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A Lipidomics Study of Malaysian Sea Urchin Gonads: Species Influence on Lipid Composition

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ABSTRACT

Sea urchin is always touted as a promising alternative marine source for dietary essential fatty acids. Often overlooked is that their lipid composition can vary significantly depending on the species and their feeding preferences. Understanding that every sea urchin species, together with their feeding behaviour, may offer unique nutritional benefits helps to optimise the utilisation of sea urchin as a reliable source of marine fatty acids. This study aimed to measure the gonad index, lipid content, and composition in the gonads of two sea urchin species live in coral reef and seagrass habitats in Malaysia. The gonad's lipid was extracted using the Folch extraction method, followed by the esterification process before being analysed using GC-FID. The gonad index was calculated based on the percentage of gonads relative weight as a proportion of total body weight. The gonad indexes of both *Diadema setosum* (Leske, 1778) 6.74 ± 2.49% and *Salmacis sphaeroides* (Linnaeus, 1758) 6.68 ± 2.30% were found to be statistically indifferent ($p>0.05$). Both *D. setosum* and *S. sphaeroides* had a negative allometric growth ($b < 3$). The major saturated fatty acids found in both species were C16:0 and C14:0 while the major unsaturated fatty acids (UFAs) were C16:1 and C18:3. In the gonads of both species, n-6 UFAs were the most abundant, followed by n-9 and n-3 UFAs. In *D. setosum*, n-6 UFAs comprised 74.77%, n-9 UFAs 21.76%, and n-3 UFAs 3.47%. In contrast, *S. sphaeroides* had 52.13% n-6, 22.48% n-9, and a notably higher proportion of n-3 UFAs at 25.39%. *S. sphaeroides* lacked two types of fatty acids that were present in *D.*

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setosum, specifically C17:1 (heptadecenoic acid) and C20:1n-9 (gondoic acid). This study reveals the different composition of fatty acids in the gonads of *D. setosum* collected from coral reef areas and *S. sphaeroides* collected from seagrass areas of Malaysian water. Recognising these differences is crucial when evaluating sea urchins as a promising alternative source of healthy dietary fats.

Keywords: *Diadema setosum*, fatty acids, gonad index, *Salmacis sphaeroides*

INTRODUCTION

Echinoderms such as sea urchins are recognised as an important source of various metabolites which is important for human biological activities (Zhukova, 2022). Accumulation of fatty acids in sea urchin gonads has drawn attention to it as a promising source of essential fatty acids. The gonad consists of two types of cells, reproductive cells and storage cells known as nutritive phagocytes. The nutritive phagocytes store proteins, carbohydrates, and fatty acids for gametogenesis (Kumari et al., 2013). Polyunsaturated fatty acids are vital components that not only determine the nutritional value of sea urchins but also ensure optimal growth and reproduction of sea urchins (Kabeya et al., 2017). Information on the fatty acids composition of Malaysian sea urchins is lacking even though their numbers are abundant locally.

Fatty acids are an important group of dietary components for humans and animals. Omega-3 and Omega-6 polyunsaturated fatty acids (PUFAs) for example, cannot be synthesised in the body and must be supplemented through diet for normal physiological functions (Marcus, 2019). The majority of global polyunsaturated fatty acids (PUFAs) are obtained from marine oily fish such as salmon, sardines, herring, and mackerel. Unfortunately, the availability of PUFAs derived from marine ecosystems is decreasing due to the combined effects of overfishing and climate change (Hixson & Arts, 2016). Wild fisheries are failing to keep up with global population growth, with many declining exponentially (Hicks et al., 2019). Modelling predicts a 10–58 % loss of docosahexaenoic acid (DHA) availability from capture fisheries by 2100 as higher temperatures suppress algal PUFA production and the deficit transfers up the food web (Colombo et al., 2020). While aquaculture attempts to address this shortfall, they are also heavy users of PUFAs supplies. Surprisingly, there is a significant disparity between PUFAs' supply and demand, with estimates indicating a deficit in the range of million tonnes (Jovanovic et al., 2021). Experimental work further shows that juvenile salmon fed PUFA-deficient diets under warm conditions suffer reduced somatic growth and mitochondrial efficiency (Závorka et al., 2021).

Sea urchins present a contrasting, potentially climate-robust source of long-chain PUFAs. Unlike most finfish, echinoids possess Δ5/Δ8 desaturase pathways that enable de novo synthesis of C20 PUFAs from shorter C18 precursors, thereby buffering their PUFA output against fluctuations at the base of the food web (Kabeya et al., 2017). Several species also exhibit genetic or physiological capacity to maintain gamete quality and fatty-acid-rich gonads under near-future pH and temperature scenarios (M. W. Kelly et al., 2013; Uboldi et al., 2023). Moreover, urchins occupy low trophic levels and can be reared on macroalgal, microalgal or plant-based feeds, minimising reliance on dwindling fish-oil resources. These traits position sea urchins as a complementary, and possibly more resilient provider of nutritionally important PUFAs as climate change progresses. Data on the fatty acids composition of the sea urchin gonad is vital in determining the nutritional and economic value of local sea urchin species (Anedda et al., 2021; Chen et al., 2010).

