

FIRST REPORT OF THE AMINO ACID AND FATTY ACID COMPOSITION OF JELLYFISH (*LOBONEMOIDES ROBUSTUS* STIASNY, 1920) COLLECTED DURING JELLYFISH BLOOM ALONG THE COX'S BAZAR COAST, BANGLADESH

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Received: Dec. 18, 2024. Revised: Feb. 08, 2024. Accepted: Feb. 13, 2024. Published online: Mar. 18, 2024

ABSTRACT. Jellyfish (JF) are essential to marine ecosystems. However, JF that increases rapidly can have negative effects. On 3-4 August 2022, a significant JF (*Lobonemoides robustus* Stiasny, 1920) bloom was observed along Cox's Bazar coast (from Najdirartek to Sabrang) in Bangladesh. The goal of the current investigation was to identify the fatty acids (FAs) and amino acids (AAs) of *L. robustus*. The AAs were determined using liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis, while the FAs were determined using a gas chromatographic system with a flame ionisation detector. The most prevalent

AA was glycine. The most common FA was linoleic acid (C18:3) (0.43%), followed by myristic acid (0.12%), cis-9-oleic acid (0.18%), gamma-linolenic acid (0.24%), and heptadecanoic acid (0.29%). Based on its AA and FA contents, *L. robustus* can be a great candidate for the potentially sustainable manufacture of nutraceutical, cosmeceutical, and biomedical natural products to improve health and well-being. In addition, the edible *L. robustus* could be exported to other countries, thus way it can play a major role in achieving a blue economy.

Keywords: amino acids; bloom; blue economy; fatty acids; jellyfish.



Cite: Islam, Md.T.; Bhuyan, Md.S.; Khan, M.; Kunda, M.; Akter, S.; Kundu, N.K. First report of the amino acid and fatty acid composition of jellyfish (*Lobonemoides robustus* Stiasny, 1920) collected during jellyfish bloom along the Cox's Bazar coast, Bangladesh. *Journal of Applied Life Sciences and Environment* 2024, 57 (1), 107-122. <https://doi.org/10.46909/alse-571126>

INTRODUCTION

The oceans are a nearly untapped reservoir of biochemicals that cover 70% of Earth's surface. They are home to over 194,000 known species of microorganisms, flora, and fauna (Primavera *et al.*, 2019), but between 2011 and 2017, only a tiny number of these marine creatures were utilised, yielding roughly 9,000 unique natural compounds (Romano *et al.*, 2022). Among these marine organisms are JF, a generic term that refers to medusae of the phylum Cnidaria, specifically the class Scyphozoa. Many people value JF for their elegant appearance, but they are also feared for their severe stings. Compared with other taxa, cnidarians have been subjected to relatively little natural product exploitation (Das *et al.*, 2023; Haider *et al.*, 2022).

Globally, JF populations seem to have risen in the last few decades. The overall increase and its causes are unclear because JF abundance is not routinely monitored (Brotz *et al.*, 2012). The natural rhythms of JF blooms may be disrupted by several human-driven activities, including overfishing, pollution, and high temperatures (Haider *et al.*, 2022). This could result in a substantial rise in JF populations in specific coastal areas and major marine ecosystems. Only a small number of bioactive substances have been recovered from oceanic cnidarians; the majority of natural goods are derived from benthic cnidarians. However, there are many significant potential human uses for the natural compounds that pelagic cnidarians synthesise (Fonseca *et al.*, 2023). Substantial scientific data supports the idea that JF are valuable

bioresources for a variety of high-end applications such as human food; feed for cultivated species; and the discovery of untapped bioactive compounds for use in pharmaceutical, cosmetic, nutraceutical, and other biotechnological applications (Das and Patel, 2020; Duarte *et al.*, 2022; Romano *et al.*, 2022).

FAs are the building blocks of lipids. They are divided into saturated fatty acids (SFAs), which lack double bonds between carbons, and unsaturated fatty acids (Ulrich *et al.*, 2011), including monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), classified based on the number and location of double bonds (Monroig *et al.*, 2022). FAs are essential parts of cells and are involved in digestion, signaling pathways, somatic development, and breeding (Yao *et al.*, 2020). Arachidonic acid (ARA) (20:4(ω -6)), eicosapentaenoic acid (EPA) (20:5(ω -3)), and docosahexaenoic acid (DHA) (22:6(ω -3)), also referred to as ω 6 and ω 3 FAs, are three very important PUFAs (Crawford *et al.*, 2023). Despite considerable interspecific variability, PUFAs are often more prevalent than SFAs and MUFAs in the FAs composition of scyphomedusae (Duarte *et al.*, 2022).

