 0.8^{a}	$9.2 \pm 0.9^{a,b}$	6.9 ± 3.6^{a}	$14.5 \pm 5.3^{a-d}$	$17.8 \pm 6.3^{b-d}$	23.3 ± 2.6^{d}	$20.8 \pm 3.5^{c,d}$	16.1 ± 6.8
C10:0	$0.2 \pm 0.0^{a,b}$	$0.2 \pm 0.1^{a,b}$	0.3 ± 0.1^{b}	$0.2 \pm 0.0^{a,b}$	$0.3 \pm 0.1^{a,b}$	$0.2 \pm 0.1^{a,b}$	$0.2 \pm 0.0^{a,b}$	0.1 ± 0.0^{a}	$0.3 \pm 0.1^{a,b}$	$0.2 \pm 0.0^{a,b}$	$0.2 \pm 0.0^{a,b}$	$0.2 \pm 0.1^{a,b}$	0.2 ± 0.0
C12:0	$0.1 \pm 0.2^{a,b}$	$0.1 \pm 0.1^{a,b}$	$0.0 \pm 0.0^{a,b}$	0.1 ± 0.1^{b}	$0.0 \pm 0.0^{a,b}$	$0.0 \pm 0.0^{a,b}$	$0.0 \pm 0.0^{a,b}$	0.0 ± 0.0^{a}	$0.0 \pm 0.0^{a,b}$	$0.0 \pm 0.0^{a,b}$	$0.0 \pm 0.0^{a,b}$	$0.0 \pm 0.0^{a,b}$	0.0 ± 0.0
C14:0	$4.7 \pm 0.8^{c-g}$	$5.2 \pm 1.6^{d-g}$	$5.3 \pm 2.7^{e-g}$	$2.9 \pm 0.2^{a-e}$	$2.3 \pm 0.2^{a-c}$	0.7 ± 0.0^{a}	$1.8 \pm 0.6^{a,b}$	$1.5 \pm 1.1^{a,b}$	$2.8 \pm 1.9^{a-d}$	$4.0 \pm 2.0^{b-f}$	6.8 ± 0.8^{g}	$5.8 \pm 0.9^{f,g}$	3.6 ± 1.9
C15:0	$0.4 \pm 0.2^{d,e}$	0.4 ± 0.1^{e}	$0.3 \pm 0.0^{c-e}$	$0.2 \pm 0.1^{a-c}$	$0.2 \pm 0.1^{a-c}$	0.1 ± 0.0^{a}	$0.1 \pm 0.0^{a,b}$	0.1 ± 0.1^{a}	$0.2 \pm 0.1^{a-c}$	$0.3 \pm 0.0^{b-d}$	$0.3 \pm 0.0^{b-d}$	$0.2 \pm 0.1^{a-c}$	0.2 ± 0.1
C16:0	$9.9 \pm 4.1^{d-f}$	11.6 ± 3.9^{f}	$10.5 \pm 5.0^{e,f}$	$5.1 \pm 2.1^{a-d}$	$3.6 \pm 0.5^{a,b}$	2.2 ± 0.2^{a}	$3.8 \pm 0.5^{a-c}$	$3.2 \pm 2.3^{a,b}$	$6.1 \pm 3.0^{a-e}$	$7.8 \pm 2.9^{b-f}$	$10.8 \pm 1.1^{e,f}$	$8.9 \pm 2.0^{c-f}$	6.9 ± 3.4
C18:0	0.5 ± 0.1^{a}	0.5 ± 0.0^{a}	0.6 ± 0.1^{a}	0.7 ± 0.3^{a}	0.6 ± 0.1^{a}	0.5 ± 0.1^{a}	0.6 ± 0.1^{a}	0.4 ± 0.2^{a}	1.1 ± 0.3^{b}	1.5 ± 0.1^{c}	$1.4 \pm 0.1^{b,c}$	$1.3 \pm 0.4^{b,c}$	0.8 ± 0.4
C20:0	1.8 ± 0.6^{d}	2.1 ± 0.4^{d}	2.2 ± 0.8^{d}	$1.4 \pm 0.3^{b-d}$	$1.6 \pm 0.3^{c,d}$	0.3 ± 0.3^{a}	$0.8 \pm 0.6^{a-c}$	0.5 ± 0.4^{a}	$0.6 \pm 0.5^{a,b}$	0.4 ± 0.2^{a}	0.2 ± 0.0^{a}	0.3 ± 0.1^{a}	1.0 ± 0.7
C21:0	0.9 ± 0.3^{e}	$0.5 \pm 0.0^{c,d}$	$0.5 \pm 0.2^{b-d}$	$0.6 \pm 0.2^{d,e}$	0.6 ± 0.3^{d}	0.2 ± 0.0^{a}	0.2 ± 0.2^{a}	0.2 ± 0.1^{a}	$0.3 \pm 0.1^{a-c}$	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	$0.3 \pm 0.1^{a,b}$	0.4 ± 0.2
C22:0	$1.1 \pm 0.3^{d,e}$	1.2 ± 0.3^{e}	$0.7 \pm 0.2^{c-e}$	$0.5 \pm 0.4^{a-c}$	$0.4 \pm 0.4^{a-c}$	$0.3 \pm 0.1^{a-c}$	$0.4 \pm 0.1^{a-c}$	0.1 ± 0.0^{a}	$0.2 \pm 0.2^{a,b}$	$0.6 \pm 0.4^{b,c}$	$0.6 \pm 0.1^{b-d}$	$0.7 \pm 0.2^{c-e}$	0.6 ± 0.3
C23:0	3.7 ± 1.7^{c}	$2.0 \pm 0.9^{a-c}$	$1.9 \pm 0.4^{a-c}$	$1.4 \pm 0.4^{a,b}$	$2.0 \pm 0.2^{a-c}$	$1.3 \pm 0.4^{a,b}$	$1.3 \pm 0.4^{a,b}$	0.8 ± 0.2^{a}	$2.9 \pm 1.2^{b,c}$	$2.7 \pm 1.3^{b,c}$	$2.7 \pm 1.0^{b,c}$	$3.0 \pm 1.5^{b,c}$	2.1 ± 0.9
C24:0	$0.1 \pm 0.0^{c,d}$	$0.1 \pm 0.1^{b-d}$	0.2 ± 0.1^{d}	$0.1 \pm 0.1^{a-d}$	$0.0 \pm 0.0^{a-c}$	$0.0 \pm 0.0^{a,b}$	0.0 ± 0.0^{a}	$0.1 \pm 0.0^{a-d}$	$0.1 \pm 0.1^{a-d}$	0.2 ± 0.0^{d}	0.1 ± 0.0^{d}	$0.1 \pm 0.0^{c,d}$	0.1 ± 0.0
MFA	$11.3 \pm 3.1^{c,d}$	12.4 ± 3.4^{d}	$11.0 \pm 2.6^{c,d}$	6.6 ± 1.4^{b}	6.5 ± 0.6^{b}	2.6 ± 0.1^{a}	6.4 ± 1.5^{b}	$6.0 \pm 2.7^{a,b}$	$8.1 \pm 2.2^{b,c}$	6.6 ± 1.3^{b}	12.4 ± 1.7^{d}	$9.7 \pm 1.9^{b-d}$	8.3 ± 3.0
C14:1	$0.3 \pm 0.1^{b,c}$	$0.4 \pm 0.1^{c,d}$	$0.3 \pm 0.2^{b,c}$	$0.2 \pm 0.1^{a-c}$	$0.1 \pm 0.0^{a,b}$	0.0 ± 0.0^{a}	$0.2 \pm 0.0^{a-c}$	$0.2 \pm 0.1^{a-c}$	$0.2 \pm 0.1^{a-c}$	$0.2 \pm 0.1^{a-c}$	0.7 ± 0.2^{e}	$0.5 \pm 0.1^{d,e}$	0.3 ± 0.2
C16:1	2.3 ± 0.7^{b}	3.5 ± 1.0^{c}	1.9 ± 0.8^{b}	1.8 ± 0.4^{b}	$1.4 \pm 0.3^{a,b}$	0.5 ± 0.1^{a}	$1.5 \pm 0.6^{a,b}$	$1.4 \pm 1.1^{a,b}$	$1.6 \pm 1.1^{a,b}$	$1.5 \pm 0.5^{a,b}$	3.7 ± 0.1^{c}	$2.7 \pm 0.2^{b,c}$	2.0 ± 0.9
C17:1	1.8 ± 0.5^{c}	1.8 ± 0.4^{c}	1.9 ± 0.4^{c}	0.9 ± 0.2^{b}	0.9 ± 0.1^{b}	$0.5 \pm 0.2^{a,b}$	0.8 ± 0.5^{b}	$0.5 \pm 0.2^{a,b}$	1.1 ± 0.7^{b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.9 ± 0.7
C18:1n9t	$3.2 \pm 2.3^{b-e}$	4.3 ± 0.9^{e}	$3.4 \pm 1.6^{c-e}$	$1.4 \pm 0.2^{a-c}$	$1.2 \pm 0.4^{a,b}$	0.4 ± 0.0^{a}	0.7 ± 0.2^{a}	1.1 ± 0.9^{a}	$1.6 \pm 1.1^{a-c}$	$1.7 \pm 1.0^{a-d}$	$3.7 \pm 0.9^{d,e}$	$3.3 \pm 1.0^{c-e}$	2.2 ± 1.3
C18:1n9c	$0.8 \pm 0.5^{b,c}$	0.3 ± 0.2^{a}	$0.4 \pm 0.3^{a-c}$	$0.7 \pm 0.1^{a-c}$	$0.7 \pm 0.2^{a-c}$	$0.4 \pm 0.1^{a,b}$	$0.8 \pm 0.2^{a-c}$	$0.5 \pm 0.1^{a-c}$	$0.8 \pm 0.4^{b,c}$	$0.9 \pm 0.1^{c,d}$	1.3 ± 0.1^{d}	$0.6 \pm 0.0^{a-c}$	0.7 ± 0.3
C20:1	0.3 ± 0.1^{a}	0.2 ± 0.0^{a}	0.1 ± 0.1^{a}	0.3 ± 0.2^{a}	0.5 ± 0.1^{a}	0.5 ± 0.3^{a}	0.3 ± 0.5^{a}	0.5 ± 0.4^{a}	$0.8 \pm 0.7^{a,b}$	1.6 ± 0.4^{c}	$1.4 \pm 0.4^{b,c}$	1.7 ± 0.8^{c}	0.7 ± 0.5
C22:1n9	$2.7 \pm 0.7^{c,d}$	$1.9 \pm 1.7^{a-d}$	3.0 ± 0.7^{d}	$1.3 \pm 0.5^{a-c}$	$1.7 \pm 0.4^{a-d}$	0.4 ± 0.4^{a}	$2.1 \pm 1.2^{b-d}$	$1.7 \pm 0.4^{a-d}$	$2.0 \pm 1.5^{a-d}$	$0.7 \pm 0.2^{a,b}$	$1.5 \pm 0.3^{a-d}$	$0.8 \pm 0.2^{a,b}$	1.7 ± 0.8
PUFA	12.6 ± 4.1^{d}	$11.0 \pm 1.3^{c,d}$	$10.5 \pm 0.9^{b-d}$	$9.3 \pm 1.2^{b,c}$	$7.5 \pm 0.4^{a,b}$	4.7 ± 1.5^{a}	$7.5 \pm 0.6^{a,b}$	$7.5 \pm 1.3^{a,b}$	$9.2 \pm 1.5^{b,c}$	$7.6 \pm 0.8^{a,b}$	$9.5 \pm 0.4^{b,c}$	$8.2 \pm 1.6^{b,c}$	8.7 ± 2.0
C20:2	$0.3 \pm 0.2^{a-c}$	$0.1 \pm 0.0^{a,b}$	$0.2 \pm 0.1^{a,b}$	$0.1 \pm 0.1^{a,b}$	$0.1 \pm 0.0^{a,b}$	0.1 ± 0.0^{a}	$0.1 \pm 0.1^{a,b}$	$0.3 \pm 0.3^{a-c}$	$0.3 \pm 0.3^{a-c}$	0.6 ± 0.3^{c}	$0.4 \pm 0.0^{b,c}$	$0.3 \pm 0.1^{a-c}$	0.2 ± 0.1
C22:2	0.2 ± 0.1^{c}	$0.2 \pm 0.1^{b,c}$	$0.1 \pm 0.1^{a-c}$	$0.1 \pm 0.0^{a,b}$	$0.2 \pm 0.1^{a-c}$	$0.1 \pm 0.0^{a,b}$	$0.1 \pm 0.1^{a,b}$	0.0 ± 0.0^{a}	$0.1 \pm 0.1^{a-c}$	$0.1 \pm 0.0^{a-c}$	$0.1 \pm 0.0^{a-c}$	$0.1 \pm 0.0^{a-c}$	0.1 ± 0.1
ω-6													
C18:2n6t	$0.3 \pm 0.1^{b,c}$	0.4 ± 0.2^{c}	$0.2 \pm 0.0^{a,b}$	$0.2 \pm 0.2^{a-c}$	$0.1 \pm 0.0^{a,b}$	0.0 ± 0.0^{a}	$0.2 \pm 0.1^{a-c}$	$0.1 \pm 0.1^{a,b}$	$0.2 \pm 0.2^{a-c}$	$0.2 \pm 0.1^{a-c}$	$0.2 \pm 0.0^{a,b}$	$0.2 \pm 0.0^{a-c}$	0.2 ± 0.1
C18:2n6c	$0.5 \pm 0.3^{b,c}$	$0.3 \pm 0.0^{a-c}$	0.6 ± 0.1^{c}	0.6 ± 0.4^{c}	$0.4 \pm 0.3^{a-c}$	0.1 ± 0.0^{a}	0.1 ± 0.1^{a}	$0.1 \pm 0.1^{a,b}$	$0.2 \pm 0.0^{a,b}$	$0.2 \pm 0.0^{a,b}$	$0.2 \pm 0.1^{a,b}$	$0.2 \pm 0.1^{a,b}$	0.3 ± 0.2
C18:3n6	2.9 ± 0.7^{b}	2.8 ± 0.5^{b}	2.7 ± 0.7^{b}	1.4 ± 0.2^{a}	1.0 ± 0.2^{a}	0.5 ± 0.1^{a}	1.1 ± 0.5^{a}	0.9 ± 0.4^{a}	1.4 ± 0.7^{a}	1.3 ± 0.2^{a}	1.4 ± 0.5^{a}	1.2 ± 0.5^{a}	1.5 ± 0.8
C20:3n6	$0.3 \pm 0.3^{a,b}$	$0.2 \pm 0.2^{a,b}$	$0.1 \pm 0.1^{a,b}$	$0.3 \pm 0.2^{a,b}$	$0.4 \pm 0.2^{a,b}$	0.7 ± 0.9^{b}	0.0 ± 0.0^{a}	$0.4 \pm 0.4^{a,b}$	$0.6 \pm 0.2^{a,b}$	$0.2 \pm 0.0^{a,b}$	$0.2 \pm 0.0^{a,b}$	$0.2 \pm 0.1^{a,b}$	0.3 ± 0.2
C20:4n6	$2.4 \pm 0.3^{b,c}$	$2.5 \pm 0.2^{b,c}$	$2.2 \pm 0.1^{b,c}$	$2.2 \pm 0.1^{b,c}$	2.1 ± 0.0^{b}	1.0 ± 0.0^{a}	2.1 ± 0.1^{b}	2.1 ± 0.0^{b}	$2.3 \pm 0.3^{b,c}$	2.1 ± 0.5^{b}	2.8 ± 0.4^{c}	$2.6 \pm 0.9^{b,c}$	2.2 ± 0.4
ω-3													
C18:3n3	2.1 ± 1.3^{b}	1.8 ± 0.5^{b}	$1.7 \pm 0.9^{a,b}$	$1.5 \pm 0.3^{a,b}$	$1.5 \pm 0.1^{a,b}$	0.7 ± 0.0^{a}	$1.1 \pm 0.0^{a,b}$	$1.0 \pm 0.4^{a,b}$	$1.3 \pm 0.3^{a,b}$	$1.2 \pm 0.1^{a,b}$	$1.1 \pm 0.8^{a,b}$	$1.1 \pm 0.0^{a,b}$	1.3 ± 0.4
C20:3n3	0.7 ± 1.1^{b}	$0.3 \pm 0.1^{a,b}$	$0.2 \pm 0.0^{a,b}$	$0.1 \pm 0.1^{a,b}$	$0.2 \pm 0.1^{a,b}$	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.2
C20:5n3	2.4 ± 0.1^{h}	2.0 ± 0.0^{f}	2.0 ± 0.1^{f}	1.6 ± 0.1^{e}	0.7 ± 0.0^{b}	0.4 ± 0.0^{a}	2.1 ± 0.1^{f}	1.4 ± 0.1^{d}	1.5 ± 0.1^{e}	1.2 ± 0.1^{c}	2.3 ± 0.1^{g}	1.7 ± 0.1^{e}	1.6 ± 0.6
C22:6n3	$0.5 \pm 0.4^{a-c}$	$0.5 \pm 0.0^{a-c}$	$0.5 \pm 0.3^{a-c}$	$1.1 \pm 0.3^{b,c}$	$0.9 \pm 0.4^{a-c}$	1.1 ± 0.8^{c}	$0.6 \pm 0.0^{a-c}$	$1.1 \pm 0.4^{a-c}$	1.1 ± 0.3^{c}	0.4 ± 0.1^{a}	$0.7 \pm 0.1^{a-c}$	$0.4 \pm 0.1^{a,b}$	0.7 ± 0.3
Total	47.3 ± 14.5^{e}	47.2 ± 10.3^{e}	$44.0 \pm 12.3^{d,e}$	$29.2 \pm 5.1^{b,c}$	$25.6 \pm 2.3^{a-c}$	13.2 ± 2.3^{a}	$23.1 \pm 2.8^{a,b}$	$20.4 \pm 7.5^{a,b}$	$31.8 \pm 7.5^{b-d}$	$32.0 \pm 6.6^{b-d}$	$45.1 \pm 4.6^{d,e}$	$38.7 \pm 6.7^{c-e}$	33.1 ± 11.4