Previously, Parvez et al., (2016) reported several species of sea urchin in Malaysian waters, including *Diadema setosum* (Leske) and *Salmacis sphaeroides* (Linnaeus). The study of the lipid composition of the two selected sea urchin species, *D. setosum* and *S. sphaeroides*, is driven by their different habitats and diet preferences. Understanding these species' lipid composition is critical since it directly correlates with their diets in the chosen environments. *D. setosum* is common in Malaysian tropical waters. It is distinguishable for having long black spines, orange coloured anus ring with blue or green spots on genital plates that are different from other sea urchins. *D. setosum* is commonly found in various parts of Malaysia, particularly in rocky areas, coral reefs, and artificial reefs. In contrast, *S. sphaeroides* is typically associated with seagrass meadows, especially in the Straits of Johor. It has very different physical characteristics compared to *D. setosum* because it has a white-greenish shell body, and its spines are very short. The main purpose of the present paper is to describe fatty acids composition of *D. setosum* and *S. sphaeroides* collected from different habitat in Malaysian water. Moreover, we discuss the gonad index and length-weight relationship of both species to understand their growth pattern.

MATERIALS AND METHODS

Study Area

Diadema setosum samples were collected from the waters of Pulau Hujung in the Johor district of Mersing (2˚29’22” N; 103˚56’55.4” E; labelled 1 in Figure 1). This species is commonly found on coral reefs. *Salmacis sphaeroides*, on the other hand, was discovered in Tanjung Adang on the Johor Strait (1˚19’49.1” N; 103˚33’52.1” E; labelled 2 in Figure 1). This species was discovered scattered within the shoal seagrass area.

Sample Collection and Preparation

A total of 48 *D. setosum* individuals were collected in the coral reef area, and 37 *S. sphaeroides* individuals were collected in the seagrass meadows. Samples were then brought back to the laboratory in ice-filled containers for further analysis.

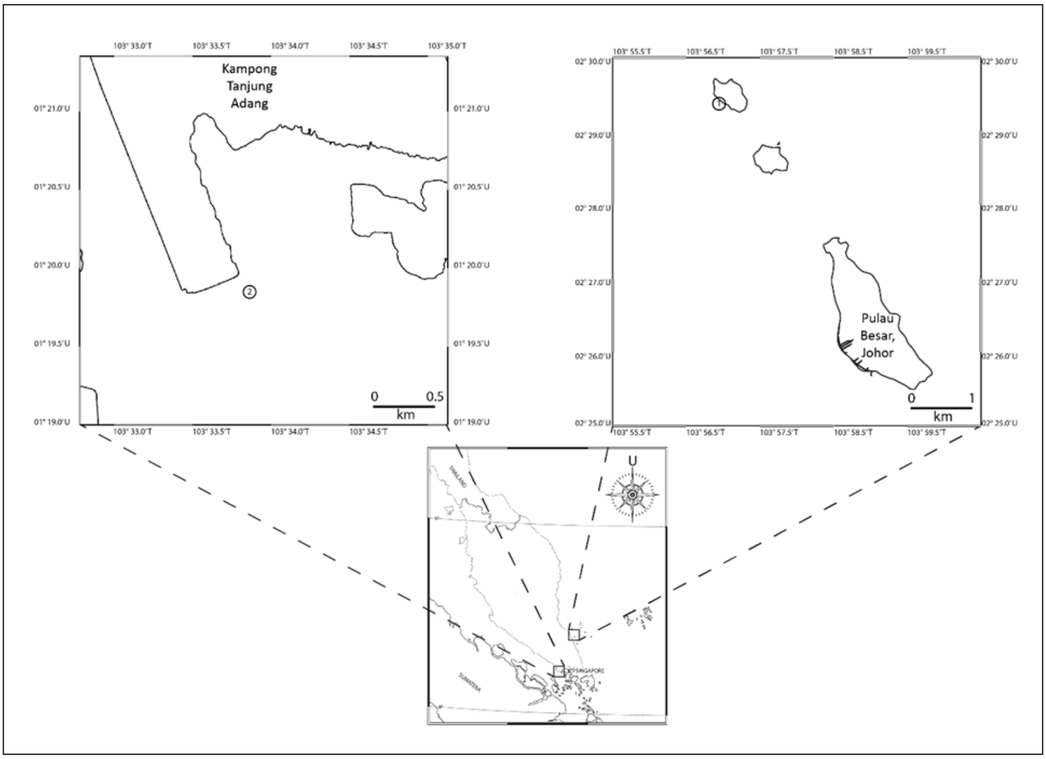

Figure 1. Map of sea urchins collection; (1): Pulau Hujung, Johor, where *Diadema setosum* samples are collected; (2): Tanjung Adang, Johor, where *Salmacis sphaeroides* are collected

Gonad Index

Each sea urchin was processed individually. Test length and body wet weight (including spines) were recorded first. The test was then opened, the gonads excised and weighed. All measurements were recorded on the same line of the data sheet, ensuring that test length, total wet weight and gonad wet weight corresponded unambiguously to the same specimen. Sub-samples of gonad tissue were retained for lipid and fatty-acid analyses. The GI of the sea urchins was calculated as a ratio of gonad wet weight over body wet weight (Grant & Tyler, 1983).

$$\text{Gonad Index (GI)} = \frac{\text{Gonad wet weight (g)}}{\text{Body wet weight (g)}} \times 100$$

The length-weight relationship was calculated using the formula below:

$$W = aL^b$$

where,

W = weight (g),
L = length (cm),
a and b = regression coefficient and slope respectively.