AAs have numerous functions, including an important contribution to the creation of hydrogen bonds and the stability of the collagen triple helix structure and thermal behaviour (Xu *et al.*, 2019). It is normal for marine creatures to have low levels of AAs, which causes collagen to denature at lower thermal denaturation temperatures (Barzideh *et al.*, 2014). The essential amino acids (EAAs) are histidine (His), isoleucine (Ile), leucine (Leu), lysine

(Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), valine (Val), and tryptophan (Trp); the conditionally EAAs comprise arginine (Arg), cystine (Cys), tyrosine (Tyr), glycine (Gly), proline (Pro), and serine (Ser); and the non-essential acids (NEAAs) include aspartic acid (Asp), glutamic acid (Glu), and alanine (Ala) (dos Santos, 2013).

During 3–4 August 2022, numerous dead JF of the species *L. robustus* were found along the shore at Cox's Bazar, Bangladesh. They were carried onto the beach at high tide and they stuck in the sand deposit during low tide. According to Kitamura and Omori (2010), *L. robustus* are marketed as 'white-type' JF and are typically seen in huge quantities during certain seasons. They live along the Bay of Bengal (BoB) coast and may be harvested for export or human use. No scientists in Bangladesh have yet researched the biochemical composition of *L. robustus*. Hence, the purpose of the current investigation was to ascertain *L. robustus*'s AA and FA content. This information could increase the export of *L. robustus* and contribute to the blue economy of Bangladesh.

MATERIALS AND METHODS

Study area

The current study was conducted in the following areas: the Sabrang coast, the Patuarteck coast, the Shamlapur coast, the Bangladesh Oceanographic Research Institute (BORI) beach, Inani Beach, the Daria Nagar coast, and Bangladesh Fisheries Development Corporation (BFDC) Ghat. Each site is located along the Cox's Bazar shore, which is part of the BoB coast (Figure 1). Samples were

collected on 3–4 August 2022 during a massive *L. robustus* bloom.

Sample collection and preservation

Using hand gloves, a total of 14 *L. robustus* samples (average weight 30 kg) were collected from each sampling site during the peak JF occurrence. The samples were collected in plastic buckets (due to their large size, only one specimen per bucket) and cleaned onsite with seawater. The samples were transported to BORI's Biological Oceanography Laboratory after being preserved in 10% formalin (Haider *et al.*, 2022). The specimens had minimal damage and were in generally good condition. Along with live specimens, photographs and videos were captured in the field for species identification. As soon as possible after capture, specimens were photographed to capture their natural hue (Haider *et al.*, 2022).

Determination of amino acids (AAs)

Preparation of stock solution and intermediate stock solution

A stock solution of 2500 μM of AAs was prepared in methanol and water (50:50, v/v), sonicated for 1 min, and stored at -4°C . The stock solution was diluted in methanol and water (50:50, v/v) to produce solutions containing 2.0–100 μM of AAs. These solutions were filtered with a 0.232- μm syringe filter (PTFE).

Sample preparation

A 10–100 mg sample was weighed in a 15 ml tube. Then, 2 mL of 6 N HCl was added, and the mixture was incubated at 120°C for hours. Following digestion, the solvent was removed and the sample was resuspended in methanol and water (50:50, v/v; 2 mL).