* Values in the same column with different superscripts are significantly different at $p < 0.05$ (n = 10).

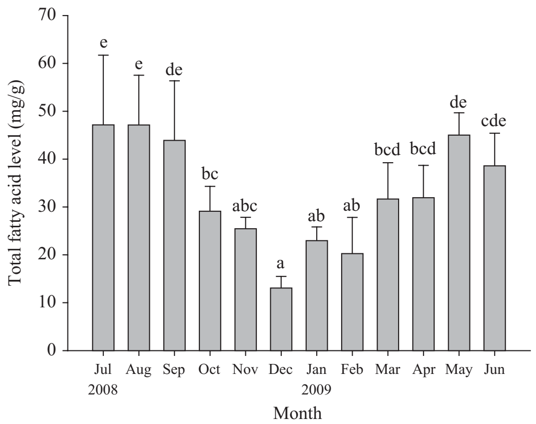

Fig. 2. Seasonal variation of total fatty acid levels in the gonads of sea urchin *Tripneustes gratilla*. * Values with different superscripts are significantly different at $p < 0.05$ (n = 10).

(EPA, C20:5n3) are the main poly-unsaturated fatty acids (PUFA) in the sea urchin *T. gratilla* gonad. The seasonal variation of total SFA level (Fig. 3) is similar to that of total fatty acids (Fig. 2), and the value of the SFA level was the highest in April (55.6 ± 6.3%) and the lowest in February (34.0 ± 5.1%) (Fig. 3). The variations of total PUFA level seems to contrast with that of the SFA levels, showing the highest level in February (36.7 ± 3.7%) and the lowest in May (21.0 ± 0.9%). The variation of MFA is not significant, but the MFA level is the highest in February (29.3 ± 1.8%) and the lowest in December (20.1 ± 4.6%). The seasonal variations of the main compositions in SFA, MFA and PUFA are shown in Fig. 4. The levels of C16:0 and C18:0 in SFA are lower in winter than other seasons. In contrast, the C20:4n6 and C20:5n3 levels in PUFA are higher in winter than in other seasons.

Table 4. The seasonal change of fatty acid composition (%) of *Tripneustes gratilla* gonad.