Lipid Extraction and Fatty Acid Analyses

The lipid content of gonad samples was determined using the Folch extraction method (Folch et al., 1957), which involved diluting homogenised oven-dried samples in chloroform/methanol (2:1 v/v) solutions, followed by lipid separation using a rotary evapourator. For both *D. setosum* and *S. sphaeroides*, dried gonads from three randomly selected individuals (sex-independent) were pooled to obtain 100 g dry mass, constituting one analytical replicate; this procedure was performed three times, yielding three independent replicates per species (Ghisaura et al., 2016). The end weight of the Folch extractions was used to calculate the total lipid content of the gonad samples. The lipid samples were then transesterified with a sodium methoxide-hexane mixture. The FAME samples were then analysed with a Shimadzu Gas Chromatography GC-2010 (Japan) equipped with a Flame Ionisation Detector (FID) and auto sampler using a standard Supelco 37 component FAME mix (Sigma Aldrich, Germany). The thickness of the film, length, and diametre of the chromatography column were 0.25 µm, 30 m, and 0.32 µm, respectively. Helium was used as the carrier gas at a flow rate of 40 cm^3min^{-1} at 250 ˚C for optimum fatty acids peak reading.

Statistical Analysis

The Shapiro-Wilk test was used to calculate data normality, and the t-test was used to compare the differences between *D. setosum* and *S. sphaeroides*. The Pearson correlation coefficient was used to assess the strength of the correlation between the two species' gonad index and fatty acid composition. MINITAB 7.0 software was used for statistical analysis as well as to generate length and weight frequency graphs, while Origin 6.0 software was used to compute the sea urchin's length-weight relationship. All data were presented in the form of mean ± standard deviation.

RESULTS AND DISCUSSION

Gonad Index

Table 1 shows the results of this study for gonad index (GI), wet weight, diametre, and gonad weight. The GI of *D. setosum* (6.74 ± 2.49%) and *S. sphaeroides* (6.68 ± 2.30%) did not differ significantly ($p > 0.05$), but the average wet weight and test diametre of *S. sphaeroides* were significantly higher than those of *D. setosum*. The average wet weight of S. *sphaeroides* was more than twice that of *D. setosum*, and its test diametre was significantly larger ($p < 0.01$). The gonad wet weight of *S. sphaeroides* was greater than that of *D. setosum*.

Table 1
Size, wet weight, weight of gonad, and gonad index (mean ± standard deviation)

Species	Gonad Index (%)	Average Wet Weight (g)	Average Test Diameter (mm)	Average Gonad Wet Weight (g)
Diadema setosum	6.74 ± 2.49	36.53 ± 13.51	44.52 ± 6.01	2.33 ± 0.94
Salmacis sphaeroides	6.68 ± 2.30	83.37 ± 23.40	60.70 ± 3.45	5.58 ± 2.54

Both *D. setosum* and *S. sphaeroides* had a negative allometric growth ($b < 3$) (Figure 2), which is consistent with the findings of Khong et al. (2012), Choo et al. (2021) and Vafidis et al. (2021). Sea urchin test diametre grows faster than its weight, possibly indicating an adaptation to quickly reach a larger size and reduce vulnerability to fish predation. *D. setosum* had an average wet weight of 36.53 ± 13.51 g and a test diametre of 44.52 ± 6.01 mm (Figure 3(a)(b)). The gonads collected from *D. setosum* had an average wet weight of 2.33 ± 0.94 g and were mostly pale yellow. Meanwhile, *S. sphaeroides* had an average wet weight of 83.37 ± 23.40 g and an average test diametre of 60.70 ± 3.45 mm (Figure 3(c)(d)). *S. sphaeroides* had a higher average gonad wet weight than *D. setosum*, at 5.58 ± 2.54 g. *S. sphaeroides*' gonad colour was mostly pale yellow. Pale yellow gonads indicate maturity of the gonads (Kobayashi & Nakamura, 1967).

Nutritive phagocytes in sea urchin gonads serve as nutrient storage for reproduction. During the spawning cycle, the gonad index serves as an indicator of gonad maturation and development (Amytaz et al., 2013; Jinadasa et al., 2016; Kaneko et al., 2012). In this study, the average body size of *D. setosum* was smaller than in Chen et al.'s (2010) study in Hong Kong waters. Despite nearly identical individual weights, the *S. sphaeroides* size in this study was larger than that in Chen et al. (2010).