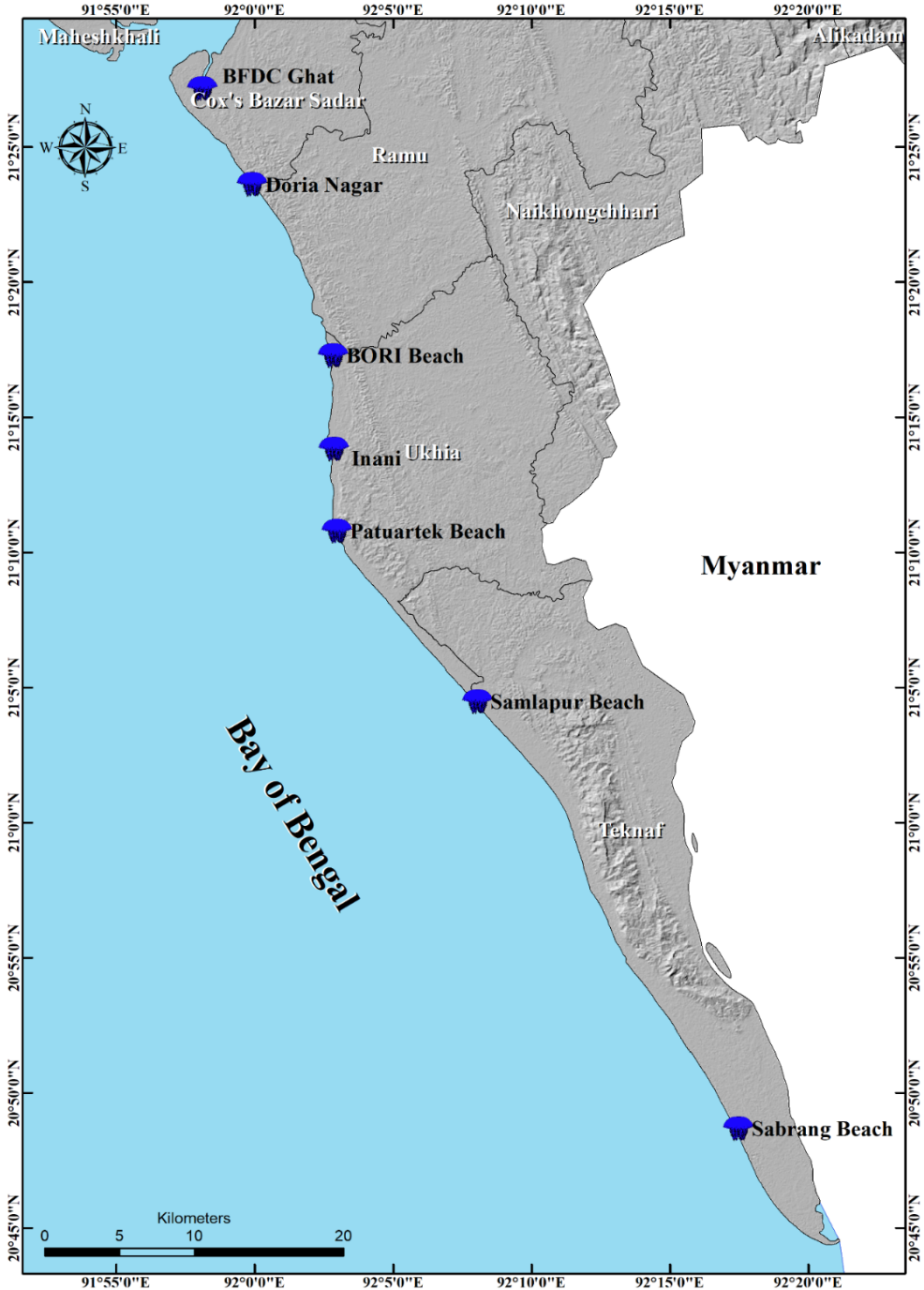


Figure 1 – The map displays the jellyfish collection points (denoted by blue color jellyfish)

Analytical conditions

The liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis used an ultra-fast liquid chromatography system (Shimadzu Corporation, Kyoto, Japan) with binary pumps, an autosampler, an on-line degassing unit, and a column oven connected to a Shimadzu LCMS-8050 triple quadrupole system mass spectrometer, which has an electrospray ionisation (ESI) source. Twenty genetically encoded AAs were subjected to an improved gradient elution method using a novel combined mode.

LC and MS conditions

The AA analysis required an Intra 100 × 3 mm, 3 µm column that was kept at 35°C. The mobile phase comprised solution A (acetonitrile [can], tetrahydrofuran [THF], 25 mM NH₄HCO₃, and HCO₂H, 9:75:16:0.3, v/v) and solution B (can and 100 mM NH₄HCO₃, 20:80, v/v). The elution programme was 0% B (0–3.0 min), 0%–17% B (3.0–9.0 min), 17%–100% B (9.0–16.0 min), 100% B (16.0–22.0 min), and 0% B (22.0 min) at a flow rate of 0.6 mL/min. The chromatographic injection volume was 10 µL, and the AAs were retained for approximately 22 min.