	2008 Jul	Aug	Sep	Oct	Nov	Dec	2009 Jan	Feb	Mar	Apr	May	Jun	Average
SFA	49.5 ± 3.1^{c-e*}	50.6 ± 1.5^{c-e}	51.2 ± 4.0^{c-e}	45.6 ± 4.9^{b-d}	45.3 ± 2.8^{b-d}	43.8 ± 6.4^{b,c}	39.8 ± 3.1^{a,b}	34.0 ± 5.1^{a}	45.7 ± 7.3^{b-d}	55.6 ± 6.3^{e}	51.6 ± 0.7^{c-e}	53.8 ± 3.2^{d,e}	47.2 ± 6.1
C10:0	0.5 ± 0.1^{a-c}	0.3 ± 0.2^{a}	0.6 ± 0.2^{a-d}	0.8 ± 0.3^{c,d}	1.0 ± 0.2^{d}	1.5 ± 0.5^{e}	0.8 ± 0.1^{a-d}	0.6 ± 0.3^{a-d}	0.8 ± 0.1^{b-d}	0.7 ± 0.2^{a-d}	0.4 ± 0.1^{a,b}	0.6 ± 0.1^{a-d}	0.7 ± 0.3
C12:0	0.2 ± 0.2^{a,b}	0.1 ± 0.2^{a}	0.0 ± 0.0^{a}	0.5 ± 0.5^{b}	0.2 ± 0.1^{a}	0.1 ± 0.2^{a}	0.1 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.1^{a}	0.1 ± 0.1^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.1
C14:0	10.0 ± 1.9^{a-c}	11.1 ± 1.6^{b,c}	12.1 ± 3.3^{b,c}	10.0 ± 1.1^{a-c}	9.0 ± 0.2^{a,b}	5.2 ± 1.0^{a}	7.9 ± 3.6^{a,b}	7.2 ± 3.0^{a,b}	8.9 ± 4.7^{a,b}	12.4 ± 3.4^{b,c}	15.0 ± 0.5^{c}	14.9 ± 4.4^{c}	10.3 ± 3.0
C15:0	0.8 ± 0.1^{c}	0.9 ± 0.2^{c}	0.7 ± 0.2^{a-c}	0.7 ± 0.1^{a-c}	0.9 ± 0.2^{c}	0.8 ± 0.1^{a-c}	0.6 ± 0.1^{a}	0.6 ± 0.1^{a}	0.5 ± 0.1^{a}	0.8 ± 0.1^{b,c}	0.6 ± 0.1^{a,b}	0.6 ± 0.2^{a}	0.7 ± 0.1
C16:0	20.9 ± 3.5^{b-d}	24.4 ± 1.1^{d}	23.9 ± 2.4^{d}	17.4 ± 3.0^{a-c}	13.9 ± 0.4^{a}	16.3 ± 3.8^{a,b}	16.5 ± 3.6^{a,b}	15.7 ± 4.5^{a,b}	19.2 ± 5.8^{a-d}	24.3 ± 3.6^{d}	23.9 ± 2.0^{d}	22.9 ± 1.5^{c,d}	20.0 ± 3.9
C18:0	1.1 ± 0.3^{a}	1.0 ± 0.3^{a}	1.5 ± 0.7^{a,b}	2.4 ± 1.1^{a-c}	2.2 ± 0.5^{a-c}	3.9 ± 0.5^{c,d}	2.7 ± 0.2^{a-c}	2.2 ± 2.1^{a-c}	3.4 ± 2.2^{b-d}	4.7 ± 0.8^{d}	3.0 ± 0.2^{a-d}	3.3 ± 0.5^{b-d}	2.6 ± 1.1
C20:0	3.8 ± 0.6^{c-f}	4.5 ± 0.8^{d-f}	4.9 ± 0.7^{e,f}	4.8 ± 0.5^{e,f}	6.1 ± 0.6^{f}	2.3 ± 1.4^{a-e}	3.4 ± 2.9^{b-f}	2.2 ± 3.2^{a-e}	2.0 ± 1.2^{a-d}	1.3 ± 0.6^{a-c}	0.5 ± 0.1^{a}	0.8 ± 0.2^{a,b}	3.1 ± 1.8
C21:0	1.9 ± 0.8^{c,d}	1.1 ± 0.3^{a-c}	1.2 ± 0.8^{a-c}	2.2 ± 0.3^{d}	2.3 ± 0.9^{d}	1.7 ± 0.3^{b-d}	0.7 ± 0.7^{a,b}	0.8 ± 0.1^{a,b}	1.0 ± 0.6^{a-c}	0.6 ± 0.0^{a}	0.4 ± 0.1^{a}	0.7 ± 0.3^{a}	1.2 ± 0.7
C22:0	2.3 ± 0.4^{c}	2.5 ± 0.2^{c}	1.7 ± 0.4^{a-c}	1.6 ± 1.1^{a-c}	1.7 ± 1.4^{a-c}	2.0 ± 1.0^{b,c}	1.5 ± 0.4^{a-c}	0.4 ± 0.3^{a}	0.5 ± 0.6^{a,b}	1.8 ± 1.4^{a-c}	1.4 ± 0.4^{a-c}	1.9 ± 1.0^{a-c}	1.6 ± 0.6
C23:0	7.7 ± 1.6^{b-f}	4.2 ± 2.6^{a,b}	4.3 ± 0.7^{a-c}	4.9 ± 1.0^{a-d}	7.8 ± 0.2^{c-f}	9.6 ± 4.5^{f}	5.5 ± 1.5^{a-e}	4.0 ± 1.0^{a}	9.1 ± 3.0^{e,f}	8.4 ± 0.1^{d-f}	6.0 ± 0.0^{a-e}	7.7 ± 0.1^{b-f}	6.6 ± 2.0
C24:0	0.3 ± 0.0^{a}	0.3 ± 0.1^{a}	0.3 ± 0.1^{a}	0.3 ± 0.3^{a}	0.2 ± 0.1^{a}	0.2 ± 2.2^{a}	0.1 ± 0.0^{a}	0.3 ± 0.1^{a}	0.3 ± 0.2^{a}	0.5 ± 2.8^{a}	0.3 ± 1.4^{a}	0.3 ± 2.8^{a}	0.3 ± 0.1
MFA	23.9 ± 0.7^{a-c}	26.2 ± 2.2^{b-d}	25.0 ± 1.5^{b-d}	22.7 ± 0.7^{a,b}	25.4 ± 0.4^{b-d}	20.1 ± 4.6^{a}	27.7 ± 3.2^{c,d}	29.3 ± 1.8^{d}	25.5 ± 3.8^{b-d}	20.7 ± 2.3^{a}	27.4 ± 1.4^{c,d}	25.1 ± 0.9^{b-d}	24.9 ± 2.7
C14:1	0.5 ± 0.3^{a,b}	0.8 ± 0.1^{b}	0.6 ± 0.3^{a,b}	0.6 ± 0.3^{a,b}	0.4 ± 0.1^{a,b}	0.1 ± 0.1^{a}	0.9 ± 0.3^{b,c}	0.8 ± 0.5^{b}	0.7 ± 0.2^{b}	0.6 ± 0.3^{a,b}	1.5 ± 0.4^{d}	1.3 ± 0.4^{c,d}	0.7 ± 0.4
C16:1	4.8 ± 1.4^{a-c}	7.3 ± 0.9^{b,c}	4.3 ± 0.9^{a,b}	6.2 ± 0.2^{a-c}	5.4 ± 0.8^{a-c}	3.4 ± 1.6^{a}	6.4 ± 3.5^{a-c}	7.0 ± 3.2^{a-c}	5.1 ± 2.8^{a-c}	4.7 ± 1.6^{a-c}	8.2 ± 0.7^{c}	6.8 ± 1.6^{a-c}	5.8 ± 1.4
C17:1	3.9 ± 0.5^{b}	3.8 ± 1.1^{b}	4.2 ± 0.8^{b}	3.2 ± 0.2^{b}	3.6 ± 0.2^{b}	3.6 ± 2.0^{b}	3.6 ± 2.3^{b}	2.6 ± 1.1^{b}	3.3 ± 2.0^{b}	0.3 ± 0.0^{a}	0.2 ± 0.0^{a}	0.3 ± 0.0^{a}	2.7 ± 1.5
C18:1n9t	6.7 ± 2.6^{a-c}	9.1 ± 0.6^{c}	7.8 ± 1.8^{b,c}	4.8 ± 0.6^{a,b}	4.6 ± 2.1^{a,b}	2.9 ± 1.5^{a}	3.1 ± 1.3^{a}	5.3 ± 3.8^{a-c}	5.1 ± 3.6^{a-c}	5.3 ± 1.8^{a-c}	8.2 ± 1.4^{b,c}	8.5 ± 1.4^{b,c}	5.9 ± 2.1
C18:1n9c	1.7 ± 1.4^{a-d}	0.6 ± 0.8^{a}	1.0 ± 1.1^{a,b}	2.4 ± 0.2^{b-d}	2.7 ± 0.6^{c,d}	3.0 ± 0.2^{c,d}	3.3 ± 0.3^{d}	2.6 ± 0.8^{c,d}	2.7 ± 1.3^{c,d}	2.8 ± 0.9^{c,d}	2.9 ± 0.5^{c,d}	1.6 ± 0.3^{a-c}	2.3 ± 0.8
C20:1	0.6 ± 0.4^{a}	0.5 ± 0.1^{a}	0.3 ± 0.3^{a}	1.1 ± 0.3^{a,b}	1.9 ± 0.7^{a-d}	4.1 ± 2.5^{b-d}	1.5 ± 2.3^{a-c}	2.5 ± 2.6^{a-d}	2.4 ± 2.4^{a-d}	4.9 ± 2.1^{d}	3.0 ± 0.7^{a-d}	4.4 ± 1.4^{c,d}	2.3 ± 1.6
C22:1n9	5.7 ± 1.5^{a-c}	4.1 ± 3.1^{a-c}	6.8 ± 2.3^{a-c}	4.4 ± 0.7^{a-c}	6.8 ± 0.9^{a-c}	3.0 ± 3.7^{a,b}	8.9 ± 5.2^{c}	8.5 ± 4.6^{b,c}	6.2 ± 4.6^{a-c}	2.1 ± 0.1^{a}	3.4 ± 0.5^{a-c}	2.2 ± 0.4^{a}	5.2 ± 2.3
PUFA	26.6 ± 3.8^{a-c}	23.2 ± 0.7^{a,b}	23.8 ± 2.6^{a,b}	31.7 ± 5.0^{c,d}	29.3 ± 2.3^{b,c}	36.1 ± 5.5^{d}	32.5 ± 1.9^{c,d}	36.7 ± 3.7^{d}	28.8 ± 3.9^{b,c}	23.7 ± 5.7^{a,b}	21.0 ± 0.9^{a}	21.1 ± 2.4^{a}	27.9 ± 5.5
C20:2	0.6 ± 0.5^{a}	0.3 ± 0.0^{a}	0.5 ± 0.1^{a}	0.4 ± 0.2^{a}	0.4 ± 0.1^{a}	0.4 ± 0.7^{a}	0.5 ± 0.3^{a}	1.4 ± 3.0^{a}	0.8 ± 1.1^{a}	1.7 ± 1.1^{a}	1.0 ± 0.1^{a}	0.9 ± 0.2^{a}	0.7 ± 0.4
C22:2	0.4 ± 0.1^{a,b}	0.4 ± 0.1^{a,b}	0.3 ± 0.2^{a}	0.2 ± 0.1^{a}	0.6 ± 0.2^{b}	0.4 ± 0.1^{a,b}	0.3 ± 0.3^{a,b}	0.2 ± 0.1^{a}	0.4 ± 0.3^{a,b}	0.4 ± 0.1^{a,b}	0.3 ± 0.1^{a}	0.2 ± 0.1^{a}	0.4 ± 0.1
ω-6													
C18:2n6t	0.5 ± 0.3^{a}	0.8 ± 0.2^{a}	0.3 ± 0.0^{a}	0.8 ± 0.6^{a}	0.6 ± 0.1^{a}	0.3 ± 0.2^{a}	0.9 ± 0.5^{a}	0.6 ± 0.2^{a}	0.8 ± 0.6^{a}	0.6 ± 0.5^{a}	0.3 ± 0.1^{a}	0.5 ± 0.0^{a}	0.6 ± 0.2
C18:2n6c	1.0 ± 0.4^{a}	0.7 ± 0.1^{a}	1.3 ± 0.7^{a,b}	2.3 ± 1.6^{b}	1.5 ± 1.0^{a,b}	0.5 ± 0.3^{a}	0.4 ± 0.4^{a}	0.6 ± 0.4^{a}	0.5 ± 0.1^{a}	0.5 ± 0.0^{a}	0.5 ± 0.1^{a}	0.5 ± 0.2^{a}	0.8 ± 0.6
C18:3n6	6.1 ± 1.6^{b}	5.8 ± 0.1^{a,b}	6.0 ± 2.0^{b}	4.7 ± 1.2^{a,b}	4.1 ± 0.3^{a,b}	4.0 ± 2.1^{a,b}	4.9 ± 2.0^{a,b}	4.2 ± 0.4^{a,b}	4.3 ± 2.2^{a,b}	4.2 ± 0.5^{a,b}	3.2 ± 1.1^{a}	3.2 ± 0.9^{a}	4.6 ± 1.0
C20:3n6	0.6 ± 0.5^{a}	0.4 ± 0.4^{a}	0.3 ± 0.0^{a}	0.9 ± 0.7^{a}	1.4 ± 1.0^{a}	5.2 ± 5.2^{b}	0.0 ± 0.0^{a}	2.1 ± 1.1^{a}	1.8 ± 0.0^{a}	0.7 ± 0.2^{a}	0.4 ± 0.0^{a}	0.5 ± 0.1^{a}	1.2 ± 1.4
C20:4n6	5.2 ± 0.6^{a}	5.2 ± 0.2^{a}	5.1 ± 0.2^{a}	7.4 ± 0.2^{b-c}	8.3 ± 0.1^{c,d}	8.0 ± 0.2^{b-c}	9.0 ± 0.3^{d,e}	10.1 ± 0.2^{e}	7.2 ± 0.9^{b-c}	6.5 ± 2.7^{a-c}	6.1 ± 0.6^{a,b}	6.7 ± 1.8^{a-c}	7.1 ± 1.6
ω-3													
C18:3n3	4.5 ± 1.3^{b-d}	3.8 ± 0.3^{a-c}	3.8 ± 1.2^{a-d}	5.2 ± 0.6^{c,d}	5.8 ± 0.1^{d}	5.2 ± 1.4^{c,d}	4.7 ± 0.9^{b-d}	5.2 ± 0.9^{c,d}	4.2 ± 1.2^{a-d}	3.9 ± 0.4^{a-d}	2.4 ± 2.0^{a}	2.9 ± 0.1^{a,b}	4.3 ± 1.0
C20:3n3	1.5 ± 1.7^{b}	0.6 ± 0.2^{a,b}	0.4 ± 0.1^{a}	0.5 ± 0.3^{a}	0.7 ± 0.3^{a,b}	0.3 ± 0.3^{a}	0.1 ± 0.2^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.3 ± 0.1^{a}	0.2 ± 0.0^{a}	0.3 ± 0.1^{a}	0.5 ± 0.4
C20:5n3	5.1 ± 0.1^{e,f}	4.2 ± 0.2^{c,d}	4.6 ± 0.1^{d,e}	5.6 ± 0.5^{f}	2.6 ± 0.0^{a}	3.2 ± 0.3^{b}	9.1 ± 0.6^{h}	6.7 ± 0.8^{g}	4.8 ± 0.4^{d,e}	3.7 ± 0.2^{b,c}	5.0 ± 0.1^{e,f}	4.3 ± 0.1^{c,d}	4.9 ± 1.7
C22:6n3	1.1 ± 0.8^{a}	1.0 ± 0.2^{a}	1.1 ± 1.2^{a}	3.7 ± 1.1^{a,b}	3.3 ± 2.0^{a,b}	8.7 ± 3.7^{c}	2.6 ± 0.8^{a,b}	5.2 ± 3.6^{b}	3.6 ± 0.2^{a,b}	1.2 ± 0.3^{a}	1.6 ± 0.4^{a}	1.1 ± 0.5^{a}	2.9 ± 2.3

* Values in the same column with different superscripts are significantly different at $p < 0.05$ (n = 10).