Differences in the size of the test diametre and body weight of this organism may be due to the availability of food in the area or competition with other individuals (Foster et al., 2015; Levitan, 1988, 1991; Minor & Scheibling, 1997). Tanjung Adang, Johor has a different availability of macroalgal than Pulau Hujung, Johor. Tanjung Adang's seagrass meadow supports a dense algae canopy, with quadrat surveys reporting 83–100% cover dominated by rhizophytic and calcareous genera such *as Caulerpa, Halimeda, and Gracilaria* (Zakaria et al., 2019). By contrast, Reef Check observations at Pulau Hujung show nutrient-indicator algae were present on 2.8% of the surveyed reef substrate, indicating a very sparse fleshy-algae cover (Reef Check Malaysia, 2025). Shunula and Ndibalema (1986) stated that sea urchin preferred several species of algae such as *Galaxaura oblongata* ((J.Ellis & Solander) J.V. Lamouroux), *Padina gymnospora* ((Kützing) Sonder), *Halymenia* *venusta* (Børgesen), *Dictyota sp*. (J.V. Lamouroux), and *Eucheuma striatum* (F.Schmitz). As a result, the gonad index of both species may differ due to dietary differences.

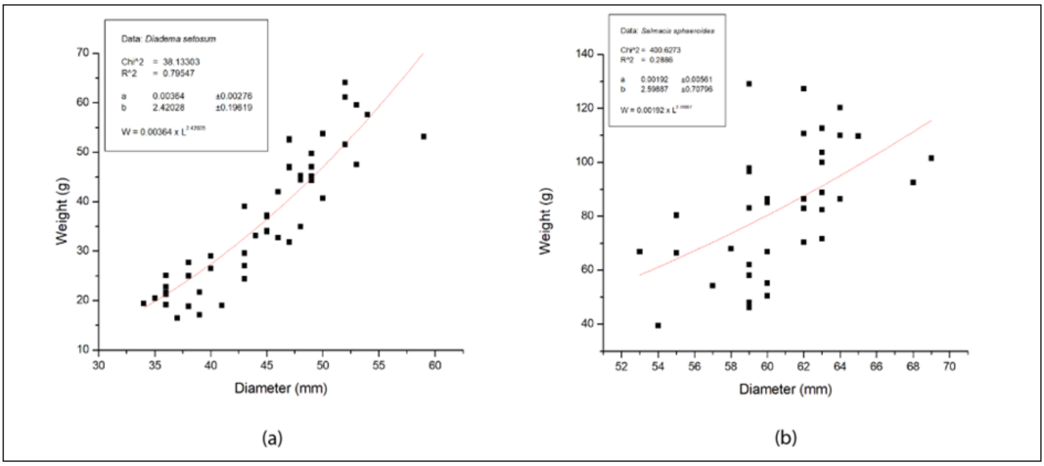

Figure 2. (a) Length-weight relationship of *D. setosum* (b<3); (b) Length-weight relationship of *S. sphaeroides* (b<3)

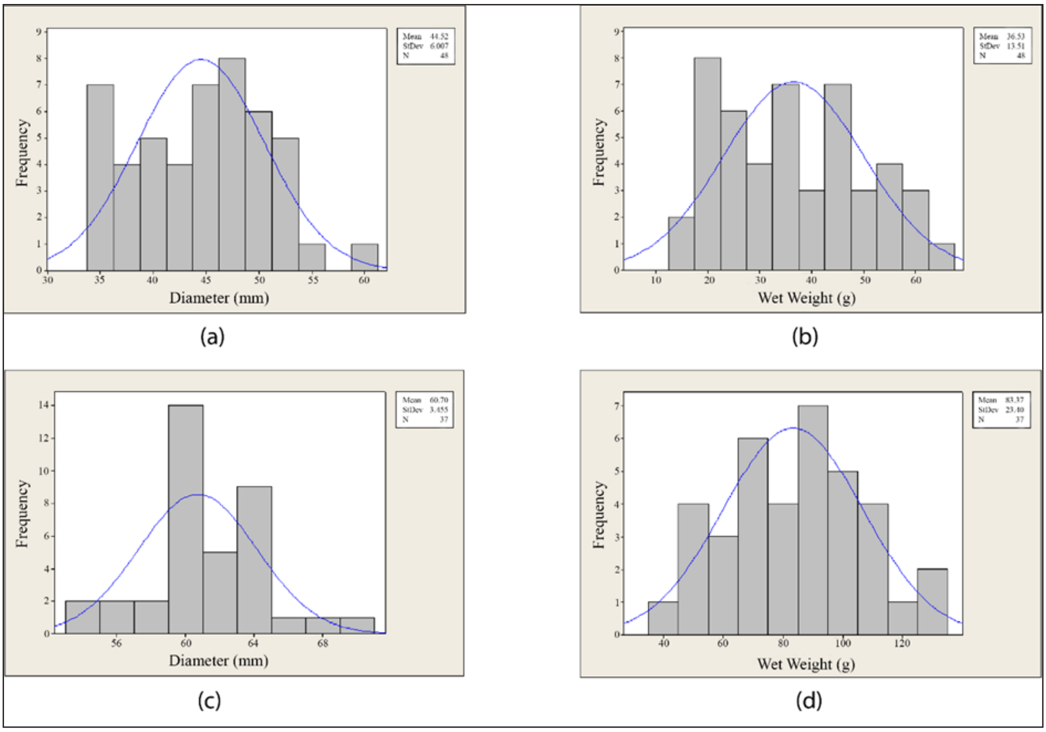

Figure 3. (a) Length frequency of *D. setosum*; (b) Weight frequency of *D. setosum*; (c) Length frequency of *S. sphaeroides*; (d) Weight frequency of *S. sphaeroides*