Table 1 presents the MS acquisition conditions, and *Table 2* presents the multiple reaction monitoring (MRM) transition events of the AAs.

Determination of fatty acids (FAs)

A Shimadzu GC 2010 Plus gas chromatographic apparatus with a flame ionisation detector was utilised to identify FAs. One hundred milligrams of C₆H₆O₃ and 2 mL of ethanol were added to a flask containing 100–200 mg of material and thoroughly mixed. Then, 10 mL of 8.3 M

HCl was added and the contents were stirred. The flask was incubated in a water bath heated to 70–80°C for 40 min, with gentle shaking every 10 min. Then, the flask was allowed to cool to ambient temperature (20–25°C). While stirring carefully, enough ethanol was added to fill the flask's bottom reservoir.

After adding 20 mL of diethyl ether and 20 mL of petroleum ether, the flask was centrifuged at 600 rpm for 5 min (if a centrifuge is not available, then the contents should be allowed to settle for at least 1 h until the upper layer is transparent). In a steam bath, the top layer was removed and the ether was evaporated.

After dissolving the residue in 2–3 mL of CHCl₃ and 2–3 mL of (C₂H₅)₂O, the mixture was shifted to a 3-dram glass vial and dried in a water bath at 40°C. Then, 1 mL of toluene and 2 mL of 7% BF₃ methanol were added. The vial was closed with a screwcap top with a teflon/silicone septum. The vial was heated in an oven to 100°C for 45 min, with gentle shaking every 10 min. The vial was cooled to room temperature (20–25°C). After adding 1 mL hexane, 5 mL water, and 1 g Na₂SO₄, the vial was shaken. Then, the upper layer was transferred to a new vial containing 1 g of Na₂SO₄ for gas chromatography.

RESULTS AND DISCUSSION

JF represents a vital part of marine food webs. Although their function as consumers has long been recognised, they are also consumed by a diverse range of species (Schaub *et al.*, 2023).

Amino acids (AAs) in *L. robustus*

In general, *L. robustus* had low EAA levels. The most abundant EAA is Gly

(Figure 2), followed by Glu, Asp, Thr, and Pro. The results of this investigation are consistent with those of Khong *et al.* (2016) and Hsieh *et al.* (2001). According to Khong *et al.* (2016), JF, regardless of the body area, contains roughly 33% EAAs, 46% conditionally EAAs, and 21% NEAAs. Kogovšek *et al.* (2014) reported that in JF, Asp, Lys, Arg, Gly, and Glu are the most abundant AAs per unit of dry mass, accounting for over half of the entire pool of AAs.

Consistent with our findings, Gly is the most prevalent AA in scyphomedusae.

This EAA is one of the major structural units of collagen (Merquiol *et al.*, 2019). Cheng *et al.* (2017) and Kittiphattanabawon *et al.* (2005) showed that Gly is the most prevalent AA in JF collagen. Although there were no statistically significant differences, Wakabayashi *et al.* (2016) reported a higher EAA content in *Aurelia aurita* compared with *Chrysaora pacifica*. Pro, Ala, Leu, Phe, Ile, and Val are other EAAs that are present in good concentrations (Table 3).

Table 1 – The mass spectrometry acquisition conditions

Parameters	State
Run time	22 minutes
Ion polarity	Positive ion mode
Ion source	Atmospheric pressure electrospray ionisation
Capillary voltage (kV)	4.0
Block temperature	400°C
Desolvation line temperature	300°C
CID gas	Argon (270 kPa)
Nebulising gas flow	N ₂ , 1.5 L/min
Drying gas flow	N ₂ , 15.0 L/min
Heating gas flow	10 L/min
Interface temperature	300°C

Table 2 – The multiple reaction monitoring (MRM) transition events of the amino acids