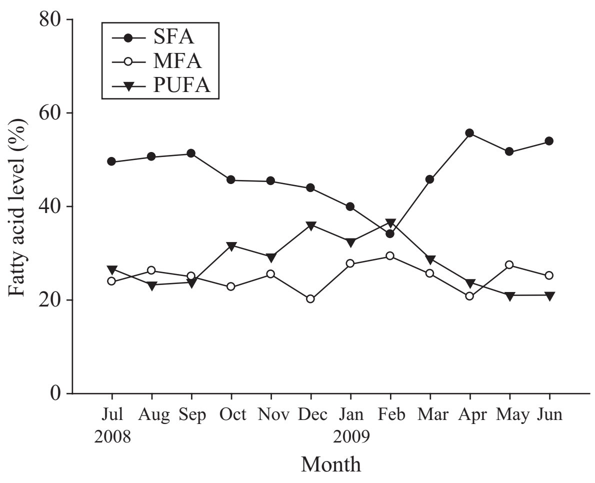

Fig. 3. The seasonal change of SFA, MFA and PUFA levels of *Tripneustes gratilla* gonad.

The level of total FAA in the sea urchin *T. gratilla* gonad is shown in Table 5. The overall average content of FAA was 858.9 ± 277.6 mg/100 g. The amount of total FAA of sea urchin gonad in November was higher than other months (1538.4 ± 41.3 mg/100 g) and the lowest in August (596.5 ± 54.8 mg/100 g) (Fig. 5). The dominant FAA in sea urchin gonad was glycine, and the average content was 304.1 ± 192.8 mg/100 g. Free amino acids like glutamic acid, alanine, proline and arginine were also abundant compared to other FAAs, the content were 110.8 ± 12.9 mg/100 g, 118.2 ± 18.3 mg/100 g, 76.4 ± 45.8 mg/100 g and 67.2 ± 42.9 mg/100 g, respectively. Fig. 6 shows the variation of FAAs which are effective on the flavor of the sea urchin gonad. The highest content of flavor effective FAA was glycine, it showed the highest value in November (846.3 ± 33.0 mg/100 g). The contents of alanine, glutamic acid and methionine were higher from spring to summer, while valine and arginine were higher in the winter.

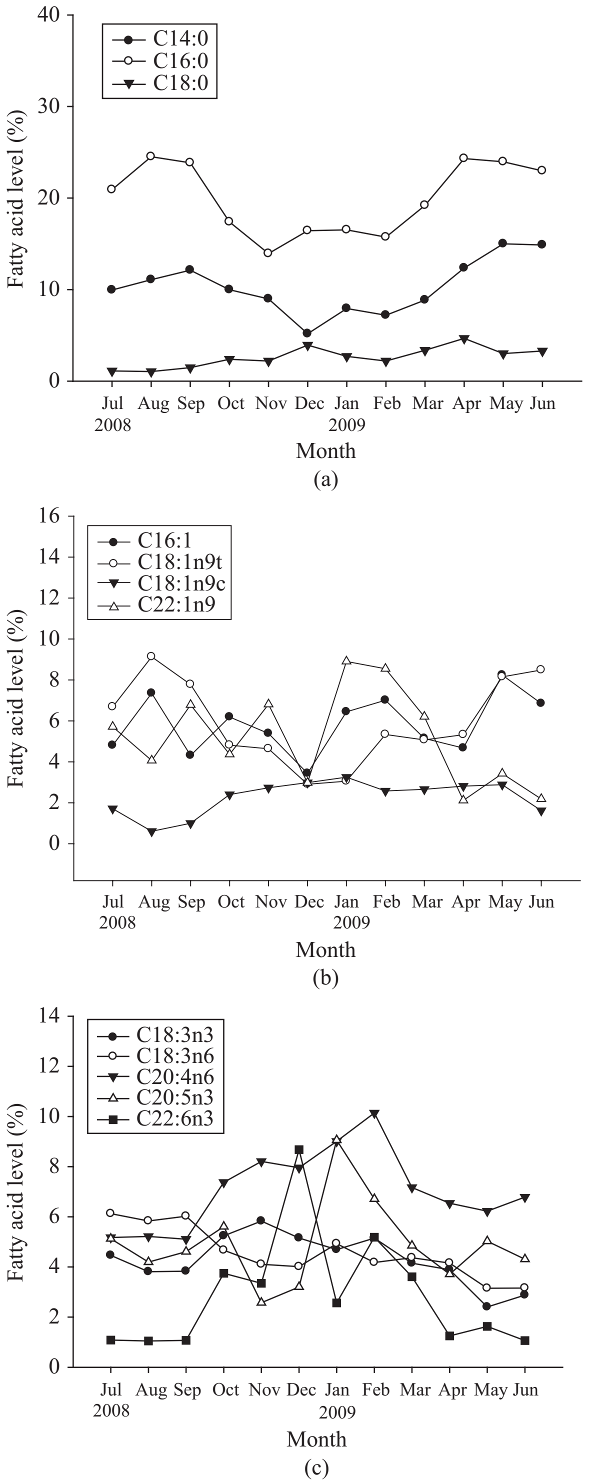

Fig. 4. Seasonal variations of (a) SFA (C14:0, C16:0), (b) MFA (C16:1, C18:1n9t, C22:1) and (c) PUFA (C20:4n6, C20:5n3) levels of *Tripneustes gratilla* gonad.

IV. DISCUSSION

The variation of GI could be one of the factors to determine the sea urchin growing stage, and to reflect the nutrient value of the diet. The increase of GI value was mainly affected by the accumulation of nutritive phagocyte [3]. In pre-mature stage, sea urchin gonad was mainly filled with nutritive phagocyte, so the growth rate of sea urchin gonad was faster than body growth. Once the sea urchin moved to mature stage, nutritive phagocyte supplied energy for gemetogenesis, and the nutritive phagocyte then shrank. When sperm or egg was released from sea urchin, the GI were decreased sharply [46, 47]. In this study, GI showed increased manner from February to July and significantly decreased in August, while the total body weight was increased in the same time.

Although color does not directly affect the nutrient value of sea urchin gonad, it may change the consuming behavior of customers. In foreign country, sea urchin gonad is mainly served as the raw material for sushi, so the quality of sea urchin gonad will directly affect the price. Sea urchin gonad color will be affected by many factors like gender, species and diet. For example, the gonad color of sea urchins is different between *Evechinus chloroticus* and *P. miliaris* [20, 44]. The color of sea urchin in bright yellow or bright orange was thought to be high commercial value, but less acceptance with pale white or tan [8, 42]. The gonad color distribution of green sea urchin *S. droebachiensis* is from light yellow to deep orange or red, once the color turn into pale white or brown, the price of gonad will decrease [38]. In this study, colorimeter was used here to determine the color of sea urchin gonad. The gonad color of Taiwan's sea urchin *T. gratilla* showed bright yellow to bright orange from April to August, and presented in pale white from October to December. It suggested that the gonad color of Taiwan's sea urchin *T. gratilla* has higher acceptance in summer.