Water temperature fluctuations have also been shown to influence the development of marine organisms (Hernandez et al., 2020). Sea urchins *Paracentrotus lividus* reared at temperatures higher than 22 °C had lower somatic and gonadal growth, as well as slower gonad maturation (Santos et al., 2020). The average water temperature in Malaysia is around 28 to 30°C all year, while in Hong Kong and Nagasaki, it is between 18 to 28°C and 16 to 25°C, respectively. In this study, the average gonad index of *D. setosum* was lower than that of the same species in Hong Kong and Nagasaki waters. As reported by Chen et al. (2010), the average diametre (66.1 mm) and individual weight of *D. setosum* (142.9 g) in Hong Kong waters showed a higher gonad index of 11.9% compared to *S. sphaeroides* (4.5%). Furthermore, the gonad index of *D. setosum* in Nagasaki is 12.9% (Kaneko et al., 2012), which is comparable to the range found in Hong Kong water. The variation in GI values may be due to differences in environmental conditions such as habitat environment and diet (Chen et al., 2010). Sea urchin reproductive patterns vary greatly depending on their geographical location, with moonlight, tidal cycles, urchin age and size, and food availability all having an impact on gametogenesis and spawning behaviour. In tropical locations, the species reproduces throughout the year, with varied peak seasons. In temperate places, spawning occurs during the summer, most likely because of seawater temperatures above 25°C, which cause gametogenesis (Vafidis et al., 2021). The spawning season factor can also influence the gonad index value. A higher gonad index suggests that the gonad is maturing and spawning (Bronstein et al., 2016). Alsaffar and Lone (2000) suggested that temperature could influence sea urchin gametogenesis, particularly in areas with high temperature fluxes.

Lipid Compositions

The total lipid in the gonad was higher in *D. setosum*, at 0.17 ± 0.02 g per 1.26 ± 0.46 g of gonad dry weight, than in *S. sphaeroides*, at 0.10 ± 0.03 g per 0.51 ± 0.12 g of gonad dry weight. However, *S. sphaeroides* had higher lipid per dry weight than *D. setosum*, with ratios of 0.20 g/g and 0.14 g/g, respectively, but the difference was not significant ($p > 0.05$). The differences in lipid content in the gonads of *S. sphaeroides* and *D. setosum* observed in this study could be attributed to differences in habitat and food availability in the surrounding area. A diet high in protein, lipids, and energy promotes healthy somatic and gonadal growth (Carboni et al., 2013). According to Arafa et al. (2012), animal lipid composition is not fixed, and diet and growth can have a significant impact on fatty acid profile. Furthermore, seasonality, water temperature, breeding cycles, and the quality and availability of food in the area all influence the biochemical composition of the gonad (Anedda et al., 2021; Arafa et al., 2012; Hughes et al., 2005). Sea urchins are extremely sensitive to environmental changes, and they tend to migrate vertically to a deeper area to avoid unsuitable environmental conditions (Muthiga & McClanahan, 2013). The breeding activities of sea urchins are poorly understood, and few studies have been conducted, particularly in tropical areas. As a result, little information is available to determine the factors influencing lipid content in sea urchin gonads in Malaysian waters.

The lipid composition of *D. setosum* and *S. sphaeroides* differed slightly. C17:1 and C20:1n-9 fatty acids were not found in *S. sphaeroides* but were found in *D. setosum* (Table 2). The composition of saturated fatty acids (SFA) in the gonads of *S. sphaeroides* was similar to *D. setosum*, but the concentration of fatty acids was quite different. *S. sphaeroides* had a higher SFA concentration than *D. setosum*, according to this study. In both species' gonads, the main SFA composition was C16:0 and C14:0 fatty acids, with C16:0 being more dominant (>50%).