Amino acid	Type	m/z	Retention time (min)	MRM event
Serine	Target	106.10>60.20	1.707	7:MRM(+)
Glycine	Target	76.00>30.00	1.732	16:MRM(+)
Glutamine	Target	147.00>84.10	1.723	6:MRM(+)
Lysine	Target	147.00>84.10	1.731	15:MRM(+)
Aspartic acid	Target	134.10>73.90	1.720	3:MRM(+)
Histidine	Target	156.10>110.10	1.761	11:MRM(+)
Threonine	Target	120.10>74.00	1.758	8:MRM(+)
Alanine	Target	90.10>44.10	1.785	1:MRM(+)
Arginine	Target	175.10>70.10	1.786	2:MRM(+)
Glutamic acid	Target	148.10>84.10	1.804	4:MRM(+)
Proline	Target	116.10>70.10	1.934	17:MRM(+)
Valine	Target	118.20>72.00	2.177	10:MRM(+)
Methionine	Target	150.10>56.10	2.380	13:MRM(+)
Leucine	Target	132.10>86.30	2.979	12:MRM(+)
Isoleucine	Target	132.10>86.30	3.180	12:MRM(+)
Tyrosine	Target	182.10>136.20	3.244	9:MRM(+)
Phenylalanine	Target	166.10>120.10	4.583	14:MRM(+)

Amino acid and fatty acid composition of jellyfish (*Lobonemoides robustus* Stiasny, 1920)

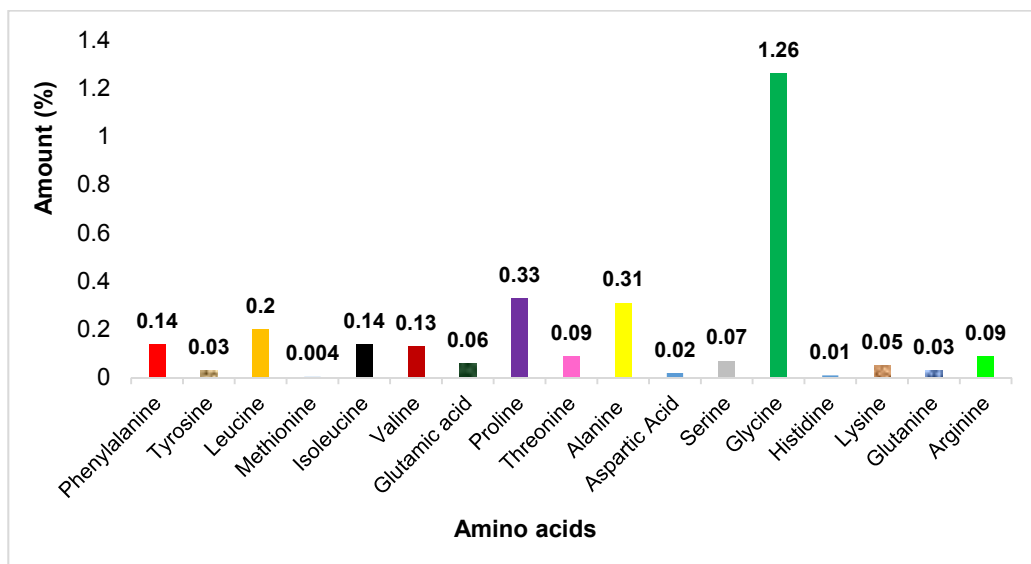


Figure 2 – Amino acid percentages in *Lobonemoides robustus*

Compared with Semaestomeae, Rhizostomeae have more EAAs (Merquiol *et al.*, 2019). Only *Cotylorhiza tuberculata* and *Rhizostoma pulmo* contain significant levels of His; in other scyphomedusae, this EAA is either absent or very low (Table 3). *L. robustus* also contains detectable amounts of Thr, Arg, Ser, Glu, and Lys (Figure 2).

Compared with the AA composition of rat tail collagen, JF had a low Pro content and higher Glu and Ala contents (Derkus *et al.*, 2016). *Rhopilema hispidum* gelatine has notably high Gly (18.90%), Pro (8.15%), and hydroxyproline (13.93%) contents (Table 3) (Cho *et al.*, 2014). *Chrysaora* sp. has a low concentration of Pro and hydroxyproline (Barzideh *et al.*, 2014). According to De Rinaldis *et al.* (2021), the most prevalent AAs in *Cassiopea andromeda* are Glu, Gln, and Gly. This species contains 15.68 g of these AAs per 100 g lyophilised sample, more than twice as much as *R. pulmo* (6.1 ± 0.09 g