Fatty acid and free amino acid composition of sea urchin gonad varied with species, food intake and environment [4]. In this study, the sea urchin gonad in Taiwan's species *T. gratilla* showed the most abundant SFA (C14:0 and C16:0), the most abundant PUFA (C20:5n3 and C20:4n6), and the most abundant MFA (C16:1 and C18:1). The C16:0 values were the highest. Other fatty acids such as C20:0, C23:0, C22:1 and C18:3n3 were also abundant. Although numerous factors affect the levels of fatty acids, C14:0, C16:0, C20:4n6 and C20:5n3 are still reported as the main fatty acids in sea urchin gonad [15, 16]. Several sea urchin feeding experiments have suggested that the composition of dairy diets and culture areas are significant enough to influence the fatty acid variation of sea urchin gonad. Some reports indicated that different diets would effect odd chain fatty acid accumulation. In the other hand, the wild species of sea urchins such as purple sea urchin and green sea urchin accumulate odd chain FA in their gonad. The accumulation of odd carbon FA (C15;0, C21;0, C23;0 and C17:1) might be mainly from their diet. The increase of lipid level may have been associated with gonad maturation. It is also possible that the variation of total lipid level through seasonal changes resulted from long term metabolic adjustment [17]. Cook *et al.* [9] found that the EPA levels in the gonad of sea urchin *Paracentrotus lividus* fed

Table 5. The seasonal change of contents of free amino acids and dipeptides (mg/100g) of the gonad of *Tripneustes gratilla.*