The composition of saturated and unsaturated fatty acids was discovered to be different in both species (Figure 4). The average concentration of unsaturated fatty acid (UFA) in *D. setosum* gonads was 46.16%. C20:4n-6 was the dominant UFA concentration in the gonads of this species by 19.55%. The monounsaturated fatty acid (MUFA) compositions in the gonads of *D. setosum* were C14:1, C16:1, C17:1, C18:1n-9, and C20:1n-9, while the

Table 2
Lipid compositions in the gonad of D. setosum *and* S. sphaeroides

Fatty acids (common name)	**Concentration (%)**	
	Diadema setosum	***Salmacis sphaeroides***
C6:0 (Caproic acid)	0.23 ± 0.07	2.47 ± 1.88
C12:0 (Lauric acid)	0.08 ± 0.02	0.06 ± 0.02
C13:0 (Tridecylic acid)	0.05 ± 0.01	0.1 ± 0.09
C14:0 (Myristic acid)	6.3 ± 1.60	16.2 ± 3.57
C14:1 (Myristoleic Acid)	0.08 ± 0.01	0.04 ± 0.04
C15:0 (Pentadecylic acid)	1.65 ± 0.10	1.77 ± 0.29
C16:0 (Palmitic acid)	34.1 ± 3.94	46.91 ± 5.85
C16:1 (Palmitoleic acid)	7.92 ± 0.40	4.22 ± 2.95
C17:0 (Margaric acid)	1.31 ± 0.07	1.13 ± 0.27
C17:1 (Heptadecenoic acid)	0.08 ± 0.03	ND
C18:0 (Stearic acid)	5.69 ± 0.42	9.54 ± 1.64
C18:1n-9 (Oleic acid)	4.46 ± 0.40	2.85 ± 2.09
C18:2n-6 (Linoleic Acid)	4.37 ± 0.84	2.2 ± 0.93
C18:3n-6 (γ-Linolenic acid)	1.41 ± 0.26	0.54 ± 0.47
C18:3n-3 (α-Linolenic acid)	1.32 ± 0.46	3.22 ± 1.37
C20:0 (Arachidic acid)	4.42 ± 0.48	4.89 ± 5.52
C20:1n-9 (Gondoic acid)	3.83 ± 0.32	ND
C20:2 (Eicosadienoic acid)	3.16 ± 0.27	1.11 ± 1.04
C20:4n-6 (Arachidonic acid)	19.55 ± 4.27	2.76 ± 4.25

Note. ND = Not detected

PUFA composition was C18:2n-6, C18:3n-6, C18:3n-3, C20:2, and C20:4n-6 (Table 2). The MUFA concentration of *D. setosum* (16.37%) was lower than the PUFA concentration (29.79%) (Figure 4). Meanwhile, the concentration of UFA in *S. sphaeroides*' gonads was lower than that of *D. setosum*, which was 16.95%. The MUFA concentration (16.37%) was lower than the PUFA concentration (29.79%). Meanwhile, the concentration of UFA in *S. sphaeroides*' gonads was lower than that of *D. setosum*, which was 16.95%. Both C17:1 and C20:1n-9 were not found in *S. sphaeroides* gonads.

D. setosum and *S. sphaeroides* gonads have very different UFA compositions and concentrations. This study discovered that *D. setosum* contained more UFA than *S. sphaeroides*. C20:4n-6 and C16:1 were the major UFAs in the *D. setosum* gonads, with C20:4n-6 being the more dominant. In the gonads of *S. sphaeroides*, the major UFAs were C16:1 and C18:3n-3. Omega-6 (n-6) fatty acids were found in higher concentrations in the gonads of both species, followed by omega-9 (n-9) and omega-3 (n-3) fatty acids (Figure 5). In *D. setosum*, n-6 UFA is the major component of UFAs at 74.77%, followed by 21.76% of n-9 UFA and 3.47% n-3 UFA. On the other hand, *S. sphaeroides* was found to

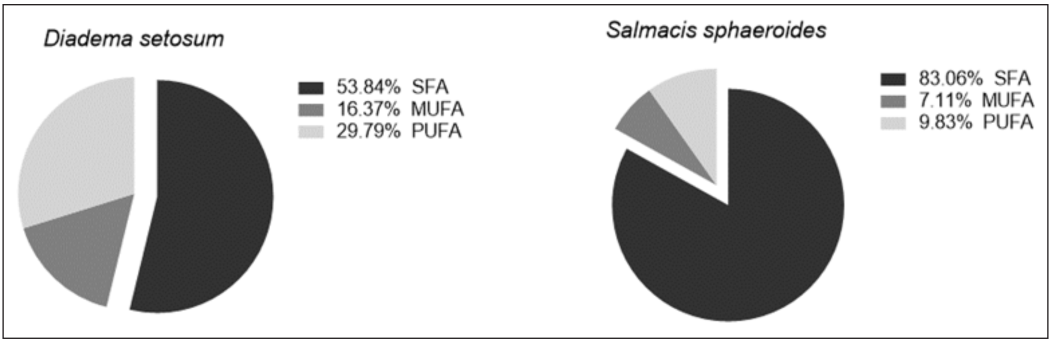

Figure 4. The composition of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in the gonad of *D. setosum* and *S. sphaeroides*, respectively