per 100 g lyophilised sample) and *Pelagia noctiluca* (8.1 ± 0.3 g per 100 g lyophilised sample) samples analysed in parallel. De Rinaldis *et al.* (2021) also reported high levels of Ala and taurine in *C. andromeda*, namely 0.96 g per 100 g dry weight. The contents of the main AAs of wild JF gonad and cultured JF gonad – Glu, Lys, Gly, Asp, and Leu – are 51.47% and 52.52% of the total AA content, respectively. Asp and Glu are often present in enzyme-active sites and are crucial for preserving the solubility and ionic nature of proteins (Yu *et al.*, 2014). Stabili *et al.* (2018) found free AAs in a gonadal extract from *R. pulmo*. The ovaries of this species may provide an abundant supply of AAs for pharmacological and nutraceutical purposes. Additionally, the ovaries may provide proteins needed for the creation of novel nutritional supplements intended to sustain fish.

Amino acid and fatty acid composition of jellyfish (*Lobonemoides robustus* Stiasny, 1920)

Species	<i>Lobonemoides robustus</i>		<i>Aurelia sp.</i>		<i>Rhizostoma pulmo</i>		<i>Rhopilema hispidum</i>		<i>Rhopilema hispidum</i>		<i>Rhizostoma pulmo</i>		<i>Pelagia noctiluca andromeda</i>		<i>Cassiopea coerulea</i>		<i>Aurelia esculentum</i>		<i>Rhopilema esculentum</i>		
	W***	W***	W	W	W***	W	W***	W	W***	W	W***	W	W***	W	W***	W	W***	W	W***	W	
Amino acids																					
Phenylalanine	0.14	1.0	40	19.3	2.16	2.16	9.3	1.7	4.58	8	2.73	26.0									
Tyrosine	0.03	0.8	16	4.50	1.72	7.6	1.5	2.74	5	1.85	23.8										
Leucine	0.20	3.5	38	22.2	3.79	9.1	2.3	5.84	33	4.15	43.5										
Methionine	0.004	0.2	6	-	2.81	4.6	-	1.38	6	0.98	20.9										
Isoleucine	0.14	2.3	75	10.7	2.99	5.5	1.2	3.52	24	2.79	26.5										
Valine	0.13	2.8	62	19.4	2.24	4.9	2.1	4.91	30	2.97	62.9										
Glutamic acid	0.06	-	88	60.9	10.4	10.5	4.8	93	7.43	77.9											
Proline	0.33	7.8	113	81.5	6.93	3.9	3.0	6.04	78	1.41	119.3										
Threonine	0.09	2.7	26	19.9	4.30	5.0	2.1	3.32	31	2.32	32.0										
Alanine	0.31	7.6	99	68.8	6.11	3.9	2.6	5.98	87	2.87	85.1										
Aspartic acid	0.02	8.3	69	54.6	6.79	3.2	-	-	88	4.23	77.7										
Serine	0.07	3.8	-	25.3	3.44	6.7	1.5	5.07	44	2.56	96.8										
Glycine	1.26	32.2	301	189.0	19.2	5.3	10.5	10.73	321	4.25	184.1										
Histidine	0.01	1.0	-	6.2	3.28	5.6	0.4	2.13	2	1.14	12.9										
Lysine	0.05	2.8	34	24.9	3.22	6.9	4.4	6.62	35	4.65	32.1										
Glutamine	0.03	9.3	-	-	-	5.0	-	-	-	-	-										
Arginine	0.09	6.	-	56.2	8.85	2.0	2.7	6.34	48	1.68	25.3										
References	Present study	Miki et al., 2015	Derkus et al., 2016	Cho et al., 2014	Ab Aziz et al., 2020	De Domenico et al., 2021	Kogovšek et al., 2014	De Rinaldis et al., 2021	Rigby and Hafey, 1972	Yu et al., 2014	Zhuang et al., 2009										

*Values in mg of AA per g of dry matter. **Values in mg of AA per g of protein. ***Values in percentages. ****Values in µg of AA per mg of dry biomass. *****Values in mg/100 mg of dry biomass. *****Values in g/kg dry biomass. B, bell; OA, oral arms; M, mesogloea; G, gonad; W, whole body.