	2008						2009						
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Average
Taurine	$20.6 \pm 5.9^{a*}$	25.2 ± 3.2^{a}	$27.3 \pm 2.7^{a,b}$	$34.2 \pm 8.4^{a-c}$	86.3 ± 22.0^{d}	44.8 ± 4.0^{c}	47.4 ± 8.4^{c}	26.8 ± 1.0^{a}	$32.1 \pm 0.7^{a-c}$	$42.3 \pm 6.3^{b,c}$	44.2 ± 5.1^{c}	$32.2 \pm 6.7^{a-c}$	35.7 ± 17.3
Aspartic acid	$3.1 \pm 2.8^{a,b}$	$1.8 \pm 0.1^{a,b}$	$1.7 \pm 0.3^{a,b}$	$1.8 \pm 0.4^{a,b}$	$3.7 \pm 0.7^{a,b}$	$3.9 \pm 0.9^{a,b}$	$3.1 \pm 3.0^{a,b}$	1.2 ± 0.0^{a}	$2.7 \pm 0.8^{a,b}$	$3.9 \pm 1.2^{a,b}$	4.4 ± 0.7^{b}	4.1 ± 2.2^{b}	2.7 ± 1.1
Threonine	3.6 ± 1.7^{a}	2.2 ± 1.1^{a}	2.5 ± 1.3^{a}	3.0 ± 0.4^{a}	14.1 ± 1.2^{c}	5.2 ± 2.6^{a}	$6.7 \pm 1.0^{a,b}$	$6.0 \pm 3.7^{a,b}$	$8.0 \pm 7.9^{a-c}$	$9.2 \pm 4.4^{a-c}$	$12.3 \pm 6.1^{b,c}$	$6.9 \pm 3.3^{a,b}$	6.1 ± 3.8
Serine	$7.3 \pm 1.7^{a,b}$	$7.6 \pm 0.6^{a,b}$	$11.8 \pm 1.5^{a,b}$	$11.7 \pm 1.4^{a,b}$	29.3 ± 4.7^{b}	$13.1 \pm 4.6^{a,b}$	$11.0 \pm 14.7^{a,b}$	4.1 ± 2.9^{a}	$5.5 \pm 3.9^{a,b}$	$14.2 \pm 4.5^{a,b}$	17.1 ± 10.4^{b}	$15.8 \pm 6.9^{a,b}$	11.4 ± 6.7
Glutamic acid	$121.5 \pm 8.5^{b,c}$	$115.3 \pm 0.0^{b,c}$	$116.5 \pm 0.3^{b,c}$	$108.7 \pm 0.1^{a,b}$	94.1 ± 10.2^{a}	$131.2 \pm 11.9^{c,d}$	$128.2 \pm 19.7^{a-c}$	$111.1 \pm 2.9^{a,b}$	$116.6 \pm 5.3^{b,c}$	$133.6 \pm 20.5^{c,d}$	142.9 ± 9.6^{d}	$120.8 \pm 5.7^{b,c}$	110.8 ± 12.9
Sarcosine	0.1 ± 0.2^{a}	0.0 ± 0.0^{a}	0.2 ± 0.3^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.1 ± 0.2^{a}	0.0 ± 0.0^{a}	0.0 ± 0.1
Glycine	$215.6 \pm 16.2^{a,b}$	159.4 ± 19.5^{a}	150.4 ± 46.1^{a}	166.5 ± 82.4^{a}	846.3 ± 33.0^{f}	$297.4 \pm 10.0^{b,c}$	429.2 ± 3.1^{e}	$233.2 \pm 51.8^{a,b}$	$276.4 \pm 64.4^{b,c}$	$334.0 \pm 68.3^{c,d}$	442.8 ± 82.7^{e}	$401.5 \pm 33.2^{d,e}$	304.1 ± 192.8
Alanine	$120.2 \pm 1.1^{a-d}$	$113.8 \pm 4.3^{a-c}$	$119.2 \pm 6.2^{a-d}$	$114.8 \pm 0.9^{a-c}$	100.2 ± 11.2^{a}	$143.5 \pm 10.8^{d-f}$	$143.0 \pm 32.4^{d-f}$	$106.8 \pm 4.8^{a,b}$	$129.0 \pm 19.0^{b-e}$	$148.4 \pm 22.5^{e,f}$	158.2 ± 12.2^{f}	$139.7 \pm 15.4^{c-f}$	118.2 ± 18.3
Citrulline	1.1 ± 1.6^{a}	0.1 ± 0.2^{a}	0.2 ± 0.2^{a}	0.0 ± 0.0^{a}	$4.4 \pm 2.9^{b,c}$	0.1 ± 0.2^{a}	0.8 ± 0.5^{a}	0.5 ± 0.3^{a}	4.9 ± 3.5^{c}	1.6 ± 0.9^{a}	$2.1 \pm 1.3^{a,b}$	1.4 ± 0.7^{a}	1.3 ± 1.7
Histidine	$2.4 \pm 1.2^{a,b}$	$2.0 \pm 1.7^{a,b}$	$1.6 \pm 0.2^{a,b}$	$1.6 \pm 0.5^{a,b}$	2.8 ± 1.1^{b}	3.0 ± 1.9^{b}	$2.2 \pm 1.1^{a,b}$	0.6 ± 0.8^{a}	$1.6 \pm 0.0^{a,b}$	$2.0 \pm 1.6^{a,b}$	3.4 ± 1.1^{b}	2.9 ± 0.6^{b}	2.0 ± 0.8
Valine	$19.5 \pm 2.5^{a-c}$	$19.4 \pm 2.7^{a-c}$	$16.3 \pm 1.5^{a-c}$	$14.8 \pm 0.2^{a,b}$	40.1 ± 0.8^{d}	$17.6 \pm 1.3^{a-c}$	66.8 ± 13.6^{e}	10.2 ± 1.4^{a}	26.7 ± 2.1^{c}	$26.3 \pm 3.4^{b,c}$	$24.5 \pm 7.7^{b,c}$	$23.3 \pm 12.8^{b,c}$	23.5 ± 15.1
Cystine	0.4 ± 0.6^{a}	0.0 ± 0.0^{a}	0.5 ± 0.7^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.5 ± 0.6^{a}	0.3 ± 0.2^{a}	2.5 ± 1.8^{b}	0.0 ± 0.0^{a}	0.4 ± 0.3^{a}	0.0 ± 0.0^{a}	0.4 ± 0.7
Methionine	62.1 ± 0.0^{f}	4.2 ± 0.2^{a}	5.7 ± 0.0^{a}	4.1 ± 0.2^{a}	$8.8 \pm 0.4^{a,b}$	28.2 ± 0.6^{c}	60.9 ± 2.7^{f}	14.1 ± 0.0^{b}	47.9 ± 0.2^{e}	37.3 ± 5.4^{d}	46.8 ± 7.8^{e}	42.7 ± 4.9^{e}	27.9 ± 22.2
Isoleucine	$2.0 \pm 0.7^{a,b}$	$1.9 \pm 1.4^{a,b}$	$1.9 \pm 1.0^{a,b}$	$2.1 \pm 0.4^{a,b}$	6.7 ± 0.6^{c}	$2.0 \pm 1.5^{a,b}$	$2.9 \pm 1.0^{a,b}$	0.7 ± 0.5^{a}	$4.5 \pm 2.7^{b,c}$	4.2 ± 2.1^{b}	$4.4 \pm 1.5^{b,c}$	3.5 ± 0.8^{b}	2.8 ± 1.7
Leucine	2.4 ± 0.8^{a}	2.6 ± 2.0^{a}	2.9 ± 1.2^{a}	3.9 ± 0.3^{a}	$5.1 \pm 0.3^{a,b}$	2.5 ± 1.1^{a}	10.3 ± 10.5^{b}	1.2 ± 0.9^{a}	3.4 ± 1.4^{a}	$5.3 \pm 1.4^{a,b}$	3.6 ± 0.9^{a}	3.7 ± 1.7^{a}	3.6 ± 2.3
Tyrosine	$6.0 \pm 4.8^{a,b}$	$3.5 \pm 3.9^{a,b}$	$2.3 \pm 1.2^{a,b}$	$2.2 \pm 0.1^{a,b}$	7.2 ± 1.4^{b}	$3.5 \pm 2.2^{a,b}$	$5.4 \pm 4.8^{a,b}$	1.2 ± 0.8^{a}	$3.6 \pm 2.3^{a,b}$	$4.1 \pm 2.9^{a,b}$	6.7 ± 1.8^{b}	$4.8 \pm 2.5^{a,b}$	3.9 ± 1.9
Phenylalanine	$3.1 \pm 1.8^{a,b}$	1.7 ± 1.2^{a}	2.4 ± 1.3^{a}	2.8 ± 1.0^{a}	$3.6 \pm 0.3^{a,b}$	2.3 ± 1.3^{a}	6.4 ± 5.2^{b}	1.0 ± 0.4^{a}	1.8 ± 0.8^{a}	2.6 ± 0.9^{a}	2.3 ± 1.4^{a}	$3.4 \pm 1.7^{a,b}$	2.6 ± 1.4
Hydroxyproline	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	1.2 ± 1.7^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.1 ± 0.4
Proline	$48.4 \pm 3.4^{a,b}$	31.2 ± 0.1^{a}	$52.2 \pm 3.7^{a,b}$	$77.1 \pm 0.7^{a-c}$	$97.6 \pm 2.1^{a-c}$	$157.9 \pm 5.4^{b,c}$	$74.5 \pm 5.3^{a,b}$	$68.6 \pm 86.6^{a,b}$	187.2 ± 179.2^{c}	$82.3 \pm 44.7^{a-c}$	$61.9 \pm 12.3^{a,b}$	$54.8 \pm 8.8^{a,b}$	76.4 ± 45.8