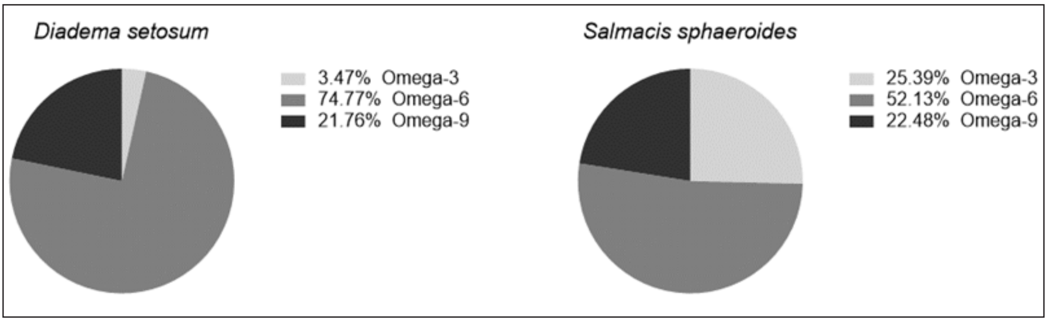

Figure 5. The composition of *unsaturated fatty acids* (UFAs) omega class in the gonad of *D. setosum* and *S. sphaeroides*, respectively

have different pattern of UFA composition. The UFA composition reveals that n-6 PUFA make up half of the overall composition, while n-3 and n-9 UFAs each contribute to a quarter of the total.

The lipid composition of sea urchin gonads is frequently linked to its nutritional diet and the biochemistry of its environment. Carboni et al. (2012) found that different macroalgal diets had a significant effect on sea urchin growth, which they attributed to the nutritional properties of the diet. He also claimed that the lipid composition of its larvae could indicate specific lipid uptake during the embryonic developmental stages. *D. setosum* and *S. sphaeroides* have slightly different lipid compositions because C17:1 and C20:1n-9 were not found in *S. sphaeroides* gonads but were present in *D. setosum*. These two fatty acids were known for their medicinal properties. Interestingly, heptadecenoic acid (C17:1) and its salts have been described as high-value-added compounds known for their anti-inflammatory effects and their efficacy in treating prophylaxis, psoriasis, allergies, and autoimmune diseases (Al Sahyouni et al., 2022). Fan et al. (2022) discovered that gondoic acid (C20:1n-9) can reduce reactive oxygen species levels and inhibit the PKCθ/ERK/STAT3 signalling pathways, thereby combating liver inflammation. The difference could be due to the availability of food in the area. Individual sea urchins collected in the Mediterranean region and those collected on the Atlantic coast had significantly different gonad lipid profiles (Martínez-Pita et al., 2010).

In the gonads of *D. setosum* and *S. sphaeroides*, the SFA is dominant. SFA like C16:0 and C14:0 found in the gonads of these two species are very similar to those found in other echinoids. C16:0, also known as palmitic acid, is a type of SFA that is found in both animals and plants. Many studies have been conducted to explain the biochemical composition of PUFA in sea urchins, which primarily consists of a-linoleic acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acid, and arachidonic acid. It helps the organisms' normal growth and reproduction, and it is an important nutrient in determining the nutritional value of their gonads (Ahmed et al., 2021; Wang et al., 2020). Arachidonic acid (ARA) C20:4n-6 was the dominant UFA concentration in the gonads of *D. setosum*. Dietary ARA has been relatively disregarded due to the emphasis on DHA and EPA. However, over the last two decades, research on ARA has intensified, revealing its critical involvement in growth, reproduction, stress tolerance, pigmentation, immunology, lipid deposition, and bone formation (Xu et al., 2022). ARA is a naturally occurring antiviral molecule whose absence may increase vulnerability to COVID-19 (Hoxha, 2020). ARA is the most abundant long chain PUFA in human milk, and it, along with DHA, is important as a neuroprotective nutrient throughout the life cycle to prevent neurodegenerative diseases associated with ageing (Salem & Van Dael, 2020; Sambra et al., 2021).

Liyana-Pathirana et al. (2002) reported that PUFAs content in the gonad increased during cold climatic conditions with a simultaneous decrease in saturated fatty acids (SFAs) content and conversely during the summer season. Moreover, the differences between SFAs and PUFAs content might be due to the adaptation of these organisms to prevent extreme desiccations due to the warm environment. In this study, the SFAs content in gonads of both sea urchins was higher than 50% in total lipid. This condition is due to the tropical area that always receives maximum sunlight, but the environmental temperature of the environment is not directly affected. The effects of changes in water temperature are still unknown, but the change in photoperiod may correspond to the changes in the total lipid content (Anedda et al., 2021; Hernandez et al., 2020).

Based on a past study by J. R. Kelly et al. (2008), coralline algae contain higher levels of C16:0 and C20:5n-3 other than algal diets from *Agarum clathratum*, *Desmaretia viridis*, and *Saccharina longicrucis*. Hence, the difference of macroalgal populations in the area can also be one reason that the sea urchins yield different lipid compositions because coral reefs area has more coralline algae than non-calcifying algae and vice versa in seagrass meadows area (Liyana-Pathirana et al., 2002; Martínez-Pita et al., 2010). In detail, a total of 23 macroalgal genera have been reported at Tanjung Adang (Zakaria et al., 2019). While no formal macroalgae checklist exists specifically for Pulau Hujung, a comparison between the macroalgal composition of the nearby Pulau Seri Buat-Sembilang and Tanjung Adang revealed both overlapping and distinct taxonomic structures at the genus level (Zaifornoor et al., 2024; Zakaria et al., 2019). A total of nine genera were shared between the two sites, including *Caulerpa*, *Halimeda*, *Avrainvillea*, *Acanthophora*, *Amphiroa*, *Cladophora*, *Polysiphonia*, *Gracilaria,* and *Jania*. Pulau Seri Buat-Sembilang exhibited unique genera such as *Acetabularia*, *Ceratodictyon*, *Ceramium*, and *Veleroa*, while Tanjung Adang supported distinctive genera including *Bryopsis*, *Chaetomorpha*, *Codium*, *Ulva*, and *Sargassum*.