Fatty acids (FAs) in *L. robustus*

FAs are components of membranes and cell structures, but they also accumulate as energy storage units in plants and animals. They can be absorbed from food or biosynthesised by the organism (Saha and Pathak, 2021). FAs do not decompose during digestion, in contrast to other complex compounds; rather, they stay mostly unaltered or barely altered. Because they often do not change as reservoirs during normal cell metabolism (Elsamadony et al., 2021), they are regarded as traditional indicators that are used in environmental research to clarify the relationships between organisms in the food chain and to ascertain the movement of organic matter from lower trophic levels to higher trophic levels (De Troch et al., 2012). In the present study, C18:3 was the most prevalent FA (0.43%) in *L. robustus*, followed by heptadecanoic acid (0.29%), gamma-linolenic acid (0.24%), cis-9-oleic acid (0.18%), decanoic acid (0.13%), and myristic acid (0.12%) (Figure 3).

According to De Renaldis et al. (2021), PUFAs and SFAs make up roughly 48% and 44% of all FAs in *C. andromeda*, respectively, but MUFAs make up only 8% of all FAs. In terms of MUFA content, the hydroalcoholic extract and the entire JF extract have similar levels of isooleic acid, oleic acid, and palmitoleic acid. Svetashev (2019) recorded different types of omega-3 FAs in *A. aurita* and *Rhopilema esculentum*, which are the principal PUFAs; *R. esculentum* contains 1.6% of C26 PUFAs.

Ying et al. (2012) reported high 20:4(ω -6) concentrations (>10%) and

ratios of 20:5(ω -3)/20:6(ω -3) > 1 in JF. The month–diameter interaction has a substantial impact on the FA profile of *Aurelia labiata*, meaning that changes in the FA profile with diameter vary from month to month (Schaub et al., 2023). According to Wakabatake et al. (2016), of the five essential fatty acids, anandamide and 20:6(ω -3) are more abundant in *C. pacifica* than in *A. aurita*, while 20:5(ω -3) is more abundant in *A. aurita* than in *C. pacifica*. According to Leone et al. (2015), the zooxanthellate JF *Cotylorhiza tuberculata* has a considerably higher presence of ω 3 and ω 6 PUFAs. With a high percentage (62.7%) of unsaturated FAs, the FA profile of *A. aurita* from the Atlantic Ocean differs significantly from that of *A. aurita* from the Aegean Sea (Kariotoglou and Mastronicolis, 2001). *A. aurita*'s FA profile exhibits notable seasonal fluctuation, with mature medusae having the highest FA levels. Furthermore, the moon jelly contains multiple critical FAs – 20:4(ω -6), 20:5(ω -3), and 20:6(ω -3) – likely to support its essential physiological activities (Stenvers et al., 2020).

The majority of FAs in *Catostylus tagi* are PUFAs, followed by MUFAs and SFAs. According to Moris et al. (2009), there is a considerable increase in the concentration of ARA, EPA, 20:4 ω 6, DHA (about 32%) in the oral arms and umbrellas of JF (mostly 20:5 ω 3). There have been similar findings in *Rhizostoma luteum* (Prieto et al., 2018), where almost half of the FAs are PUFAs, mainly ω 3 C18:3, essential ω 6 C18:2, and ω 6 C20:4 acids. Stabili et al. (2018) described the presence of PUFAs, diunsaturated fatty acids (DUFAs), MUFAs, and SFAs in the gonads of *R. pulmo*.

Amino acid and fatty acid composition of jellyfish (*Lobonemoides robustus* Stiasny, 1920)