Tryptophan	$1.8 \pm 0.8^{a-c}$	$2.6 \pm 0.7^{a-c}$	$3.3 \pm 0.6^{a-d}$	$3.8 \pm 1.2^{b-d}$	$1.3 \pm 0.3^{a,b}$	1.0 ± 0.7^{a}	$1.2 \pm 1.2^{a,b}$	5.8 ± 4.1^{d}	$4.2 \pm 0.4^{c,d}$	$3.9 \pm 0.9^{b-d}$	$2.4 \pm 1.1^{a-c}$	$3.1 \pm 0.7^{a-c}$	2.7 ± 1.4
Arginine	41.8 ± 19.4^{a}	46.7 ± 21.7^{a}	78.4 ± 36.6^{b}	$125.6 \pm 5.3^{c,d}$	111.3 ± 6.8^{c}	$122.6 \pm 14.4^{c,d}$	149.0 ± 9.2^{d}	23.3 ± 2.3^{a}	34.0 ± 4.2^{a}	44.8 ± 17.4^{a}	$53.7 \pm 24.0^{a,b}$	42.0 ± 12.9^{a}	67.2 ± 42.9
Hydroxylysine	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.4 ± 0.6^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.1
Ornithine	$0.8 \pm 0.5^{a,b}$	$1.2 \pm 0.2^{a-c}$	$1.4 \pm 1.2^{b,c}$	0.4 ± 0.1^{a}	$1.1 \pm 0.1^{a-c}$	0.4 ± 0.2^{a}	5.5 ± 0.3^{d}	$0.8 \pm 0.3^{a,b}$	$1.4 \pm 0.3^{b,c}$	1.9 ± 0.4^{c}	$1.1 \pm 0.3^{a-c}$	$1.2 \pm 0.7^{a-c}$	1.3 ± 1.3
Lysine	$20.9 \pm 5.2^{b-d}$	$10.5 \pm 9.5^{a-c}$	$8.2 \pm 2.0^{a,b}$	$8.7 \pm 2.1^{a,b}$	$22.8 \pm 2.1^{c,d}$	6.1 ± 1.4^{a}	26.6 ± 18.7^{d}	4.3 ± 2.1^{a}	$8.4 \pm 3.4^{a,b}$	$17.3 \pm 5.8^{a-d}$	$21.9 \pm 3.4^{c,d}$	$20.6 \pm 4.1^{b-d}$	13.6 ± 7.7
Cystathionine	0.5 ± 0.7^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	1.3 ± 1.8^{a}	13.9 ± 3.1^{c}	$8.0 \pm 1.1^{b,c}$	$8.2 \pm 8.8^{b,c}$	$4.2 \pm 3.0^{a,b}$	$5.4 \pm 6.3^{a,b}$	0.5 ± 0.8^{a}	0.0 ± 0.0^{a}	0.3 ± 0.2^{a}	3.2 ± 4.5
phosphoethanolamine	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.3 ± 0.5^{c}	$0.2 \pm 0.2^{b,c}$	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.1
Anserine	0.5 ± 0.7^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.5 ± 0.7^{a}	1.4 ± 2.0^{a}	0.0 ± 0.0^{a}	7.6 ± 10.8^{b}	1.9 ± 2.7^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.1 ± 0.2^{a}	0.9 ± 2.2
Carnosine	31.5 ± 12.0^{b}	$28.0 \pm 24.9^{a,b}$	$19.8 \pm 5.0^{a,b}$	$19.9 \pm 2.7^{a,b}$	$15.5 \pm 0.6^{a,b}$	$8.9 \pm 4.9^{a,b}$	$22.9 \pm 29.8^{a,b}$	$15.2 \pm 10.7^{a,b}$	3.3 ± 2.4^{a}	$24.4 \pm 9.7^{a,b}$	33.9 ± 14.3^{b}	32.5 ± 12.9^{b}	19.7 ± 9.5
Urea	$2.0 \pm 0.7^{a,b}$	$2.5 \pm 1.3^{a,b}$	4.2 ± 2.3^{b}	$1.1 \pm 1.5^{a,b}$	0.5 ± 0.8^{a}	0.0 ± 0.0^{a}	9.8 ± 3.0^{c}	0.0 ± 0.0^{a}	4.1 ± 3.6^{b}	4.3 ± 1.4^{b}	$2.2 \pm 1.1^{a,b}$	4.2 ± 2.3^{b}	2.7 ± 2.7
Phosphoserine	7.8 ± 1.6^{b}	$9.5 \pm 3.8^{b,c}$	$12.0 \pm 1.0^{c,d}$	13.9 ± 1.7^{d}	7.4 ± 0.6^{b}	3.2 ± 0.1^{a}	3.4 ± 1.3^{a}	1.5 ± 0.5^{a}	1.5 ± 0.8^{a}	7.6 ± 1.4^{b}	7.4 ± 2.2^{b}	$9.9 \pm 0.6^{b,c}$	6.6 ± 4.0
Aminoadipic acid	$0.1 \pm 0.1^{a,b}$	$0.2 \pm 0.0^{a,b}$	0.0 ± 0.0^{a}	$0.3 \pm 0.1^{a,b}$	2.9 ± 0.1^{e}	1.8 ± 0.7^{d}	1.4 ± 0.0^{c}	0.0 ± 0.0^{a}	0.5 ± 0.4^{b}	$0.3 \pm 0.2^{a,b}$	0.0 ± 0.0^{a}	$0.1 \pm 0.1^{a,b}$	0.6 ± 0.9
Aminobutyric Acid	$1.5 \pm 1.3^{b,c}$	$0.8 \pm 0.1^{a-c}$	$0.9 \pm 0.2^{a-c}$	$0.8 \pm 0.1^{a-c}$	2.0 ± 1.6^{c}	$1.5 \pm 0.1^{b,c}$	1.7 ± 0.7^{c}	$0.3 \pm 0.2^{a,b}$	0.0 ± 0.0^{a}	$1.2 \pm 0.2^{a-c}$	$0.7 \pm 0.1^{a-c}$	$1.5 \pm 1.2^{b,c}$	1.0 ± 0.6
Ammonia	2.1 ± 0.5^{a}	2.1 ± 0.2^{a}	2.2 ± 0.3^{a}	$2.5 \pm 0.1^{a,b}$	2.1 ± 0.4^{a}	1.8 ± 0.2^{a}	2.0 ± 0.1^{a}	4.6 ± 3.2^{c}	$4.2 \pm 0.3^{b,c}$	$2.3 \pm 0.4^{a,b}$	1.9 ± 1.2^{a}	$3.0 \pm 0.7^{a-c}$	2.4 ± 0.9
B-Alanine	1.0 ± 0.6^{a}	0.4 ± 0.6^{a}	1.5 ± 1.0^{a}	0.4 ± 0.6^{a}	$2.7 \pm 1.2^{a,b}$	$3.1 \pm 3.0^{a,b}$	2.3 ± 2.5^{a}	5.8 ± 4.1^{b}	$3.1 \pm 0.5^{a,b}$	$3.2 \pm 1.7^{a,b}$	1.2 ± 0.5^{a}	1.4 ± 0.9^{a}	2.0 ± 1.5
Aminoisobutyric	0.4 ± 0.6^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	2.9 ± 4.2^{b}	0.6 ± 0.8^{a}	0.7 ± 1.1^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.2 ± 0.3^{a}	0.4 ± 0.8
Aminobutyric Acid	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.1 ± 0.2^{a}	0.2 ± 0.3^{a}	2.3 ± 3.0^{b}	0.1 ± 0.1^{a}	0.5 ± 0.7^{a}	$0.7 \pm 0.6^{a,b}$	0.4 ± 0.2^{a}	$1.7 \pm 1.1^{a,b}$	$1.2 \pm 0.3^{a,b}$	0.5 ± 0.8
1-Methylhistidine	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	$0.1 \pm 0.0^{a,b}$	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.3 ± 0.2^{c}	1.0 ± 0.0^{d}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	$0.2 \pm 0.1^{b,c}$	0.1 ± 0.3
3-Methylhistidine	$1.8 \pm 2.6^{a,b}$	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	2.4 ± 2.2^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.3 ± 0.4^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	$1.0 \pm 0.4^{a,b}$	$0.7 \pm 0.2^{a,b}$	0.2 ± 0.1^{a}	0.5 ± 0.8
Ttotal	754.2 ± 6.4	596.5 ± 54.8	647.6 ± 37.6	730.6 ± 92.3	1538.4 ± 41.3	1019.4 ± 62.0	1242.6 ± 37.5	656.7 ± 160.9	925.2 ± 93.8	964.4 ± 88.9	1106.9 ± 174.2	983.2 ± 64.3	858.9 ± 277.6