Recent studies have shown that dietary macroalgae can significantly influence the lipid profiles of sea urchins, with implications for growth, reproductive health, and aquaculture performance. For example, *Strongylocentrotus droebachiensis* and *S. purpuratus* assimilate EPA and ARA from macroalgal sources such as *Ulva*, *Nereocystis*, and *Pyropia*, integrating these lipids into somatic tissues and faeces (Schram et al., 2018). Juvenile *Tripneustes gratilla* fed brown macroalgae, *Undaria pinnatifida*, exhibited higher PUFA levels, specific growth rate, and feed conversion efficiency compared to those fed green (*Ulva pertusa*) or red (*Gloiopeltis furcata*) macroalgae (Floreto et al., 1996). Furthermore, *Paracentrotus lividus* fed with natural kelp exhibited higher EPA concentrations compared to those fed a pellet diet, while DHA levels were higher in pellet-fed individuals (Carboni et al., 2013). Overall, these findings demonstrate that the choice of macroalgal diet can significantly affect the fatty acid composition of sea urchins. However, due to the lack of detailed data on macroalgal diversity and distribution at the study locations, future research should prioritise mapping the occurrence and fatty acid composition of native macroalgae.

In Martínez-Pita et al. (2010)'s study, the ovaries of *Paracentrotus lividus* (Lamarck) and *Arbacia lixula* (Linnaeus) had higher percentages of 14:0, 16:0, 16:1n-7, 18:2n-6, 18:3n-3 and 18:4n-3 than in the male reproductive organs (testes), and the levels of 18:0, 22:1n-9, 20:4n-6 and 22:5n-3 were found to be lower. These differences may indicate the different needs of males and females during gametogenesis (Poorbagher et al., 2010) so the sea urchins collected in this study were most likely dominated by males than females. The concentration and composition of PUFAs detected in *D. setosum* and *S. sphaeroides* gonad in this study was quite different from other studies, but the concentration of n-6 was high, especially in *D. setosum*. However, we may deduce that changing the food of sea urchins can modify the lipid composition of their gonads. Based on the size and lipid yields obtained in this investigation, these species may be commercially viable.

CONCLUSION

This study examines the physical characteristics and fatty acid profiles of two sea urchin species, *D. setosum* and *S. sphaeroides*, and highlights their potential nutritional benefit. The results show significant differences in average wet weight and test diametre, with *S. sphaeroides* exhibiting substantially higher values than *D. setosum*. Despite the physical size disparities, both species' gonad indexes (GI) were not significantly different, indicating equal reproductive investments. *D. setosum* and *S. sphaeroides* both exhibit a negative allometric growth pattern, with the test diametre increasing faster than the overall weight. This negative allometry is unique to sea urchins and serves as an adaptive technique for rapidly achieving a bigger size, minimising vulnerability to predators.

Sea urchins are well-known for their high lipid content, with a focus on essential fatty acids such as EPA and DHA. However, diet and environmental conditions influence the specific fatty acid composition of their gonads, and sea urchins from different locations may have distinct fatty acid profiles. This study discovered significant differences in the fatty acid profiles of *D. setosum* and *S. sphaeroides*, which reflect their respective habitats and dietary sources. *D. setosum*, which lives on coral reefs, and *S. sphaeroides*, which lives in seagrass meadows, both have distinct fatty acid compositions. The study also highlights the importance of diet and environmental conditions in determining the fatty acid composition of sea urchins. Consistently, prior studies have shown that the type of macroalgae consumed can significantly influence their lipid and fatty acid profiles. By controlling their diets, they can change the nutritional value and lipid composition of their gonads, making them more useful as human and animal dietary supplements. However, due to limited data on the distribution and biochemical profiles of native macroalgae, future studies are needed to characterise these resources and optimise their use in nutrition-focused applications. This adaptability demonstrates sea urchins' potential as valuable resources for improving human nutrition and health. Furthermore, given the abundance of these species in Malaysian waters, the findings suggest new avenues for developing alternative lipid sources. Comprehensive research on these species has the potential to lead to the development of functional foods and dietary supplements that take advantage of their rich and diverse fatty acid content. Finally, sea urchins from different locations exhibit distinct fatty acid profiles, likely shaped by varying environmental conditions. The different biochemical compositions found in *D. setosum* and *S. sphaeroides* highlight the importance of geographical and ecological factors in determining the nutritional value of sea urchins, emphasising the need for tailored approaches to their use in human and animal nutrition.

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