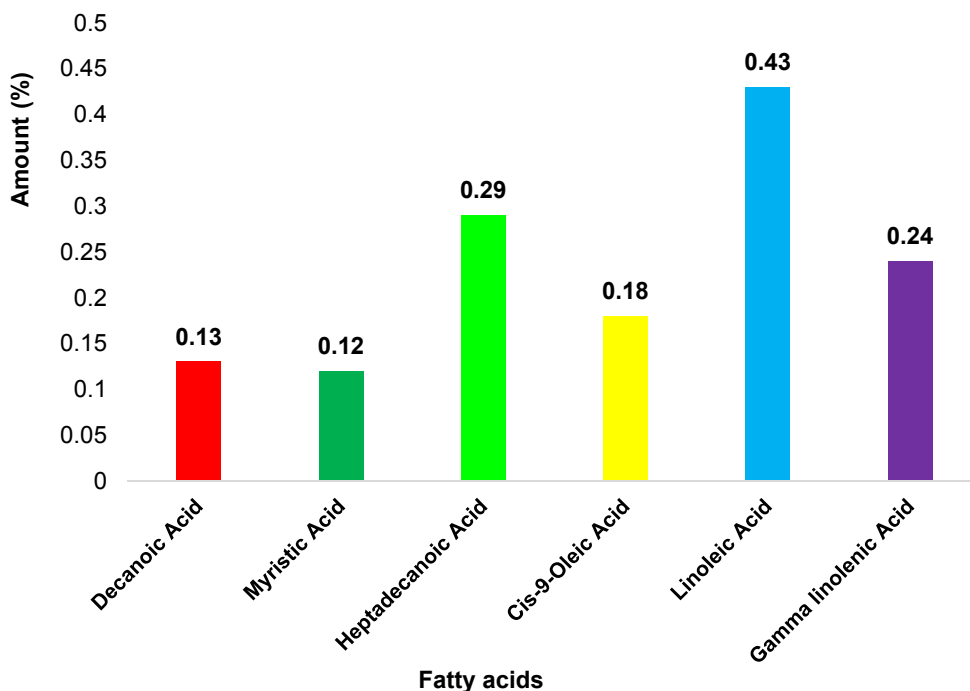


Figure 3 – Fatty acid contents in *Lobonemoides robustus*

While the overall fatty acid concentration of *P. noctiluca* changes according to the body area, MUFAs and PUFAs make up 15% and 14%–19% of the total, respectively, and there are no sex differences (Costa *et al.*, 2019). In comparison, Leone *et al.* (2015) discovered that in JF, SFAs (55%–70%) dominate, followed by PUFAs (25%–30%) and MUFAs (4%–15%).

The gonads of *R. pulmo* have ω 3 PUFAs, primarily DHA and EPA, which suggests that these molecules could be extracted from them and used in the pharmaceutical industry (Stabili *et al.*, 2018). DHA and EPA have anti-inflammatory and antioxidant properties and may be used in treatment plans for mental health issues and memory

impairments brought on by neuroinflammation (Apetz *et al.*, 2014). Additionally, considering that fish diets are typically supplemented with extra EPA and DHA, the gonads of *R. pulmo* may provide these necessary FAs that could be extracted and then added to the fish feed (Stabili *et al.*, 2018).

According to Khong *et al.* (2016), EAAs, conditionally EAAs, and non-EAAs account for 33%, 46%, and 21% of the total amino acids (TAAs), respectively, in JF species. According to Yu *et al.* (2014), the TAAs in *R. esculentum* gonads are made up of 40.70–42.89% EAAs, 47.39%–50.12% taste AAs, and 66.55%–66.92% medicinal AAs. According to Leone *et al.* (2015), the proportion of EAAs of the TAA

content in *Aurelia* sp., *R. pulmo*, and *C. tuberculata* is 31.4%, 50.8%, and 53.6%, respectively.

These findings suggest that JF may find usage as a functional food and dietary supplement (Raposo *et al.*, 2022).

CONCLUSIONS

The search for substitute sources of bioactive chemicals to take the place of overfished resources is a pressing need for modern society. JF are important sources of AAs and FAs. In the present study, we found that *L. robustus* is rich in Gly. The most common FAs are linoleic acid, myristic acid, cis-9-oleic acid, gamma-linolenic acid, and heptadecanoic acid. Our data indicate that *L. robustus* could be a sustainable source of AAs and FAs for use in manufacturing natural nutraceutical, cosmeceutical, and biomedical products. Moreover, in Southeast Asia, *L. robustus* is commonly used for food. The commercially valuable *L. robustus* could be exported to other countries and contribute to a blue economy.

Author Contributions: Conceptualisation, Methodology, Funding acquisition, Supervision: MTI; Conceptualisation, Methodology, Statistical analysis, Writing - original draft: MSB; Literature survey, SA; Analysis, review and editing, MK, NKK; Review and editing: MSB, MK, SA. All authors have read and agreed to the published version of the manuscript.

Data availability: This study's findings are supported by the data presented in the report.

Acknowledgments: The authors are grateful to the Bangladesh Oceanographic Research Institute for providing this research facility. Authors also express heartfelt thanks to Bangladesh Reference Institute for Chemical

Measurements (BRiCM) for providing chemical analysis facilities.

Conflicts of Interest: The authors declare no competing interests.

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Academic Editor: Dr. Isabela Maria Simion

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