* Values in the same column with different superscripts are significantly different at $p < 0.05$ (n = 10).

with high levels of EPA was higher than the control. Liu *et al*. [30] also noted that the sea urchin *P. miliaris*, having been fed a diet rich in C23:0, showed an increase of C23:0 in the gonad lipids. C14:0 and C16:0 are abundant in sea urchin gonad and they are usually initiators of the synthesis of many long-chain fatty acids, making them valuable energy sources during the reproductive period. In this study, C14:0 and C16:0 were higher from spring to summer and the total SFA level showed a similar trend. These results indicate that the reproduction period of Taiwan's sea urchin *T. gratilla* is from spring to summer. For organisms, saturated fatty acids are important for reproduction regulation, and they play a role in physiological functions. Fatty acids can offer energy for sea urchin growth during the reproductive season and can protect juveniles from oxidative damages [39, 45]. However, unsaturated fatty acids such as C20:5n3 (EPA) and C20:4n6 were also found in abundance in the sea urchin gonads in this study. Recently, Liyana-Pathirana *et al*. [31] also reported C20:4n6 and EPA as major PUFAs in the sea urchin *S. droebachiensis* gonad. Other results suggest that sea urchins can self-synthesize C20:4n6 or EPA, even though their diets are deficient in of those nutrients [10, 28]. González-Durán *et al*. [17] suggested that *S. droebachiensis* gonad can elongate and desaturate C18:2n6 to C20:4n6 and C18:3n3 to C20:5n3. Unsaturated fatty acids

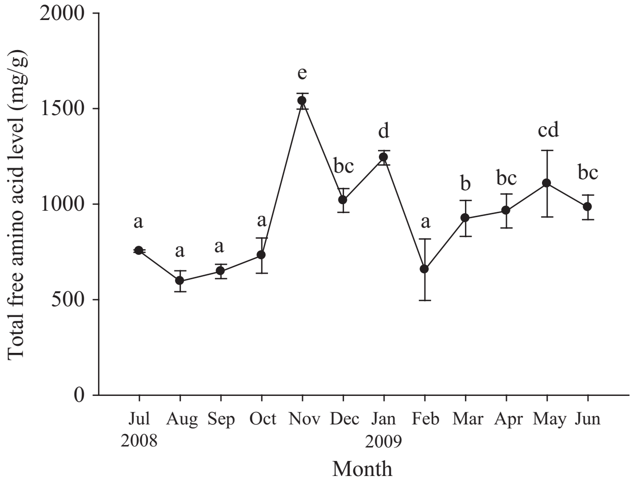

Fig. 5. The seasonal change of total free amino acid level of the *Tripneustes gratilla* gonad. * Values with different superscripts are significantly different at $p < 0.05$ (n = 10).

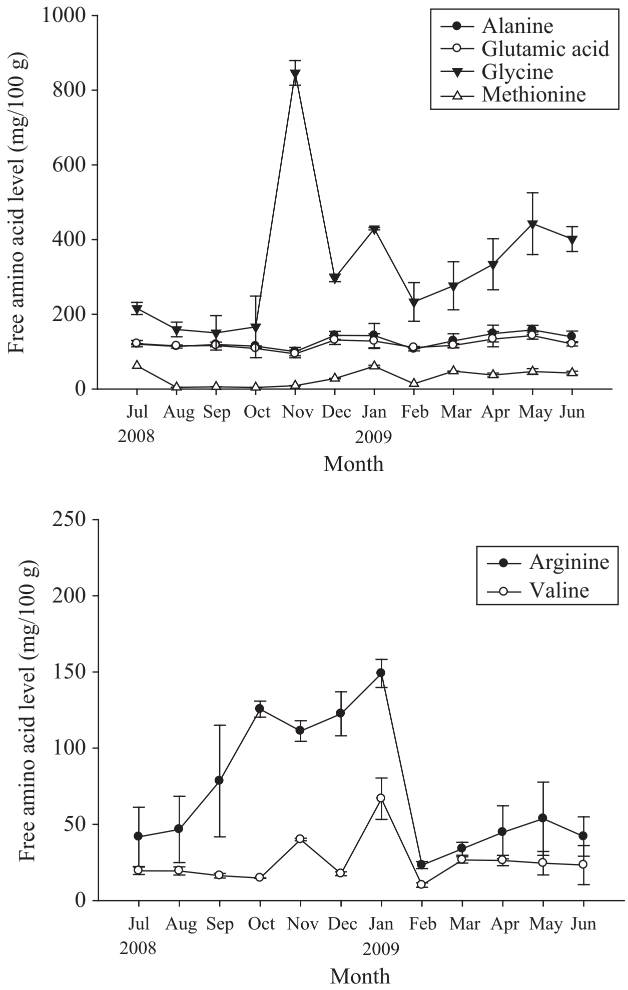

Fig. 6. The seasonal change of free amino acid level of alanine, glutamic acid, glycine, methionine, arginine and valine in gonad of *Tripneustes gratilla*.

play important roles in many aspects in invertebrates, for instance, in osmotic regulation and as a regulator of fertility. Another report indicated that long-chain unsaturated fatty acids are important for sea urchin gamete development and the accumulation of energy for juveniles [34]. Moreover, PUFAs can protect sea urchins from oxidative damage [9]. Other functions of unsaturated fatty acids, such as EPA, may regulate the osmosis in sea urchins [11], and arachidonic acid may affect Na^+/H^+ exchange and neutral amino acid transport in sea urchin eggs [7].

This study analyzed the content of FAA, and indicated that glycine was the most abundant FAA in Taiwan's sea urchin *T. gratilla*, the contents of other FAA like glutamic acid, alanine, proline, arginine and lysine were also abundant in this study. FAA mainly affect the flavor and physiological functions of seafood [18, 19], and FAA content was affected by many factors like seasonal change and diets [35]. In purple sea urchin *Anthocidaris crasispina*, glycine content was higher in artificial diet group than wild species, FAA such as valine, methionine, lysine and arginine were also increased, but aspartic acid, glutamic acid, proline and alanine had no significant change [34]. In developing stage, the glycine content was increased during mature stage, but valine, alanine, histidine, lysine and arginine were decreased in this period [33]. In other research indicated the glycine content was decreased in green sea urchin *Hemicentrotus pulcherrimus* during mature stage, which means the FAA content will differed between species. In this study, the FAA content of glycine started to decrease in June, and the lowest value was found in September. This result suggested the glycine content was decreased in Taiwan's sea urchin *T. gratilla* when the reproduction season was coming. Glycine content can also reflect the environment osmotic pressure [48]. In this study, glycine content was suddenly increased in November. Considering the rainfall from September to December in Taiwan, the rainfall in late October is significantly decreased, it suggested that sea urchin may regulate the glycine content to prevent from dehydrating in a high osmotic pressure environment. Other FAA like valine, histidine, lysine, thronine, glutamic acid, methionine, isoleucine, leucine and tyrosine were served as a energy source for gametogenisis or peptide and protein synthesis [33]. Free amino acid content also affect the flavor of sea urchin gonad, Fuke and Ueda [14] indicated the main flavor effective components of FAA in sea urchin gonad are glycine, glutamic acid, alanine, methionine, valine and arginine. In this research, glycine, glutamic acid, alanine and methionine, which offer umami, sweetness and aftertaste, were higher in summer. On the other hand, valine and arginine, which offer bitterness taste, were lower in summer but higher in winter.

V. CONCLUSIONS

In conclusion, the fatty acid and FAA compositions from the gonad of sea urchin *T. gratilla* varied with season. The total fatty acid level was higher from spring to summer and generally decreased from autumn. Free amino acid level was higher in spring and decreased in summer. Considering the factor of GI content, it indicates the mature stage of sea urchin *T. gratilla* is from May to August, and the gonad color is more acceptable in this period. But in August to September, the GI content was significantly decreased. Therefore, it offers a consult for consumer that the better period for consuming sea urchin *T. gratilla* gonad is from May to July.

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